A Transglucosylase of *Streptococcus bovis*

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1. A transglucosylase has been separated from the α -amylase of *Streptococcus bovis* by chromatography of the cell extract on DEAE-cellulose. 2. The transglucosylase can synthesize higher maltodextrins from maltotriose, but maltose, isomaltose and panose do not function as donors. 3. Iodine-staining polysaccharide may be synthesized from maltotriose provided that glucose is removed. Synthesis from maltohexaose results in dextrins of sufficient chain length to stain with iodine, but again maltodextrins of longer chain length are formed when glucose is removed from the system. 4. The transglucosylase degrades amylose in the presence of a suitable acceptor, transferring one or more glucosyl residues from the non-reducing end of the donor to the non-reducing end of the acceptor. With [^{14}C]glucose as acceptor the maltodextrins produced were labelled in the reducing glucose unit only. 5. The acceptor activities of 25 sugars have been compared with that of glucose. Maltose has 50%, methyl α -glucoside has 15%, isomaltose and panose each has 8% and sucrose has 6% of the accepting efficiency of glucose. Mannose and sorbose also had detectable activity. With the exception of maltose all these sugars produced a different series of dextrins from that obtained with glucose. 6. It was concluded that *S. bovis* transglucosylase transfers α -(1 $\rightarrow$ 4)-glucosidic linkages in the same manner as D-enzyme, but some differences in specificity distinguish the two enzymes. Unlike D-enzyme, *S. bovis* transglucosylase can transfer glucosyl units, producing appreciable amounts of maltose both during synthesis from maltotriose and during transfer from amylose to glucose. 7. No evidence was found that the transglucosylase was extracellular. The enzyme is cell-bound, and is released by treatment of the cells with lysozyme and by suspension of the spheroplasts in dilute buffer. 8. The transglucosylase may be responsible for the storage of intracellular iodophilic polysaccharide that occurs when the cells are grown in the presence of suitable carbohydrate sources.

Several reports have appeared on the ability of the 'viridans' group of streptococci to store intracellular iodophilic polysaccharide. Hobson & Mann (1955) described the formation of iodophilic polysaccharide when group D streptococci from the rumen were grown in agar media containing maltose, maltotriose, amylose dextrins, amylopectin or glycogen. Gibbons & Kapsimalis (1963) found that a strain of *Streptococcus mitis* isolated from human dental plaque could store iodophilic polysaccharide when these organisms were grown on glucose. Dunican & Seeley (1962) showed that *Streptococcus equinus* could form iodophilic polysaccharide when suspended in buffered maltose. Synthesis did not occur when the cells were grown on glucose or on starch. The cells did not grow on starch medium unless a small quantity of glucose was added; starch was then stored in the cells. Dunican & Seeley (1962) suggested that the intracellular polysaccharide was synthesized in a

similar manner to the synthesis of polysaccharide by the amylomaltase of *Escherichia coli*. Crowley & Jevons (1955) also concluded that the synthesis of a starch-like polysaccharide from maltose by strains of *Streptococcus pyogenes* was carried out by an amylomaltase. Hobson & Mann (1955) considered that a similar reaction operated in *Streptococcus bovis*, and that, since maltotriose was most likely an intermediate in the formation of polysaccharide, only glucose units were added to the growing molecules, and not maltose units as such.

In the present paper the preparation of a cell-bound transglucosylase from *S. bovis* is described. The properties of this enzyme are such that, though, in common with other transglucosylases, it can synthesize iodine-staining polysaccharide, it differs in some respects from both D-enzyme (Peat, Whelan & Rees, 1953, 1956a) and from amylomaltase (Monod & Torriani, 1950; Wiesmeyer & Cohn, 1960).

MATERIALS AND METHODS

Carbohydrates. Waxy-maize starch was prepared by the method of Schoch (1957) from hand-sorted single-cross Tapicorn seed kindly given by the Bear Hybrid Corn Co., Ill., U.S.A. Waxy-maize β -dextrin was prepared from the starch by the action of sweet-potato β -amylase (crystalline) purchased from the Worthington Biochemical Corp., Freehold, N.J., U.S.A. Amylose was prepared from potato starch by the method of Hobson, Pirt, Whelan & Peat (1951). [^{14}C]Glucose was purchased from The Radiochemical Centre, Amersham, Bucks. Maltotriose was prepared as described by Peat, Whelan & Kroll (1956b). Maltotetraose and maltoheptaose were isolated by paper chromatography from the products of the reaction between potato amylose and crystalline α -amylase from *Bacillus subtilis*. Isomaltose and panose were prepared by the action of the transglucosylase of *Aspergillus oryzae* on maltose. They were isolated from the reaction mixture by preparative paper chromatography. Isopanose was a gift from Dr J. H. Pazur. All other carbohydrates were obtained from commercial sources and were purified by paper chromatography when necessary. ATP was supplied by the Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A.

Enzymes. Hexokinase (crystalline) was purchased from C. F. Boehringer und Soehne, Mannheim, W. Germany. Pullanase was a gift from Dr W. J. Whelan. Lysozyme (crystalline) was a product of California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.

Cell extract. The cell extract was prepared as described by Walker (1965).

