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MULTI-AUTHOR REVIEW

Remarkable evolutionary relatedness among the enzymes and proteins from the α-amylase family

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Abstract The α-amylase is a ubiquitous starch hydrolase catalyzing the cleavage of the α-1,4-glucosidic bonds in an endo-fashion. Various α-amylases originating from different taxonomic sources may differ from each other significantly in their exact substrate preference and product profile. Moreover, it also seems to be clear that at least two different amino acid sequences utilizing two different catalytic machineries have evolved to execute the same α-amylolytic specificity. The two have been classified in the Cabohydrate-Active enZyme database, the CAZy, in the glycoside hydrolase (GH) families GH13 and GH57. While the former and the larger α-amylase family GH13 evidently forms the clan GH-H with the families GH70 and GH77, the latter and the smaller α-amylase family GH57 has only been predicted to maybe define a future clan with the family GH119. Sequences and several tens of enzyme specificities found throughout all three kingdoms in many taxa provide an interesting material for evolutionarily oriented studies that have demonstrated remarkable observations. This review emphasizes just the three of them: (1) a close relatedness between the plant and archaeal α-amylases from the family GH13; (2) a common ancestry in the family GH13 of animal heavy chains of heteromeric amino acid transporter rBAT and 4F2 with the microbial α-glucosidases; and (3) the unique sequence features in the primary structures of amylomaltases from the genus *Borrelia* from the family GH77. Although the three examples cannot represent an exhaustive list of exceptional topics worth to be interested in, they may demonstrate the importance these enzymes possess in the overall scientific context.

Keywords α-Amylase family GH13 · Plant and archaeal α-amylases · Heavy-chains of rBAT and 4F2 proteins · Family GH77 amylomaltases of borrelian origin · Evolutionary relatedness

Abbreviations

CAZy	Carbohydrate-Active enZymes
CBM	Carbohydrate-binding module
CGTase	Cyclodextrin glucanotransferase
CSR	Conserved sequence region
DPE	Disproportionating enzyme
GH	Glycoside hydrolase
hcHATs	Heavy chains of the heteromeric amino acid transporters

Introduction

α-Amylase (EC 3.2.1.1) represents probably the best known and most deeply studied amylolytic enzyme [1–6]. It catalyzes the hydrolytic cleavage of the α-1,4-glucosidic linkages in starch and related α-glucans in an endo-fashion employing the retaining reaction mechanism. Its evolution started to attract the serious scientific interest approximately 25 years ago when it became clear that there is a group of starch hydrolases and related enzymes possessing closely related functions within the frame of homologous amino acid sequences [7–9]. It was, for example, the

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enzyme cyclodextrin glucanotransferase (CGTase; EC 2.4.1.19) sharing with the α-amylase the first step of the catalyzed reaction, which in the CGTase proceeds not with the molecule of water like in the α-amylase but with a molecule of a saccharide to be transferred [10]. For CGTases, it was thus revealed that, despite obvious differences between an α-amylase and a CGTase, they exhibit interesting sequence similarities with α-amylases [11] that previously might even complicate the correct assignment of the α-amylase/CGTase specificity [12–14] as well as the answer concerning their evolutionary history [15]. The other closely related enzyme was the so-called maltohexaohydrolase (EC 3.2.1.98), i.e., the exo-amylase producing maltohexaose [16]. The discovery of a completely novel enzyme, the neopullulanase (EC 3.2.1.135) in 1989 [17], able to perform both hydrolysis and transglycosylation of both the α-1,4- and α-1,6-glucosidic linkages [18–20], can be considered as a milestone in creating the overall view of the group of sequentially and functionally related amylolytic enzymes. Thus, also from the evolutionary point of view, a new enzyme family, named according to its leading member as the α-amylase family, has been established [20–24].

Interestingly, from the very beginning the α-amylase family has offered various surprises or at least the phenomena deserving a special attention. For example, even the α-amylases originating from various sources (roughly from *Bacteria*, *Archaea* and *Eucarya*) may differ from each other quite substantially in their substrate preference and/or product profile although they are still active towards and produce various α-glucans [1]. Some of them have been confirmed to be able to transglycosylate to a small, limited extent, e.g., the α-amylase from *Pseudoalteromonas haloplanktis* [25].

Historically, the establishment of the four conserved sequence regions (CSRs) among the primary structures of α-amylases in 1986 [26] has played an unique role, especially as one of the requirements for an enzyme to become a member of the family [20]. Soon after the four CSRs were observed in other enzymes (members) of the developing α-amylase family [8, 9, 20, 22] and completed by the three additional CSRs [27–29]. Currently, it has been recommended to use as many as possible of the seven defined CSRs [30, 31] to best characterize a protein as a member of the α-amylase family, mainly if there are any doubts concerning the exact enzyme specificity [1, 32].

Almost simultaneously with the gradual appearance and final definition of the α-amylase enzyme family in the literature early in 1990s [7–9, 20–24], a pioneering study was published [33] delivering the newly developed concept of classification of glycoside hydrolases (GHs) to sequence-based families, placing the group of enzymes known already at that time as the α-amylase family into the family GH13. After a few published updates [34, 35], the increasing system of GH families has been incorporated into the current web-server database CAZy (Carbohydrate-Active enzymes) [36], the α-amylase enzyme specificity being potentially found also in families GH57, GH119 and even GH126 [1, 37]. Nevertheless, the family GH13 represents the main α-amylase family [1] and with approximately 31,500 sequences and more than 30 different enzyme specificities (Table 1) it belongs—among 129 GH functional families created until now—to the largest GH families at all [37]. On a higher level of hierarchy, it forms the clan GH-H together with families GH70 and GH77 [31, 37, 38]; the former of these two families covers various glucansucrases with typically circularly permuted primary structure with respect to that seen in the main family GH13 [39–41], whereas the latter is the monospecific family of 4-α-glucanotransferases known also as amylomaltase and disproportionating enzyme (DPE) in prokaryotes and eukaryotes, respectively [42–44]. On the lower level of hierarchy, in 2006 the family GH13, reflecting also the previous efforts [45], was officially divided into 35 subfamilies by the CAZy curators [46]; currently the number of GH13 subfamilies has reached 41 [37, 47, 48] and it will very probably raise in the future [49].

As far as the family GH13 is concerned in its entirety, it has become evident that the same enzyme specificity (e.g., the α-amylase or pullulanase) may exist in several separated groups or GH13 subfamilies, but on the other hand some other specificities (e.g., oligo-1,6-glucosidase, α-glucosidase and dextran glucosidase) may exist altogether within a single group or GH13 subfamily. The attractiveness of the α-amylase clan GH-H for scientists has been strengthened also by classifying into the family the heavy-chains of heteromeric amino acid transporters, known as rBAT protein and 4F2 antigen (i.e., neither amylolytic enzymes, nor any enzymes at all) that, being typically of animal origin, are evolutionary related to bacterial α-glucosidases [50]. The present review aims to deliver the updated view of a few perhaps most remarkable evolutionary relatedness observed within the α-amylase family.

The family GH13 α-amylases from plants and archaea

While the first amino acid sequences of the α-amylases from *Archaea* have become available only at the end of 1990s [51–55], the primary structures of their first plant counterparts were determined at least 10–15 years earlier [56–60]. Moreover, the three-dimensional structure of the high p*I* isozyme of the barley α-amylase AMY-2 was solved a few years before [61] the first archaeal α-amylase

Table 1 Members of the α-amylase GH families

Enzyme class	Enzyme	EC no.	GH family	GH13 subfamily
Hydrolases	α-Amylase	3.2.1.1	13, 57, 119, 126	1, 5, 6, 7, 15, 20, 24, 27, 28, 32, 36, 37
	Oligo-1,6-glucosidase	3.2.1.10	13	23, 31
	α-Glucosidase	3.2.1.20	13	17, 21, 23, 30, 31, 40
	α-Galactosidase	3.2.1.22	57	
	Pullulanase	3.2.1.41	13	12, 13, 14
	Amylopullulanase	3.2.1.1/41	13, 57	12, 14, 39
	Cyclomaltodextrinase	3.2.1.54	13, 57	20, 36
	Maltotetraose-forming amylase	3.2.1.60	13	19
	Isoamylase	3.2.1.68	13	11, 14
	Isoamylase/4-α-glucanotransferase	3.2.1.68/2.4.1.25	13	11
	Dextran glucosidase	3.2.1.70	13	31
	Trehalose 6-phosphate hydrolase	3.2.1.93	13	29
	Maltohexaose-forming amylase	3.2.1.98	13	5, 19
	Maltotriose-forming amylase	3.2.1.116	13	2, 32
	Maltogenic amylase	3.2.1.133	13, 57	2, 20
	Neopullulanase	3.2.1.135	13	20
	Maltooligosyltrehalose threhalohydrolase	3.2.1.141	13	10
	Sucrose hydrolase	3.2.1.–	13	4
	Maltopentaose-forming amylase	3.2.1.–	13	5
	Glycogen degrading enzyme	3.2.1.–	13	12
	Cyclic α-maltosyl-1,6-maltose hydrolase	3.2.1.–	13	20
Transferases	Amylosucrase	2.4.1.4	13	4
	Dextransucrase	2.4.1.5	70	
	Sucrose phosphorylase	2.4.1.7	13	18
	Glucan branching enzyme	2.4.1.18	13, 57	8, 9
	Cyclodextrin glucanotransferase	2.4.1.19	13	2
	4-α-Glucanotransferase	2.4.1.25	13, 57, 77	
	Glucan debranching enzyme	2.4.1.25/3.2.1.33	13	11, 20, 25
	Alternansucrase	2.4.1.140	70	
	α-1,3-Glucan synthase	2.4.1.183	13	22
	α-1,4-Glucan: phosphate α-maltosyltransferase	2.4.99.16	13	3
	Sucrose-6-phosphate phosphorylase	2.4.1.–	13	18
	α-Transglucosidase	2.4.1.–	13	23
	Isocyclomaltooligosaccharide glucanotransferase	2.4.1.–	13	
	α-4,6-Glucanotransferase	2.4.1.–	70	
	Reuteran sucrase	2.4.1.–	70	
	α-1,6/α-1,2-Branching glucansucrase	2.4.1.–	70	
Isomerases	Isomaltulose synthase	5.4.99.11	13	31
	Maltooligosyltrehalose synthase	5.4.99.15	13	26
	Trehalose synthase	5.4.99.16	13	16, 33
HAT proteins	hc-rBAT protein	–	13	35
	4F2hc antigen	–	13	34

sequence from *Pyrococcus furiosus* was announced [51, 52]. The barley α-amylase AMY-2 structure [61], solved later also as a complex with acarbose [62], was at that time only the fourth known α-amylase tertiary structure [30], in addition to those from *Aspergillus oryzae* [63, 64], *Aspergillus niger* [65, 66] and *pig pancreas* [67, 68]. At present, with regard to plant and archaeal α-amylases, tertiary structures have been solved and published also for

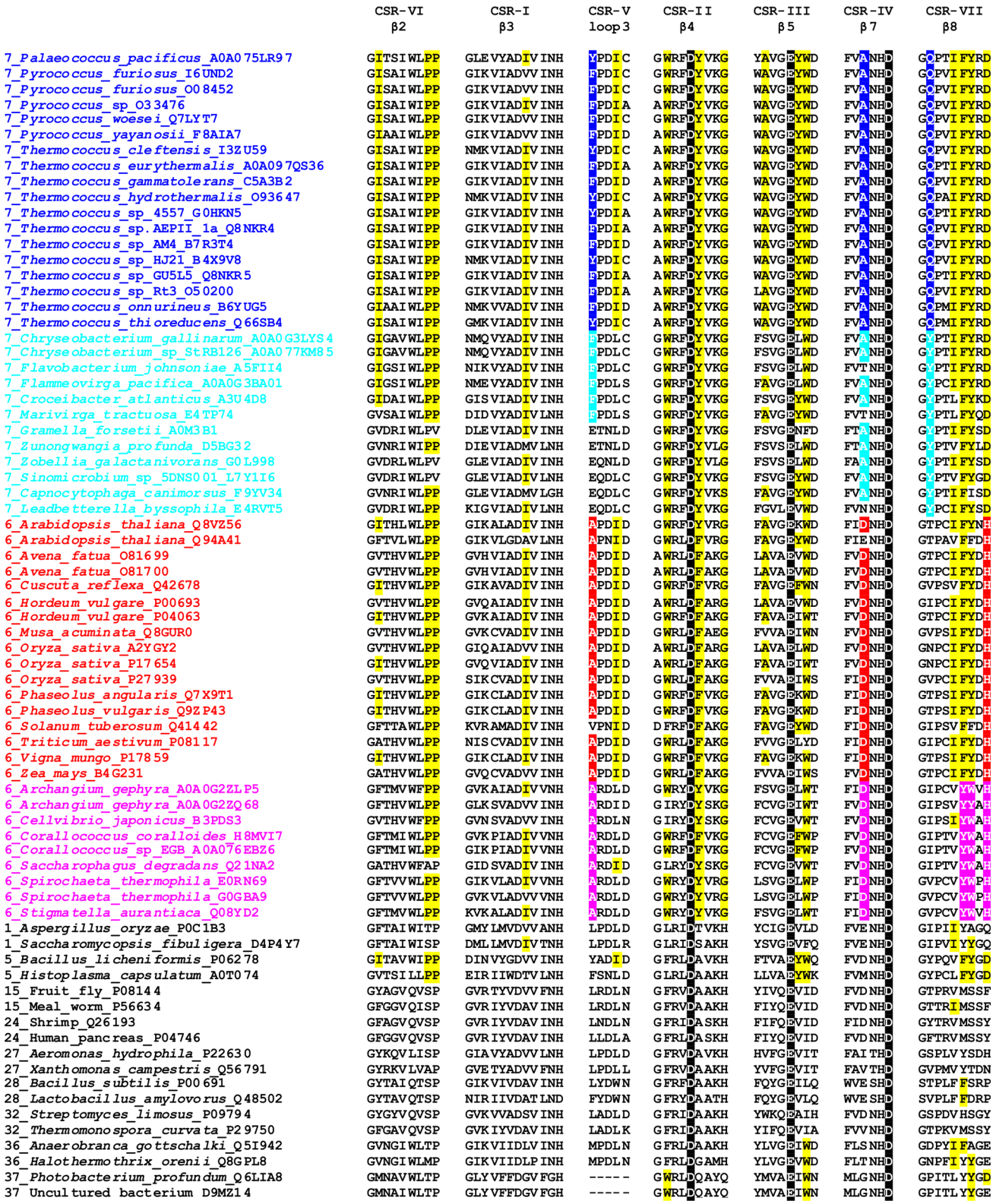
the low p*I* isozyme of the barley α-amylase AMY-1 [69, 70], the α-amylase from rice [71, 72] as well as the α-amylase from *Pyrococcus woesei* [73].

From the evolutionary point of view, before the α-amylases from archaea were known, plant α-amylases formed a compact cluster neighboring with the liquefying α-amylases from bacilli [28]. As documented by various evolutionarily oriented studies [73–78] this has remained true until now with a significant upgrading of the original picture [28] illustrating that the cluster of archaeal α-amylases shares the branch with that of plant counterparts [53, 79]. According to the CAZy nomenclature [46], the α-amylases from plants and archaea have been assigned the subfamily number GH13_6 and GH13_7, respectively; the bacterial homologues representing mostly liquefying α-amylases from bacilli being classified within the subfamily GH13_5. It should be pointed out that all these three subfamilies of α-amylases are mutually very closely related and the exact branching pattern among them in the evolutionary tree may depend on the aligned segment of their amino acid sequences (i.e., just CSRs, the catalytic domain or the entire sequence, etc.) [76].

