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Metabolism of the Reserve Polysaccharide of *Streptococcus mitis*

PROPERTIES OF α-(1→6)-GLUCOSIDASE, ITS SEPARATION FROM TRANSGLUCOSYLASE, AND THE ACTION OF THE TWO ENZYMES ON BRANCHED OLIGOSACCHARIDES

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1. An α-(1→6)-glucosidase has been separated from cell extracts of *Streptococcus mitis*. The enzyme was freed from transglucosylase by adsorption of the latter on retrograded amylose. 2. The enzyme was detected in five of the six strains of *S. mitis* that were studied; α-(1→6)-glucosidase was not found in strain RB1633, a strain that did not store polysaccharide. 3. The glucosidase could act on compounds in which α-glucose is joined through an α-(1→6)-bond to either a maltosaccharide or an isomaltosaccharide. 6^2-α-Glucosylmaltose (panose) and 6^3-α-glucosylmaltotriose were hydrolysed more rapidly and isomaltodextrins more slowly than isomaltose. 4. Transferring activity towards isomaltose and panose was appreciable when the concentration of substrate was 2% or higher. 5. The enzyme had no action on α-(1→4)-glucosidic linkages. 6-α-Maltodextrinylglucoses were hydrolysed only after transglucosylase action had attenuated them to isomaltose.

Several strains of *Streptococcus mitis* can synthesize intracellular iodophilic polysaccharide (Walker, 1966). Gibbons & Kapsimalis (1963), who worked with a strain from human dental plaque, considered that the polysaccharide was a glycogen–amylopectin-type polymer. These organisms should therefore have an enzymic system for debranching the reserve polysaccharide. In the present paper an α-(1→6)-glucosidase that is present in cell extracts of all the strains of *S. mitis* that store reserve polysaccharide is described. The enzyme has a different specificity from the amylo-(1→6)-glucosidase of muscle, but both enzymes will remove single glucosyl units joined by an α-(1→6)-linkage to a maltodextrin. The possibility that *S. mitis* α-(1→6)-glucosidase may function together with transglucosylase in the degradation of glycogen is discussed.

MATERIALS AND METHODS

Carbohydrates. Isomaltose and 6^2-α-glucosylmaltose (panose) were prepared by the action of the transglucosylase of *Aspergillus oryzae* on maltose. Isomaltodextrins were isolated from a partial acid hydrolysate of dextran (Pharmachem Corp., Bethlehem, Pa., U.S.A.). Isopanose, 6-α-maltotriosylglucose and 6-α-maltotetraosylglucose were prepared by the action of *S. mitis* transglucosylase on amylose and isomaltose. 6^2-α-Maltosylmaltose and 6^2-α-maltotriosylmaltose were synthesized in a similar reaction with panose as acceptor (Walker, 1966). The branched dextrins 6^3-α-glucosylmaltotriose, 6^3-α-glucosylmaltotetraose, 6^3-α-maltosylmaltotetraose and 6^3-α-maltosylmaltotriose were isolated from the products of the reaction between salivary α-amylase and waxy-maize starch. 6^3-α-Glucosylmaltopentaose was prepared by the addition of a glucose residue to the main chain of 6^3-α-glucosylmaltotetraose with rabbit muscle glycogen synthetase. α-(1→6)-Glucosyl α-Schardinger dextrin (Taylor & Whelan, 1966) was a gift from Professor W. J. Whelan. All the carbohydrates were pure, as determined by paper chromatography.

Enzymes. Glucose oxidase (pure) and horseradish peroxidase were obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio, U.S.A. Human salivary α-amylase was isolated by the method of Fischer & Stein (1961). *S. mitis* transglucosylase was purified as described by Walker (1966). Glycogen synthetase was prepared from rabbit muscle by method 2 of Villar-Palasi, Rosell-Perez, Hizukuri, Huijing & Larner (1966).

Cell extracts. The cell extracts were prepared as described by Walker (1966).

Determination of α-(1→6)-glucosidase activity. The activity digest (total volume 0·5ml.), which contained isomaltose (1mg.), 0·2M-phosphate buffer, pH6·4 (0·2ml.), and enzyme, was incubated at 35° for 1hr. The reaction was terminated by the addition of 0·5M-tris–HCl buffer, pH7·0 (0·7ml.). Glucose oxidase reagent (0·8ml.) dissolved in 0·5M-tris–HCl buffer as described by Dahlqvist (1961) was added, and the mixture was incubated at 35° for 1hr. The colour that developed was measured by reading E_{420} in cells of 1cm. light-path in a Unicam spectrophotometer.