Chromatography of the cell extract on DEAE-cellulose. The dialysed cell extract (30 ml.) was applied to a column (1 cm. $\times$ 25 cm.) packed with DEAE-cellulose (Brown Co., Berlin, N.H., U.S.A.) that had been thoroughly washed with 0.01 M-phosphate buffer, pH 6.4. The column was eluted with increasing concentrations of phosphate buffer, the gradient being linear between 0.01 and 0.40 M. α -Amylase, sucrose phosphorylase and α -galactosidase were eluted with 0.12 M-, 0.21 M- and 0.22 M-phosphate buffer respectively and were well separated from the transglucosylase, which had peak activity eluted with 0.29 M-phosphate buffer.

Determination of transglucosylase activity. The digest (0.5 ml.) contained amylose (0.5 mg.), glucose (2.5 mg.), tris-maleate buffer, pH 7.5 (10 mM), and enzyme (0.1 ml.). A control digest was incubated without glucose. After 30 min. at 30° portions (0.1 ml.) from the digest and control were stained with iodine [0.1 ml. of a solution that contained iodine (2 g./l.) and potassium iodide (20 g./l.)] and diluted to 10 ml. The extinction at 660 m μ was read in 2 cm. cells in a Unicam spectrophotometer. The value obtained in the digest was subtracted from the control to which no acceptor had been added, and the result was a measure of the transferring ability of the enzyme.

Paper chromatography. Maltodextrins were separated on Whatman no. 3MM paper during irrigation with ethyl acetate-pyridine-water (10:4:3, by vol.) for 48 hr. The papers were dipped in silver nitrate-NaOH (Trevelyan, Proctor & Harrison, 1950). Glucose was separated from sorbitol in ethyl methyl ketone-acetic acid-water saturated with boric acid, (9:1:1, by vol.), as described by Rees & Reynolds (1958), and the dipping reagents contained pentaerythritol (Frahm & Mills, 1959).

Analysis of carbohydrates. Maltodextrins separated by

chromatography on paper were eluted from the appropriate areas of paper with water (1 ml.) and hydrolysed with 1.5 N- H_2SO_4 at 100°. The reducing power (as glucose) was measured by the Nelson (1944) method. [^{14}C]Maltodextrins were located by radioautography and determined by the primary cysteine- H_2SO_4 method of Dische, Shettles & Osnos (1949).

RESULTS

Action of *Streptococcus bovis* cell extracts on starch and maltotriose. The relative activities of the dialysed cell extract on 1% starch and on 0.4% maltotriose were measured at pH 6.5 and 39°. Measurement of the increase in reducing power with the Shaffer & Hartmann (1921) reagent showed that both substrates were hydrolysed, maltose being liberated from starch at 4 times the rate from maltotriose. In another experiment a cell extract prepared from *S. bovis* was dialysed against distilled water to remove all traces of phosphate. The ratio of maltose produced from starch and from maltotriose in this case was 4.9. Walker & Hope (1964) found that the extracellular α -amylase of *S. bovis* hydrolysed maltotriose very slowly (about 200 times slower than its rate of action on starch), and Walker (1965) showed that the cell-bound amylase had similar properties. The rapid action of the cell extract on maltotriose could not therefore be attributed to α -amylase.

Chromatography of *Streptococcus bovis* cell extracts on DEAE-cellulose: action of the eluates on amylose, maltotriose and maltose. Walker (1965) showed that the major cell-bound amylase of *S. bovis* was eluted from DEAE-cellulose with 0.12 M-phosphate buffer. When the DEAE-cellulose column was eluted with higher concentrations of phosphate buffer in the present work, and the fractions obtained were incubated with maltotriose for 20 hr., there was no significant increase in the reducing power of the digests, but analysis by paper chromatography revealed that a disproportionation of the linkages in maltotriose had occurred in all tubes containing enzyme eluted with 0.2–0.4 M-phosphate buffer. In addition to glucose and maltose there was a spot of equal intensity to maltose in a position corresponding to maltotetraose. These results suggested that the cell extract contained a transglucosylase that could synthesize maltodextrins from maltotriose, and that the apparent hydrolysis of maltotriose by *S. bovis* cell extracts was due to the action of α -amylase on the products of transglucosylase activity. A test for transglucosylase activity in the fractions was made by incubating a portion with amylose in the presence and absence of glucose. When glucose was absent there was negligible action on amylose, the fall in iodine-staining capacity of the polysaccharide being only small. In the presence of glucose there was a rapid

fall in iodine stain, and chromatograms showed the production of maltose, maltotriose and higher maltodextrins up to maltoheptaose. These dextrins were formed by transfer from amylose to the accepting glucose molecule. The transglucosylase was therefore similar in its action to D-enzyme, the transglucosylase found in potatoes: both enzymes could transfer α -(1 $\rightarrow$ 4)-glucosidic linkages. None of the fractions from the columns had any action on maltose. D-enzyme, similarly, cannot act on maltose, and this property distinguishes these two enzymes from the amylomaltase of *E. coli*. The fractions eluted with 0.29–0.31 M-phosphate buffer were pooled and used for all subsequent work with the transglucosylase. Although the cell extract could be dialysed with little loss of transglucosylase activity, the fractions eluted from DEAE-cellulose were more sensitive and were used without dialysis where possible.