The pronounced relatedness between the plant and archaeal α-amylases is remarkable not only due to their long taxonomical distance (eukaryotic plants and prokaryotic archaea) but also when differences in their thermostability (or the temperature optimal for their enzymatic activity) are considered [1]. The close positions in the evolutionary tree obviously reflect the similarities throughout their amino acid sequences, especially within the CSRs (Fig. 1) described first in detail in 1999 [79]. While the sequence similarities shared by both plant and archaeal α-amylases represent something that distinguishes both subfamilies from remaining GH13 α-amylases, there have to be some additional sequence features that are unique for each group, i.e., that discriminate the two subfamilies from each other, just these unique sequence features should be used in the efforts aimed at identifying the factors that could be responsible for the hyperthermostability of archaeal α-amylases (or, on the other hand, the low thermostability of the α-amylases from plants). Thus, for example, the two residues deserving the attention in the α-amylase from *Thermococcus hydrothermalis* could be as follows (Fig. 1): (1) the first position of the CSR-V, i.e., Tyr184Ala (184_YPDIC in the *T. hydrothermalis* α-amylase versus 146_APDID in the barley high p*I* α-amylase isozyme), because among the archaea it is occupied only by either phenylalanine or tyrosine (i.e., an aromatic residue); and (2) the third position of the CSR-IV, i.e., Ala308Asp (306_FVANHD versus 284_FVDNHD), because there is invariantly conserved alanine among the archaea in comparison with invariant aspartic acid in plants.

Recently, some hypothetical bacterial (i.e., not archaeal) α-amylases from genome sequencing projects were assigned to the "archaeal" subfamily GH13_7 [1, 37]. Currently, in the CAZy database, all these belong to the phylum Flavobacteria [37]. It is, however, worth mentioning that even a simple BLAST search [80] using the flavobacterial GH13_7 α-amylase from *Sinomicrobium* sp. 5DNS001 [81] as a query retrieves hypothetical bacterial α-amylases with GH13_7 sequence features, but not belonging to Flavobacteria. Anyhow, the moderately thermostable *Sinomicrobium* sp. 5DNS001 α-amylase [81], clearly homologous to its archaeal hyperthermostable counterparts (Fig. 2), may add to our understanding of the rules that have governed the evolution of plant, (flavo)-bacterial and archaeal α-amylases and the factors that are responsible for their thermostability differences [76, 79, 81–83]. In analogy with the above-mentioned features in which the plant and archaeal α-amylases differ from each other, it makes sense to try to identify the features in the amino acid sequences of (flavo)-bacterial α-amylases that are well conserved but simultaneously well discriminating them from their archaeal counterparts (Fig. 1). One example is the second position of the CSR-VII, i.e., Tyr363Gln (362_GYPTVFYGD in the *Sinomicrobium* sp. 5DNS001 α-amylase versus 330_GQPAIFYRD in the *T. hydrothermalis* α-amylase isozyme), because both the tyrosine and the glutamine are invariantly conserved among the respective groups of bacterial and archaeal α-amylases. Thus, a silico analysis as detailed as possible focused on comparison of amino acid sequences is of special importance, especially if, at the tertiary structure level the individual representatives of both GH13_6 and GH13_7 subfamilies look very similar and obviously without any substantial differences (Fig. 2).

It is worth mentioning that, in addition to archaeal α-amylases from the class Thermococci (namely the two genera *Pyrococcus* and *Thermococcus*) classified within the subfamily GH13_7, there are several potential α-amylases produced by halophilic archaea (the class Halobacteria) deserving the attention that, however, until now have not been assigned any GH13 subfamily [37]. Some of them simply may not be true α-amylases, e.g., those from *Natronococcus amylolyticus* [84, 85] and *Haloarcula japonica* [86] producing mainly maltotriose and maltose, respectively. For others, the ambiguity arises from the fact that the studied sequences originate from genome projects [87], i.e., they are still hypothetical amylolytic enzymes. Interestingly, for the sequence of the halophilic α-amylase from *Haloarcula hispanica* [88] the fold recognition server Phyre-2 [89] revealed the *Bacillus stearothermophilus* α-amylase from the subfamily GH13_5 [90] as the best structural template.


CSR-VI β2
CSR-I β3
CSR-V loop3
CSR-II β4
CSR-III β5
CSR-IV β7
CSR-VII β8
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7_Pyrococcus_furiosus_I6UND2
7_Pyrococcus_furiosus_O08452
7_Pyrococcus_sp_O33476
7_Pyrococcus_woesei_Q7LYT7
7_Pyrococcus_yayanosii_F8AIA7
7_Thermococcus_cleftensis_I3ZU59
7_Thermococcus_eurythermalis_A0A097QS36
7_Thermococcus_gammatolerans_C5A3B2
7_Thermococcus_hydrothermalis_O93647
7_Thermococcus_sp_4557_G0HKN5
7_Thermococcus_sp._AEPII_1a_Q8NKR4
7_Thermococcus_sp_AM4_B7R3T4
7_Thermococcus_sp_HJ21_B4X9V8
7_Thermococcus_sp_GU5L5_Q8NKR5
7_Thermococcus_sp_Rt3_O50200
7_Thermococcus_onnurineus_B6YUG5
7_Thermococcus_thioreducens_Q66SB4
7_Chryseobacterium_gallinarum_A0A0G3LYS4
7_Chryseobacterium_sp_StRB126_A0A077KM85
7_Flavobacterium_johnsoniae_A5FII4
7_Flammeovirga_pacifica_A0A0G3BA01
7_Croceibacter_atlanticus_A3U4D8
7_Marivirga_tractuosa_E4TP74
7_Gramella_forsetii_A0M3B1
7_Zunongwangia_profunda_D5BG32
7_Zobellia_galactanivorans_G0L998
7_Sinomicrobium_sp_5DNS001_L7Y1I6
7_Capnocytophaga_canimorsus_F9YV34
7_Leadbetterella_byssophila_E4RVT5
6_Arabidopsis_thaliana_Q8VZ56
6_Arabidopsis_thaliana_Q94A41
6_Avena_fatua_O81699
6_Avena_fatua_O81700
6_Cuscuta_reflexa_Q42678
6_Hordeum_vulgare_P00693
6_Hordeum_vulgare_P04063
6_Musa_acuminata_Q8GUR0
6_Oryza_sativa_A2YGY2
6_Oryza_sativa_P17654
6_Oryza_sativa_P27939
6_Phaseolus_angularis_Q7X9T1
6_Phaseolus_vulgaris_Q9ZP43
6_Solanum_tuberosum_Q41442
6_Triticum_aestivum_P08117
6_Vigna_mungo_P17859
6_Zea_mays_B4G231
6_Archangium_gephyra_A0A0G2ZLP5
6_Archangium_gephyra_A0A0G2ZQ68
6_Cellvibrio_japonicus_B3PDS3
6_Corallococcus_coralloides_H8MVI7
6_Corallococcus_sp_EGB_A0A076EBZ6
6_Saccharophagus_degradans_Q21NA2
6_Spirochaeta_thermophila_E0RN69
6_Spirochaeta_thermophila_G0GBA9
6_Stigmatella_aurantiaca_Q08YD2
1_Aspergillus_oryzae_P0C1B3
1_Saccharomycopsis_fibuligera_D4P4Y7
5_Bacillus_licheniformis_P06278
5_Histoplasma_capsulatum_A0T074
15_Fruit_fly_P08144
15_Meal_worm_P56634
24_Shrimp_Q26193
24_Human_pancreas_P04746
27_Aeromonas_hydrophila_P22630
27_Xanthomonas_campestris_Q56791
28_Bacillus_subtilis_P00691
28_Lactobacillus_amylovorus_Q48502
32_Streptomyces_limosus_P09794
32_Thermomonospora_curvata_P29750
36_Anaerobranca_gottschalki_Q5I942
36_Halothermothrix_orenii_Q8GPL8
37_Photobacterium_profundum_Q6LIA8
37 Uncultured bacterium D9MZ14

◀**Fig. 1** Comparison of seven CSRs of the family GH13 α-amylases with focus on the subfamilies GH13_6 and GH13_7. These CSRs cover mostly individual β-strands of the catalytic TIM-barrel domain [32]. Each α-amylase in the list is characterized by the GH13 subfamily number [46], the source of origin (the organism) and the accession number from the UniProt database [221]. The α-amylases from the subfamilies GH13_6 and GH13_7 were collected from the actual CAZy database [37], whereas the set of representative α-amylases from the GH13 remaining subfamilies was prepared according to previous in silico studies [74–79]. The catalytic triad is signified by *black*-and-*white* inversion. Sequence features characteristic of the GH13_7 archaeal α-amylases are highlighted in *yellow*. The features typical specifically for each group of α-amylases within the two GH13 subfamilies, i.e., (1) archaea and (flavo)-bacteria within GH13_7; and (2) plants and bacteria within GH13_6, which may discriminate the individual groups from each other are emphasized by respective colors

Animal transport proteins rBAT and 4F2 versus the microbial GH13 α-glucosidases

A sequence resemblance of heavy-chains of the heteromeric amino acid transporters (hcHATs), the rBAT protein and the 4F2 antigen, to α-glucosidases from the α-amylase family was originally revealed already in 1992 in two independent studies [91, 92]. However, for a few subsequent years it was, in fact, not seriously taken into account by researchers engaged in the enzymology of the α-amylase family. The main reason was that the hcHAT proteins obviously do not obey all the criteria defined for an enzyme to be a member of the α-amylase family [93, 94]. The eventual evolutionary relatedness between the hcHATs and the α-amylase family enzymes was first studied in a detail in 1997 [95] when the attention was paid mainly to domain B, protruding typically in the family GH13 out of the catalytic $(\beta/\alpha)_8$-barrel (simply called the TIM-barrel [1, 30, 31]) between the strand β3 and the helix α3 [61, 63, 67, 72, 96–104], which in the rBAT protein is present, whereas the 4F2 antigen lacks it (Fig. 3a). Both the rBAT protein and 4F2 antigen belong to the so-called solute carrier families SLC7 (the light chain) and SLC3 (the heavy chain); the respective hetero-chains being connected by a disulphide bridge [105–109]. Both groups have been accepted as members of the CAZy α-amylase family GH13 members and received the subfamily numbers GH13_34 and GH13_35 for the heavy chains of 4F2 antigens and rBAT proteins, respectively [46]. HAT proteins are responsible for transport of various types of amino acids across the plasma membrane in animals, namely in mammals, and their defects may lead to a failure in amino acids re-absorption and digestion (e.g., cystinuria, lysinuric protein intolerance) [107–109]. While the light chain has nothing to do with the family GH13 (it is a hydrophobic transmembrane protein consisting of 12 α-helices), the heavy subunit consists of the intracellular N-terminus, a transmembrane region and a large extracellular C-terminal part that exhibits the unambiguous sequence-structural similarity with α-glucosidases from the α-amylase family GH13 [91, 92, 110, 111]. Thus, the transportation activity is operated by the light subunit, whereas the heavy chain functions as a chaperone to help to orient the light chain to a proper position in the plasma membrane [112–114]. Note that, only the heavy subunit in its C-terminal part exhibits clear sequence similarities with the members of the α-amylase family [50, 91, 92, 95, 103, 104].

Interestingly, however, the heavy chains of both the rBAT protein and the 4F2 antigen display the best similarity to the α-amylase family GH13 enzymes in the parts of their sequences that are not involved in the catalytic action (Fig. 3b), i.e., in CSR-VI (the strand β2 of the catalytic TIM-barrel) and CSR-VII (the strand β8) [50]. While the heavy chain of the 4F2 antigen does not possess the segment corresponding with the domain B [50, 95, 103], the heavy subunit of the rBAT protein contains the entire domain B that is, moreover, very closely related to its counterpart seen in the members of the so-called oligo-1,6-glucosidases [45, 48, 95], sharing even the sequence fingerprint QPDLN of the CRS-V [50, 95]. The evidently closer relationships between the enzymes from the oligo-1,6-glucosidase subfamily and the heavy-chains of the rBAT proteins is reflected also in the presence of catalytic residues in the transport proteins, although in all cases the entire GH13 catalytic triad is not conserved completely. This feature could even be traced among the potential sequences of the heavy subunits of the rBAT proteins originated from basal metazoa [50]. For the heavy chain of the 4F2 antigen, no α-glucosidase activity has been detected [103], which is in agreement with a lack of residues that would correspond to the catalytic triad of the members of the α-amylase family [50, 103, 104], i.e., the aspartic acid as the catalytic nucleophile at the strand β4 (the CSR-II), the glutamic acid as the proton donor at the strand β5 (the CSR-III) and the aspartic acid as the transition-state stabilizer at the strand β7 (the CSR-IV) [1, 31].

With regard to sequence-structural resemblance of heavy chains of both the rBAT protein and 4F2 antigen, it is worth mentioning that, for example, the human 4F2 heavy chain lacks not only domain B, but also a stretch of about 40 amino acid residues succeeding the strand β4, i.e., it possesses a very short loop 4 connecting the strand β4 to helix α4 [50], whereas in both the oligo-1,6-glucosidase and the human rBAT heavy chain (both having also the entire domain B [93, 102]) the loop connecting the strand β4 to the helix α4 is longer (Fig. 3c). It is of note that even the GH13 neopullulanase subfamily members [45, 115], possessing shorter domain B [95], also lack the longer excursion of the loop 4 segment, but currently it is still unknown whether or not the domain B in GH13 oligo-1,6-glucosidase subfamily (and also in rBATs) operates in