The amount of glucose released was obtained by comparison with a standard curve. One unit of enzyme was defined as the amount that liberated 1 μmole of glucose in the 0·5 ml. activity digest.

Paper chromatography. Maltodextrins and isomaltodextrins were separated on Whatman no. 3MM paper by irrigation with ethyl acetate–pyridine–water (10:4:3, by vol.) for 48 hr. The papers were dipped in $AgNO_3$–NaOH (Trevelyan, Procter & Harrison, 1950).

Separation of α-(1→6)-glucosidase from transglucosylase. All the enzymes in the cell extract that could act on amylose were removed by adsorption on retrograded amylose. The method was based on the procedure of Baum & Gilbert (1953) for the purification of potato phosphorylase. A cell extract of *S. mitis* strain 439 (10 ml.) was treated at 0° with '50% ethanol' (cf. Whelan, 1955) to bring the ethanol concentration to 14%. The precipitate was removed by centrifuging, the supernatant was treated with retrograded amylose (1 ml. containing 10 mg.) and the ethanol concentration was adjusted to 11%. After the mixture had been stirred for 10 min. the precipitate (ppt. I) was removed by centrifuging, and the supernatant was again treated with amylose (6·0 ml. containing 60 mg.). The ethanol concentration was brought to 8%, the mixture was stirred for 10 min. and the precipitate (ppt. II) was removed by centrifuging. This procedure was repeated once more to give ppt. III and a final supernatant (32 ml.) that contained 73% of the original glucosidase activity. Two-thirds of the transglucosylase activity was adsorbed on ppt. I, and the remainder was on ppt. II. This enzyme was eluted from the precipitates with 0·05% maltotriose and the recovery was complete. The supernatant, containing α-(1→6)-glucosidase, was passed through a grade 3 sintered-glass filter to remove traces of amylose, and was then dialysed overnight against 0·01 M-phosphate buffer, pH 6·4. This preparation, enzyme *A*, contained 1·23 units of enzyme/ml.

A second preparation, enzyme *B*, was prepared from a cell suspension that was five times more concentrated than that used for enzyme *A*. The ethanol concentration was raised to 28%, and ten times more retrograded amylose was added. The final supernatant was dialysed to remove ethanol, and the protein was precipitated by the addition of 4 vol. of saturated $(NH_4)_2SO_4$, pH 7·0. The precipitate was dissolved in 0·01 M-citrate buffer, pH 6·5 (17 ml.), and dialysed overnight against the same buffer. The solution contained 10 units of enzyme/ml. The enzyme retained 75% of its activity after being stored in the refrigerator for 12 months, but it was not stable to freezing and thawing.

RESULTS

Effect of various conditions on α-(1→6)-glucosidase activity. Various amounts of isomaltose were incubated with the enzyme in activity digests as described in the Materials and Methods section. A concentration of 0·2% isomaltose was a suitable excess of substrate (Fig. 1) for the amount of enzyme that was generally used in this work. Conditions were so arranged that no more than 20 μg. of glucose was released in 1 hr., corresponding to 2% hydrolysis of isomaltose.

The enzyme was incubated with isomaltose at various pH values. The pH optimum for activity of the enzyme was between pH 5·7 and pH 6·5 (Fig. 2). The effect of temperature on the reaction was studied between 18° and 43°. Although the rate of reaction increased with temperature up to 40° (Fig. 3), some inactivation occurred after 1 hr. at this temperature, and 35° was chosen for experiments with α-(1→6)-glucosidase.