Effect of various conditions on transglucosylase activity. The dialysed enzyme was incubated with amylose and glucose, as described in the Materials and Methods section, at various pH values. The pH optimum was between 6.8 and 7.5 (Fig. 1). The effect of temperature on the reaction was studied between 20° and 45°. The optimum temperature was 30°; at 25° and 35° the reaction proceeded at 85% of the optimum rate. The amount of glucose in the activity digest was varied from 0.025 to 2.5 mg., and the accepting ability of maltose was compared with that of glucose (Fig. 2). With both sugars maximum accepting ability was achieved with 2.5 mg. but maltose was only half as efficient as glucose.

Activities of various carbohydrates as acceptors. Of 25 sugars tested, ten had acceptor activity (Table 1).

Methyl α -D-glucoside had 15% of the activity of glucose; the dextrins produced had different R_F values from the maltodextrins, and reacted more slowly with the silver nitrate reagent, as expected for non-reducing sugars. Two transglucosylase-activity digests were incubated at 30°; in one digest methyl α -D-glucoside (2.5 mg.) replaced glucose. After 6 hr. both digests were achroic, and they were heated at 100° for 2 min. to stop the reaction. The solutions were deionized with Bio-Deminrolit and concentrated to dryness. A portion of each was

Fig. 2. Determination of the weight of glucose and maltose required for maximum accepting efficiency. Transglucosylase-activity digests (each 0.5 ml.) containing various weights of glucose (●) or maltose (○) were incubated for 30 min. at 30° and pH 7.5.

Fig. 1. Effect of pH on transglucosylase activity. The activity digests were incubated at 30° for 6 hr. (○) and 22 hr. (●).

Table 1. *Acceptor activity of sugars that will replace glucose as acceptor of glucosyl residues in the reaction between amylose and transglucosylase*

The following sugars (10 mg.) replaced glucose in transglucosylase-activity digests, and the incubation was continued for 4 hr. Controls without amylose were incubated to show by paper chromatography that no hydrolysis of the disaccharides or panose occurred. The fall in iodine stain of amylose was a measure of acceptor activity, and glucose was given a value of 100.

Sugar	Activity (%)
Glucose	100
Maltose	50
Methyl α -D-glucoside	15
Panose	8
Isomaltose	6
Sucrose	6
L-Sorbose	12
D-Mannose	11
$\alpha\alpha$ -Trehalose	2
Gentiobiose	2

applied to paper and treated with a 10% solution of benzylamine in methanol (3 μ l.), as described by Bayly & Bourne (1953). The paper was irrigated with butanol-ethanol-water-aq. ammonia (sp.gr. 0.88) (45:12:20:1, by vol.) for 21 hr. Ninhydrin spray revealed glucose and many spots of lower R_F values in the digest containing glucose as acceptor. No spots were seen when methyl α -D-glucoside was the acceptor. Therefore no maltodextrins were produced in this digest and the fall in iodine stain of amylose was entirely due to transfer of glucose residues to methyl α -D-glucoside, showing that the reducing group of the acceptor need not be free.

The products obtained when sucrose (10 mg.) replaced glucose in the transglucosylase-activity digest were separated by paper chromatography. The rate of movement relative to glucose (R_{Glc}) of sucrose and the three main products were 0.67, 0.38, 0.23 and 0.13 respectively. The sugars were eluted with water, hydrolysed at 100° for 30 min. with 0.5% oxalic acid, deionized and examined by paper chromatography. The sugar having R_{Glc} 0.38 gave maltose and fructose on hydrolysis, and the other two sugars gave maltotriose and fructose, and maltotetraose and fructose respectively. Thus, when sucrose was the acceptor, the transglucosylase transferred glucose units from amylose to produce 4 α - α -glucosylsucrose, 4 α - α -maltoyslucrose and 4 α - α -maltotriyslucrose.

The oligosaccharides produced by transfer of glucose residues from amylose to isomaltose and to panose were isolated by chromatography on paper followed by elution with water. Since both isomaltose and panose have two glucosyl residues that are unsubstituted at position 4, there was a possibility that the transglucosylase could transfer to either or both of these positions. Summer & French (1956) obtained two homologous series of branched oligosaccharides by transfer of glucose units from α -Schardinger dextrin to isomaltose and panose in a *Bacillus macerans* amylase coupling reaction. This enzyme made use of both the possible positions for attachment of glucose residues to form new α -(1 $\rightarrow$ 4)-glucosidic linkages. The *S. bovis* transglucosylase, on the other hand, transferred glucose units to only one.

