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Reviews

The mechanism of glutamine-dependent amidotransferases

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Abstract. Glutamine-dependent amidotransferases have been known for more than 30 years. The mechanism by which these enzymes generate ammonia from the glutamine amide nitrogen and transfer it to seven different chemical classes of acceptors has been the subject of intense scrutiny for the last 5 years. The increasing number of biochemical and structural studies dealing with amidotransferases and with mechanistically related enzymes has disclosed the dichotomy of the mechanisms within these enzymes for achieving the glutamine amide bond cleavage. Some of them use a catalytic Cys/His/Glu triad similar to serine protease, whereas the aminoterminal cysteine of the others is believed to play the same function. The transfer of ammonia from the glutamine site to the acceptor site which must operate in a concerted manner has been demonstrated in two cases to involve channelling but is still matter of investigation.

Key words. Glutamine-dependent amidotransferase; catalytic mechanism; ammonia transfer.

Introduction

One of the main metabolic pathways for incorporation of nitrogen into biological molecules utilizes the amido group on the side chain of glutamine. The enzymes that catalyse the transfer of the amido nitrogen of glutamine to an acceptor substrate (S) to produce one molecule of glutamate and one molecule of an aminated product (P), equation (1a), have been called glutamine-dependent amidotransferases (Gn-AT). This peculiar reaction requires neither utilization of cofactor nor hydrolysis of ATP. However, for some Gn-AT, the whole catalysed transformation also includes either one preliminary step equation (1b), to form the activated acceptor substrate S′ at the expense of one molecule of ATP, or one subsequent step equation (1c), to further transform the aminated product P into a more stable final product P′ when necessary. Sixteen glutamine-dependent amidotransferases have been identified to date [1] (see tables 1a–c). They are involved in the biosynthesis of nucleotides (purines and pyrimidines), amino acids (tryptophan, histidine, as-

S + H_2N ···COOH / O= NH_2 —**1a**, H_2O→ H_2N ···COOH / O= OH + P (1)

1b ATP (S ⇌ S')

S : acceptor substrate ; P : nitrogen containing product

1c (P ⇌ P')

S' P'

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Table 1a. Class I glutamine-dependent amidotransferases.

Enzyme	Product	Biosynthetic route	Peculiarities
Anthranilate synthase (EC 4.1.3.27)	COOH, NH_2	tryptophan	-
Carbamoyl phosphate synthetase (EC 6.3.5.5)	O, H_2N, O(P)	arginine pyrimidines (UTP, CTP)	nitrogen transfer + phosphorylation of the 'aminated' product complex glutamine:enzyme isolated X-ray structure solved
CTP synthetase (EC 6.3.4.2)	NH_2, N, O, N, ribose triphosphate	CTP	complex glutamine:enzyme isolated
Formylglycinamidine synthetase (EC 6.3.5.3)	HN, N–CHO, H, NH, O, (P)O, HO OH	purines (AMP, GMP)	complex glutamine:enzyme isolated
GMP synthetase (EC 6.3.5.2)	O, HN, N, H_2N, N, N, ribose phosphate	GMP	X-ray structure solved
Imidazole glycerol phosphate synthase (EC: n.d.)		histidine	nitrogen transfer + cyclocondensation of the 'aminated' product
Aminodeoxychorismate synthase (EC: n.d.)	OH, H, N, O(P), OH, N	folic acid	part of the *p*-aminobenzoate synthase complex complex glutamine:enzyme isolated no glutaminase activity detected

paragine, glutamate), aminated sugars (glucosamine), coenzymes (nicotinamide adenine dinucleotide (NAD), folic acid and coenzyme B_{12}) and antibiotics (chloramphenicol). Furthermore, 4-amino-3,4-dideoxy-7-phospho-D-arabino-heptulosonate synthase (aminoD-AHP synthase, table 1c), which is involved in the biosynthesis of the antibiotic rifamycin B by the yeast *Nocardia mediterranei* [2] might also catalyse an amidotransferase reaction, but the experimental proof is still lacking.

The goal of this review is not to duplicate the excellent overview written 5 years ago [1] to which the reader should refer to for specific information about any Gn-AT. Rather, our purpose is to give an insight into the intriguing mechanism of the amidotransferase reaction (eq. 1a), focusing on transversal properties of these enzymes and summarizing the last years' progress. After 3 decades of considerable work on this challenging question, very recent structural determinations have significantly improved our understanding of this process.

Problems with nomenclature and classification of the Gn-AT

One remarkable feature about Gn-At is that their two-(or three)-substrate transformation (eq. 1) may be regarded from different points of view. Hence, today's literature searches are often complicated by the existence of several

Table 1b. Class II glutamine-dependent amidotransferases.

Enzyme	Product	Biosynthetic route	Peculiarities
Glutamine-PRPP amidotransferase (EC 2.4.2.14)		purines (AMP, GMP)	NH_3-dependent higher than glutamine-dependent activity X-ray structure solved
Glucosamine 6-phosphate synthase EC 2.6.1.16)		hexosamines	nitrogen transfer + isomerisation of the 'aminated' product NH_3 not substrate X-ray structures of both separated GAT and synthase domains solved
Asparagine synthetase (EC 6.3.1.1)		asparagine	NH_3-dependent higher than glutamine-dependent activity glutaminase activity higher than glutamine-dependent synthase activity
Glutamate synthase (EC 1.4.1.13)		glutamic acid	nitrogen transfer + reduction of the 'aminated' product flavine as a cofactor NADPH as electron donor

names for a single enzyme. Furthermore, some of these names do not clearly display the amidotransferase nature of the catalysed reaction, especially when the stress is only put on the net synthesis of the aminated product, (synthases or synthetases), or on the peculiar aspect of the subsequent transformation of the aminated product, (eq. 1c), which may, for instance, be isomerisation or reduction. It now appears that the name of the enzyme should reflect the characteristic aspect of the reaction which is the transfer of the nitrogen group from glutamine. Therefore, systematic names of the following type have been proposed for each Gn-AT: 'glutamine:[name of substrate S (or S′)] amidotransferase', e.g. 'L-glutamine:D-fructose-6-P amidotransferase' for the enzyme usually called 'D-glucosamine-6-P synthase'. The term *amidotransferases* (or *transamidase*) has been adopted to distinguish these enzymes from the aminotransferases or transaminases (sub-subclass 2.6.1), which catalyse the well-known pyridoxal 5′-phosphate-dependent transfer of the particular α-amino nitrogen from amino acids to α-keto acids (often α-ketoglutarate)[1].
Besides, most Gn-AT have been classified by only taking into account reactions 1b or 1c instead of the characteristic reaction 1a (eq. 1). Hence, this newly recognized family of enzymes is still scattered today throughout the International Union of Biochemistry and Molecular Biology's official classification [3]. Only the five of them that use ATP have been brought together as sub-subclass 6.3.5 (C–N ligases using glutamine as nitrogen donor), but the other Gn-AT cannot be considered as ligases since they do not hydrolyze ATP. Rather, a specific sub-subclass should be created for amidotransferases, likely in the subclass 2.6 that is among the transferases transferring nitrogenous groups. The need for a change of classification is especially acute for glucosamine-6-P synthase, which is obviously, for the reasons mentioned above, an intruder in the aminotransferase sub-subclass (E.C. 2.6.1)[2].

