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THE PURIFICATION AND PROPERTIES OF DEXTRANSUCRASE FROM *Streptococcus sanguis* ATCC 10558*

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ABSTRACT

Dextransucrase has been purified from the culture fluids of *S. sanguis* 10558 by a combination of hydroxylapatite, ion-exchange, and gel-filtration steps. Two active proteins were isolated with specific activities approaching one order of magnitude higher than other preparations reported. The enzymes have mol. wt. on the order of 100 000 and exhibit pH optima between 5.8 and 6.2. In addition, detailed analysis of one of the enzymes indicates that the enzyme undergoes two ionizations that are important for activity. One pK is at 4.4 and the second at 7.4. The structures of dextrans produced by the two enzymes have been examined by p.m.r. spectroscopy, and a substantial degree of similarity was observed, with only minor differences in the proportion of α-(1→3) and α-(1→6) bonds. No evidence could be obtained that either of the enzymes was capable of catalyzing a rearrangement of α-(1→6) to α-(1→3) bonds.

INTRODUCTION

The observed correlation between the utilization of sucrose by the cariogenic bacteria and caries formation has stimulated interest in the manner in which the sugar is metabolized. The process by which these bacteria utilize sucrose to form an insoluble polysaccharide, and to produce substantial quantities of organic acids appears to be essential to the cariogenic process[1]. The reaction that is of interest is the initial step in sucrose utilization in which a D-glucosyl or D-fructosyl residue is transferred to a growing polymer, while simultaneously releasing monomeric D-fructose or D-glucose residues. The monosaccharides are subsequently utilized by the organisms for acid production. The enzymes that catalyze this first step are D-glycosyltransferases and are specific for either D-fructosyl or D-glucosyl residues depending upon the species of organisms. The D-fructosyl-transferase (levansucrase EC 2.4.1.10) has been characterized by Dedonder and assoc.[2–4]. The D-glucosyltransferase, dextransucrase

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[sucrose:(1→6)-α-D-glucan 6-α-D-glucosyltransferase, EC 2.4.1.5] has not been as well characterized, although a number of investigators[5–16] have been engaged in studies on it. We have attempted to study the enzyme derived from *S. sanguis* as a model for the reaction. The study has been initiated by the development of a purification procedure that permits good yield of homogeneous enzyme fractions. Such preparations will permit us to carry out studies on the mechanism of catalysis. We wish to report our findings and the properties of the purified enzyme preparations.

RESULTS

Enzyme purification. — The purification procedure described in the Experimental section employs an initial adsorption to hydroxylapatite, and subsequent elution with increasing concentration of phosphate buffer. The procedure is a modification of that described by Newbrun and Carlsson[5]. We have observed that adsorption to the gel is more complete and reproducible (see Table I), when the culture fluid has been subjected to prior dialysis as described, and when the pH is adjusted to 5.5 during adsorption. The requirement for dialysis may be related to the relatively high concentration of organic acids produced during cell growth.

The first fractionation step on DEAE-cellulose, carried out at pH 6.0, resulted in the separation of two protein peaks that contained enzyme activity (Fig. 1). A third peak was also observed; however, this peak was relatively minor and quite variable from one preparation to the next, and no further work with it was performed. The first peak (A) appeared to be eluted from the column with the nonretained materials, while the second peak (B) was eluted at a concentration of 0.05M sodium chloride.

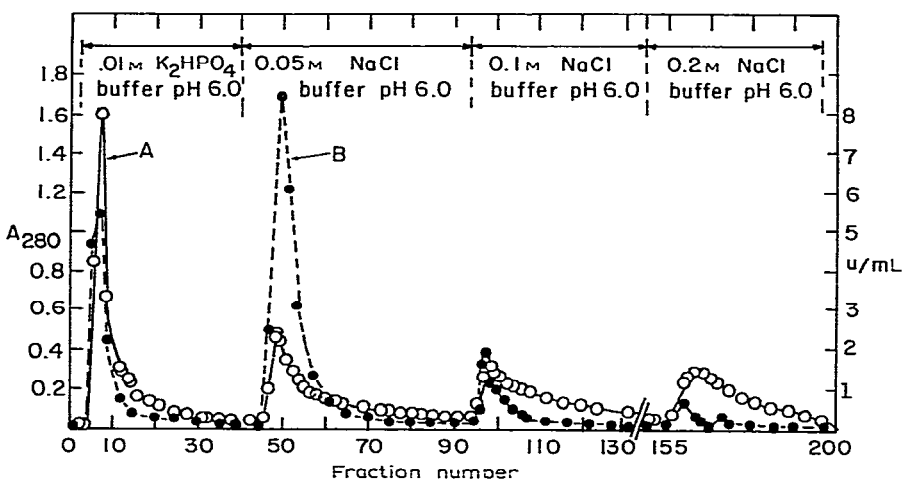

Fig. 1. Chromatography on DEAE-cellulose at pH 6.0. A solution of dextransucrase with a conductivity of 0.4 kA/Vm was applied to a 1.5×35 cm column, pH 6.0, of DEAE-cellulose, and eluted batchwise with 0.01M phosphate buffer (280 mL), pH 6.0, followed by 0.05M NaCl (415 mL), 0.1M NaCl(480 mL), and 0.2M NaCl (330 mL) in the same buffer. Enzyme activity (●----●) was measured as described in Methods, and protein (○ —— ○) was measured by A_{280}.