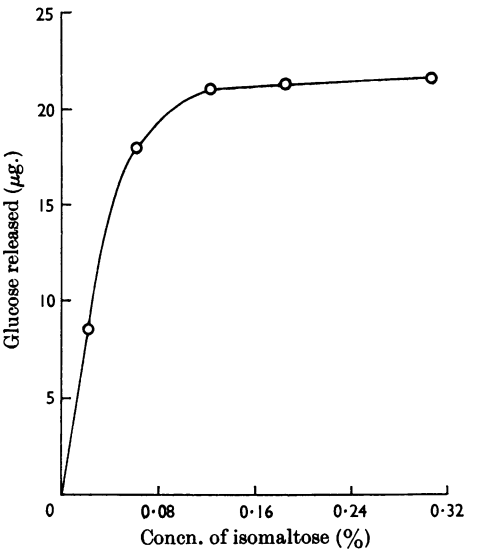

Fig. 1. Dependence of the reaction rate on isomaltose concentration. α-(1→6)-Glucosidase (0·12 unit of enzyme *A*) was incubated for 1 hr. with various amounts of isomaltose in a total volume of 0·5 ml.; for full details see the text.

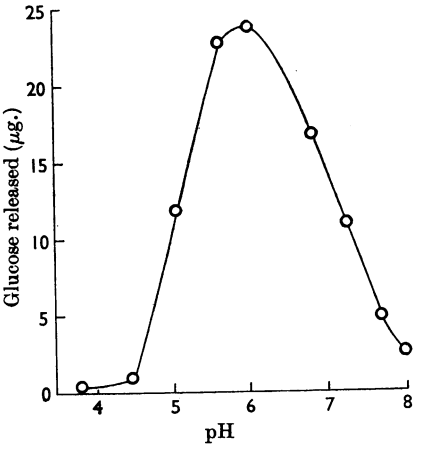

Fig. 2. Effect of pH on α-(1→6)-glucosidase activity. The digests were incubated at 35° for 1 hr.; for full details see the text.

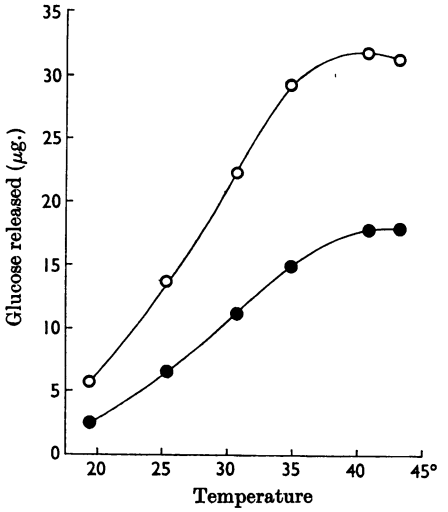

Fig. 3. Effect of temperature on α-(1→6)-glucosidase activity. The digests were incubated at pH 6·0 for 0·5 hr. (●) and 1 hr. (○). For full details see the text.

Table 1. *Effect of ammonium molybdate and tris on the hydrolysis of isomaltose*

Enzyme *B* (0·5 unit) was incubated with isomaltose (1 mg.) in the usual activity digest (0·5 ml.) in the presence of the inhibitors shown below. After 45 min. at 35°, 0·5 M-tris (0·6 ml.) and glucose oxidase reagent (0·9 ml.) were added. For details of the activity digest, see the Materials and Methods section.

Inhibitor	Glucose released (μg.)	Inhibition (%)
None	24·74	—
Ammonium molybdate (2%, w/v)	0	100
Tris (50 mM)	0·40	98·4
Tris (35 mM)	0·64	97·4
Tris (20 mM)	0·64	97·4
Tris (5 mM)	2·16	91·2

When the enzyme was present in digests containing 2% (w/v) isomaltose, paper chromatography revealed the presence, in addition to glucose, of a spot with the same R_F value as isomaltotriose. With a lower concentration of isomaltose (0·34%) the extent of transglucosylation was far less marked.

The effect of tris on the activity of α-(1→6)-glucosidase was determined by including various concentrations of tris in the standard activity digests. The lowest concentration tested, 5 mM, gave 91% inhibition. The enzyme was completely inhibited by 2% (w/v) ammonium molybdate. The glucose controls for the experiment with ammonium molybdate also contained the inhibitor in order to allow for the small inhibition of glucose oxidase reagent by ammonium molybdate. The results are shown in Table 1.

Table 2. *Hydrolysis of isomaltose by a cell extract of* S. mitis *strain S3*

The reaction was followed by determining the increase in reducing power (Nelson, 1944), and glucose release was measured with glucose oxidase. Full details are given in the text.