The two possible structures for the trisaccharide obtained by transfer to isomaltose are shown in Fig. 3 (A and B). These oligosaccharides were isolated by Sowa, Blackwood & Adams (1963) from a partial acid hydrolysate of pullulan, and the R_{Glc} and M_{Glc} values (where M_{Glc} is the migration of a substance relative to that of glucose) published by these authors for their oligosaccharides A and B are shown in Table 2, together with the results for the trisaccharide obtained in the present work, and for an authentic specimen of isopanose provided by Dr J. H. Pazur. The trisaccharide moved the same

Table 2. *Electrophoretic mobilities and R_{Glc} values of trisaccharides that contain both α -(1 $\rightarrow$ 4)- and α -(1 $\rightarrow$ 6)-glucosidic linkages*

The values for oligosaccharide A (isopanose), reduced oligosaccharide A and oligosaccharide B, i.e. *O*- α -D-glucopyranosyl-(1 $\rightarrow$ 4)-*O*-[α -D-glucopyranosyl-(1 $\rightarrow$ 6)]-D-glucose, are those of Sowa, Blackwood & Adams (1963). The paper electrophoresis of oligosaccharides A and B was carried out in 0.1M-sodium tetraborate. Ionophoresis of reduced oligosaccharide A and reduced trisaccharide was carried out in 0.1M-sodium molybdate adjusted to pH 5 (Bourne, Hutson & Weigel, 1959). The trisaccharide was obtained by transfer of a glucosyl residue from amylose to isomaltose by *S. bovis* transglucosylase.

	R_{Glc}	M_{Glc}	M_{Sorbitol}
Oligosaccharide A	0.16	0.64	—
Reduced oligosaccharide A	—	—	0.6
Oligosaccharide B	0.24	0.50	—
Trisaccharide	0.20	0.58	—
Reduced trisaccharide	—	—	0.57
Isopanose	0.20	0.58	—
Panose	0.24	0.12	—

Fig. 3. Possible structures for the dextrins formed on transfer of glucosyl residues from amylose to isomaltose and to panose. ○, Glucose unit; ●, reducing glucose unit; —, α -(1 $\rightarrow$ 4)-link; ↓, α -(1 $\rightarrow$ 6)-link.

distance as isopanose during paper chromatography and paper electrophoresis in 0.05 M-borax. The higher M_{Glc} value of oligosaccharide A (isopanose) compared with oligosaccharide B is to be expected for a trisaccharide with the glucose unit at the reducing end substituted only at position 6.

The trisaccharide and isopanose both gave strong colour reactions with aniline oxalate, a reagent that gives a weak reaction with sugars such as panose and maltose in which the reducing glucose moiety is substituted at position 4.

The tetrasaccharide in the series produced by transfer to isomaltose was rapidly and completely hydrolysed by a dilute solution of β -amylase to give maltose and isomaltose. Thus structure C (Fig. 3) was ruled out, and this proved that the transglucosylase did not attach glucosyl residues to both the unsubstituted 4-positions. By comparison with isopanose, the expected structure for the tetrasaccharide was D, and this conclusion was supported

Fig. 4. The R_M values, i.e. $\log[(1/R_A) - 1]$, of the dextrans formed by transfer of glucosyl residues from amylose to isomaltose (●) and to panose (O). R_A is (distance moved by dextrin)/(distance moved by acceptor).

by the R_M values for this series of oligosaccharides (Fig. 4). A relationship exists between the chromatographic mobilities and the degree of polymerization of homologous series of oligosaccharides (French & Wild, 1953), and the straight-line plot of R_M , i.e. $\log[(1/R_A) - 1]$, where R_A is the distance moved by the oligosaccharide relative to the acceptor, proves that the transglucosylase had synthesized a homologous series of α -(1 $\rightarrow$ 4)-linked oligosaccharides each having an α -(1 $\rightarrow$ 6)-glucosidic linkage at the reducing end. The pentasaccharide in the series yielded maltose and isopanose on β -amylolysis, and the hexasaccharide gave maltose and isomaltose. The amount of β -amylase used in these experiments was such that maltotriose was not attacked. The oligosaccharides (0.5 mg.) were incubated for 4 hr. at 35° with dilute β -amylase (β_2 solution, 0.05 ml.; cf. Walker & Whelan, 1960) at pH 5.0 in a total volume of 0.5 ml. Similar digests contained 0.5 mg. of maltotriose and maltohexaose. All the oligosaccharides with the exception of maltotriose were completely hydrolysed, and thus the tetrasaccharide 6- α -maltotriosylglucose was not hydrolysed much slower than maltohexaose.

It was expected that the glucose residues transferred to panose would be attached in a similar position to that ascertained for isomaltose, so that the tetrasaccharide formed in the amylose-panose coupling reaction would be 6²- α -maltosylmaltose (E). The alternative structure would be 6²- α -glucosylmaltotriose (F). The products obtained on hydrolysis of the α -(1 $\rightarrow$ 6)-glucosidic linkage with pullulanase proved the tetrasaccharide to have the structure shown for E. Pullulanase (30 mg.) was extracted with two 0.5 ml. portions of 0.02 M-phosphate buffer, pH 6.8, as described by Bender & Wallenfels (1961). A portion (0.35 ml.) of the extract was incubated with the tetrasaccharide (280 μ g.) for 5 hr. at 39°. The digest was then analysed by paper chromatography and found to contain maltose only. The complete absence of glucose and maltotriose proved that the transglucosylase could attach glucosyl units only at the non-reducing end of panose to give a 6²- α -maltosylmaltose. Thus with panose as acceptor the transglucosylase produced a homologous series of α -(1 $\rightarrow$ 4)-linked oligosaccharides having an α -(1 $\rightarrow$ 6)-glucosidic linkage penultimate to the reducing end.