Common properties

Reaction irreversibility

For all Gn-AT, the whole transformation seems to be an irreversible process [4] due to the irreversibility of the amidotransferase reaction (eq. 1a). For some enzymes, however, the subsequent step (eq. 1c) has been shown to be reversible, e.g. the reduction of the intermediary imine for glutamate synthase [5].

Use of exogenous ammonia

Almost every Gn-AT can accept exogenous ammonia as a nitrogen donor and therefore display an ammonia-dependent activity (eq. 2).

[1] No confusion should either be made with amidinotransferases which catalyse the transfer of an amidine group NH_2–CH=NH_2^+.

[2] The problem with glucosamine-6-P synthase is that its classification has already changed once, since it was first regarded as an isomerase (for this Gn-AT, reaction 1c is of aldose/ketose isomerization type).

Table 1c. Non classified glutamine-dependent amidotransferases.

Enzyme	Product	Biosynthetic route	Peculiarities
NAD synthetase (EC 6.3.5.1)	(nicotinamide, N-linked) ribose-5'-diphosphoadenosine	NAD^+	-
Arylamine synthetase (EC: n.d.)	n.d.	chloramphenicol (antibiotic)	- -
Glu-ARNt^{Gln} amidotransferase (EC: n.d.)	NH_2, H_2N, ARNt, O, O	Gln-ARNt^{Gln}	asparagine is an alternate nitrogen donor GAT domain displays amidase signature[11]
Cobyrinic acid *a*,*c*-diamide synthetase (EC: n.d.)	COBYRINATE: *a* H_2NOC, *b* CO_2H, *c* $CONH_2$, *d* CO_2H, *e* CO_2H, *f* HO_2C, *g* HO_2C	cobalamine (coenzyme B_{12})	transfer of 2 nitrogen atoms on acceptor substrate
Cobyric acid synthetase (EC: n.d.)	COBYRINATE: *a* H_2NOC, *b* $CONH_2$, *c* $CONH_2$, *d* $CONH_2$, *e* $CONH_2$, *f* HO_2C, *g* H_2NOC	cobalamine (coenzyme B_{12})	transfer of 4 nitrogen atoms on acceptor substrate
AminoDAHP synthase (EC: n.d.)	COOH, =O, CH_2, H_2N, OH, OH, O(P)	ansamycines mitomycines (antibiotics)	nucleophilic transfer + nucleophilic addition on the 'aminated' product

$$\mathbf{S} + NH_3(+ATP) \longrightarrow \mathbf{P}(+AMP+PPi) \qquad (2)$$

Whereas the glutamine-dependent activity is optimal at neutral pH, the NH_3-dependent activity is optimal at higher pH, e.g. 8.5 for *Escherichia coli* imidazole glycerol phosphate synthase [6], at which the unprotonated ammonia concentration is high.

At neutral pH, for most Gn-AT, it is a minor activity that represents as low as a few percent of the glutamine-dependent activity, e.g. for bacterial formylglycinamidine synthetase [7] and glutamate synthase [8]. For Glu-tRNA^{Gln} amidotransferase [9] and yeast NAD synthetase [10], both activities are comparable. It is noteworthy that *E. coli* glutamine-phosphoribosyl pyrophosphate (PRPP) amidotransferase [11] and human asparagine synthetase [12] have greater ammonia- than glutamine-dependent activity, while glucosamine-6-phosphate synthase [13] and *R. capsulatus* glutamate synthase [14] are the only Gn-AT that possess no ammonia-dependent activity.

The values of K_m and k_{cat}/K_m at neutral pH clearly show that glutamine is by far the best amino nitrogen donor for most of the amidotransferases. However, the NH_3-dependent reaction has been shown to be functional in vivo for anthranilate synthase [15], glutamine-PRPP

amidotransferase [16] and carbamoyl phosphate synthetase [17]. But for *E. coli* aminodeoxychorismate synthase [1] and *Azospirillum brasilense* glutamate synthase [18], the K_m values for ammonia (0.2–0.5 M) are so high that ammonia cannot be a substrate in vivo.

As the ammonia-dependent activity is absent for some Gn-At, and as its extent is highly variable among the rest of the family, its occurrence was once believed to be fortuitous[3]. On the other hand, regardless of its variability, this widespread property might be significant for the mechanism of the amidotransferase reaction: it could indicate that the transferred group is actually NH_3. We shall examine this point later and see how very recent structural results on some Gn-At seem to support this idea.

The amidotransferase activity seems to be highly specific for glutamine utilization; besides ammonia, no other nitrogen donor has been reported, except for the case of Glu-tRNA^{Gln} amidotransferase, which accepts three nitrogen donor substrates: glutamine, ammonia and asparagine, in decreasing order of activity [9].

Glutaminase activity

Early studies (see ref. 1) have shown that almost every Gn-AT is able to hydrolyse glutamine (glutaminase activity) in the absence of the acceptor substrate (eq. 3).

$$\text{Glutamine} \xrightarrow{H_2O} \text{Glutamate} + NH_3 \qquad (3)$$

This amidohydrolase activity can also be detected on substrates that do not support nitrogen transfer, such as γ-hydroxamate glutamic acid [19] or γ-glutamyl-*para*-nitroanilide (fig. 1) [20]. This activity is generally very low, and has not even been detected for aminodeoxychorismate synthase or *R. capsulatus* glutamate synthase. It amounts to only a few percent of the glutamine-dependent activity for most of the other enzymes, except for asparagine synthetase [12], for which it is the major activity.

Gn-AT are modular enzymes

As first predicted by primary sequence alignments, Gn-AT are organized in domains bearing glutamine and substrate S (or S′) binding sites[4]. The domain containing the glutamine site has been called the GAT domain (glutamine amide transfer domain [1]) and possesses glutaminase activity (eq. 3). The domain containing the acceptor substrate site is called the synthase (or synthetase) domain and is generally able to catalyse the ammonia-dependent reaction (eq. 2). Although GAT domains are highly homologous throughout the Gn-AT family (although of two types, as will be discussed below), synth(et)ase domains are all different, since they bind different substrates S and S′, and since these substrates bear different chemical functions as acceptors of nitrogen group.