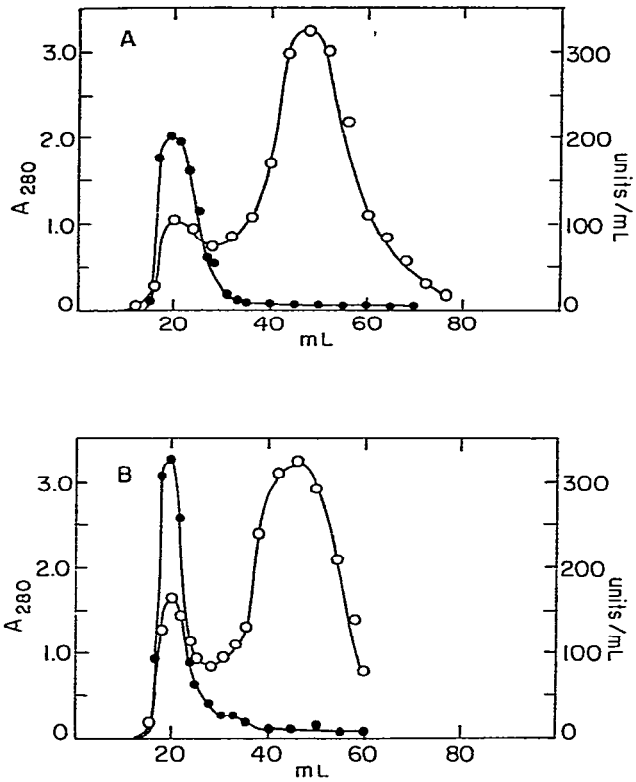

Fig. 2. Gel-filtration of dextransucrase: (A) Enzyme-fraction A from a DEAE I column was applied to a 1.1×51 cm column of Bio-Gel P-100, and eluted with 0.01M phosphate buffer, pH 6.0. (B) Enzyme-fraction B from a DEAE I column was applied to a 1.1×51 cm column of Bio-Gel P-100, and eluted with 0.01M phosphate buffer, pH 6.0; ● —— ●, units/mL; ○ —— ○, A_{280}.

When these enzymes were dialyzed and applied to similar DEAE-cellulose columns, their elution behavior remained similar to that observed on the initial column.

Both enzyme fractions obtained from the DEAE-cellulose column were excluded from Bio-Gel P-100 columns, as shown in Fig. 2 (A and B), and were effectively separated from the bulk of the contaminating material absorbing at 280 nm. While many of the low-mol.-wt. contaminants were removed in the first two steps, the residual peptides and peptones derived from the growth medium were the major components that were removed in this step. Subsequent to the Bio-Gel P-100 step, the two enzymes were chromatographed on DEAE-cellulose at pH 6.8. The behavior of enzyme A on DEAE-cellulose at pH 6.8 is shown in Fig. 3A. As in the case of the chromatography at pH 6.0, it appeared to pass directly through the column. However, additional extraneous protein was retained on the column, and was eluted when the ionic strength was increased. The behavior of enzyme B under these conditions was not reproducible from one preparation to another. A major portion of the enzyme was always eluted with the nonretained materials, but it was also observed that variable amounts of