Neither isomaltose nor panose could act as a donor in the reaction with transglucosylase. The enzyme had no action on these sugars in the absence of an added donor.

The following sugars had no detectable acceptor activity when incubated for 4 hr. in the transglucosylase activity digest: galactose, lactose, melibiose, melezitose, raffinose, sorbitol, L-fucose, fructose, L-arabinose, L-rhamnose, glycerol, cellobiose, glucose 1-phosphate and glucose 6-phosphate.

Synthesis of maltodextrins by transglucosylase action. A digest containing glucose (25 mg.), amylose (25 mg.) and transglucosylase (1 ml.) in 6 ml. was incubated under toluene for 24 hr. at 30°. The mixture was then heated to inactivate the enzyme, and centrifuged to remove retrograded amylose. The supernatant was deionized and concentrated to dryness. Water (1.2 ml.) was added, and portions (0.42 and 0.60 ml.) were spread along the starting line of two paper chromatograms. The first paper was irrigated for 2 days, and the second for 3 days. The maltodextrins were eluted and determined as described in the Materials and Methods section. The yields (Table 3) of maltose, maltotriose and maltotetraose were comparable. This is in contrast with the results of a similar experiment with D-enzyme (Walker & Whelan, 1957), where the yield of maltose was only 25% of that of maltotriose.

[¹⁴C]Maltodextrins were prepared in a similar digest, containing [¹⁴C]glucose (25 mg., 0.025 mc), amylose (21.5 mg.) and transglucosylase (1 ml.) in 6 ml. After incubation for 24 hr. at 30° the iodine-

Table 3. *Yield of maltodextrins formed by transfer of glucosyl residues from amylose to glucose*

Details of the digest are given in the text. The maltodextrins were separated by chromatography on paper. The second paper was irrigated for a longer time to allow better separation of the higher maltodextrins. Values are given for the weight (mg.) in the volume (1.2 ml.) of water in which the digest contents were dissolved after being concentrated to dryness.

Dextrin	Weight (mg.)		
	Paper 1	Paper 2	Mean
Glucose	15	—	15
Maltose	2.3	—	2.3
Maltotriose	2.7	2.6	2.6
Maltotetraose	2.0	2.0	2.0
Maltopentaose	1.7	1.7	1.7
Maltohexaose	—	1.7	1.7
Maltoheptaose	—	1.2	1.2

Table 4. *Yield and radioactivity of maltodextrins formed by the transfer of glucosyl residues from amylose to [¹⁴C]glucose*

Details of the digest are given in the text. The maltodextrins were separated by paper chromatography and eluted with water. Portions of the solutions were analysed for carbohydrate content and radioactivity.

Dextrin	Yield (mg.)	Sp.	Degree of
		radioactivity (counts/sec./mg.)	polymerization × sp. radioactivity
Glucose	17.54	20500	20500
Maltose	2.63	11725	23450
Maltotriose	2.87	6045	18135
Maltotetraose	2.02	4654	18616
Maltopentaose	1.58	4044	20220
Maltohexaose	1.62	3410	20460

staining capacity of the amylose was 9% of the original, and the digest was heated at 100° for 2 min., then deionized and concentrated to dryness. The mixture was dissolved in water and applied to the origin of a paper chromatogram. The yields of [¹⁴C]maltodextrins are shown in Table 4. The radioactivity of the dextrins was measured in an Ekco liquid-scintillation counter N644A used with an N530G scaler (Ekco Electronics Ltd., Southend-on-Sea, Essex). The specific activity of the maltodextrins varied inversely as the degree of polymerization, and, since the value obtained by multiplying the specific activity with degree of polymerization was constant, it was probable that each maltodextrin contained only one labelled glucose residue.

The position of the label in each maltodextrin

Table 5. *Products of the action of D-enzyme and Streptococcus bovis transglucosylase on maltotriose*

The values for D-enzyme are from Peat *et al.* (1956b), who separated the maltodextrins on charcoal-Celite. *S. bovis* transglucosylase (1 ml.) was incubated with maltotriose (46.5 mg.) in 3 ml. After 24 hr. a portion (0.65 ml.) was withdrawn, and the products of the reaction were separated by paper chromatography.

Product	D-enzyme	<i>S. bovis</i> enzyme
	Yield (g.) from 10 g. of maltotriose	Yield (mg.) from 10 mg. of maltotriose
Glucose	1.52	0.63
Maltose	0.143	0.71
Maltotriose	1.95	3.55
Maltotetraose	1.48	1.14
Maltopentaose	1.23	0.79
Maltohexaose	0.676	0.55

was determined by reduction of the dextrin (200 µg.) with sodium borohydride (7 mg.) in 0.3 ml. for 4 hr. at room temperature. The solution was then made 0.5 N with respect to sulphuric acid, and the reduced dextrin was hydrolysed at 100° for 4 hr. After deionization and removal of boric acid as methyl borate, glucose and sorbitol were separated by paper chromatography. A radioautograph showed that radioactivity was associated with sorbitol only. The glucose and sorbitol areas were eluted with water, and measurements of the radioactivity in the eluates proved that less than 2.5% of the total counts was associated with glucose in each case.