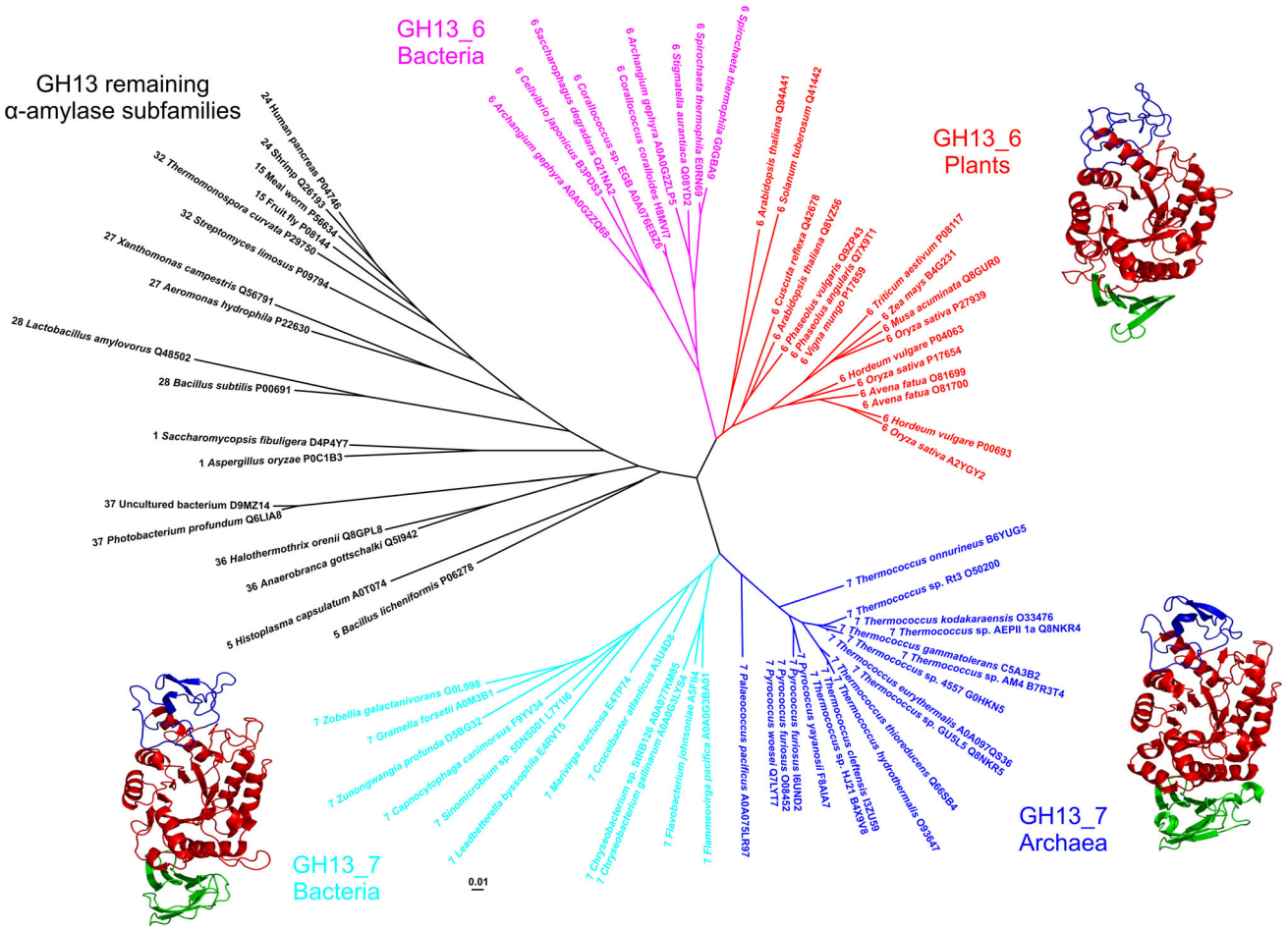
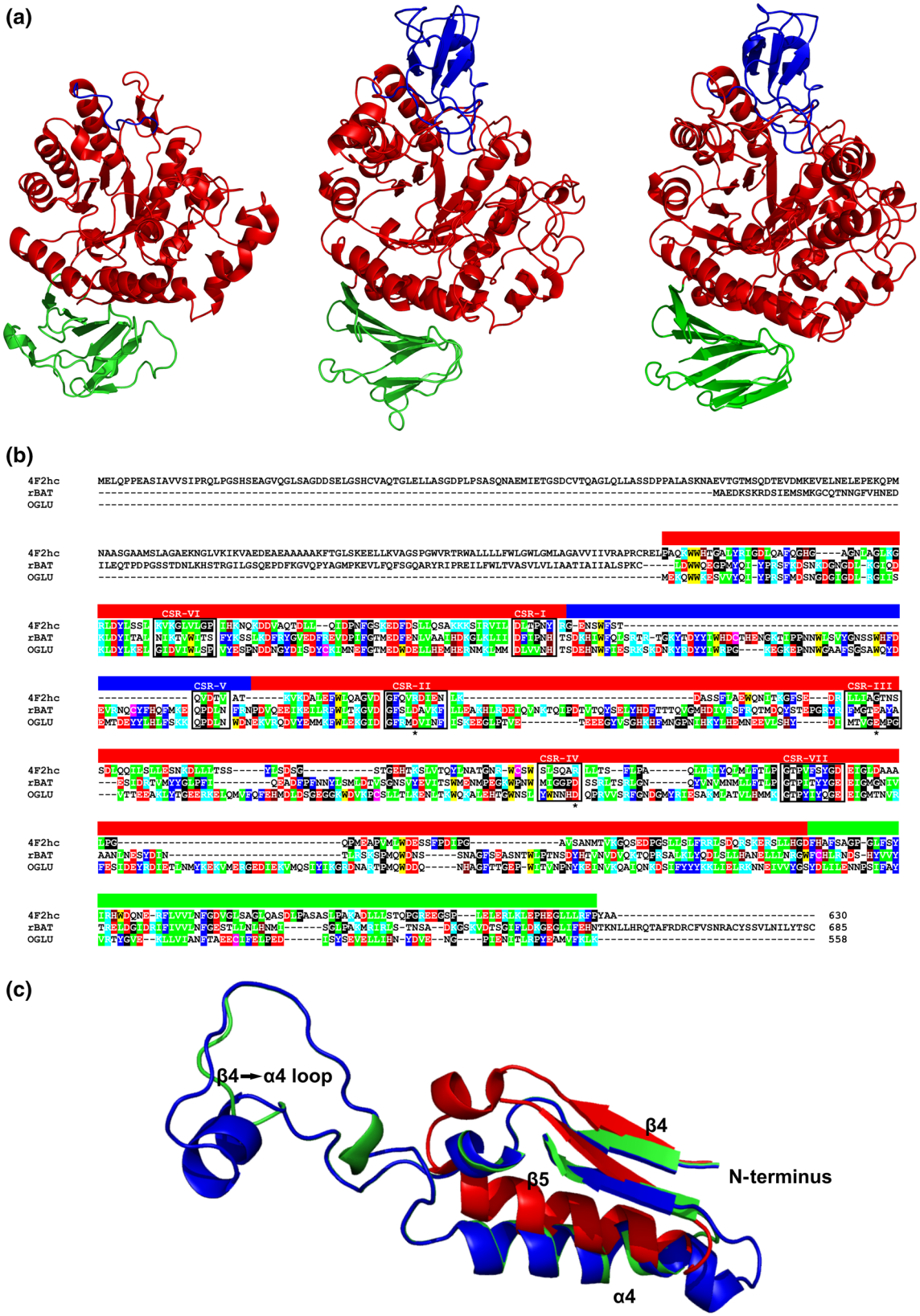


Fig. 2 Evolutionary tree of the family GH13 α-amylases with focus on the subfamilies GH13_6 and GH13_7. The tree is based on the alignment of seven conserved sequence regions, shown in Fig. 1. The tree was calculated using the neighbor-joining clustering [222] implemented in the Clustal-W2 phylogeny package [223] available at the European Bioinformatics Institute's web-site (http://www.ebi.ac.uk/), and then displayed with the program iTOL [224]. Tertiary structures of representatives of flavobacterial, archeal and plant family GH13 α-amylases are shown near their clusters in the tree. Sources of the α-amylases: *Sinomicrobium* sp. 5DNS001 [81] (subfamily GH13_7; flavobacteria); *Pyrococcus woesei* [72] (subfamily GH13_7; archaea); *Hordeum vulgare*—barley high pI isozyme AMY-2 [61] (subfamily GH13_6; plants). The archaeal and plant α-amylases are experimentally solved crystal structures retrieved from the Protein Data Bank (PDB) [225] under the PDB codes 1MWO and 1AMY, respectively. The flavobacterial α-amylase is a tertiary structure model obtained at the fold recognition server Phyre-2 [89] for its amino acid sequence (UniProt accession number: L7Y1I6; residues: Gly52-Gly477) based on the *P. woesei* α-amylase structure (1MWO) as template. The individual domains are colored as follows: catalytic $(\beta/\alpha)_8$-barrel—*red*, domain B—*blue*, domain C—*green*. The structural models were displayed with the program PyMol [226]

conjunction with the prolonged loop 4 (the β4 to α4 connection) [50]. It seems, however, that the consecutive loss of domain B in the heavy subunits of the 4F2 antigens might be connected with adequate shortening of the loop4, since this observation can be generalized to all 4F2 heavy-chains proteins [50]. Noticeably, similar heteromeric amino acid transporter system known in mammals was observed in insect [116, 117], nematodes [118] and schistozomes [119]. According to the in silico analysis of hcHATs and their enzymatic counterparts from the α-amylase family GH13 [50], a protein that could be close to the common ancestor of hcHATs and GH13 α-glucosidases might be represented by the hypothetical GH13-like protein from the cnidarian starlet sea anemone *Nematostella vectensis*.

Due to the obviously closer similarity of the heavy chains of rBAT proteins to the family GH13 α-glucosidases than that of those of 4F2 antigens, it is not easy to draw an unambiguous scenario of their evolution [95]. Based on a detailed in silico analysis [50], two different scenarios should be considered (Fig. 4). One of the possibilities means that the division of hcHATs from the enzymes of the α-amylase family might occur in one single event in basal Metazoa and subsequent split to rBAT proteins and 4F2 antigens in chordates. The above-mentioned higher similarity of the rBAT proteins to the GH13 enzymes than to


(a)
(b)
4F2hc
rBAT
OGLU
CSR-VI
CSR-I
CSR-V
CSR-II
CSR-III
CSR-IV
CSR-VII
(c)
β4→α4 loop
β4
β5
N-terminus
α4

◀**Fig. 3** **a** Comparison of tertiary structures of the family GH13 hcHAT proteins from animals and α-glucosidase from bacteria. Sources of the proteins: *Homo sapiens* 4F2hc antigen [103] (subfamily GH13_34; *left*); *Homo sapiens* rBAT protein [111] (subfamily GH13_35; *middle*); *Bacillus cereus* oligo-1,6-glucosidase [96] (subfamily GH13_31; *right*). **b** Amino acid sequence alignment of the same three proteins: human 4F2hc antigen [103] (UniProt accession No.: P08195-1); human rBAT protein [111] (Q07837); *Bacillus cereus* oligo-1,6-glucosidase (OGLU) [96] (P21332). Color code for the selected residues: W, *yellow*; F, Y—*blue*; V, L, I—*green*; D, E—*red*; R, K—*cyan*; H—*brown*; C—*magenta*; G, P—*black*. The seven characteristic CSRs are boxed and marked above the alignment. The *colored lane* above the alignment blocks means the three domains shown in **a**. The catalytic triad is signified by *asterisks* under the alignment. **c** Structural overlay emphasizing the longer loop 4 connecting the strand β4 to the helix α4 in the oligo-1,6-glucosidase (*green*) and rBAT protein (*blue*) in comparison to a very short version present in the 4F2hc antigen (*red*). The structures were superimposed using the MultiProt web-server [227] (http://bioinfo3d.cs.tau.ac.il/MultiProt/); the overlap being characterized by 280 corresponding C_α-atoms and the RMSD value of 0.96 Å. The human 4F2hc antigen and bacterial oligo-1,6-glucosidase are experimentally solved crystal structures retrieved from the PDB [225] under the PDB codes 2DH3 and 1UOK, respectively. The human rBAT protein is a tertiary structure model obtained at the fold recognition server Phyre-2 [89] for its amino acid sequence (UniProt accession number: Q07837; residues: Asp116-Glu649) based on the *B. cereus* oligo-1,6-glucosidase structure (1UOK) as template. The individual domains are colored as follows: catalytic $(\beta/\alpha)_8$-barrel—*red*, domain B—*blue*, domain C—*green*. The structures were visualized with the program PyMol [226]

the 4F2hc antigens could be explained by a selection pressure acting against change upon rBAT (and not upon 4F2hc), to preserve its eventual enzymatic capabilities, although until now no evidence has been delivered concerning the enzymatic activity of any rBAT protein [50]. Alternatively, the 4F2hc protein could be experiencing accelerated evolution toward some new function, accompanied by changes in both sequence and structure, while rBAT has retained its original function, remained mostly unchanged and thus similar to GH13 enzymes. The other eventuality assumes two independent branching events: one of the 4F2 antigens in the basal Metazoa and the other one of the rBAT proteins directly from enzymes of oligo-1,6-glucosidase subfamily of the α-amylase family in chordates. Since chordates obviously do not possess enzymes of the oligo-1,6-glucosidase subfamily [45, 48, 50], their α-glucosidases counterparts could have been transformed into the heavy chains of the rBAT proteins [120]. Both scenarios (Fig. 4) reflect the ancestry of both rBAT proteins and 4F2 antigens anchored within the GH13 α-amylase family, the difference being only in the way leading from the GH13 enzymes either to rBAT and 4F2 together or to rBAT and 4F2 separately [50]. At present, it seems that the hcHATs are found in animals starting from basal Metazoa, but it is not possible to exclude that in the future some new sequences of hcHAT-like proteins of non-metazoan origin become available [121].

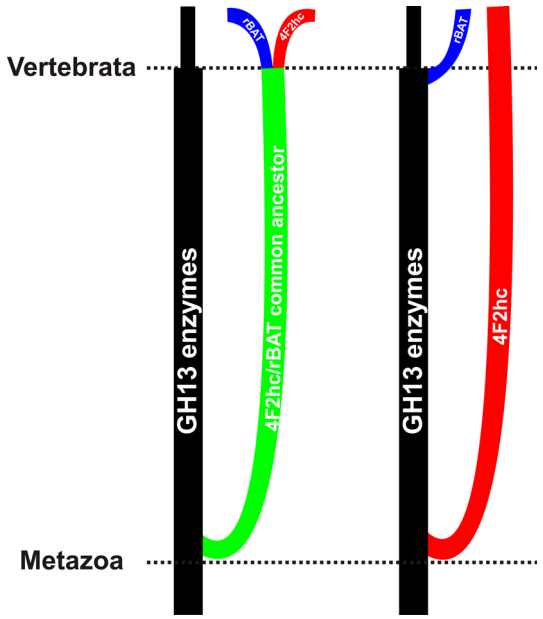


Fig. 4 Evolutionary scenarios of hcHAT proteins with respect to the α-amylase family GH13. The *left* eventuality considers a single-event division of both rBAT proteins and 4F2hc antigens from the enzymes of the α-amylase family in basal Metazoa with a subsequent split to rBAT proteins and 4F2 antigens in higher animals like chordates. The *right* possibility counts with two independent evolutionary events leading first to separation of the 4F2hc antigens from the α-amylase family enzymes in the basal Metazoa and second to recruitment of the rBAT proteins from enzymatic members of the oligo-1,6-glucosidase subfamily in higher animals

The family GH77 amylomaltases from borreliae

The family GH77, together with families GH13 and GH70, is a member of the α-amylase clan GH-H [31, 37]. As already mentioned above, it is, in contrast to the main α-amylase family GH13, a monospecific family, i.e., it contains only one enzyme specificity of 4-α-glucanotransferase (EC 2.4.1.25) [122]. The trivial name of the 4-α-glucanotransferase within the family GH77 has been distinguished with regard to taxonomy—while in prokaryotes (both *Bacteria* and *Archaea*) the name amylomaltase has been used, the name disproportionating enzyme (DPE) has been established in eukaryotes (mainly in plants and green algae) [42–44, 123–135]. In general, the 4-α-glucanotransferase catalyzes, employing the retaining reaction mechanism, the intermolecular transglycosylation of α-1,4-glucans, i.e., it transfers a glucan chain from one α-glucan to another one or within a single linear glucan molecule to produce a cyclic α-1,4-glucan [42–44, 136–138]. Currently, the family counts almost 3000 sequenced members with absolute domination of *Bacteria* accompanied approximately equally by a few dozens each from *Archaea* and *Eucarya* [37].