It has been confirmed that the distinction between the GAT domain and the synth(et)ase domain is a topological reality. In bovine asparagine synthetase, although located on the same polypeptide chain, the two sites have been shown to be topologically distinct, since several monoclonal antibodies selectively inhibit either the glutaminase or the ammonia-dependent synth(et)ase activity [21]. For bacterial glucosamine-6-P synthase, controlled chymotrypsin hydrolysis cleaves the peptide chain into catalytically active GAT and synthase domains which, furthermore, can be separately expressed and characterized [20, 22].

Although GAT and synth(et)ase domains can be precisely distinguished on the primary sequence of every Gn-AT, their relative arrangement is highly variable [1]. They can be situated either on the same polypeptide chain (cytidine triphosphate (CTP) synthetase, formylglycinamidine synthetase, guanosine monophosphate (GMP) synthetase, Gln:PRPP amidotransferase, glucosamine-6-P synthase, asparagine synthetase), or on distinct subunits in heterodimeric Gn-AT such as anthranilate synthase [23], bacterial aminodeoxychorismate synthase [24], bacterial imidazole glycerol-P synthase [25] and bacterial carbamoyl-P synthetase [26], or even situated on subunits from distinct enzymes (joined together in a multienzymatic complex). Different arrangements may even be found for a single enzyme, depending only on its origin (e.g. anthranilate synthase, carbamoyl-P synthetase). Recently, a 29-amino acid sequence of mammalian carbamoyl-P synthetase was identified to function as a linker between GAT and synthetase domains whose deletion gives a protein that is still active, but lacks allosteric sensitivity [27]. Similarly, after fusion of the two subunits of *E. coli* carbamoyl-P synthetase, the resulting protein is active but unresponsive to ornithine, an allosteric inhibitor of the native enzyme [28]. Therefore, the high variability of the relative arrangements between the two Gn-AT domains may reflect the complex regulation of these enzymes, which is specific in each organism; indeed, most Gn-AT are known to be key enzymes for their biosynthetic pathway, and many effectors have been reported [1].

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[3] As will be seen later, the acceptor function is electrophilic, and NH_3 could be only an occasional substrate with no implication on the amidotransferase mechanism. In this case, water, or other nucleophiles in solution, might be expected to add to the acceptor substrate, but there is no report of the formation of the corresponding products. Besides, it is not clear whether the ammonia-dependent activity is a vestige of an ancestral enzyme activity that only used ammonia and would have evolved to use glutamine.

[4] This modular organization within the amidotransferase family has been recently suggested [90, 52].

Figure 1. Alternate substrates for glutaminase activity and main inactivators.

A catalytic cysteine residue in the GAT domain

Chemical labelling and site-directed mutagenesis experiments have shown that all Gn-AT have a cysteine residue that is essential for catalytic activity. Gn-AT are rapidly inactivated by thiol reagents like iodoacetamide and by glutamine analogues (fig. 1) like 6-diazo-5-oxo-L-norleucine (DON) [29–33], 6-chloro-5-oxo-L-norleucine (chloroketone) [34, 35], azaserine [29, 32, 36, 37] or acivicine [38]. Assays that test for enzyme thiol groups before and after inactivation indicate that the cited compounds act by forming a covalent adduct with the thiol group of a cysteine residue whose position may then be identified by radiolabelling.

As a general rule, when this residue is chemically modified or replaced by site-directed mutagenesis, the resulting protein loses both glutamine-dependent synth(et)ase and glutaminase activities. On the contrary, the ammonia-dependent synth(et)ase activity (when existing) is generally unaffected, and may even be stimulated in some cases (e.g. Gln:PRPP amidotransferase [29]). These results show that the catalytic cysteine residue is involved in the generation of the nitrogen group to be transferred from glutamine.

Kinetic mechanisms of the Gn-AT transformation

The order of substrate binding and product release has not been studied for all amidotransferases, and most of the available results are incomplete [1]. Nevertheless, a common feature, compatible with a concerted nitrogen group transfer, can be seen: all kinetics determined to date are ordered mechanisms, in which both glutamine and acceptor substrate are bound to their respective sites before the nitrogen transfer can take place. Two sets of figures can be distinguished. For formylglycinamidine synthetase [7] and carbamoyl-P synthetase [39], glutamine is bound first, whereas for glucosamine-6-P synthase [40], asparagine synthetase[5] [41], glutamate synthase [42, 43], aminodeoxychorismate synthase [44], and anthranilate synthase [23], the acceptor substrate is bound before glutamine.

[5] The most recent data for asparagine synthetase (class II) correct earlier published results indicating that glutamate was released before the acceptor was bound (implying that native ammonia was generated and remained bound to the enzyme).

Evidence of substrate-induced conformational changes

Several indications point to there being a major change in the conformation of the molecule during the catalytic cycle.

In heterodimeric bacterial carbamoyl phosphate synthetase, the affinity for glutamine of the GAT domain-bearing subunit is considerably increased in the presence of the synthetase subunit. First, it has been proposed that this could be due to partial binding of glutamine to the synthetase subunit [26]; nevertheless it could also reflect a change in the rate-determining step of the reaction which could render the Michaelis constant higher or lower than the dissociation constant of the enzyme:glutamine complex.

The presence of substrate S′ or of ATP has been shown to enhance the glutaminase activity of Gln-PRPP amidotransferase [45], Glu-tRNA^{Gln} amidotransferase [9], aminodeoxychorismate synthase [46], anthranilate synthase [23] and carbamoyl-P synthetase [26], which suggests that conformational changes occur upon occupation of the acceptor domain. However, for glucosamine-6-P synthase and Gln:PRPP amidotransferase, none of the reversible competitive inhibitors of the synth(et)ase site such as acyclic glucosamine-6-P or carbocyclic ribose-PP analogues increase glutaminase activity [47, 48], whereas for formylglycinamidine synthetase, glutaminase activity is observed only with some analogs of acceptor substrate [49].

Gn-AT inactivation by glutamine analogues is also influenced by events occurring at the synth(et)ase site: NAD synthetase inhibition by azaserine (fig. 1) requires the presence of the acceptor substrate and ATP [10]; the inactivation of anthranilate synthase [23], glutamine:PRPP amidotransferase [29], glutamate synthetase [33] and other enzymes by DON (fig. 1) are stimulated by the acceptor substrate in a concentration-dependent fashion.

Figure 2. Active sites of (*a*) GMP synthetase and (*b*) Gln:PRPP amidotransferase.

There is also an impact on the stoichiometry of alkylation. For example, only one molecule of DON per oligomer causes total inactivation of homodimeric glucosamine-6-P synthase [31], as if alkylation of one monomer caused the locking of the other monomers in an inactive conformation.

The noncatalytic cysteine residue 248 of *E. coli* carbamoyl-P synthetase, which lies in the subunit containing the GAT domain, can be affinity-labelled with *N*-ethylmaleimide (NEM) only once the enzyme has bound MgATP and bicarbonate[6], revealing that a change in conformation has been induced on binding of these substrates [50].

Glutamine site

Two types of GAT domains

The primary sequences of the GAT domains are of two types, and these formed the initial basis for dividing the Gn-AT into two subfamilies, classes I and II[7].