Action of transglucosylase on maltotriose. When D-enzyme was incubated with maltotriose a rapid disproportion of α-(1→4)-glucosidic linkages occurred within 0.5 hr. to give maltopentaose, glucose and a small amount of maltotetraose (Peat *et al.* 1956a). Thereafter all these products increased in amount and spots of lower and zero *R_F* values appeared. Only a trace of maltose was formed. Peat *et al.* (1956b) have published results for the yields of maltodextrins obtained when maltotriose was treated with D-enzyme and the products were separated on charcoal-Celite. These results are shown in Table 5, and are compared with the yields of maltodextrins obtained when the transglucosylase of *S. bovis* was incubated with maltotriose for a similar time. Fig. 5 shows the rate at which maltodextrins were synthesized when maltotriose (46 mg. in 2 ml.) was incubated with the transglucosylase. Although a quantitative chromatogram was not prepared until the reaction had continued for 4 hr., qualitative chromatograms were examined at 30 min. intervals, and these also showed that maltotetraose and maltopentaose were produced in approximately equal amounts from the beginning of the reaction. Maltose and maltotetra-

Fig. 5. Synthesis of maltodextrins from maltotriose. Glucose (○), maltose (●), maltotetraose (Δ) and maltopentaose (▲) were separated by quantitative paper chromatography.

ose were present in equimolar proportions. These results were interpreted as showing that the transglucosylase could transfer glucosyl and maltosyl residues with equal facility. This was in contrast with D-enzyme, which transfers maltosyl residues preferentially, producing maltopentaose and glucose, but no maltose, as the first products of the reaction with maltotriose.

Further proof that *S. bovis* transglucosylase could break both linkages of maltotriose was provided by incubating the enzyme (0.55 ml.) with maltotriose (4.6 mg. in 0.2 ml.) and [¹⁴C]glucose (1.46 mg. in 0.25 ml.) for 24 hr. at 30°. At intervals portions (0.1 ml.) were removed and prepared for chromatography. A radioautograph of the developed chromatogram showed maltotriose as the only radioactive product after 30 min. incubation with the enzyme; there was insufficient radioactive maltose to allow detection by this method. Elution of the appropriate area of the chromatogram yielded a maltose solution that was radioactive (Table 6). Thereafter both maltose and maltotriose increased in radioactivity, but the ratio of counts in maltotriose to maltose decreased with time (Table 6). The yield of maltose approached that of maltotriose because the radioactive maltotriose, unlike the maltose, could be used as a donor in synthetic

Table 6. Transfer of glucosyl and maltosyl residues from maltotriose to [¹⁴C]glucose

Details of the digest are given in the text. Maltose and maltotriose produced at various times of incubation were separated by paper chromatography, and detected on the paper by radioautography. The sugars were eluted with water and their radioactivities determined.

Time of incubation (hr.)	Radioactivity (counts/100 sec.)		Radioactivity of maltotriose Radioactivity of maltose
	Maltose	Maltotriose	
0.5	277	1302	4.7
1.2	559	2359	4.2
2	889	3280	3.7
4	1543	5230	3.4
7	2462	6582	2.7

reactions. At 24 hr. the incorporation of radioactivity from glucose into maltodextrins was 27%. Since transfer of a maltosyl residue to [¹⁴C]glucose yielded labelled maltotriose, and transfer of a glucosyl residue yielded labelled maltose, the experiment proved that the transglucosylase was capable of transferring both glucosyl and maltosyl residues in its reaction with maltotriose.

Peat *et al.* (1956a) observed products that stained orange with iodine when D-enzyme reacted with high concentrations of maltotriose. No iodine-staining polysaccharide was obtained when *S. bovis* transglucosylase was incubated with 0.10 M-maltotriose. A possible explanation for this difference is the appreciable amount of maltose produced by *S. bovis* transglucosylase. Maltose, being a good acceptor, would have the effect of lowering the average degree of polymerization of the maltodextrins produced in the reaction.

Synthesis of iodine-staining polysaccharides. Walker & Whelan (1959) observed that amylose was synthesized from maltotetraose by D-enzyme when the glucose produced during the disproportionation was removed by a hexokinase ATP trap. In the absence of the trap no iodine-staining maltodextrins were produced from the low concentration of maltotetraose used in the experiment. The results of similar experiments with *S. bovis* transglucosylase acting on maltotriose and on maltohexaose are shown in Fig. 6. When hexokinase and ATP were added to the digests, iodine-staining products were obtained from maltotriose, and the synthesis of polysaccharide from maltohexaose was greatly enhanced. The addition of glucose to a maltoheptaose digest incubated without ATP and hexokinase when it had reached equilibrium resulted in a fall in iodine stain.