From the structural point of view, since the family GH77 is a member of the α-amylase clan GH-H, the GH77

4-α-glucanotransferase possesses a typical catalytic $(\beta/\alpha)_8$-barrel (TIM-barrel). It contains just more insertions in comparison with the family GH13 TIM-barrel having, in fact, only domain B protruding out of the barrel in the place of the loop 3 connecting the strand β3 to the helix α3 (Fig. 2). The family GH77 4-α-glucanotransferase TIM-barrel has thus three subdomains called B1, B2 and B3 (Fig. 5), of which B1 and B3 correspond with domains B and C in the family GH13, while the subdomain B2 is unique for the family GH77 [44, 139–143]. It is, however, worth mentioning that although the subdomain B3 may play the role of the C-terminal family GH13 domain C succeeding the catalytic TIM-barrel, it is not an antiparallel β-sandwich (a Greek key motif) seen typically in the GH13 (see Figs. 2, 5).

Within the family GH77, the amylomaltases from the genus *Borrelia* obviously play a special (evolutionary) role [122]. Originally, based on the complete genome sequence of the Lyme disease spirochaete *Borrelia burgdorferi* [144], an in silico analysis published in 2003 [145] delivered a remarkable observation of mutations in several conserved and functionally important positions in the *B. burgdorferi* hypothetical amylomaltase encoded by the gene *malQ*. The importance of the eventual mutations in the hypothetical GH77 amylomaltase was strengthened by the fact that, one of the residues was the otherwise throughout the entire clan GH-H invariantly conserved arginine in the position *i-2* with respect to catalytic nucleophile (aspartic acid) located at the strand β4 of the catalytic TIM-barrel, i.e., in the CSR-II [145]. The possible implications of remarkable in silico observations thus evoked a serious interest for experimental confirmation. The gene *malQ* from *B. burgdorferi* was cloned and expressed in *Escherichia coli* and the recombinant amylomaltase was shown to exhibit not only all the unique sequence features seen in the hypothetical MalQ but also the amylomaltase activity [129].

According to the most recent study focused on analysis of all available amylomaltase sequences from borreliae within the context of the entire family GH77 [122], 32 such sequences can be, in fact, divided into a few groups. Basically, there are only two major groups with respect to the presence/absence of the above-mentioned functional arginine, which—if naturally substituted—only lysine has been observed to replace it (Fig. 5c). It is the position of the Arg291 in the amylomaltase from *Thermus aquaticus* [43] mutated to Lys306 in the *B. burgdorferi* counterpart [129]. It should be pointed out that with regard to domain composition and length, all 32 amylomaltases from borreliae resemble typical bacterial GH77 amylomaltases represented by the enzyme from *T. aquaticus*. Nevertheless, some of those with the arginine to lysine mutation contain additional substitutions in several other well-conserved positions. On the other hand, some of those, possessing the original arginine, keep also those well-conserved positions (Fig. 6) characteristic for typical prokaryotic amylomaltases of non-borrelian origin [145]. Besides there are also some that exhibit an intermediary character. This phenomenon as a whole makes the amylomaltases from borreliae a unique evolutionary lineage in

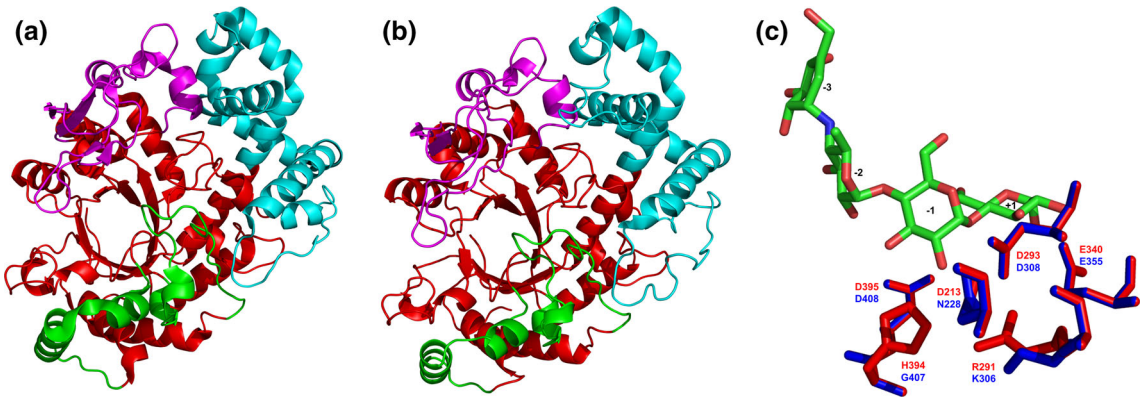
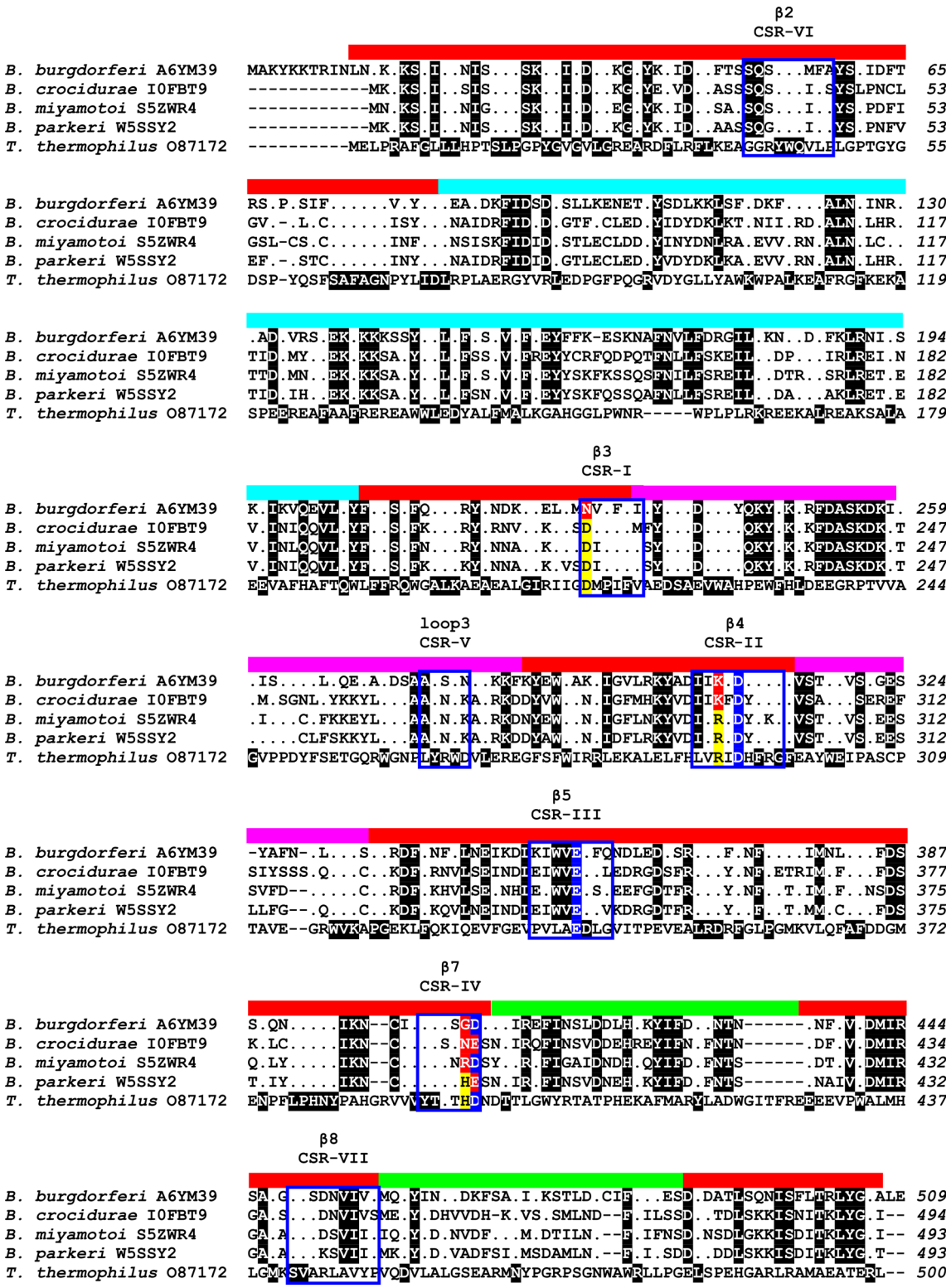


Fig. 5 Tertiary structures of the family GH77 amylomaltases from bacteria and their comparison. Sources of the amylomaltases: **a** *Thermus aquaticus* [139]; **b** *Borrelia burgdorferi* [129]. **c** Structural overlay focusing on the active-site residues with complexed acarbose in the *Thermus* amylomaltase (*red*) compared with the naturally mutated corresponding residues in the *Borrelia* amylomaltase (*blue*). The structures were superimposed using the MultiProt web-server [227] (http://bioinfo3d.cs.tau.ac.il/MultiProt/); the overlap being characterized by 488 corresponding C_α-atoms and the RMSD value of 0.18 Å. The *Thermus* amylomaltase is experimentally solved crystal structure retrieved from the PDB [225] under the PDB code 1ESW, whereas the *Borrelia* amylomaltase is a tertiary structure model obtained at the homology modeling server SwissModel [228] for its amino acid sequence (UniProt accession number: A6YM39; residues: Asn12-Ala507) based on the *T. aquaticus* amylomaltase structure (1CWY [44]) as template. The individual domains are colored as follows: catalytic $(\beta/\alpha)_8$-barrel—*red*, subdomain B1—*cyan*, subdomain B2—*magenta*, subdomain B3—*green*. The exact positions of displayed active-site residues in the amino acid sequence can be identified in the alignment shown in Fig. 6. Based on the accepted nomenclature [229] the acarbose occupies the subsites from −3 to +1. The structures were visualized with the program PyMol [226]


β2
CSR-VI
B. burgdorferi A6YM39 MAKYKKTRINLN.K.KS.I..NIS...SK..I.D..KG.YK.ID..FTSSQS...MFAYS.IDFT 65
B. crocidurae I0FBT9 ----------MK.KS.I..SIS...SK..I.D..KG.YE.VD..ASSSQS...I.SYSLPNCL 53
B. miyamotoi S5ZWR4 ----------MN.KS.I..NIG...SK..I.D..EG.YK.ID..SA.SQS...I..YS.PDFI 53
B. parkeri W5SSY2 ----------MK.KS.I..NIS...SK..I.D..KG.YK.ID..AASSQG...I..YS.PNFV 53
T. thermophilus O87172 ----------MELPRAFGLLLHPTSLPGPYGVGVLGREARDFLRFLKEAGGRYWQVLPLGPTGYG 55
B. burgdorferi A6YM39 RS.P.SIF.....V.Y...EA.DKFIDSD.SLLKENET.YSDLKKLSF.DKF....ALN.INR. 130
B. crocidurae I0FBT9 GV.-.L.C.....ISY...NAIDRFID.D.GTF.CLED.YIDYDKLKT.NII.RD.ALN.LHR. 117
B. miyamotoi S5ZWR4 GSL-CS.C.....INF...NSISKFIDID.STLECLDD.YINYDNLRA.EVV.RN.ALN.LC.. 117
B. parkeri W5SSY2 EF.-.STC.....INY...NAIDRFIDID.GTLECLED.YVDYDKLKA.EVV.RN.ALN.LHR. 117
T. thermophilus O87172 DSP-YQSFSAFAGNPYLIDLRPLAERGYVRLEDPGFPQGRVDYGLLYAWKWPALKEAFRGFKEKA 119
B. burgdorferi A6YM39 .AD.VRS.EK.KKKSSY..L.F.S.V.F.EYFFK-ESKNAFNVLFDRGIL.KN..D.FKLRNI.S 194
B. crocidurae I0FBT9 TID.MY..EK.KKSA.Y..L.FSS.V.FREYYCRFQDPQTFNLLFSKEIL..DP...IRLREI.N 182
B. miyamotoi S5ZWR4 TTD.MN..EK.KKSA.Y..L.F.S.V.F.EYYSKFKSSQSFNLLFSREIL..DTR..SRLRET.E 182
B. parkeri W5SSY2 TID.IH..EK.KKSA.Y..L.FSN.V.F.EYYSKFQSSQAFNLLFSREIL..DA...AKLRET.E 182
T. thermophilus O87172 SPEEREAFAAFREREAWWLEDYALFMALKGAHGGLPWNR-----WPLPLRKREEKALREAKSALA 179
β3
CSR-I
B. burgdorferi A6YM39 K.IKVQEVL.YF..S.FQ...RY.NDK..EL.MNV.F.I.Y...D...YQKY.K.RFDASKDKI. 259
B. crocidurae I0FBT9 V.INIQQVL.YF..S.FK...RY.RNV..K..SD....MFY...D....QKY.K.KFDASKDK.T 247
B. miyamotoi S5ZWR4 V.INLQQVL.YF..S.FN...RY.NNA..K...DI....SY...D....QKY.K.KFDASKDK.T 247
B. parkeri W5SSY2 V.INIQQVL.YF..S.FK...KY.NNA..K.VSDI....SY...D....QKY.K.RFDASKDK.T 247
T. thermophilus O87172 EEVAFHAFTQWLFFRQWGALKAEAEALGIRIIGDMPIEVAEDSAEVWAHPEWFHLDEEGRPTVVA 244
loop3
CSR-V
β4
CSR-II
B. burgdorferi A6YM39 .IS....L.QE.A.DSAA.S.N..KKFKYEW.AK.IGVLRKYADIIK.D....VST..VS.GES 324
B. crocidurae I0FBT9 .M.SGNL.YKKYL...AA.N.KA.RKDDYVW..N.IGFMHKYVDIIKFDY....VSA...SEREF 312
B. miyamotoi S5ZWR4 .I...C.FKKEYL...AA.N.KA.RKDNYEW..N.IGFLNKYVDI.R.DY.K..VST..VS.EES 312
B. parkeri W5SSY2 .....CLFSKKYL...AA.N.KA.RKDDYAW..N.IDFLRKYVDI.R.DY....VST..VS.EES 312
T. thermophilus O87172 GVPPDYFSETGQRWGNPLYRWDVLEREGFSFWIRRLEKALELFHLVRIDHFRGFEAYWEIPASCP 309
β5
CSR-III
B. burgdorferi A6YM39 -YAFN-.L...S..RDF.NF.LNEIKDIKIWVE.FQNDLED.SR...F.NF....IMNL...FDS 387
B. crocidurae I0FBT9 SIYSSS.Q...C..KDF.RNVLSEINDIEIWVE..LEDRGDSFR...Y.NF.ETRIM.F...FDS 377
B. miyamotoi S5ZWR4 SVFD--.....C..RDF.KHVLSE.NHIE.WVE.S.EEFGDTFR...Y.NF..T.IM.F..NSDS 375
B. parkeri W5SSY2 LLFG--.Q...C..KDF.KQVLNEINDIEIWVE..VKDRGDTFR...Y..F..T.MM.C...FDS 375
T. thermophilus O87172 TAVE--GRWVKAPGEKLFQKIQEVFGEVPVLAEDLGVITPEVEALRDRFGLPGMKVLQFAFDDGM 372
β7
CSR-IV
B. burgdorferi A6YM39 S.QN.....IKN--CI....SGD...IREFINSLDDLH.KYIFD..NTN------.NF.V.DMIR 444
B. crocidurae I0FBT9 K.LC.....IKN--C.....S.NESN.IRQFINSVDDEHREYIFN.FNTN------.DF.V.DMIR 434
B. miyamotoi S5ZWR4 Q.LY.....IKN--C.....NRDSY..R.FIGAIDNDH.QYIFD.FNTS------.DT.V.DMIR 432
B. parkeri W5SSY2 T.IY.....IKN--C.....HESN.IR.FINSVDNEH.KYIFD.FNTS------.NAIV.DMIR 432
T. thermophilus O87172 ENPFLPHNYPAHGRVVVYT.THDNDTTLGWYRTATPHEKAFMARYLADWGITFREEEEVPWALMH 437
β8
CSR-VII
B. burgdorferi A6YM39 SA.G..SDNVIV.MQ.YIN..DKFSA.I.KSTLD.CIE...ESD.DATLSQNISFLTRLYG.ALE 509
B. crocidurae I0FBT9 GA.S...DNVIVSME.Y.DHVVDH-K.VS.SMLND--F.ILSSD..TDLSKKISNITKLYG.I-- 494
B. miyamotoi S5ZWR4 GA.A...DSVII.IQ.Y.D.NVDF...M.DTILN.--F.IFNSD.NSDLGKKISDITKLYG.I-- 493
B. parkeri W5SSY2 GA.A...KSVII.MK.Y.D.VADFSI.MSDAMLN.--F.I.SDD..DDLSKKISDITKLYG.T-- 493
T. thermophilus O87172 LGMKSVARLAVYFVQDVLALGSEARMNYPGRPSGNWAWRLLPGELSPEHGARLRAMAEATERL-- 500