Class I contains seven Gn-AT (table 1a) and class II, four (table 1b). Among the remaining six Gn-AT (table 1c), cobyrinate *a*,*c*-diamide synthetase, cobyrate synthetase and Glu-tRNA^{Gln} amidotransferase (table 1c) have been temporarily placed apart because their GAT domains could not be properly aligned with any type. The amino acid sequences of the last three of these enzymes (NAD synthetase, arylamine synthetase and perhaps amino-DAHP synthase) have not yet been determined.

Alignment of the amino acid sequences of the GAT domains of class I and class II amidotransferases initially led to the selection of a limited number of conserved residues (other than the catalytic cysteine) that could be involved in catalysis. Their exact role has been investigated by using site-directed mutagenesis to examine the suggestion of a catalytic triad [51]. Several mutations led to a loss of activity, but in most cases it was difficult to draw any clear conclusion from the kinetics of the mutated proteins, consistent with the likely conformational changes occurring during the catalytic cycle. Hence, studies on amidotransferases have recently focused on the three-dimensional (3D) structure of these proteins, and the first reports of X-ray structures have clarified the differences between class I and class II GAT domains in short order.

The cysteine proteinase-type GAT domain of class I Gn-AT

From comparisons of the primary sequences, it has been deduced that the typical class I GAT domain is about 200 residues long and includes three conserved regions that contain an invariant glycine residue each [1].

The 3D structures of *E. coli* GMP synthetase binding AMP and PP_i inorganic pyrophosphate [52] and *E. coli* carbamoyl-P synthetase binding ATP, Mn^{++} and the allosteric activator ornithine [53] at a resolution of 2.2 and 2.8 Å, respectively, have confirmed the presence of a catalytic triad, first suggested by Zalkin [51] and predicted from primary sequence alignments (fig. 2a). In addition to the cysteine residue already found essential, the catalytic triad is formed by a histidine residue and a glutamate residue: Cys^{86}-His^{181}-Glu^{183} for *E. coli* GMP

[6] Remarkably, it is possible, under controlled conditions, to label Cys248 in *E. coli* carbamoyl-P synthetase without altering the catalytic residue Cys269, so that enzyme activity can still be measured. It is difficult to study the catalytic cysteine in this way in most other amidotransferases because the catalytic cysteine is too sensitive to *N*-ethylmaleimide.

[7] The 'class I/class II' designation is replacing the old ones: 'G-type/F-type', or 'trpG/purF'. These refered to *trpG* and *purF*, the genes encoding respectively anthranilate synthase and glutamine-PRPP amidotransferase, which represent each subfamily.

synthetase and Cys^{259}-His^{353}-Glu^{355} for *E. coli* carbamoyl phosphate synthetase.

The presence of a histidine residue to activate the catalytic cysteine thiol by forming a thiol–imidazolium ion pair and/or to protonate the nitrogen leaving group had been postulated by several authors. From kinetic analyses of mutant enzymes, this residue had also been proposed to act in the hydrolysis of the putative intermediate thiol ester by general acid–base catalysis [54]. The role of the glutamate residue is probably to maintain the catalytic histidine residue in a correct orientation, as shown by the GMP synthetase structure, where one His^{181} imidazole nitrogen interacts with Glu^{183} and the other with Cys^{86} thiol [52]. Class I Gn-AT primary sequence alignments predict that the corresponding catalytic residues could be Glu^{170} in *E. coli* aminodeoxychorismate synthase [55] and Glu^{172} in *Serratia marcescens* anthranilate synthase [56], although evidence has not yet been provided by site-directed mutagenesis.

The probability that the main function of the class I GAT domain is glutamine amide hydrolysis is strengthened by the presence of an oxyanion hole whose role is to stabilize the negative charge on the oxygen atom of the tetrahedral intermediate that would result from attack of the cysteine residue on glutamine. For *E. coli* GMP synthetase, the oxyanion hole is formed by the peptide backbone alpha N-atoms of Tyr^{87} and Gly^{59} (fig. 2a) [52]. 3D structures also show that most of the conserved residues in class I GAT domains are situated on the border of the glutamine site, even if this site does not seem to be completely formed in the crystallized conformation of both enzymes[8].

GMP synthetase catalytic triad and oxyanion hole have been quite satisfactorily superimposed on the corresponding structural elements of papain, a cysteine proteinase that possesses a Cys-His-Asn triad [52]. However, the reason the third residue of the Gn-AT triad (Glu) differs from that of cysteine proteases (Asn) is still unknown. Although asparagine and glutamate residues are assumed to play a similar role in both cases for similar amidohydrolase reactions, the difference in their pK_a might reflect a fundamental difference between the catalytic mechanisms [57]. Future site-directed mutagenesis experiments should explore this point.

The Ntn hydrolase-type GAT domain of class II Gn-AT

The main characteristic of class II Gn-AT is the specific location of the catalytic cysteine at the amino terminus of the peptide chain.

Three 3D structures of class II GAT domains have been reported recently: the 3 Å-resolved structure of AMP-complexed *Bacillus subtilis* glutamine:PRPP amidotransferase [58], the 2.3 Å-resolved structures of DON-labelled *E. coli* glutamine:PRPP amidotransferase [45] and the 1.8 Å-resolved structure of glutamate-binding *E. coli* glucosamine-6-P synthase GAT domain (expressed alone) [59]. The most striking result given by these structures is that, contrary to class I Gn-AT, there is no catalytic triad (fig. 2b), as catalytic residues other than Cys^{1} failed to be discovered. Despite early postulates on the histidine requirement for hydrolytic activity, alignments of increasing numbers of sequences have eventually shown that class II amidotransferases have no conserved histidine residue, although the pH dependence of glutamate synthase [33], the sensitivity of glucosamine-6-P synthase to diethylpyrocarbonate [60] and mutant studies of glutamine-PRPP amidotransferase [51] and of glucosamine-6-P synthase (M.-A. Badet-Denisot, unpublished data) have shown that at least one histidine residue plays a major role in the function of these GAT domains.

The only remarkable structural element observed in the 3D structures is the oxyanion hole facing the catalytic Cys^{1} thiol: for *E. coli* glutamine:PRPP amidotransferase, it is formed of the nitrogen atoms from the Gly^{103} and Asn^{102} side chain, which interact with the carbonyl oxygen atom of DON; similarly, in *E. coli* glucosamine-6-P synthase Gly^{99} and Asn^{98} interacts with a δ oxygen of glutamate. In these two cases, glutamine-anchoring residues could be identified (e.g. Arg^{73} and Asp^{123} for glucosamine-6-P synthase), and as for class I GAT domains, it could be seen that the glutamine site is bordered by class II invariant residues (e.g. Arg^{26}, Arg^{72}, Pro^{86} and Asp^{127} for *E. coli* Gln:PRPP amidotransferase) [58, 59].