Action of Streptococcus bovis transglucosylase on amylopectin and β-dextrin. The transglucosylase had

Fig. 6. Synthesis of iodine-staining oligosaccharides from maltoheptaose (□) and maltohexaose (Δ). The digests (0.5 ml.) contained substrate (10 mM), tris-maleate buffer, pH 7.5 (5 mM), magnesium chloride (10 mM) and transglucosylase (0.4 ml.). After incubation for 52 hr. (↓), glucose (2.5 mg.) and more enzyme (0.12 ml.) were added to the maltoheptaose digest. The effect of removing glucose with hexokinase and ATP was studied in a maltohexaose digest (▲) and a maltotriose digest (○) as above that also contained ATP (3 mg.) and crystalline hexokinase (0.025 ml. of a 1:370 dilution). The iodine stain of portions (0.05 ml.) of the digests in 0.4 ml. was read at 490 mμ on the Unicam spectrophotometer. Extinction values higher than 1 were obtained by dilution.

Fig. 7. Relative action of *S. bovis* transglucosylase on amylose (●), amylopectin (Δ) and β-dextrin (○) in the presence of glucose (for details of the activity digests see the Materials and Methods section). There was no fall in iodine stain of waxy-maize starch (□) when glucose was omitted from the transglucosylase-activity digest.

Table 7. Relative action of transglucosylase on amylose and on maize starch

The digests (1 ml.) contained substrate (1 mg.), glucose (5 mg.) and transglucosylase (0.2 ml.). Portions (0.1 ml.) were withdrawn at intervals for the determination of iodine stain.

Time (hr.)	Iodine stain (% of original)	
	Starch	Amylose
0.5	65.1	63.6
1	55.6	48.9
2	46.9	30.8
3.5	40.9	—
5	36.3	12.5

little action on waxy-maize starch (amylopectin) or on β-dextrin. Digests in which amylopectin and β-dextrin replaced amylose as donor to glucose were incubated with transglucosylase, and the results (Fig. 7) showed that the reaction of the enzyme with these branched substrates was very slow compared with amylose. When the transglucosylase was incubated with starch and glucose (Table 7) the rate of fall in iodine stain was comparable to that of amylose during the first 30 min. Later the rate of action on starch became slower, showing that the enzyme had far less affinity for amylopectin than for amylose.

Comparison of the amylase and transglucosylase activities of Streptococcus bovis cells. Walker (1965) presented some evidence that the cell-bound α-amylase of *S. bovis* was an intracellular enzyme. *S. bovis* cells therefore had two enzymes that could degrade amylose. A measure of the relative activity of the two systems was obtained by incubating portions, taken from the peak fractions of these enzymes eluted from DEAE-cellulose, with amylose. The enzymes were tested under the optimum conditions for their activity: pH 6 and 39° for the amylase, and at pH 7.5 and 30° with glucose as acceptor for the transglucosylase. A similar rate of fall in the iodine stain of amylose occurred in both digests (Table 8).

Location of the transglucosylase. Some information on the location of transglucosylase activity in the cells was obtained by studying the release of enzyme that occurred during the preparation of cell lysates with egg-white lysozyme. A 16 hr. culture of *S. bovis* was washed twice with 0.05 M-ammonium acetate containing magnesium sulphate (8 mM) and calcium acetate (2.5 mM). The cells were resuspended in the same solution at a cell density of 4 mg. dry wt./ml. Lysozyme (0.5 mg./ml.) was added, and the digest was incubated overnight at 38°. The extinction of the suspension was un-

Table 8. *Degradation of amylose by the cell-bound amylase and transglucosylase of Streptococcus bovis*

The amylase digest (0.5 ml.) contained amylose (0.5 mg.), tris-maleate buffer, pH 6.0 (10 mM), and enzyme (0.125 ml.). The transglucosylase digest (0.5 ml.) contained amylose (0.5 mg.), tris-maleate buffer, pH 7.5 (10 mM), glucose (2.5 mg.) and enzyme (0.125 ml.). A control was incubated without glucose. Portions were withdrawn at intervals for determinations of iodine stain. The original iodine stain of amylose gave an extinction value of 0.583.

Time (hr.)	Fall in iodine stain	
	Amylase	Transglucosylase
0.5	0.103	0.123
1	0.170	0.191
2	0.263	0.296
3	—	0.350
4	0.381	0.363

changed during this time, but rhamnose was released into the medium, showing that lysis of the cell wall was taking place. The digest was then centrifuged, and a portion (5 ml.) of the supernatant was freed from salts and sugars by gel-filtration on Sephadex G-25 (Pharmacia, Uppsala, Sweden). The protein eluted from the Sephadex column was pooled, and a portion (0.5 ml.) was incubated with maltotriose (4 mg.) at pH 7.5 and 30° overnight. The digest was desalted and concentrated, and a portion was examined by paper chromatography. In addition to glucose, maltose and maltotriose, heavy spots corresponding to maltotetraose and maltopentaose, and weaker spots of maltohexaose and maltoheptaose, were seen. Thus, in spite of the presence of amylase in the enzyme system, the oligosaccharides produced from maltotriose by transglucosylase activity were able to accumulate under the conditions of the experiment.