Time of incubation (hr.)	Hydrolysis (%)	
	Measured by reducing power	Measured by glucose oxidase
0·5	11·9	10·5
1·25	19·7	18·4
2·25	26·1	27·3
3·75	—	36·9
5·75	38·4	42·6
8·0	55·2	55·6

Hydrolysis of isomaltose. Glucose could arise either from transglucosylation or from hydrolysis of isomaltose, and the determination of glucose with glucose oxidase does not distinguish between these possibilities. Accordingly, the increase in reducing power that occurred during the hydrolysis of isomaltose was determined with the Nelson (1944) reagent. Isomaltose (8·55 mg.) was incubated with a dialysed cell extract of *S. mitis* strain S3 (1·25 ml.) and 0·4 M-phosphate buffer, pH 6·4 (0·12 ml.), in a 2·5 ml. digest. At intervals, portions were withdrawn for the determination of reducing power and glucose release. The results (Table 2) showed that the hydrolysis of isomaltose could be determined accurately with glucose oxidase. Glucose release during the reaction was therefore mainly due to hydrolysis. The reaction was also followed by paper chromatography; at no stage did isomaltotriose appear in other than trace amounts.

Comparison of α-(1→6)-glucosidase activity in various strains of S. mitis. Dialysed cell extracts (0·1 ml.) were incubated with isomaltose (3 mg.) and 0·4 M-phosphate buffer, pH 6·4 (0·05 ml.), in a total volume of 0·5 ml. At intervals portions (0·1 ml.) were withdrawn, diluted to 1 ml., boiled and incubated with glucose oxidase reagent (3 ml.) for 1 hr. at 35°. The results (Table 3) showed that the four strains of *S. mitis* that store reserve polysaccharide (Walker, 1966) contained α-(1→6)-glucosidase, whereas strains FW 251 and RB 1633, which do not store polysaccharide, had low activity and no activity respectively.

Action of α-(1→6)-glucosidase on isomaltodextrins. The isomaltodextrins were incubated with enzyme *A* from strain 439 (0·1 ml.) in standard activity

Table 3. *α-(1→6)-Glucosidase activity and concentration of reserve polysaccharide in various strains of* S. mitis

The experimental conditions for the enzymic digests are described in the text. The concentration of reserve polysaccharide in the various strains (Walker, 1966) is given in terms of glucose content (%) of acid-hydrolysed cells. The cells had been grown on medium containing 1% of glucose. When the cells were grown on medium containing 0·1% of glucose, the glucose content of all the strains was in the range 2·2–2·8%.

Strain	Glucose released (μg./μg. of N)			Polysaccharide content of cells (%)
Time.....	2hr.	5hr.	7hr.	
439	2·3	5·7	7·7	10·1
FW225	2·1	5·1	7·4	16·7
S3	2·8	5·8	7·0	37·1
FW213	1·4	3·3	4·2	28·6
FW251	0·2	0·9	1·4	1·3
RB1633	0	0	0	2·8

Table 4. *Relative activity of α-(1→6)-glucosidase on isomaltodextrins*

The experimental conditions are described in the text.

Dextrin	Glucose released (μg.)	Relative rate of hydrolysis
Isomaltose	20·2	100
Isomaltotriose	9·5	94
Isomaltotetraose	6·8	68
Isomaltopentaose	6·7	66

digests. The release of glucose is shown in Table 4.

In a second experiment, isomaltotriose, isomaltotetraose and isomaltopentaose (0·5mg.) were each incubated overnight with enzyme *B* (0·04ml.) in a final volume of 0·25ml. Paper chromatography betrayed marked transglucosylation in all the digests. The isomaltopentaose digest, for example, contained significant amounts of isomaltohexaose as well as the expected isomaltotetraose, isomaltotriose and glucose, and a small amount of isomaltose. Thus the transferring activity of the enzyme was apparent in dilute solutions of isomaltodextrins.

Action of α-(1→6)-glucosidase on oligosaccharides containing both α-(1→4)- and α-(1→6)-glucosidic linkages. The oligosaccharides (1·0mg.) were incubated in activity digests containing 0·123unit of enzyme for 1hr. The results for substrates that would yield glucose on hydrolysis are given in Table 5. In addition, it was shown by paper chromatography that 6^2-α-maltosylmaltose, 6^3-α-maltosylmaltotriose, 6^3-α-maltosylmaltotetraose and 6^2-α-maltotriosylmaltose were not substrates for the enzyme.