It is worth noting that, besides glutamate synthase, all class II Gn-AT are homooligomers. In glutamate synthase, the flavin-containing small subunit provides the electrons required for the reduction of the intermediary imine P into the final product P′ (glutamate) [61], as a 1c-type reaction (see eq. 1)[9]. The influence of oligomerization on the enzymatic mechanism remains to be elucidated.

As the 3D structures clearly show that no residue is close enough to enhance the nucleophilicity of Cys^{1}, it has been proposed that this role could be played by the Cys^{1}

[8] A recent paper describes the structure of the glutamine-PRPP amidotransferase locked in an active conformation by affinity-labelled DON and a carbocyclic acceptor substrate analogue [91]. This conformation permits the transfer of the NH3 intermediate via a 20-Å channel connecting the GAT domain and the acceptor binding site.

[9] Glutamate synthase definitely possesses class II characteristic features. However, difficulties with computer alignments have arisen from an amino acid sequence insertion in the GAT domain (at the N-terminus of the whole-protein sequence) which is clearly related to its tendency to catalyse the imine reduction step.

Figure 3. Proposed catalytic mechanism of glutamine hydrolysis by class II amidotransferases. Amino acid residues are numbered according to glucosamine-6-P synthase numerotation.

α-amino group (i.e. the protein terminal amine function) through a relay with a water molecule, as shown on figure 3 [59]. There is still no proof of this hypothesis, since i the *E. coli* glutamine:PRPP structure, Cys[1] is engaged in a covalent bond with DON, and in *E. coli* glucosamine-6-P synthase, Cys[1] thiol is pointing out of the site, due to electrostatic repulsion with the carboxylate group of bound glutamate.

The hydrolytic function of the class II GAT domain has been established by superimposition, with remarkable agreement between the catalytic elements identified so far (nucleophilic residue and oxyanion hole), with the structures of four other hydrolytic enzymes also each having an N-terminal nucleophilic catalytic residue ([62]; table 2). These proteins, called Ntn hydrolases (Ntn: N-terminal nucleophile), all display a characteristic folding and are likely to share a common mechanism of action, though the nucleophilicities of the catalytic residues (Cys, Ser, Thr) are different. In such a common mechanism, the specific N-terminus location might be highly significant. It could give flexibility to the catalytic residue, allowing it to point toward or away from the active site, as seen with the glutamate-binding glucosamine-6-P synthase structure. This flexibility could be enhanced by the glycine residue at position 2 of the primary sequence. Otherwise, the N-terminus amino group could, as mentioned above, play a catalytic role similar to that of the triad histidine residue; in this case, one could assume that no glutamate residue is needed, either because the pK_a of the amino group doesn't need to be adjusted like that of histidine imidazole or because no repositionning is needed due to the covalent link of the amino group to the nucleophilic residue. The hypothesis that the N-terminus nucleophile mechanism might be equivalent to the triad mechanism for amidohydrolase activity remains though to be demonstrated.

Before the absence of a catalytic triad was demonstrated for class II Gn-AT, the hypothesis of an analogy with cysteine proteases had been raised. As peptide nitriles are strong inhibitors of cysteine proteases, but not substrates (or at an almost undetectable level [63]), the nitrile analogue of glutamine (fig. 6a) has been synthesized and tested on glucosamine-6-P synthase and asparagine synthetase; unexpectedly, it behaves differently in each case. For glucosamine-6-P synthase, there is no inhibition, but an enzyme-catalysed hydrolysis yielding glutamate [64]. Interestingly, no formation of aminated product was detected, which suggests that (i) ammonia generated in situ was not transferred to the acceptor substrate, and (ii) glutamine is not an intermediate of the hydrolysis, because if it were, it should have undergone the amidotransferase reaction. Thus, hydrolysis of this nitrile compound by glucosamine-6-P synthase seems to follow a mechanism analogous to that of nitrilases, enzymes that hydrolyse nitriles without forming an intermediary amide and, interestingly, also possess a catalytic cysteine residue [65]. On the contrary, asparagine synthetase exhibits nitrile hydratase activity, i.e. catalysis of conversion of this nitrile compound into the amide, and very consistent with observations made on papain, a cysteine protease [66], this activity could be increased by a single point mutation in the oxyanion hole.

Table 2. N-terminal nucleophile amidohydrolases identified from X-ray structures.

Enzyme	Substrate	Nucleophile	Reference
Glycosylasparaginase	GlcNAc-Asn	Thr	[87]
Proteasome	Proteins	Thr	[88]
Penicillin acylase	Penicillin G	Ser	[89]
Gln:PRPP amidotransferase	Gln	Cys	[58]
GlcN-6-P synthase	Gln	Cys	[59]

Amination of acceptor substrate

Although every Gn-AT utilizes glutamine most likely to transfer the same nitrogen group (probably ammonia), the nitrogen function formed on the product molecule varies largely throughout the Gn-AT family, but depends only on the chemical function present on the substrate S or S′, which will be now referred to as the acceptor functionsc (eq. 4). Seven different types of acceptor functions have been distinguished and reported in figure 4, giving an alternate Gn-AT subclassification. These seven types can be distributed in turn into three cases, according to the nature of the preliminary step 1b that serves to activate the acceptor function (in every case, at the expense of one molecule of ATP).

$$\text{Acceptor} + NH_3 \longrightarrow \text{Product} \qquad (4)$$

Gn-AT transformations involving hydroxyl phosphorylation as step 1b

This type of Gn-AT-catalysed activation occurs for the formation of a hemiaminal from a hemiacetal group (fig. 4, pathway 1), a carbamate from a carbonate (pathway 2), an amidine from a secondary amide (pathway 3) or a primary amide from a carboxylic acid (pathway 4). During reaction 1a, the phosphate group is displaced by the nucleophilic action of the transferred nitrogen group. Note that in pathway 3, the hydroxyl group is formed by tautomerization of the amide function, then phosphorylated.

Gn-AT transformations involving adenylylation as step 1b

This type of activation occurs for the formation of guanidine from urea (pathway 5), or a primary amide from a carboxylic acid (pathway 6). The adenylate group is displaced by the nucleophilic action of the transferred nitrogen group, which is an alternate to pathway 4.

Gn-AT catalysing pathways 5 and 6 are considered as belonging to a family of ATP-pyrophosphatase homologues [67].

Gn-AT transformations not utilizing ATP

For the formation of an $\alpha,\beta:\gamma,\delta$ unsaturated amine from a Michael acceptor (pathway 7), or the transformation of a ketone into an amine function (pathway 8), no substrate activation step 1b seems to be required: none of these enzymes uses ATP.

In pathway 7 there is a conjugated 1–6 addition after the formation of the leaving group (protonated carbinol or enzyme nucleophile); this step might be regarded as a non ATP-utilizing 1b step.