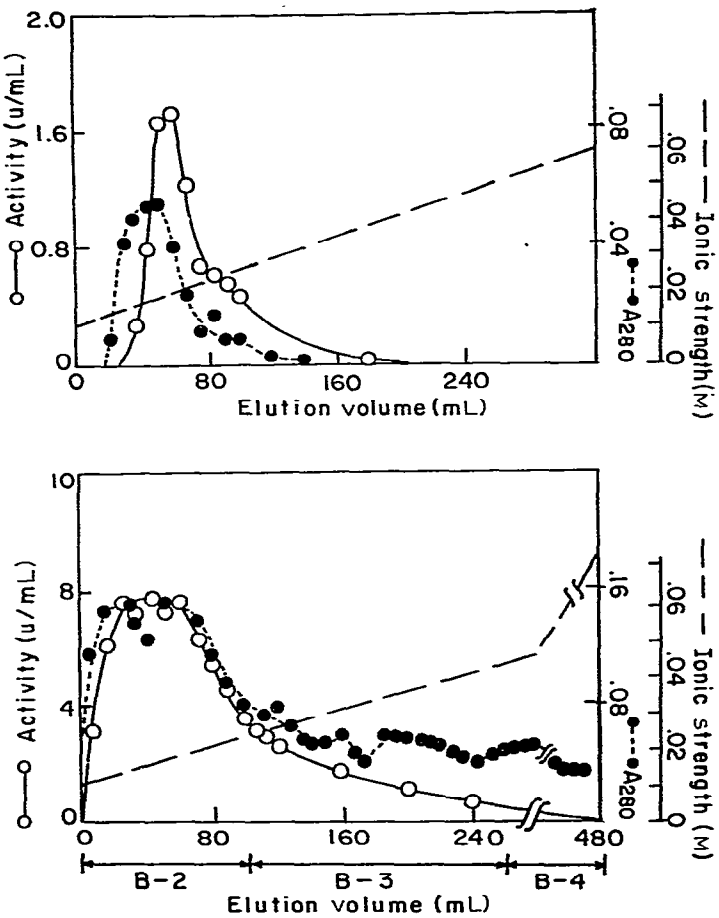

Fig. 3. Chromatography on DEAE-cellulose at pH 6.8. Enzyme fractions A (3A) (upper fig.) and B (3B) (lower fig.) were applied to 1.1 × 20 cm columns of DEAE cellulose equilibrated at pH 6.8. Elutions were carried out with a linear gradient (600 mL) from 0.01M to 0.1M NaCl, and 4-mL fractions were collected. The gradient was initiated following a 40-mL wash of the column with 0.01M phosphate buffer, pH 6.8. Fraction B-1 was collected in a single fraction, and is not shown in 3B; ●---●, units/mL; ○ —— ○, A_{280}.

enzyme could be retained, and eluted at ionic strengths ranging from ~0.05 to 0.08M (see Fig. 3B).

The purification described here permitted a total recovery of 13% of the original activity in the two separated fractions. Recovery from each of the chromatographic steps ranged between 28 and 95%, as seen in Table I. Losses of activity up to 40% were frequently encountered in steps requiring concentration. These losses were taken into account in the calculation of the overall recovery. A variety of concentration techniques were studied, such as pressure filtration with Pelicon filters (Millipore), hollow-fiber devices (Bio-Rad Laboratories), and readsorption and elution from hydroxylapatite. The results observed proved to be quite variable and in general not as good as the procedures described.

TABLE I

SUMMARY OF PURIFICATION OF DEXTRANSUCRASE

Steps	*Activity*[a]	*Spec. activ.*[b]	*D.p.*[c]	*Recovery* (%)
Crude product (original)	10,400	0.09	1	100
Crude product (dialyzed)	8,789	0.09	1	84.5
Hydroxylapatite				
0.2M Phosphate wash at pH 6.0	2,684	2.10	22	25.8
0.5M Phosphate wash at pH 6.8	5,629	3.40	36	54.1
Total	8,313			80
DEAE-cellulose at pH 6.0				
Fraction A	1,271	24.0	266	12.2
Fraction B	4,075	12.6	140	39.2
Total	5,346			51.4
Bio-gel P-100				
Fraction A	573		313	5.5
Fraction B				
1st Chromatography	2,235	37.8	420	21.5
2nd Chromatography	1,886	44.6	496	18.1
Total[d]	2,459			24
DEAE-cellulose at pH 6.8				
Fraction A	389	197	2190	3.7
Fraction B				
B-1	136	296	3280	1.3
B-2	446	202	2250	4.3
B-3	300	28	309	2.9
B-4	100	6.3	70	1.0
Total	1,371			13.2

[a]Total enzyme activities (units) as measured by method A. [b]Specific activity (unit/mg of protein). [c]Degree of purification (Spec. activ./spec. activ. of crude product). [d]Sum of Fraction A and Fraction B (2nd chromatography).

The data shown in Table I summarize the purification procedure. It can be seen that the procedure affords about a 2200-fold purification with regard to enzyme A, which has a final specific activity of 197 units per mg of protein. The B enzyme has a variable specific activity, but reaches levels of 202 and 296, and thus represents an increase in purity of some 2200-to 3300-fold relative to the crude enzyme.

Stability. – The enzyme at all stages of purification could be stored at 0 to 4° in the presence of 0.01 % sodium azide. In the more highly purified stages, fractions A and B displayed quite different stabilities. Fraction A was observed to be much more labile, losing activity at a rate of between 25 to 30 % per week when stored at 0 to 4°. Freezing and thawing produced extensive losses of activity, *i.e.*, 75 to 90 %. Fraction B only lost activity at a rate of less than 10 % per week when stored at 0 to 4°, and was virtually stable to freezing and thawing.