In a similar experiment the cells were incubated with lysozyme in the presence of 0.6 M-sucrose for 4 hr. at 38°. The spheroplasts were then washed once with 0.6 M-sucrose and resuspended in ammonium acetate for 30 min. at room temperature. The residue was removed by centrifuging, and a portion of the supernatant was tested for transglucosylase activity as described above. Maltodextrins were again formed from maltotriose, showing that transglucosylase had been released on lysis of the spheroplasts. The transglucosylase was therefore located either on or within the cytoplasmic membrane. The enzyme was also detected on or within the cytoplasmic membrane in cells that had been grown in a medium in which glucose replaced starch.

DISCUSSION

The action pattern of *S. bovis* transglucosylase resembled those of D-enzyme (Walker & Whelan, 1957) and of amylomaltase (Wiesmeyer & Cohn, 1960) in one respect. All three enzymes transferred glucosyl residues from the non-reducing end of the donor to the non-reducing end of the acceptor. With *S. bovis* transglucosylase this was proved as follows. First, transfer to methyl α -D-glucoside showed that the reducing group of the acceptor was not required to be free. Secondly, the maltodextrins produced in the reaction between amylose and [14 C]glucose were labelled only at the reducing end. Acid hydrolysis of the reduced maltodextrins gave radioactive sorbitol and unlabelled glucose. Some radioactivity would have been detected in the glucose had there been any transfer from the reducing end of the donor.

The end-labelled maltodextrins were used to study the action pattern of *S. bovis* α -amylase (Walker, 1965). This amylase degraded amylose to give maltotriose and maltose as the main products, and was unusual in producing far more maltotriose than maltose. Maltotriose was also the main product of the action of the amylase on maltodextrins, and studies with the end-labelled maltodextrins revealed that with maltohexaose, maltopentaose and maltotetraose the main linkage to be hydrolysed was the third from the non-reducing end. The transglucosylase of *S. bovis* could therefore utilize the main product of α -amylase action, maltotriose, as a substrate for the synthesis of oligosaccharides. It was conceivable that the production of intracellular iodophilic storage polysaccharide was mediated by this transglucosylase. The enzyme was capable of synthesizing maltodextrins of sufficiently high chain length to stain with iodine from maltotriose, provided that glucose was removed.

The transglucosylase could not use maltose as a donor substrate, and *S. bovis* cell extracts had no detectable action on maltose. The only function found for maltose was that of acceptor. With amylose as donor, maltose was 50% as efficient an acceptor as glucose. It is not known whether this reaction alone could utilize all the maltose in the cell. Maltose was produced both during synthesis from maltotriose and by transfer of glucosyl residues from amylose to glucose. With D-enzyme, the amount of maltose formed in such reactions was so small that it was suggested by Walker & Whelan (1957) that maltose arose either from traces of amylolytic activity or from the dimerization of glucose. Such explanations did not account for the appreciable amounts of maltose produced by *S. bovis* transglucosylase. Maltose obtained by transfer of a glucosyl residue from amylose to [14 C]glucose

was labelled in the reducing glucose unit only, and the specific activity was half that of the radioactive glucose used as acceptor (Table 4). The main amylase in the cell extract was removed by separation on DEAE-cellulose, and the peak fractions of transglucosylase activity chosen for this work were virtually free from amylase. Little maltose could have arisen from amylase activity, and therefore it was concluded that *S. bovis* transglucosylase, in contrast with D-enzyme, could transfer a single glucosyl residue from either amylose or maltotriose to glucose, forming maltose.

Peat, Whelan & Jones (1957) tested the capacities of nearly 50 sugars and related compounds to act as acceptors of glycosyl radicals transferred from maltodextrins by D-enzyme. Many of these were tested as acceptors for *S. bovis* transglucosylase in the present work (Table 1). The main differences between the two enzymes were that maltose was a better acceptor for *S. bovis* enzyme, and methyl α -D-glucoside was better for D-enzyme. Xylose was a good acceptor for D-enzyme, but the small acceptor activity in *S. bovis* transglucosylase-activity tests disappeared after traces of glucose were removed from the xylose by paper chromatography. Isomaltose had no acceptor activity in the D-enzyme disproportionation. Sucrose, trehalose, D-mannose and L-sorbose were effective acceptors for both enzymes. Mannose is also an acceptor for the transfer of glucosyl residues from amylose by amylomaltase (Wiesmeyer & Cohn, 1960).

The ability of the transglucosylase to transfer glucose residues to isomaltose and to panose provided a useful means of synthesizing a series of oligosaccharides having an α -(1 $\rightarrow$ 6)-glucosidic linkage at the reducing end and penultimate to the reducing end respectively. Since isomaltose and panose could act as acceptors, it is probable that the branched limit dextrins produced by amylase action on amylopectin could also function as acceptors.

Bailey (1959) reported the isolation of dextran-sucrase from cell-free culture fluids of *S. bovis* grown on sucrose or glucose in the presence of carbon dioxide. This enzyme converted sucrose into fructose and an unbranched polysaccharide, dextran, in which the glucosidic linkages were α -(1 $\rightarrow$ 6). The present work has therefore provided a second example of the ability of *S. bovis* to syn-

thesize polysaccharide by transferring glucosyl residues.

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