In the case of 6^2-α-glucosylmaltose (panose) and 6^3-α-glucosylmaltotriose the experiment was repeated with double the concentration of substrate. The same amount of glucose was released and the hydrolysis was linear for 2hr. It was ascertained

Table 5. *Relative activity of α-(1→6)-glucosidase on oligosaccharides containing α-(1→4)-glucosidic linkages*

The extent of the reaction was measured with glucose oxidase; full details are given in the text. 6-α-Glucosyl α-Schardinger dextrin was incubated with enzyme for 4hr. in order to improve the accuracy of the result. After 24hr. a portion of the digest was shown by chromatography to contain glucose.

Substrate	Glucose released (μg./hr.)	Relative rate of hydrolysis
6-α-Glucosylglucose (isomaltose)	23·2	100
6^2-α-Glucosylmaltose (panose)	18·6	160
6^3-α-Glucosylmaltotriose	12·8	110
6^3-α-Glucosylmaltotetraose	0	0
6-α-Maltosylglucose (isopanose)	0	0
6-α-Maltotriosylglucose	0	0
6-α-Maltotetraosylglucose	0	0
6-α-Glucosyl α-Schardinger dextrin	1	9

that the increased activity with panose compared with isomaltose was not due to transglucosylase impurity reacting with the maltose liberated during the reaction; no glucose was released when maltose replaced isomaltose in the standard activity digest.

The oligosaccharides that had not been hydrolysed under the standard conditions were subjected to higher concentrations of enzyme (1·5units) for 20hr., and then the composition of the digests was analysed by paper chromatography. Again there was no release of glucose from 6^3-α-glucosylmaltotetraose or from the 6-α-maltodextrinylglucoses. There was, however, some hydrolysis of 6-α-maltotriosylmaltose. This activity was not inhibited by

0·05M-tris, and was found to be due to a trace of pullulanase activity associated with the α-(1→6)-glucosidase (G. J. Walker, unpublished work).

Brown (1964) reported that the rate of hydrolysis of the α-(1→6)-linkage in 6^3-α-glucosylmaltopentaose by amylo-(1→6)-glucosidase was eightfold greater than that of 6^3-α-glucosylmaltotetraose. This indicated the effect on the enzyme of the exact structure in the vicinity of the branch point. Accordingly, despite the complete absence of action of *S. mitis* α-(1→6)-glucosidase on 6^3-α-glucosylmaltotetraose, the action of this enzyme (1·5units) on 6^3-α-glucosylmaltopentaose (120μg.) was tested. After 15hr. at 35°, glucose was not detected with glucose oxidase.

The action of α-(1→6)-glucosidase on the branched tetrasaccharide 6^3-α-glucosylmaltotriose was followed to completion by measuring the increase in reducing power that developed in the digest with the Nelson (1944) reagent. At the end of the reaction, paper chromatography of the products showed only glucose and maltotriose in the digest of 6^3-α-glucosylmaltotriose. The absence of maltose indicated again that transglucosylase had been completely removed.

When the enzyme was incubated with a 2% solution of panose, transglucosylation occurred to give maltose and a tetrasaccharide that on paper chromatography in ethyl acetate–pyridine–water (10:4:3, by vol.) moved 0·41 of the distance moved by panose. This is likely to have been 6^2-α-isomaltosylmaltose, which has also been synthesized by the action of a potato transglucosylase on panose (Abdullah & Whelan, 1960).