In pathway 8, transfer of the nitrogen group to a ketone function results in the formation of an unstable imine which is further transformed (into product P′, according to equation 1, step 1c) either by cyclization for glycerol imidazole phosphate synthase, or isomerization for glucosamine-6-phosphate synthase, or reduction for glutamate synthase.

Mechanism of nitrogen transfer

Basic hypothesis for the mechanism of the amidotransferase reaction

First suggested by the existence of glutaminase activity and NH_3-dependent synth(et)ase activity, the hypothesis of a sequential mechanism involving hydrolysis of glutamine and ammonia transfer has been recently strengthened by the evidence of the modular Gn-AT organization and the amidohydrolase topology of both class I and class II GAT domains. Thus, a general four-step mechanism outlined in figure 5 has been proposed as a basic hypothesis.

If all substrates are bound by the Gn-AT, then:

Step 1. In the glutamine site: the catalytic Cys residue attacks the amide group of the substrate, leading to formation of a tetrahedral intermediate whose negative charge is stabilized by the oxyanion hole; in the acceptor site: for Gn-AT utilizing ATP, reaction 1b (eq. 1) occurs, leading to activation of acceptor substrate.

Step 2. Collapse of tetrahedral intermediate leads to the formation of native ammonia, after abstraction of a proton (from the class I Gn-AT triad histidine residue or from the class II Gn-AT N-terminal amino group), and a covalent γ-glutamyl enzyme thioester adduct (eq. 5).

Step 3. In the glutamine site: a water molecule attacks the thioester adduct, leading to the formation of a

Figure 4. Classification of the amidotransferases according to the acceptor. n.c. = nonclassified.

second tetrahedral intermediate; in the acceptor site: native ammonia is transferred to the acceptor site and attacks the electrophilic group of the acceptor substrate (an intermediate species may form, the nature of which depends on the structure of the acceptor substrate).

Step 4. In the glutamine site: collapse of the second tetrahedral intermediate leads to regeneration of the catalytic cysteine residue and release of glutamate; in the acceptor site: formation of 'aminated' product P occurs: depending on the enzyme, either P is released in the medium, or further transformed (step 1c, eq. 1) into a more stable final product P′, then released.

This basic mechanism assumes that the glutaminase and glutamine-dependent synth(et)ase activities work in the same way. It is consistent with inactivation of Gn-AT by electrophilic analogues and with the strong reversible inhibition of glucosamine-6-P by glutamate semialdehyde (fig. 6b), which, yet mainly present in solution as a cyclized imine form, is supposed to act as a transition state analogue as do aldehydes with cysteine proteases [47, 68]. The following paragraphs will summarize the studies aimed at demonstrating this hypothesis: trapping of thioester intermediates, evidence for native ammonia formation, coupling between glutamine hydrolysis and synthesis of the 'aminated' product.

[11] The recent sequence determination of *B. subtilis* Glu-tRNA^{Gln} amidotransferase [93] has not provided evidence for classification either as class I or class II amidotransferase, since the signature of either subfamily is lacking. However, it does bear the characteristic signature of amidase.

Isolation of a covalent glutamyl-enzyme adduct

The isolation of the acyl enzyme adduct after incubation of Gn-AT with glutamine has been possible only with four enzymes: formylglycinamidine synthetase [7,36], CTP synthetase [69], carbamoyl phosphate synthetase (CAD multifunctional protein) [39] and aminodeoxychorismate synthase [46], with glutamine:enzyme stoichiometry ranging from 0.3 to 1.

All attempts to isolate such an adduct with *E. coli* glucosamine-6-P synthase failed [70]; furthermore, 5-thioglutamine (fig. 6c) does not form the expected dithioester adduct (analogous to the thioester of eq. 5) that would be detected by its UV absorption [47]. Isolating a glutamyl-enzyme adduct seems to be more difficult with class II than with class I Gn-AT whose GAT domains are of triad type.

In these successful cases, the nature of the adduct is assumed to be a γ-glutamyl thioester for several reasons: upon breakdown, glutamate is released [71]; the formyl-

Figure 5. Basic catalytic mechanism for glutamine-dependent amidotransferases. In class I enzymes, B is a histidine, whereas in class II, this role is played by the NH_2 group of the amino terminal residue of the protein, the catalytic cysteine.

glycinamidine synthetase and the carbamoyl-P synthetase adducts could be attacked by exogenous ammonia to regenerate glutamine [36, 39] and by hydroxylamine to release pyrrolidone carboxylic acid as γ-glutamyl hydroxamate and its cyclic derivative [36, 72]; the failure of carbamoyl-P synthetase mutant Cys269Ser to form such an adduct was indicative of catalytic Cys^{269} involvement. Consistent with the absence of acceptor substrate in these experiments, the kinetic parameters for the formation of this adduct have been found close to those of carbamoyl-P synthetase glutaminase activity, showing that its hydrolysis is the glutaminase rate-limiting step [39].

All the observations above strongly suggest that a glutamyl thioester forms during hydrolysis of glutamine by the GAT domain. However, they still do not rule out the possibility that the amidotransferase reaction in the presence of both substrates follows another mechanism, not involving this complex. Two results seem contradictory on the matter: (i) once the adduct is formed, introduction of the other substrate causes its breakdown to glutamate, which dissociates from the site without reacting to form the aminated product [7, 71]; this leads to the conclusion that the adduct is not catalytically competent for reaction 1a (eq.1); (ii) for carbamoyl-P synthetase, both adduct formation and breakdown are accelerated by the presence of MgATP and bicarbonate [39], which on the contrary strongly suggests that the adduct is needed as an intermediate in reaction 1a (eq. 1).

Figure 6. Structures of glutamine analogues: (*a*) nitrile analogue of glutamine, (*b*) glutamate semi-aldehyde, (*c*) 5-thioglutamine, (*d*) bisubstrate analogue for glucosamine-6-P synthase.