◀**Fig. 6** Amino acid sequence comparison of family GH77 amylomaltases from borreliae and *Thermus aquaticus*. The four amylomaltases from borrelian origin represent different subgroups within the genus *Borrelia* [122] exhibiting unique mutations in several important active-site positions with respect to a typical bacterial amylomaltase represented by the one from *Thermus* [129, 145]. The sequences were retrieved from the UniProt database [221] according to their accession numbers succeeding the name of the organism. The alignment was done using the program Clustal-Omega [230] available at the European Bioinformatics Institute's web-site (http://www.ebi.ac.uk/). The unique borreliae-like positions (Asn228, Lys306 and Gly407) and the catalytic triad (Asp308, Glu355 and Asp408; *B. burgdorferi* amylomaltase numbering) are signified by *yellow*/*red* and *blue highlighting*, respectively. The positions in amylomaltases from borreliae identical to that from *Thermus* are shown as *dots*. The *black highlighting* in the *T. aquaticus* amylomaltase means that all four borrelian counterparts have the same residue in those positions. The positions that are signified by *black highlighting* in amylomaltases from borreliae are identical among them but different from those in the enzyme from *Thermus*. The seven CSRs known for the entire α-amylase clan GH-H are *boxed* and *marked* as CSR-I to CSR-VII with indicated well-accepted secondary structure elements [32]. The individual domains are indicated as a *colored lane* above the alignment blocks as follows: catalytic $(\beta/\alpha)_8$-barrel—*red*, subdomain B1—*cyan*, subdomain B2—*magenta*, subdomain B3—*green*

the family GH77 [122]. However, it should also be taken into account that until now of the 32 available GH77 amylomaltase sequences from borreliae, the MalQ from *B. burgdorferi* is the only one that has already been biochemically characterized and found enzymatically active [129, 146].

The family GH77 offers also other examples that are of interest from the evolutionary point of view, e.g., the DPE2 version of a typical plant DPE1 [42, 134, 135] possessing an insert of ~140 residues between catalytic nucleophile and proton donor [147–152] that have been found also among some bacteria [122], or a large group of additional bacterial amylomaltases represented by the enzyme from *Escherichia coli* [125] including also the well-characterized amylomaltase from *Clostridium glutamicum* [131, 153–155] that have at the N-terminus a separate carbohydrate-binding module-(CBM)-like domain [122, 142]. In any case it is evident that with regard to the family GH77 a gradual evolutionary transition has occurred among borreliae that can be characterized from the version of a typical bacterial *Thermus*-like amylomaltase to the version with progressively mutated functionally important conserved residues [122].

Other examples of interest

The main goal of this review article was to demonstrate some selected examples of remarkable evolutionary phenomena within the α-amylase clan GH-H. There are many other cases that would deserve to be mentioned, including also those seen in the second and smaller α-amylase family GH57. To mention at least a few, the non-exhaustive list could be as follows: (1) close similarity and relatedness between the family GH13 α-amylases from animals and actinomycetes [28, 74, 79] constituting a well-known group of chloride-dependent α-amylases [156, 157]; (2) existence of an intermediary group of α-amylases, classified in the subfamily GH13_36 [48], with a mixed enzyme specificity exhibiting simultaneous relatedness to various α-glucosidases and debranching enzymes [158–163] from the so-called subfamilies of oligo-1,6-glucosidase and neopullulanase [45, 46]; (3) presence of the so-called α-amylase-like homologues in the family GH57 [164–166] strikingly similar to α-amylases, but having incomplete GH57 catalytic machinery—the real α-amylases being more frequent among *Archaea*, whereas the α-amylase-like homologues are being found rather among *Bacteria* [164]; (4) the pronounced similarity between the α-amylase families GH57 and GH119 revealed by an in silico study suggesting that the members of the family GH119 share with those of the GH57 catalytic machinery, CSRs and fold of the catalytic domain [167]; (5) observation of a totally novel lineage or subfamily of glucansucrases within the circularly-permuted family GH70 [39–41, 168–170], exhibiting a close homology to the rest of the family but without any circular permutation of the catalytic TIM-barrel [171, 172]; (6) existence of various and evolutionarily independent starch/glycogen-binding domains classified as different CBM families [173–185] in, e.g., not typical amylolytic enzymes and proteins, such as glucan phosphatases—animal laforin and plant SEX-4 [186–197], AMP-activated protein kinase [198–201], genethonin-1 [202, 203], and starch synthases [204–206], glucan water dikinases [207–211] and lytic polysaccharide monooxygenases [212–214]; and (7) the so-called carbohydrate surface binding sites different from the distinct CBMs mentioned above and present in various carbohydrate-active enzymes, well represented just in the α-amylase family GH13 [69, 72, 215–219]—in the particular case of the barley α-amylase low p*I* isozyme AMY1 named as "a pair of sugar tongs" and a starch granule binding site, respectively [69, 218]. All these particular details make the α-amylases and the remaining enzyme specificities an attractive subject not only for evolutionary studies but also from the practical point of view for the approaches focused on their protein design. Even if known that some living organisms can survive without any sugars [220].

Conclusions

Due to a huge amount of accumulated sequence data, the questions concerning the structure, function and evolution of amylolytic enzymes have really become complicated,

especially when they are ambitiously treated in an effort to reach answers to complex questions. This obvious trend can simply be illustrated by a few dozens of the α-amylase family GH13 members when it was created in 1991 versus more than 30 thousand sequences classified in the family nowadays. There is, however, not only the prevalent number of sequences available complicating the situation, i.e., our knowledge, but there is also the necessity to accept the changes in minds of scientists reflecting the continuously widening scope of the α-amylase family. Thus, for example, this family was originally established as a family of starch hydrolases and related enzymes active towards the α-1,4- and α-1,6-glucosidic linkages catalyzing either the hydrolysis (EC 3) or transglycosylation (EC 2). Currently, also other bonds are attacked, e.g., those in trehalose (α-1,1-) and sucrose (α-1,2-), the enzymatic repertoire of the family members has been expanded including the isomerases (EC 5). What might originally be unbelievable, even the non-enzymatic transport proteins (rBAT and 4F2hc), lacking in most cases the GH13 catalytic machinery, have been classified constituting their respective GH13 subfamilies. One of the ways that could lead to a consolidation of the knowledge may be to continue in looking for and revealing the features in amino acid sequences and structures that would reflect the exclusivity of smaller family groups (i.e., subfamilies) of either particular substrate specificity or from the point of view of taxonomy, or both, and confirming their roles experimentally.

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References

1. Janecek S, Svensson B, MacGregor EA (2014) α-Amylase—an enzyme specificity found in various families of glycoside hydrolases. Cell Mol Life Sci 71:1149–1170
2. MacGregor EA (1988) α-Amylase structure and activity. J Protein Chem 7:399–415
3. Vihinen M, Mäntsälä P (1989) Microbial amylolytic enzymes. Crit Rev Biochem Mol Biol 24:329–418
4. Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R (2000) Advances in microbial amylases. Biotechnol Appl Biochem 31:135–152
5. Søgaard M, Ji Abe, Martin-Eauclaire MF, Svensson B (1993) α-Amylases: structure and function. Carbohydr Polym 21:137–146
6. Gupta R, Gigras P, Mohapatra H, Kumar Goswami V, Chauhan B (2003) Microbial α-amylases: a biotechnological perspective. Process Biochem 38:1599–1616
7. Svensson B (1988) Regional distant sequence homology between amylases, α-glucosidases and transglucanosylases. FEBS Lett 230:72–76
8. MacGregor EA, Svensson B (1989) A super-secondary structure predicted to be common to several α-1,4-D-glucan-cleaving enzymes. Biochem J 259:145–152
9. Jespersen HM, MacGregor EA, Sierks MR, Svensson B (1991) Comparison of the domain-level organization of starch hydrolases and related enzymes. Biochem J 280:51–55
10. Uitdehaag JC, Mosi R, Kalk KH, van der Veen BA, Dijkhuizen L, Withers SG, Dijkstra BW (1999) X-ray structures along the reaction pathway of cyclodextrin glycosyltransferase elucidate catalysis in the α-amylase family. Nat Struct Biol 6:432–436
11. Kimura K, Kataoka S, Ishii Y, Takano T, Yamane K (1987) Nucleotide sequence of the β-cyclodextrin glucanotransferase gene of alkalophilic *Bacillus* sp. strain 1011 and similarity of its amino acid sequence to those of α-amylases. J Bacteriol 169:4399–4402
12. Janecek S, Svensson B, MacGregor EA (1995) Characteristic differences in the primary structure allow discrimination of cyclodextrin glucanotransferases from α-amylases. Biochem J 305:685–686
13. Itkor P, Tsukagoshi N, Udaka S (1990) Nucleotide sequence of the raw-starch-digesting amylase gene from *Bacillus* sp. B1018 and its strong homology to the cyclodextrin glucanotransferase genes. Biochem Biophys Res Commun 166:630–636
14. Bahl H, Burchhardt G, Spreinat A, Haeckel K, Wienecke A, Schmidt B, Antranikian G (1991) α-Amylase of *Clostridium thermosulfurogenes* EM1: nucleotide sequence of the gene, processing of the enzyme, and comparison of other α-amylases. Appl Environ Microbiol 57:1554–1559
15. del-Rio G, Morett E, Soberon X (1997) Did cyclodextrin glycosyltransferases evolve from α-amylases? FEBS Lett 416:221–224
16. Tsukamoto A, Kimura K, Ishii Y, Takano T, Yamane K (1988) Nucleotide sequence of the maltohexaose-producing amylase gene from an alkalophilic *Bacillus* sp. #707 and structural similarity to liquefying type α-amylases. Biochem Biophys Res Commun 151:25–31
17. Imanaka T, Kuriki T (1989) Pattern of action of *Bacillus stearothermophilus* neopullulanase on pullulan. J Bacteriol 171:369–374
18. Kuriki T, Imanaka T (1989) Nucleotide sequence of the neopullulanase gene from *Bacillus stearothermophilus*. J Gen Microbiol 135:1521–1528
19. Kuriki T, Takata H, Okada S, Imanaka T (1991) Analysis of the active center of *Bacillus stearothermophilus* neopullulanase. J Bacteriol 173:6147–6152
20. Takata H, Kuriki T, Okada S, Takesada Y, Iizuka M, Minamiura N, Imanaka T (1992) Action of neopullulanase. Neopullulanase catalyzes both hydrolysis and transglycosylation at α-(1,4)- and α-(1,6)-glucosidic linkages. J Biol Chem 267:18447–18452
21. MacGregor EA (1993) Relationships between structure and activity in the α-amylase family of starch-metabolising enzymes. Starch/Staerke 45:232–237
22. Jespersen HM, MacGregor EA, Henrissat B, Sierks MR, Svensson B (1993) Starch- and glycogen-debranching and branching enzymes:prediction of structural features of the catalytic $(\beta/\alpha)_8$-barrel domain and evolutionary relationship to other amylolytic enzymes. J Protein Chem 12:791–805
23. Janecek S (1994) Parallel β/α-barrels of α-amylase, cyclodextrin glycosyltransferase and oligo-1,6-glucosidase versus the barrel of β-amylase: evolutionary distance is a reflection of unrelated sequences. FEBS Lett 353:119–123
24. Svensson B (1994) Protein engineering in the α-amylase family: catalytic mechanism, substrate specificity, and stability. Plant Mol Biol 25:141–157
25. Aghajari N, Roth M, Haser R (2002) Crystallographic evidence of a transglycosylation reaction: ternary complexes of a psychrophilic α-amylase. Biochemistry 41:4273–4280