Action of S. mitis *transglucosylase on* 6-α-*maltosylglucose* (*isopanose*) *and on* 6-α-*maltotriosylglucose.* The results of Table 5 indicated that α-(1→6)-glucosidase could cleave only those α-(1→6)-glucosidic linkages of unbranched oligosaccharides in which a single glucose residue is attached by its anomeric carbon atom to C-6 of a glucose or maltodextrin residue. Thus 6-α-maltosylglucose and 6-α-maltotriosylglucose were not hydrolysed. The action of *S. mitis* transglucosylase on these compounds was investigated because the removal of one or two glucosyl residues respectively by transglucosylase would expose a 6-α-glucosyl stub that would then be susceptible to the action of α-(1→6)-glucosidase. Digests containing either 6-α-maltosylglucose or 6-α-maltotriosylglucose (1mg.) were incubated with purified transglucosylase of *S. mitis* strain RB1633 in the presence and absence of glucose for 24hr. at 35°. Paper chromatography revealed a complete disproportionation of 6-α-maltotriosylglucose. When glucose was absent the major products were isomaltose, 6-α-maltosylglucose, 6-α-maltotetraosylglucose and 6-α-maltopentaosylglucose, with smaller amounts of the higher dextrins of this series. In the presence of glucose the products were the same, with small amounts of maltose and maltotriose. Since the yields of isomaltose and of 6-α-maltosylglucose were similar it was clear that the transglucosylase could transfer glucosyl or maltosyl residues with equal ease. It had been shown previously that the transglucosylase similarly transfers either glucose or maltose units from maltotriose (Walker, 1966). The transglucosylase had little action on 6-α-maltosylglucose, and the results were the same whether or not glucose was present. Only traces of isomaltose and 6-α-maltotriosylglucose were seen on the chromatogram, and it was judged that less than 1% of the substrate had reacted. Thus the α-(1→4)-glucosidic linkage next to the α-(1→6)-glucosidic linkage can be rapidly transferred by *S. mitis* transglucosylase only when it is covered by a second α-(1→4)-linkage as in 6-α-maltotriosylglucose.

Examination of transglucosylase action on oligosaccharides containing α-(1→4)- *and* α-(1→6)-*glucosidic linkages.* The action of transglucosylase on the α-(1→4)-glucosidic linkages of panose and of 6^3-α-glucosylmaltotriose was tested by incubating these dextrins (0·5mg.) and [^{14}C]glucose (0·3mg.) with transglucosylase for 48hr. The label was not incorporated into panose or 6^3-α-glucosylmaltotriose, and new products were not observed by paper chromatography. It was concluded that the transglucosylase could not transfer isomaltosyl groups.

Since the α-(1→6)-glucosidase had no action on 6^3-α-glucosylmaltotetraose, the ability of transglucosylase to transfer the glucose residue at the non-reducing end of the chain, thus producing 6^3-α-glucosylmaltotriose, was tested. The branched dextrin (0·4mg.) and glucose (0·5mg.) were incubated with transglucosylase (0·25ml.) in a 0·5ml. digest for 24hr. at 35°. The mixture was then processed for chromatography, and no products were found. The absence of maltose and 6^3-α-glucosylmaltotriose indicated that the transglucosylase had no action on 6^3-α-glucosylmaltotetraose. In a similar experiment, it was further shown that the transglucosylase could not transfer glucosyl or maltosyl residues from 6^3-α-glucosylmaltopentaose.

DISCUSSION

Several α-(1→6)-glucosidases that are similar to the *S. mitis* enzyme have been described. The intracellular isomaltodextrinase of *Lactobacillus bifidus* (Bailey & Roberton, 1962) has the most similar specificity. Both enzymes hydrolysed isomaltodextrins, the *L. bifidus* enzyme falling off in activity when the degree of polymerization exceeded five glucosyl groups. Both could act as

transferases, and both hydrolysed the α-(1→6)-linkage in panose, although the *L. bifidus* enzyme acted less readily on panose than on isomaltose.

The oligo-1,6-glucosidase extracted by Larner & McNickle (1955) from hog intestinal mucosa had a similar action on isomaltose, isomaltotriose and panose. This enzyme, however, had a rapid action on α-amylase limit dextrins, whereas the *S. mitis* enzyme would react only with 6^3-α-glucosylmaltotriose.

The isomaltase–dextranase of rat intestine (Dahlqvist, 1963) was associated with maltase activity, and the α-glucosidase of *Aspergillus niger* also hydrolysed both the α-(1→4)- and the α-(1→6)-glucosidic linkages in a variety of oligosaccharides (Pazur & Ando, 1960; Abdullah, Fleming, Taylor & Whelan, 1963). Rabbit muscle α-glucosidases have a similar specificity (Abdullah, Taylor & Whelan, 1964).