The transfer of the nitrogen group

If native ammonia is formed (see mechanism 1), it must remain trapped in the enzyme structure before reaction with the acceptor, because its diffusion away from the active site would lead to its protonation (in neutral medium) and loss of its nucleophilic character. This inference has been confirmed by studies on the effect of pH on the glutamine- and ammonia-dependent activities (CTP synthetase [69]; GMP synthetase [73]; glutamate synthetase [5]): if any native ammonia is formed during

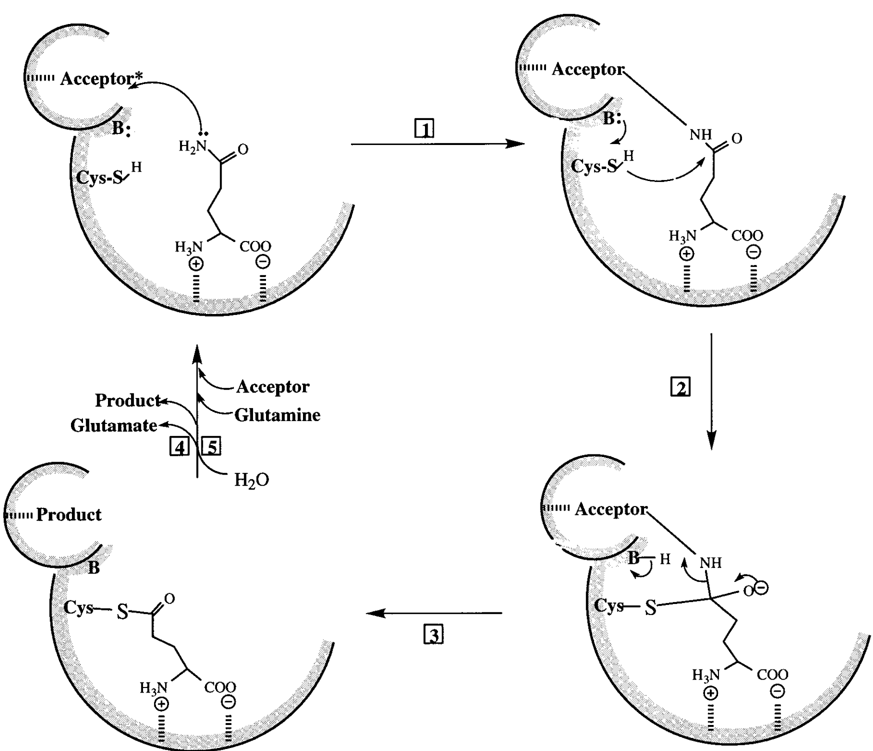

Figure 7. Alternate catalytic mechanism for glutamine-dependent amidotransferases. In class I enzymes, B is a histidine, whereas in class II, this role is played by the NH_2 group of the amino-terminal residue of the protein, the catalytic cysteine.

the reaction, it is not exchanged with exogenous ammonia. There are thus probably only two possibilities for ammonia transfer: either in a concerted way (which assumes proximity of glutamine and acceptor substrate sites), or through channelling from the donor to the acceptor site. Early postulates had proposed a specific site for ammonia, but as ammonia remains sequestered in the protein structure, existence of an inner site and channelling through the protein structure point to the same idea.

Kinetic mechanism studies show that nitrogen transfer only occurs when donor and acceptor substrates are both bound to their specific receptor sites, in keeping with the necessity for transformation efficiency. However, in the first reported 3D structures, GAT and synth(et)ase domains are not close enough for a concerted mechanism to occur. On the GMP synthetase 3D structure, they are separated by a flexible hinge domain that is open, keeping the two sites about 30 Å apart, but the authors suggested that this flexibility may allow a conformational change that brings the sites close together for concerted ammonia transfer [67]. Similarly, the *B. subtilis* glutamine:PRPP amidotransferase 3D structure shows that the two active sites are too far apart to allow concerted transfer unless a major conformational change occurs during the catalytic cycle [58].

Some results are in favour of the concerted mechanism with no formation of native ammonia. [^{15}N] nuclear magnetic resonance (NMR) studies on glutamate synthase and glucosamine-6-P synthase catalysis ([5]; Badet et al., unpublished observations) detected no intermediate species between glutamine and the nitrogen-containing product, although this absence of a signal could be due to an increase in some intermediate relaxation time due to the presence of the protein. Epitope mapping of bovine asparagine synthetase with monoclonal antibodies showed that the glutamine and aspartyl-ATP intermediate sites are contiguous [21]. Irreversible inhibitors directed against the acceptor site have provided in some cases good evidence for close proximity with the GAT domain: for instance, fructose-6-P site-directed inhibition of bacterial glucosamine-6-P synthase by *N*-iodoacetyl glucosamine-6-P was prevented by the presence of glutamine, suggesting a possible participation of the GAT domain catalytic cysteine in the inactivation process [74]. This participation was indeed demonstrated by a radiolabelling experiment on the same enzyme with the tritiated inactivator 1,2-anhydroglucitol-6-P [75].

Other results are in favour of generation and channelling of native ammonia. On chicken liver formylglycinamidine synthetase, isotopic dilution of the [^{14}C]glutamyl-enzyme adduct with nonradioactive glutamine (eq. 6) suggested an attack on the thioester by ammonia that was still bound to the complex [36].

$$\text{Glutamine} + [^{14}\text{C}]\text{-Glutamyl-Enzyme} \rightleftharpoons [^{14}\text{C}]\text{-Glutamine} + \text{Glutamyl-Enzyme} \quad (6)$$

In the *E. coli* carbamoyl phosphate synthetase structure, a long and rather narrow channel has been recently identified joining the glutamine and the carboxyphosphate sites and possibly serving to transport native ammonia [53]. A very recent structure of PRPP amidotransferase points to the same conclusion[8]. If the ammonia channel seen for carbamoyl-P synthetase [53] and glutamine PRPP amidotransferase[8] applies to the other amidotransferases, it would settle the question of how nitrogen is transferred from glutamine to the acceptor substrate.

How is coupling realized between glutamine hydrolysis and 'amination' of the acceptor substrate?

The basic mechanistic hypothesis described above assumes that glutamine hydrolysis follows the same mechanism in the absence or in the presence of the acceptor substrate, leading to the formation of aqueous ammonia or 'aminated' product, respectively. On the other hand, glutamine- and exogenous ammonia-dependent activities are also believed to follow the same mechanism. The fact that glutaminase and synth(et)ase activities are optimal only when both substrates are present indicates that they are coupled, probably for reasons of efficiency and to avoid any risk of activated acceptor hydrolysing, which could lead to waste of substrate and ATP.

This coupling phenomenon could involve conformational changes during the catalytic cycle, which is strongly consistent with the signs of substrate-induced conformational changes that have been observed for Gn-AT. Some results have been interpreted as trapping of a specific conformation where the coupling is enhanced or abolished. Cys^{248}-labelled or Cys^{248}-mutated (with a bulky residue) *E. coli* carbamoyl-P synthetase has reduced synthetase activity and increased glutaminase activity, as if it were locked in a conformation favouring the hydrolysis of glutamine, but preventing the transfer of ammonia to the acceptor [50]. A 29-amino acid sequence-deleted mammalian carbamoyl-P synthetase has been reported to be 10 times more active than the native protein, but ammonia-dependent synthetase activity is abolished: the authors thus suggest that the modified protein is fixed in an activated conformation and that the deleted sequence serves to add flexibility to allow the native protein to cycle between two conformations, an open one with low activity where exogenous ammonia has access to the acceptor substrate site, and a closed one where this access is blocked and native ammonia is directly channelled from the glutamine site to the acceptor substrate site [27]. Similar results have been observed with fused *E. coli* carbamoyl-P synthetase [28]. The suppression of the ammonia-dependent activity might also provide an explanation for its absence in glucosamine-6-P synthase, which to date has always been interpreted against any transfer mechanism involving native ammonia.