26. Nakajima R, Imanaka T, Aiba S (1986) Comparison of amino acid sequences of eleven different α-amylases. Appl Microbiol Biotechnol 23:355–360
27. Janecek S (1992) New conserved amino acid region of α-amylases in the third loop of their $(\beta/\alpha)_8$-barrel domains. Biochem J 288:1069–1070
28. Janecek S (1994) Sequence similarities and evolutionary relationships of microbial, plant and animal α-amylases. Eur J Biochem 224:519–524
29. Janecek S (1995) Close evolutionary relatedness among functionally distantly related members of the $(\alpha/\beta)_8$-barrel glycosyl hydrolases suggested by the similarity of their fifth conserved sequence region. FEBS Lett 377:6–8
30. Janecek S (1997) α-Amylase family: molecular biology and evolution. Prog Biophys Mol Biol 67:67–97
31. MacGregor EA, Janecek S, Svensson B (2001) Relationship of sequence and structure to specificity in the α-amylase family of enzymes. Biochim Biophys Acta 1546:1–20
32. Janecek S (2002) How many conserved sequence regions are there in the α-amylase family? Biologia 57(Suppl 11):29–41
33. Henrissat B (1991) A classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J 280:309–316
34. Henrissat B, Bairoch A (1993) New families in the classification of glycosyl hydrolases based on amino acid sequence similarities. Biochem J 293:781–788
35. Henrissat B, Bairoch A (1996) Updating the sequence-based classification of glycosyl hydrolases. Biochem J 316:695–696
36. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B (2009) The carbohydrate-active enZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res 37:D233–D238
37. Lombard V, Ramulu GH, Drula E, Coutinho PM, Henrissat B (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucl Acids Res 42:D490–D495
38. Henrissat B, Davies GJ (1997) Structural and sequence-based classification of glycoside hydrolases. Curr Opin Struct Biol 7:637–644
39. MacGregor EA, Jespersen HM, Svensson B (1996) A circularly permuted α-amylase-type α/β-barrel structure in glucan-synthesizing glucosyltransferases. FEBS Lett 378:263–266
40. Vujicic-Zagar A, Pijning T, Kralj S, Lopez CA, Eeuwema W, Dijkhuizen L, Dijkstra BW (2010) Crystal structure of a 117 kDa glucansucrase fragment provides insight into evolution and product specificity of GH70 enzymes. Proc Natl Acad Sci USA 107:21406–21411
41. Kralj S, Grijpstra P, van Leeuwen SS, Leemhuis H, Dobruchowska JM, van der Kaaij RM, Malik A, Oetari A, Kamerling JP, Dijkhuizen L (2011) 4,6-α-Glucanotransferase, a novel enzyme that structurally and functionally provides an evolutionary link between glycoside hydrolase enzyme families 13 and 70. Appl Environ Microbiol 77:8154–8163
42. Takaha T, Yanase M, Okada S, Smith SM (1993) Disproportionating enzyme (4-α-glucanotransferase; EC 2.4.1.25) of potato. Purification, molecular cloning, and potential role in starch metabolism. J Biol Chem 268:1391–1396
43. Terada Y, Fujii K, Takaha T, Okada S (1999) *Thermus aquaticus* ATCC 33923 amylomaltase gene cloning and expression and enzyme characterization: production of cycloamylose. Appl Environ Microbiol 65:910–915
44. Przylas I, Tomoo K, Terada Y, Takaha T, Fujii K, Saenger W, Sträter N (2000) Crystal structure of amylomaltase from *Thermus aquaticus*, a glycosyltransferase catalysing the production of large cyclic glucans. J Mol Biol 296:873–886
45. Oslancova A, Janecek S (2002) Oligo-1,6-glucosidase and neopullulanase enzyme subfamilies from the α-amylase family defined by the fifth conserved sequence region. Cell Mol Life Sci 59:1945–1959
46. Stam MR, Danchin EG, Rancurel C, Coutinho PM, Henrissat B (2006) Dividing the large glycoside hydrolase family 13 into subfamilies:towards improved functional annotations of α-amylase-related proteins. Protein Eng Des Sel 19:555–562
47. Lei Y, Peng H, Wang Y, Liu Y, Han F, Xiao Y, Gao Y (2012) Preferential and rapid degradation of raw rice starch by an α-amylase of glycoside hydrolase subfamily GH13_37. Appl Microbiol Biotechnol 94:1577–1584
48. Majzlova K, Pukajova Z, Janecek S (2013) Tracing the evolution of the α-amylase subfamily GH13_36 covering the amylolytic enzymes intermediate between oligo-1,6-glucosidases and neopullulanases. Carbohydr Res 367:48–57
49. Janecek S, Kuchtova A, Petrovicova S (2015) A novel GH13 subfamily of α-amylases with a pair of tryptophans in the helix α3 of the catalytic TIM-barrel, the LPDlx signature in the conserved sequence region V and a conserved aromatic motif at the C-terminus. Biologia 70:1284–1294
50. Gabrisko M, Janecek S (2009) Looking for the ancestry of the heavy-chain subunits of heteromeric amino acid transporters rBAT and 4F2hc within the GH13 α-amylase family. FEBS J 276:7265–7278
51. Dong G, Vieille C, Savchenko A, Zeikus JG (1997) Cloning, sequencing, and expression of the gene encoding extracellular α-amylase from *Pyrococcus furiosus* and biochemical characterization of the recombinant enzyme. Appl Environ Microbiol 63:3569–3576
52. Jorgensen S, Vorgias CE, Antranikian G (1997) Cloning, sequencing, characterization, and expression of an extracellular α-amylase from the hyperthermophilic archaeon *Pyrococcus furiosus* in *Escherichia coli* and *Bacillus subtilis*. J Biol Chem 272:16335–16342
53. Jones RA, Jermiin LS, Easteal S, Patel BK, Beacham IR (1999) Amylase and 16S rRNA genes from a hyperthermophilic archaebacterium. J Appl Microbiol 86:93–107
54. Frillingos S, Linden A, Niehaus F, Vargas C, Nieto JJ, Ventosa A, Antranikian G, Drainas C (2000) Cloning and expression of α-amylase from the hyperthermophilic archaeon *Pyrococcus woesei* in the moderately halophilic bacterium *Halomonas elongata*. J Appl Microbiol 88:495–503
55. Leveque E, Haye B, Belarbi A (2000) Cloning and expression of an α-amylase encoding gene from the hyperthermophilic archaebacterium *Thermococcus hydrothermalis* and biochemical characterisation of the recombinant enzyme. FEMS Microbiol Lett 186:67–71
56. Rogers JC, Milliman C (1983) Isolation and sequence analysis of a barley α-amylase cDNA clone. J Biol Chem 258:8169–8174
57. Rogers JC (1985) Two barley α-amylase gene families are regulated differently in aleurone cells. J Biol Chem 260:3731–3738
58. Baulcombe DC, Huttly AK, Martienssen RA, Barker RF, Jarvis MG (1987) A novel wheat α-amylase gene (α-Amy3). Mol Gen Genet 209:33–40
59. Huang N, Sutliff TD, Litts JC, Rodriguez RL (1990) Plant Mol Biol 14:655–668
60. Young TE, DeMason DA, Close TJ (1994) Cloning of an α-amylase cDNA from aleurone tissue of germinating maize seed. Plant Physiol 105:759–760
61. Kadziola A, Abe J, Svensson B, Haser R (1994) Crystal and molecular structure of barley α-amylase. J Mol Biol 239:104–121
62. Kadziola A, Søgaard M, Svensson B, Haser R (1998) Molecular structure of a barley α-amylase-inhibitor complex:implications for starch binding and catalysis. J Mol Biol 278:205–217

63. Matsuura Y, Kusunoki M, Harada W, Kakudo M (1984) Structure and possible catalytic residues of Taka-amylase A. J Biochem 95:697–702
64. Swift HJ, Brady L, Derewenda ZS, Dodson EJ, Dodson GG, Turkenburg JP, Wilkinson AJ (1991) Structure and molecular model refinement of *Aspergillus oryzae* (TAKA) α-amylase: an application of the simulated-annealing method. Acta Crystallogr B 47:535–544
65. Boel E, Brady L, Brzozowski AM, Derewenda Z, Dodson GG, Jensen VJ, Petersen SB, Swift H, Thim L, Woldike HF (1990) Calcium binding in α-amylases: an X-ray diffraction study at 2.1-Å resolution of two enzymes from *Aspergillus*. Biochemistry 29:6244–6249
66. Brady RL, Brzozowski AM, Derewenda ZS, Dodson EJ, Dodson GG (1991) Solution of the structure of *Aspergillus niger* acid α-amylase by combined molecular replacement and multiple isomorphous replacement methods. Acta Crystallogr B 47:527–535
67. Buisson G, Duee E, Haser R, Payan F (1987) Three dimensional structure of porcine pancreatic α-amylase at 2.9 Å resolution. Role of calcium in structure and activity. EMBO J 6:3909–3916
68. Qian M, Haser R, Payan F (1993) Structure and molecular model refinement of pig pancreatic α-amylase at 2.1 Å resolution. J Mol Biol 231:785–799
69. Robert X, Haser R, Gottschalk TE, Ratajczak F, Driguez H, Svensson B, Aghajari N (2003) The structure of barley α-amylase isozyme 1 reveals a novel role of domain C in substrate recognition and binding: a pair of sugar tongs. Structure 11:973–984
70. Robert X, Haser R, Mori H, Svensson B, Aghajari N (2005) Oligosaccharide binding to barley α-amylase 1. J Biol Chem 280:32968–32978
71. Ochiai A, Sugai H, Harada K, Tanaka S, Ishiyama Y, Ito K, Tanaka T, Uchiumi T, Taniguchi M, Mitsui T (2014) Crystal structure of α-amylase from *Oryza sativa*: molecular insights into enzyme activity and thermostability. Biosci Biotechnol Biochem 78:989–997
72. Linden A, Mayans O, Meyer-Klaucke W, Antranikian G, Wilmanns M (2003) Differential regulation of a hyperthermophilic α-amylase with a novel (Ca, Zn) two-metal center by zinc. J Biol Chem 278:9875–9884
73. Pujadas G, Palau J (2001) Evolution of α-amylases:architectural features and key residues in the stabilization of the $(\beta/\alpha)_8$ scaffold. Mol Biol Evol 18:38–54
74. Da Lage JL, Feller G, Janecek S (2004) Horizontal gene transfer from Eukarya to bacteria and domain shuffling: the α-amylase model. Cell Mol Life Sci 61:97–109
75. van der Kaaij RM, Janecek S, van der Maarel MJ, Dijkhuizen L (2007) Phylogenetic and biochemical characterization of a novel cluster of intracellular fungal α-amylase enzymes. Microbiology 153:4003–4015
76. Godany A, Majzlova K, Horvathova V, Vidova B, Janecek S (2010) Tyrosine 39 of GH13 α-amylase from *Thermococcus hydrothermalis* contributes to its thermostability. Biologia 65:408–415
77. Hostinova E, Janecek S, Gasperik J (2010) Gene sequence, bioinformatics and enzymatic characterization of α-amylase from *Saccharomycopsis fibuligera* KZ. Protein J 29:355–364
78. Puspasari F, Radjasa O, Noer A, Nurachman Z, Syah Y, van der Maarel M, Dijkhuizen L, Janecek S, Natalia D (2013) Raw starch degrading α-amylase from *Bacillus aquimaris* MKSC 6.2: isolation and expression of the gene, bioinformatics and biochemical characterization of the recombinant enzyme. J Appl Microbiol 114:108–120
79. Janecek S, Leveque E, Belarbi A, Haye B (1999) Close evolutionary relatedness of α-amylases from Archaea and plants. J Mol Evol 48:421–426
80. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410
81. Li C, Du M, Cheng B, Wang L, Liu X, Ma C, Yang C, Xu P (2014) Close relationship of a novel Flavobacteriaceae α-amylase with archaeal α-amylases and good potentials for industrial applications. Biotechnol Biofuels 7:18
82. Linden A, Wilmanns M (2004) Adaptation of class-13 α-amylases to diverse living conditions. ChemBioChem 5:231–239
83. Lim JK, Lee HS, Kim YJ, Bae SS, Jeon JH, Kang SG, Lee JH (2007) Critical factors to high thermostability of an α-amylase from hyperthermophilic archaeon *Thermococcus onnurineus* NA1. J Microbiol Biotechnol 17:1242–1248
84. Kobayashi T, Kanai H, Hayashi T, Akiba T, Akaboshi R, Horikoshi K (1992) Haloalkaliphilic maltotriose-forming α-amylase from the archaebacterium *Natronococcus* sp. strain Ah-36. J Bacteriol 174:3439–3444
85. Kobayashi T, Kanai H, Aono R, Horikoshi K, Kudo T (1994) Cloning, expression, and nucleotide sequence of the α-amylase gene from the haloalkaliphilic archaeon *Natronococcus* sp. strain Ah-36. J Bacteriol 176:5131–5134
86. Onodera M, Yatsunami R, Tsukimura W, Fukui T, Nakasone K, Takashina T, Nakamura S (2013) Gene analysis, expression, and characterization of an intracellular α-amylase from the extremely halophilic archaeon *Haloarcula japonica*. Biosci Biotechnol Biochem 77:281–288
87. Zorgani MA, Patron K, Desvaux M (2014) New insight in the structural features of haloadaptation in α-amylases from halophilic *Archaea* following homology modeling strategy: folded and stable conformation maintained through low hydrophobicity and highly negative charged surface. J Comput Aided Mol Des 28:721–734
88. Hutcheon GW, Vasisht N, Bolhuis A (2005) Characterisation of a highly stable α-amylase from the halophilic archaeon *Haloarcula hispanica*. Extremophiles 9:487–495
89. Kelley LA, Sternberg MJ (2009) Protein structure prediction on the Web: a case study using the Phyre server. Nat Protoc 4:363–371
90. Suvd D, Fujimoto Z, Takase K, Matsumura M, Mizuno H (2001) Crystal structure of *Bacillus stearothermophilus* α-amylase: possible factors determining the thermostability. J Biochem 129:461–468
91. Wells RG, Hediger MA (1992) Cloning of a rat kidney cDNA that stimulates dibasic and neutral amino acid transport and has sequence similarity to glucosidases. Proc Natl Acad Sci USA 89:5596–5600
92. Bertran J, Werner A, Moore ML, Stange G, Markovich D, Biber J, Testar X, Zorzano A, Palacin M, Murer H (1992) Expression cloning of a cDNA from rabbit kidney cortex that induces a single transport system for cystine and dibasic and neutrl amino acids. Proc Natl Acad Sci USA 89:5601–5605
93. Kuriki T, Imanaka T (1999) The concept of the α-amylase family: structural similarity and common catalytic mechanism. J Biosci Bioeng 87:557–565
94. Kuriki T, Takata H, Yanase M, Ohdan K, Fujii K, Terada Y, Takaha T, Hondoh H, Matsuura Y, Imanaka T (2006) The concept of the α-amylase family: a rational tool for interconverting glucanohydrolases/glucanotransferases, and their specificities. J Appl Glycosci 53:155–161
95. Janecek S, Svensson B, Henrissat B (1997) Domain evolution in the α-amylase family. J Mol Evol 45:322–331
96. Watanabe K, Hata Y, Kizaki H, Katsube Y, Suzuki Y (1997) The refined crystal structure of *Bacillus cereus* oligo-1,6-glucosidase at 2.0 Å resolution: structural characterization of