The absence of α-(1→4)-glucosidase activity and the removal of 4-glucosyltransferase activity from the α-(1→6)-glucosidase made it possible to test the separate action of these enzymes on branched dextrins. It was shown that *S. mitis* transglucosylase could remove glucosyl residues from the side chain of branched dextrins, thus exposing a 6-α-glucosyl stub that the α-(1→6)-glucosidase could then hydrolyse. If, however, one or two glucosyl residues remained on the main chain peripheral to the branch point, as in 6^3-α-glucosylmaltotetraose and 6^3-α-glucosylmaltopentaose, the glucosidase was without action on the branch linkage. *S. mitis* transglucosylase failed to remove glucosyl or maltosyl residues from the main chain of 6^3-α-glucosylmaltotetraose and 6^3-α-glucosylmaltopentaose respectively. An attempt to extend further the length of the main chain by transfer of glucosyl residues to the glucosylmaltopentaose with glycogen synthetase was not successful. The possibility still exists that, although one or two glucose units peripheral to the branch point inhibit glucosidase action, three or more glucosyl units might not cause such an obstruction. Support is given to this argument by the fact that 6-α-glucosyl α-Schardinger dextrin is a substrate for the enzyme.

Although the results described in this paper have only shown the ability of *S. mitis* α-(1→6)-glucosidase to hydrolyse the α-(1→6)-linkage of certain dextrins, the enzyme could possibly play a part in the debranching of glycogen, given the presence of the correct supporting enzymes. The enzyme is sensitive to the presence of α-(1→4)-linkages in the vicinity of the branch point, and is capable of hydrolysing the α-(1→6)-linkage in most of the branched dextrins that are not susceptible to the action of pullulanase. Its specificity differs considerably from that of amylo-1,6-glucosidase, the debranching enzyme of muscle (Illingworth & Brown, 1962). Amylo-1,6-glucosidase hydrolyses the α-(1→6)-linkage in 6^3-α-glucosylmaltotetraose, but not in 6^3-α-glucosylmaltotriose, panose or isomaltose, whereas the *S. mitis* enzyme hydrolyses 6^3-α-glucosylmaltotriose, panose and isomaltose, but not 6^3-α-glucosylmaltotetraose. Both enzymes can hydrolyse 6-α-glucosyl α-Schardinger dextrin, and the range of action of both enzymes is enlarged in the presence of transferase.

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REFERENCES

Abdullah, M., Fleming, I. D., Taylor, P. M. & Whelan, W. J. (1963). *Biochem. J.* **89**, 35P.

Abdullah, M., Taylor, P. M. & Whelan, W. J. (1964). In *Control of Glycogen Metabolism*, p. 123. Ed. by Whelan, W. J. & Cameron, M. P. London: J. and A. Churchill Ltd.

Abdullah, M. & Whelan, W. J. (1960). *Biochem. J.* **75**, 12P.

Bailey, R. W. & Roberton, A. M. (1962). *Biochem. J.* **82**, 272.

Baum, H. & Gilbert, G. A. (1953). *Nature, Lond.*, **171**, 983.

Brown, D. H. (1964). In *Control of Glycogen Metabolism*, p. 170 (Discussion). Ed. by Whelan, W. J. & Cameron, M. P. London: J. and A. Churchill Ltd.

Dahlqvist, A. (1961). *Biochem. J.* **80**, 547.

Dahlqvist, A. (1963). *Biochem. J.* **86**, 72.

Fischer, E. H. & Stein, E. A. (1961). *Biochem. Prep.* **8**, 27.

Gibbons, R. J. & Kapsimalis, B. (1963). *Arch. oral Biol.* **8**, 319.

Illingworth, B. & Brown, D. H. (1962). *Proc. nat. Acad. Sci., Wash.*, **48**, 1619.

Larner, J. & McNickle, C. M. (1955). *J. biol. Chem.* **215**, 723.

Nelson, N. (1944). *J. biol. Chem.* **153**, 375.

Pazur, J. H. & Ando, T. (1960). *J. biol. Chem.* **235**, 297.

Taylor, P. M. & Whelan, W. J. (1966). *Arch. Biochem. Biophys.* **113**, 500.

Trevelyan, W. E., Procter, D. P. & Harrison, J. S. (1950). *Nature, Lond.*, **166**, 444.

Villar-Palasi, C., Rosell-Perez, M., Hizukuri, S., Huijing, F. & Larner, J. (1966). In *Methods in Enzymology*, vol. 8, p. 378. Ed. by Neufeld, E. F. & Ginsberg, V. New York: Academic Press Inc.

Walker, G. J. (1966). *Biochem. J.* **101**, 861.

Whelan, W. J. (1955). In *Methods in Enzymology*, vol. 1, p. 194. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.