The following structural data provide additional information on possible substrate-induced conformational changes:

– In the crystalline form binding AMP and PPi, GMP synthetase does not bind glutamine, suggesting that the specific binding site only forms following a conformational change that links glutamine hydrolysis and ammonia transfer during the catalytic cycle. Similarly, the 3D structure of allosterically inhibited Gln:PRPP amidotransferase displays an unorganized glutamine site [58].

– 3D structures of the glucosamine-6-P synthase GAT domain complexed with glutamate or γ-glutamyl hydroxamate [59] and glutamine:PRPP amidotransferase inactivated by DON [45] reveal the presence of a lid loop that moves on glutamine binding to bury the occupied glutamine site in the protein structure. This might be the way Gn-AT prevent native ammonia from diffusing off the active site.

– The presence of the catalytic cysteine in an inactive conformation in the structure of the isolated glucosamine-6-P synthase GAT domain complexed with a reversible inhibitor [59] might also indicate the existence of two conformations: an active one where the catalytic cysteine thiol is pointing toward the substrate, and an inactive one where it points in the opposite direction, as allowed by its N-terminal position.

How the coupling between glutamine hydrolysis and the synthesis of the 'aminated' product is actually realized remains thus to be clarified in each case.

Other mechanisms

Although results in favour of the basic mechanism are accumulating, this hypothesis has not been definitely established. Before the report of Gn-AT 3D structures, some authors had favoured another mechanism that would not involve generation of native ammonia (since glucosamine-6-P synthase lacks ammonia-dependent synthase activity), while conserving both the nucleophilic character of nitrogen attack on the acceptor substrate and the hydrolytic properties of the GAT domain.

Richards et al. suggested a mechanism analogous to the one proposed for the N-glycosylation of asparagine residues [76] where the glutamine amide nitrogen nucleophilically attacks the acceptor. It was assumed that the enzyme first catalyses the formation of an α-hydroxyimine tautomer[10] before an attack on the electrophilic acceptor group occurs [77, 78], although this may not

[10] The general base that takes part in the nucleophilic attack could deprotonate the hydroxyl group of the α-OH-imine form more easily, as its pK is estimated at around 11, while the amide pK is about 18 [92].

be necessary, since amides can react as nucleophilic agents, especially on aldehydes under weak basic or acidic catalysis, or even without catalyst when the aldehyde serves as the solvent [79–82]. The proposed alternative mechanism would have five steps (fig. 7).

Step 1. After both acceptor substrate and glutamine are bound to their respective sites, the glutamine amide or α-hydroxy-imine tautomer attacks the acceptor to form a bisubstrate intermediate.

Step 2. The catalytic cysteine thiol attacks the [δ]-carbonyl function of the bisubstrate intermediate glutaminyl moiety, leading to the formation of a tetrahedral intermediate adduct with a negative charge on the oxygen which is stabilized by the oxyanion hole.

Step 3. The collapse of this tetrahedral adduct leads to cleavage of the C–N bond, forming, in the glutamine site, a covalent glutamyl thioester adduct, and in the acceptor site, the 'aminated' product P.

Step 4. In the glutamine site, a water molecule attacks the thioester adduct, leading to the formation of a second tetrahedral intermediate.

Step 5. Finally, the collapse of the second tetrahedral intermediate leads to the regeneration of the catalytic cysteine residue and release of glutamate; in the acceptor site either P is released into the medium, or is further transformed (step 1c, eq. 1) into a more stable final product P′, before release.

This mechanism displays some features analogous to the first one, such as tetrahedral intermediates and a glutamyl thioester adduct. But ammonia cannot occur as an intermediate in the reaction; the existence of ammonia-dependent synth(et)ase activity is interpreted as a result of diffusion of this small molecule to the acceptor site; this implies, however, that only the difference in nucleophilicity between water and ammonia should explain the lack of observation of activated acceptor hydrolysis in the absence of glutamine. On the other hand, as the glutaminase activity cannot follow the same mechanism, it is assumed that in the absence of acceptor substrate, glutamine is hydrolysed the same way as described in the basic hypothesis. Hence, different mechanisms for glutamine hydrolysis would naturally account for the differences seen in the reaction rates. This idea was supported by measurement of different kinetic ^{15}N amide isotope effects for glutamine-dependent synthetase and glutaminase reactions catalysed by asparagine synthetase [83].

To date, no more evidence has been brought forward in favour of such a mechanism. Instead, two results obtained on *E. coli* glucosamine-6-P synthase provide some arguments against it. On the one hand, the synthetic thioamide analogue of glutamine (fig. 6c) is not recognized by the enzyme [47]. It was expected to serve as a nitrogen donor since the proportion of α-(thio)hydroxy-imine tautomer is larger in thioamide than in its oxygenated homologue [84]. Hence, this result may contradict the hypothesis of glutamine tautomerism. On the other hand, bisubstrate analogues synthesized by linking a γ-glutamyl moiety to the nitrogen of 2-amino-2-deoxyglucitol-6-P (fig. 6d) all failed to display any affinity for the enzyme and, therefore, to provide any evidence for generation of a bisubstrate intermediate [47].

More strikingly, this mechanism implies that glutamine and acceptor sites are close enough together to constitute one big site anchoring the bisubstrate intermediate. However, recent work on the 3D structure of carbamoyl-P synthetase suggests that the sites are apart from one another at any time in the catalytic cycle [53]. The very recent structures of glutamine-PRPP amidotransferase trapped in an active conformation seem to discard this alternative hypothesis[8].

Conclusion

The glutamine-dependent amidotransferases constitute a recently identified family of enzymes that link nitrogen metabolism to the biosynthetic pathways of several important compounds. These enzymes are modular, and the glutamine and synth(et)ase sites may, under special circumstances, function independently. GAT domains are of two types, either including a Cys-His-Glu catalytic triad or having an N-terminal nucleophile topology; both types are obviously constituted to hydrolyse an amide function. These recent findings strongly support a sequential reaction mechanism involving hydrolysis of glutamine and transfer of native ammonia. Recent reports of 3D structures tend to show that glutamine and acceptor substrate sites are far apart and lead to the proposal that ammonia could be transferred by channelling through the protein structure in a way similar to the classical channelling of common metabolites between sequential enzyme pairs such as the tryptophan synthase bienzyme complex [85]. Otherwise, a major change of conformation should occur during the catalytic cycle to bring both sites into a proximity that enables a concerted ammonia transfer. Accumulation of 3D structures of Gn-AT in different conformations will probably soon help to determine which is the mechanism for each enzyme. One of the main objectives will be to reconstitute the conformational changes occurring during catalysis, as in the reported case of dienelactone hydrolase [86]: determining the sequence of these events might shed definite light on the mechanism of nitrogen transfer by glutamine-dependent amidotransferases.

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