proline-substitution sites for protein thermostabilization. J Mol Biol 269:142–153
97. Zhang D, Li N, Lok SM, Zhang LH, Swaminathan K (2003) Isomaltulose synthase (PalI) of *Klebsiella* sp. LX3. Crystal structure and implication of mechanism. J Biol Chem 278:35428–35434
98. Hondoh H, Saburi W, Mori H, Okuyama M, Nakada T, Matsuura Y, Kimura A (2008) Substrate recognition mechanism of α-1,6-glucosidic linkage hydrolyzing enzyme, dextran glucosidase from *Streptococcus mutans*. J Mol Biol 378:913–922
99. Shirai T, Hung VS, Morinaka K, Kobayashi T, Ito S (2008) Crystal structure of GH13 α-glucosidase GSJ from one of the deepest sea bacteria. Proteins 73:126–133
100. Kim JS, Cha SS, Kim HJ, Kim TJ, Ha NC, Oh ST, Cho HS, Cho MJ, Kim MJ, Lee HS, Kim JW, Choi KY, Park KH, Oh BH (1999) Crystal structure of a maltogenic amylase provides insights into a catalytic versatility. J Biol Chem 274:26279–26286
101. Lee HS, Kim MS, Cho HS, Kim JI, Kim TJ, Choi JH, Park C, Lee HS, Oh BH, Park KH (2002) Cyclomaltodextrinase, neopullulanase, and maltogenic amylase are nearly indistinguishable from each other. J Biol Chem 277:21891–21897
102. Hondoh H, Kuriki T, Matsuura Y (2003) Three-dimensional structure and substrate binding of *Bacillus stearothermophilus* neopullulanase. J Mol Biol 326:177–188
103. Fort J, de la Ballina LR, Burghardt HE, Ferrer-Costa C, Turnay J, Ferrer-Orta C, Uson I, Zorzano A, Fernandez-Recio J, Orozco M, Lizarbe MA, Fita I, Palacin M (2007) The structure of human 4F2hc ectodomain provides a model for homodimerization and electrostatic interaction with plasma membrane. J Biol Chem 282:31444–31452
104. Janecek S (2000) Proteins without enzymatic function with sequence relatedness to the α-amylase family. Trends Glycosci Glycotechnol 12:363–371
105. Broer S, Wagner CA (2002) Structure-function relationships of heterodimeric amino acid transporters. Cell Biochem Biophys 36:155–168
106. Hediger MA, Romero MF, Peng JB, Rolfs A, Takanaga H, Bruford EA (2004) The ABCs of solute carriers: physiological, pathological and therapeutic implications of human membrane transport proteins. Pflug Arch 447:465–468
107. Palacin M, Nunes V, Font-Llitjos M, Jimenez-Vidal M, Fort J, Gasol E, Pineda M, Feliubadalo L, Chillaron J, Zorzano A (2005) The genetics of heteromeric amino acid transporters. Physiology 20:112–124
108. Chillaron J, Roca R, Valencia A, Zorzano A, Palacin M (2001) Heteromeric amino acid transporters: biochemistry, genetics, and physiology. Am J Physiol Renal Physiol 281:995–1018
109. Chillaron J, Font-Llitjos M, Fort J, Zorzano A, Goldfarb DS, Nunes V, Palacin M (2010) Pathophysiology and treatment of cystinuria. Nat Rev Nephrol 6:424–434
110. Bertran J, Werner A, Chillaron J, Nunes V, Biber J, Testar X, Zorzano A, Estivill X, Murer H, Palacin M (1993) Expression cloning of a human renal cDNA that induces high affinity transport of L-cystine shared with dibasic amino acids in *Xenopus* oocytes. J Biol Chem 268:14842–14849
111. Lee WS, Wells RG, Sabbag RV, Mohandas TK, Hediger MA (1993) Cloning and chromosomal localization of a human kidney cDNA involved in cystine, dibasic, and neutral amino acid transport. J Clin Invest 91:1959–1963
112. Reig N, Chillaron J, Bartoccioni P, Fernandez E, Bendahan A, Zorzano A, Kanner B, Palacin M, Bertran J (2002) The light subunit of system b0, + is fully functional in the absence of the heavy subunit. EMBO J 21:4906–4914
113. Fernandez E, Jimenez-Vidal M, Calvo M, Zorzano A, Tebar F, Palacin M, Chillaron J (2006) The structural and functional units of heteromeric amino acid transporters. The heavy subunit rBAT dictates oligomerization of the heteromeric amino acid transporters. J Biol Chem 281:26552–26556
114. Rosell A, Meury M, Alvarez-Marimon E, Costa M, Perez-Cano L, Zorzano A, Fernandez-Recio J, Palacin M, Fotiadis D (2014) Structural bases for the interaction and stabilization of the human amino acid transporter LAT2 with its ancillary protein 4F2hc. Proc Natl Acad Sci USA 111:2966–2971
115. Park KH, Kim TJ, Cheong TK, Kim JW, Oh BH, Svensson B (2000) Structure, specificity and function of cyclomaltodextrinase, a multispecific enzyme of the α-amylase family. Biochim Biophys Acta 1478:165–185
116. Jin X, Aimanova K, Ross LS, Gill SS (2003) Identification, functional characterization and expression of a LAT type amino acid transporter from the mosquito *Aedes aegypti*. Insect Biochem Mol Biol 33:815–827
117. Reynolds B, Roversi P, Laynes R, Kazi S, Boyd CA, Goberdhan DC (2009) *Drosophila* expresses a CD98 transporter with an evolutionarily conserved structure and amino acid-transport properties. Biochem J 420:363–372
118. Veljkovic E, Stasiuk S, Skelly PJ, Shoemaker CB, Verrey F (2004) Functional characterization of *Caenorhabditis elegans* heteromeric amino acid transporters. J Biol Chem 279:7655–7662
119. Krautz-Peterson G, Camargo S, Huggel K, Verrey F, Shoemaker CB, Skelly PJ (2007) Amino acid transport in schistosomes: characterization of the permease heavy chain SPRM1hc. J Biol Chem 282:21767–21775
120. Gabrisko M (2013) Evolutionary history of eukaryotic α-glucosidases from the α-amylase family. J Mol Evol 76:129–145
121. Verrey F, Jack DL, Paulsen IT, Saier MH Jr, Pfeiffer R (1999) New glycoprotein-associated amino acid transporters. J Membr Biol 172:181–192
122. Kuchtova A, Janecek S (2015) *In silico* analysis of family GH77 with focus on amylomaltases from borreliae and disproportionating enzymes DPE2 from plants and bacteria. Biochim Biophys Acta 1854:1260–1268
123. Smith AM, Zeeman SC, Thorneycroft D, Smith SM (2003) Starch mobilization in leaves. J Exp Bot 54:577–583
124. Kaper T, van der Maarel MJ, Euverink GJ, Dijkhuizen L (2004) Exploring and exploiting starch-modifying amylomaltases from thermophiles. Biochem Soc Trans 32:279–282
125. Pugsley AP, Dubreuil C (1988) Molecular characterization of malQ, the structural gene for the *Escherichia coli* enzyme amylomaltase. Mol Microbiol 2:473–479
126. Goda SK, Eissa O, Akhtar M, Minton NP (1997) Molecular analysis of a *Clostridium butyricum* NCIMB 7423 gene encoding 4-α-glucanotransferase and characterization of the recombinant enzyme produced in *Escherichia coli*. Microbiology 143:3287–3294
127. Bhuiyan SH, Kitaoka M, Hayashi K (2003) A cycloamylose-forming hyperthermostable 4-α-glucanotransferase of *Aquifex aeolicus* expressed in *Escherichia coli*. J Mol Catal B Enzym 22:45–53
128. Kaper T, Talik B, Ettema TJ, Bos H, van der Maarel MJ, Dijkhuizen L (2005) Amylomaltase of *Pyrobaculum aerophilum* IM2 produces thermoreversible starch gels. Appl Environ Microbiol 71:5098–5106
129. Godany A, Vidova B, Janecek S (2008) The unique glycoside hydrolase family 77 amylomaltase from *Borrelia burgdorferi* with only catalytic triad conserved. FEMS Microbiol Lett 284:84–91
130. Lee BH, Oh DK, Yoo SH (2009) Characterization of 4-α-glucanotransferase from *Synechocystis* sp. PCC 6803 and its application to various corn starches. N Biotechnol 26:29–36
131. Srisimarat W, Powviriyakul A, Kaulpiboon J, Krusong K, Zimmermann W, Pongsawasdi P (2011) A novel amylomaltase from *Corynebacterium glutamicum* and analysis of the large-

ring cyclodextrin products. J Incl Phenom Macrocycl Chem 70:369–375
132. Hwang S, Choi KH, Kim J, Cha J (2013) Biochemical characterization of 4-α-glucanotransferase from *Saccharophagus degradans* 2-40 and its potential role in glycogen degradation. FEMS Microbiol Lett 344:145–151
133. Sawasdee S, Rudeekulthamrong P, Zimmermann W, Murakami S, Pongsawasdi P, Kaulpiboo J (2014) Direct cloning of gene encoding a novel amylomaltase from soil bacterial DNA for large-ring cyclodextrin production. Appl Biochem Microbiol 50:17–24
134. Wattebled F, Ral JP, Dauvillee D, Myers AM, James MG, Schlichting R, Giersch C, Ball SG, D'Hulst C (2003) STA11, a *Chlamydomonas reinhardtii* locus required for normal starch granule biogenesis, encodes disproportionating enzyme. Further evidence for a function of α-1,4 glucanotransferases during starch granule biosynthesis in green algae. Plant Physiol 132:137–145
135. Bresolin NS, Li Z, Kosar-Hashemi B, Tetlow IJ, Chatterjee M, Rahman S, Morell MK, Howitt CA (2006) Characterisation of disproportionating enzyme from wheat endosperm. Planta 224:20–31
136. van der Maarel MJ, van der Veen B, Uitdehaag JC, Leemhuis H, Dijkhuizen L (2002) Properties and applications of starch-converting enzymes of the α-amylase family. J Biotechnol 94:137–155
137. van der Maarel MJ, Leemhuis H (2013) Starch modification with microbial α-glucanotransferase enzymes. Carbohydr Polym 93:116–121
138. Ahmad N, Mehboob S, Rashid N (2015) Starch-processing enzymes– emphasis on thermostable 4-α-glucanotransferases. Biologia 70:709–725
139. Przylas I, Terada Y, Fujii K, Takaha T, Saenger W, Sträter N (2000) X-ray structure of acarbose bound to amylomaltase from *Thermus aquaticus*. Implications for the synthesis of large cyclic glucans. Eur J Biochem 267:6903–6913
140. Barends TR, Bultema JB, Kaper T, van der Maarel MJ, Dijkhuizen L, Dijkstra BW (2007) Three-way stabilization of the covalent intermediate in amylomaltase, an α-amylase-like transglycosylase. J Biol Chem 282:17242–17249
141. Jung JH, Jung TY, Seo DH, Yoon SM, Choi HC, Park BC, Park CS, Woo EJ (2011) Structural and functional analysis of substrate recognition by the 250s loop in amylomaltase from *Thermus brockianus*. Proteins 79:633–644
142. Weiss SC, Skerra A, Schiefner A (2015) Structural basis for the interconversion of maltodextrins by MalQ, the amylomaltase of *Escherichia coli*. J Biol Chem 290:21352–21364
143. O'Neill EC, Stevenson CE, Tantanarat K, Latousakis D, Donaldson MI, Rejzek M, Nepogodiev SA, Limpaseni T, Field RA, Lawson DM (2015) Structural dissection of the maltodextrin disproportionation cycle of the *Arabidopsis* plastidial enzyme DPE1. J Biol Chem 290:29834–29853
144. Fraser CM, Casjens S, Huang WM, Sutton GG, Clayton R, Lathigra R, White O, Ketchum KA, Dodson R, Hickey EK, Gwinn M, Dougherty B, Tomb JF, Fleischmann RD, Richardson D, Peterson J, Kerlavage AR, Quackenbush J, Salzberg S, Hanson M, van Vugt R, Palmer N, Adams MD, Gocayne J, Weidman J, Utterback T, Watthey L, McDonald L, Artiach P, Bowman C, Garland S, Fuji C, Cotton MD, Horst K, Roberts K, Hatch B, Smith HO, Venter JC (1997) Genomic sequence of a Lyme disease spirochaete, *Borrelia burgdorferi*. Nature 390:580–586
145. Machovic M, Janecek S (2003) The invariant residues in the α-amylase family: just the catalytic triad. Biologia 58:1127–1132
146. Hoon-Hanks LL, Morton EA, Lybecker MC, Battisti JM, Samuels DS, Drecktrah D (2012) *Borrelia burgdorferi* malQ mutants utilize disaccharides and traverse the enzootic cycle. FEMS Immunol Med Microbiol 66:157–165
147. Lloyd JR, Blennow A, Burhenne K, Kossmann J (2004) Repression of a novel isoform of disproportionating enzyme (stDPE2) in potato leads to inhibition of starch degradation in leaves but not tubers stored at low temperature. Plant Physiol 134:1347–1354
148. Lütken H, Lloyd JR, Glaring MA, Baunsgaard L, Laursen KH, Haldrup A, Kossmann J, Blennow A (2010) Repression of both isoforms of disproportionating enzyme leads to higher maltooligosaccharide content and reduced growth in potato. Planta 232:1127–1139
149. Chia T, Thorneycroft D, Chapple A, Messerli G, Chen J, Zeeman SC, Smith SM, Smith AM (2004) A cytosolic glucosyltransferase is required for conversion of starch to sucrose in *Arabidopsis* leaves at night. Plant J 37:853–863
150. Lu Y, Sharkey TD (2004) The role of amylomaltase in maltose metabolism in the cytosol of photosynthetic cells. Planta 218:466–473
151. Steichen JM, Petty RV, Sharkey TD (2008) Domain characterization of a 4-α-glucanotransferase essential for maltose metabolism in photosynthetic leaves. J Biol Chem 283:20797–20804
152. Ruzanski C, Smirnova J, Rejzek M, Cockburn D, Pedersen HL, Pike M, Willats WG, Svensson B, Steup M, Ebenhöh O, Smith AM, Field RA (2013) A bacterial glucanotransferase can replace the complex maltose metabolism required for starch to sucrose conversion in leaves at night. J Biol Chem 288:28581–28598
153. Srisimarat W, Murakami S, Pongsawasdi P, Krusong K (2013) Crystallization and preliminary X-ray crystallographic analysis of the amylomaltase from *Corynebacterium glutamicum*. Acta Crystallogr, Sect F: Struct Biol Cryst Commun 69:1004–1006
154. Srisimarat W, Kaulpiboon J, Krusong K, Zimmermann W, Pongsawasdi P (2012) Altered large-ring cyclodextrin product profile due to a mutation at Tyr-172 in the amylomaltase of *Corynebacterium glutamicum*. Appl Environ Microbiol 78:7223–7228
155. Rachadech W, Nimpiboon P, Naumthong W, Nakapong S, Krusong K, Pongsawasdi P (2015) Identification of essential tryptophan in amylomaltase from *Corynebacterium glutamicum*. Int J Biol Macromol 76:230–235
156. D'Amico S, Gerday C, Feller G (2000) Structural similarities and evolutionary relationships in chloride-dependent α-amylases. Gene 253:95–105
157. Da Lage JL, Danchin EG, Casane D (2007) Where do animal α-amylases come from? An interkingdom trip. FEBS Lett 581:3927–3935
158. Sivakumar N, Li N, Tang JW, Patel BK, Swaminathan K (2006) Crystal structure of AmyA lacks acidic surface and provide insights into protein stability at poly-extreme condition. FEBS Lett 580:2646–2652
159. Møller MS, Fredslund F, Majumder A, Nakai H, Poulsen JC, Lo Leggio L, Svensson B, Abou Hachem M (2012) Enzymology and structure of the GH13_31 glucan 1,6-α-glucosidase that confers isomaltooligosaccharide utilization in the probiotic *Lactobacillus acidophilus* NCFM. J Bacteriol 194:4249–4259
160. Kobayashi M, Saburi W, Nakatsuka D, Hondoh H, Kato K, Okuyama M, Mori H, Kimura A, Yao M (2015) Structural insights into the catalytic reaction that is involved in the reorientation of Trp238 at the substrate-binding site in GH13 dextran glucosidase. FEBS Lett 589:484–489
161. Saburi W, Rachi-Otsuka H, Hondoh H, Okuyama M, Mori H, Kimura A (2015) Structural elements responsible for the glucosidic linkage-selectivity of a glycoside hydrolase family 13 exo-glucosidase. FEBS Lett 589:865–869
162. Møller MS, Henriksen A, Svensson B (2016) Structure and function of α-glucan debranching enzymes. Cell Mol Life Sci. doi:10.1007/s00018-016-2241-y

163. Okuyama M, Saburi W, Mori H, Kimura A (2016) α-Glucosidases and α-1,4-glucan lyases: structures, functions, and physiological actions. Cell Mol Life Sci. doi:10.1007/s00018-016-2247-5
164. Janecek S, Blesak K (2011) Sequence-structural features and evolutionary relationships of family GH57 α-amylases and their putative α-amylase-like homologues. Protein J 30:429–435
165. Blesak K, Janecek S (2012) Sequence fingerprints of enzyme specificities from the glycoside hydrolase family GH57. Extremophiles 16:497–506
166. Blesak K, Janecek S (2013) Two potentially novel amylolytic enzyme specificities in the prokaryotic glycoside hydrolase α-amylase family GH57. Microbiology 159:2584–2593
167. Janecek S, Kuchtova A (2012) In silico identification of catalytic residues and domain fold of the family GH119 sharing the catalytic machinery with the α-amylase family GH57. FEBS Lett 586:3360–3366
168. Passerini D, Vuillemin M, Ufarte L, Morel S, Loux V, Fontagné-Faucher C, Monsan P, Remaud-Simeon M, Moulis C (2015) Inventory of the GH70 enzymes encoded by *Leuconostoc citreum* NRRL B-1299—identification of three novel α-transglucosylases. FEBS J 282:2115–2130
169. Moulis C, André I, Remaud-Simeon M (2016) GH13 amylosucrases and GH70 branching sucrases, atypical enzymes in their respective families. Cell Mol Life Sci. doi:10.1007/s00018-016-2244-8
170. Meng X, Gangoiti J, Bai Y, Pijning T, Van Leeuwen SS, Dijkhuizen L (2016) Structure-function relationships of family GH70 glucansucrase and 4,6-α-glucanotransferase enzymes, and their evolutionary relationships with family GH13 enzymes. Cell Mol Life Sci. doi:10.1007/s00018-016-2245-7
171. Gangoiti J, Pijning T, Dijkhuizen L (2016) Biochemical characterization of the *Exiguobacterium sibiricum* 255-15 GtfC enzyme representing a novel glycoside hydrolase 70 subfamily of 4,6-α-glucanotransferase enzymes. Appl Environ Microbiol 82:756–766
172. Gangoiti J, van Leeuwen SS, Vafiadi C, Dijkhuizen L (2016) The Gram-negative bacterium *Azotobacter chroococcum* NCIMB 8003 employs a new glycoside hydrolase family 70 4,6-α-glucanotransferase enzyme (GtfD) to synthesize a reuteran like polymer from maltodextrins and starch. Biochim Biophys Acta 1860:1224–1236
173. Boraston AB, Bolam DN, Gilbert HJ, Davies GJ (2004) Carbohydrate-binding modules: fine-tuning polysaccharide recognition. Biochem J 382:769–781
174. Machovic M, Janecek S (2006) Starch-binding domains in the post-genome era. Cell Mol Life Sci 63:2710–2724
175. Carvalho CC, Phan NN, Chen Y, Reilly PJ (2015) Carbohydrate-binding module tribes. Biopolymers 103:203–214
176. Christiansen C, Abou Hachem M, Janecek S, Viksø-Nielsen A, Blennow A, Svensson B (2009) The carbohydrate-binding module family 20—diversity, structure, and function. FEBS J 276:5006–5029
177. Janecek S, Svensson B, MacGregor EA (2011) Structural and evolutionary aspects of two families of non-catalytic domains present in starch and glycogen binding proteins from microbes, plants and animals. Enzyme Microb Technol 49:429–440
178. Majzlova K, Janecek S (2014) Two structurally related starch-binding domain families CBM25 and CBM26. Biologia 69:1087–1096
179. Koropatkin NM, Smith TJ (2010) SusG: a unique cell-membrane-associated α-amylase from a prominent human gut symbiont targets complex starch molecules. Structure 18:200–215
180. Cameron EA, Maynard MA, Smith CJ, Smith TJ, Koropatkin NM, Martens EC (2012) Multidomain carbohydrate-binding proteins involved in *Bacteroides thetaiotaomicron* starch metabolism. J Biol Chem 287:34614–34625
181. Peng H, Zheng Y, Chen M, Wang Y, Xiao Y, Gao Y (2014) A starch-binding domain identified in α-amylase (AmyP) represents a new family of carbohydrate-binding modules that contribute to enzymatic hydrolysis of soluble starch. FEBS Lett 588:1161–1167
182. Xu J, Ren F, Huang CH, Zheng Y, Zhen J, Sun H, Ko TP, He M, Chen CC, Chan HC, Guo RT, Song H, Ma Y (2014) Functional and structural studies of pullulanase from *Anoxybacillus* sp. LM18-11. Proteins 82:1685–1693
183. Ze X, Ben David Y, Laverde-Gomez JA, Dassa B, Sheridan PO, Duncan SH, Louis P, Henrissat B, Juge N, Koropatkin NM, Bayer EA, Flint HJ (2015) Unique organization of extracellular amylases into amylosomes in the resistant starch-utilizing human colonic Firmicutes bacterium *Ruminococcus bromii*. MBio 6:e01058–e01115
184. Foley MH, Cockburn DW, Koropatkin NM (2016) The Sus operon: a model system for starch uptake by the human gut Bacteroidetes. Cell Mol Life Sci. doi:10.1007/s00018-016-2242-x
185. Pfister B, Zeeman SC (2016) Formation of starch in plant cells. Cell Mol Life Sci. doi:10.1007/s00018-016-2250-x
186. Niittyla T, Comparot-Moss S, Lue WL, Messerli G, Trevisan M, Seymour MD, Gatehouse JA, Villadsen D, Smith SM, Chen J, Zeeman SC, Smith AM (2006) Similar protein phosphatases control starch metabolism in plants and glycogen metabolism in mammals. J Biol Chem 281:11815–11818
187. Minassian BA, Ianzano L, Meloche M, Andermann E, Rouleau GA, Delgado-Escueta AV, Scherer SW (2000) Mutation spectrum and predicted function of laforin in Lafora's progressive myoclonus epilepsy. Neurology 55:341–346
188. Ganesh S, Tsurutani N, Suzuki T, Hoshii Y, Ishihara T, Delgado-Escueta AV, Yamakawa K (2004) The carbohydrate-binding domain of Lafora disease protein targets Lafora polyglucosan bodies. Biochem Biophys Res Commun 313:1101–1109
189. Gentry MS, Dixon JE, Worby CA (2009) Lafora disease: insights into neurodegeneration from plant metabolism. Trends Biochem Sci 34:628–639
190. Gentry MS, Dowen RH 3rd, Worby CA, Mattoo S, Ecker JR, Dixon JE (2007) The phosphatase laforin crosses evolutionary boundaries and links carbohydrate metabolism to neuronal disease. J Cell Biol 178:477–488
191. Tagliabracci VS, Turnbull J, Wang W, Girard JM, Zhao X, Skurat AV, Delgado-Escueta AV, Minassian BA, Depaoli-Roach AA, Roach PJ (2007) Laforin is a glycogen phosphatase, deficiency of which leads to elevated phosphorylation of glycogen *in vivo*. Proc Natl Acad Sci USA 104:19262–19266
192. Gentry MS, Roma-Mateo C, Sanz P (2013) Laforin, a protein with many faces: glucan phosphatase, adapter protein, et alii. FEBS J 280:525–537
193. Raththagala M, Brewer MK, Parker MW, Sherwood AR, Wong BK, Hsu S, Bridges TM, Paasch BC, Hellman LM, Husodo S, Meekins DA, Taylor AO, Turner BD, Auger KD, Dukhande VV, Chakravarthy S, Sanz P, Woods VL Jr, Li S, Vander Kooi CW, Gentry MS (2015) Structural mechanism of laforin function in glycogen dephosphorylation and lafora disease. Mol Cell 57:261–272
194. Sankhala RS, Koksal AC, Ho L, Nitschke F, Minassian BA, Cingolani G (2015) Dimeric quaternary structure of human laforin. J Biol Chem 290:4552–4559
195. Kötting O, Santelia D, Edner C, Eicke S, Marthaler T, Gentry MS, Comparot-Moss S, Chen J, Smith AM, Steup M, Ritte G, Zeeman SC (2009) STARCH-EXCESS4 is a laforin-like phosphoglucan phosphatase required for starch degradation in *Arabidopsis thaliana*. Plant Cell 21:334–346
196. Vander Kooi CW, Taylor AO, Pace RM, Meekins DA, Guo HF, Kim Y, Gentry MS (2010) Structural basis for the glucan

phosphatase activity of starch excess4. Proc Natl Acad Sci USA 107:15379–15384
197. Emanuelle S, Brewer MK, Meekins DA, Gentry MS (2016) Unique carbohydrate binding platforms employed by the glucan phosphatases. Cell Mol Life Sci. doi:10.1007/s00018-016-2249-3
198. Hudson ER, Pan DA, James J, Lucocq JM, Hawley SA, Green KA, Baba O, Terashima T, Hardie DG (2003) A novel domain in AMP-activated protein kinase causes glycogen storage bodies similar to those seen in hereditary cardiac arrhythmias. Curr Biol 13:861–866
199. Polekhina G, Gupta A, Michell BJ, van Denderen B, Murthy S, Feil SC, Jennings IG, Campbell DJ, Witters LA, Parker MW, Kemp BE, Stapleton D (2003) AMPK β subunit targets metabolic stress sensing to glycogen. Curr Biol 13:867–871
200. Polekhina G, Gupta A, van Denderen BJ, Feil SC, Kemp BE, Stapleton D, Parker MW (2005) Structural basis for glycogen recognition by AMP-activated protein kinase. Structure 13: 1453–1462
201. Emanuelle S, Hossain MI, Moller IE, Pedersen HL, van de Meene AM, Doblin MS, Koay A, Oakhill JS, Scott JW, Willats WG, Kemp BE, Bacic A, Gooley PR, Stapleton DI (2015) SnRK1 from Arabidopsis thaliana is an atypical AMPK. Plant J 82:183–192
202. Janecek S (2002) A motif of a microbial starch-binding domain found in human genethonin. Bioinformatics 18:1534–1537
203. Jiang S, Heller B, Tagliabracci VS, Zhai L, Irimia JM, DePaoli-Roach AA, Wells CD, Skurat AV, Roach PJ (2010) Starch binding domain-containing protein 1/genethonin 1 is a novel participant in glycogen metabolism. J Biol Chem 285:34960–34971
204. Palopoli N, Busi MV, Fornasari MS, Gomez-Casati D, Ugalde R, Parisi G (2006) Starch-synthase III family encodes a tandem of three starch-binding domains. Proteins 65:27–31
205. Gomez-Casati DF, Martín M, Busi MV (2013) Polysaccharide-synthesizing glycosyltransferases and carbohydrate binding modules: the case of starch synthase III. Protein Pept Lett 20:856–863
206. Barchiesi J, Hedin N, Gomez-Casati DF, Ballicora MA, Busi MV (2015) Functional demonstrations of starch binding domains present in Ostreococcus tauri starch synthases isoforms. BMC Res Notes 8:613
207. Mikkelsen R, Suszkiewicz K, Blennow A (2006) A novel type carbohydrate-binding module identified in α-glucan, water dikinases is specific for regulated plastidial starch metabolism. Biochemistry 45:4674–4682
208. Glaring MA, Baumann MJ, Abou Hachem M, Nakai H, Nakai N, Santelia D, Sigurskjold BW, Zeeman SC, Blennow A, Svensson B (2011) Starch-binding domains in the CBM45 family—low-affinity domains from glucan, water dikinase and α-amylase involved in plastidial starch metabolism. FEBS J 278:1175–1185
209. Hejazi M, Steup M, Fettke J (2012) The plastidial glucan, water dikinase (GWD) catalyses multiple phosphotransfer reactions. FEBS J 279:1953–1966
210. Orzechowski S, Grabowska A, Sitnicka D, Siminska J, Felus M, Dudkiewicz M, Fudali S, Sobczak M (2013) Analysis of the expression, subcellular and tissue localisation of phosphoglucan, water dikinase (PWD/GWD3) in *Solanum tuberosum* L.: a bioinformatics approach for the comparative analysis of two α-glucan, water dikinases (GWDs) from *Solanum tuberosum* L. Acta Physiol Plant 35:483–500
211. Mahlow S, Orzechowski S, Fettke J (2016) Starch phosphorylation: insights and perspectives. Cell Mol Life Sci. doi:10.1007/s00018-016-2248-4
212. Vu VV, Beeson WT, Span EA, Farquhar ER, Marletta MA (2014) A family of starch-active polysaccharide monooxygenases. Proc Natl Acad Sci USA 111:13822–13827
213. Lo Leggio L, Simmons TJ, Poulsen JC, Frandsen KE, Hemsworth GR, Stringer MA, von Freiesleben P, Tovborg M, Johansen KS, De Maria L, Harris PV, Soong CL, Dupree P, Tryfona T, Lenfant N, Henrissat B, Davies GJ, Walton PH (2015) Structure and boosting activity of a starch-degrading lytic polysaccharide monooxygenase. Nat Commun 6:5961
214. Vu VV, Marletta MA (2016) Starch-degrading polysaccharide monooxygenases. Cell Mol Life Sci. doi:10.1007/s00018-016-2251-9
215. Cuyvers S, Dornez E, Delcour JA, Courtin CM (2012) Occurrence and functional significance of secondary carbohydrate binding sites in glycoside hydrolases. Crit Rev Biotechnol 32:93–107
216. Cockburn D, Wilkens C, Ruzanski C, Andersen S, Willum Nielsen J, Smith AM, Field RA, Willemoës M, Abou Hachem M, Svensson B (2014) Analysis of surface binding sites (SBSs) in carbohydrate active enzymes with focus on glycoside hydrolase families 13 and 77—a mini-review. Biologia 69:705–712
217. Ragunath C, Manuel SG, Venkataraman V, Sait HB, Kasinathan C, Ramasubbu N (2008) Probing the role of aromatic residues at the secondary saccharide-binding sites of human salivary α-amylase in substrate hydrolysis and bacterial binding. J Mol Biol 384:1232–1248
218. Cockburn D, Nielsen MM, Christiansen C, Andersen JM, Rannes JB, Blennow A, Svensson B (2015) Surface binding sites in amylase have distinct roles in recognition of starch structure motifs and degradation. Int J Biol Macromol 75:338–345
219. Suzuki E, Suzuki R (2016) Distribution of glucan-branching enzymes among prokaryotes. Cell Mol Life Sci. doi:10.1007/s00018-016-2243-9
220. Coutinho PM, Henrissat B (1999) Life with no sugars? J Mol Microbiol Biotechnol 1:307–308
221. UniProt Consortium (2015) UniProt: a hub for protein information. Nucleic Acids Res 43:D204–D212
222. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425
223. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948
224. Letunic I, Bork P (2007) Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics 23:127–128
225. Deshpande N, Addess KJ, Bluhm WF, Merino-Ott JC, Townsend-Merino W, Zhang Q, Knezevich C, Xie L, Chen L, Feng Z, Green RK, Flippen-Anderson JL, Westbrook J, Berman HM, Bourne PE (2005) The RCSB Protein Data Bank: a redesigned query system and relational database based on the mmCIF schema. Nucl Acids Res 3:D233–D237
226. The PyMOL Molecular Graphics System, Version 1.7.4 Schrödinger, LLC
227. Shatsky M, Nussinov R, Wolfson HJ (2004) A method for simultaneous alignment of multiple protein structures. Proteins 56:143–156
228. Arnold K, Bordoli L, Kopp J, Schwede T (2006) The SWISS-MODEL Workspace: a web-based environment for protein structure homology modelling. Bioinformatics 22:195–201
229. Davies GJ, Wilson KS, Henrissat B (1997) Nomenclature for sugar-binding subsites in glycosyl hydrolases. Biochem J 321:557–559
230. Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Söding J, Thompson JD, Higgins DG (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. Mol Syst Biol 7:539