Homogeneity and molecular weight. — The mol. wts. of Fractions A and B were

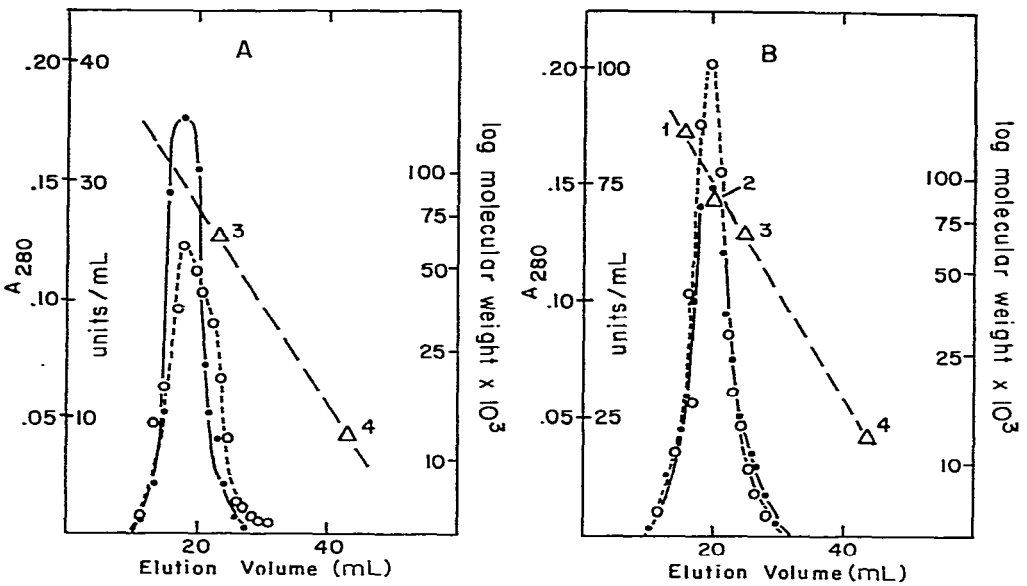

Fig. 4. Determination of molecular weight. Enzyme fractions DEAE II-A (A) and -B (B) were applied to of 1.1 × 40 cm columns Bio-Gel P-200, and eluted with 0.01M phosphate buffer, pH 6.0. Fractions of 0.8 mL were collected. The columns were calibrated with D-glucose oxidase (1), alkaline phosphatase (2), egg albumin (3), chymotrypsinogen (4), and ribonuclease (5) in decreasing order of mol. wt. The values were plotted as a function of log of mol. wt.: △ - - - - △; Enzyme activity (● —— ●) and protein (○ - - - - ○) were measured.

estimated by gel-filtration on Bio-Gel P-200 according to the procedure described by Andrews[17]. In both cases (Figs. 4A and 4B), the activity was eluted as a single peak corresponding to a mol. wt. of 102,000. As seen in Fig. 4B, only one protein peak, which superimposes with the activity, was found in Fraction B. The specific activity across the peak falls in the range of 42–50 units per absorbance unit at 280 nm. These data suggest that the enzyme preparation is homogeneous. The behavior of enzyme fraction A, on the other hand, suggests that an additional protein is present, since a distinct shoulder is observed (Fig. 4A). Estimates of the relative areas under the enzyme peak and the shoulder suggest that the enzyme is approximately 90% pure.

Effects of reagents. — Some preliminary information on the characteristics of dextransucrase was obtained by treatment with a variety of reagents (see Table II). The denaturing agents examined, guanidine and dodecyl sodium sulfate, were effective inhibitors of activity, whereas urea was less effective. EDTA had no inhibitory effect on enzyme activity up to a concentration of 0.1M, which supports the argument that the enzyme is not a metalo-enzyme. Disulfide reagents, such as 2-mercaptoethanol and dithiothreitol, produced partial inactivation of the enzyme at the concentrations examined. This may indicate that the activity is somewhat dependent upon the integrity of disulfide linkages. Whereas the thio reactive reagent *p*-mercuribenzoic acid had no effect on the activity of the enzyme, mercuric chloride caused complete inactivation.

TABLE II

EFFECTS OF VARIOUS REAGENTS ON DEXTRANSUCRASE[a]

Reagents	*Final conc. of reagent*	*Activity remaining (%)*
None	—	100
Urea	4 M	50.2
Urea	2 M	82.6
Guanidinium·Cl	3 M	0
Guanidinium·Cl	1.5 M	9.7
Dodecyl Na sulfate	6.9 mM	5.6
Dodecyl Na sulfate	0.69 mM	80.5
EDTA	0.1 M	123
EDTA	0.05 M	123
EDTA	5 mM	119
$HgCl_2$	0.13 M	0.6
Dithiothreitol	0.25 M	41.1
Dithiothreitol	25 mM	68
2-Mercaptoethanol	0.15 M	51.4
2-Mercaptoethanol	0.05 M	50.5
p-Mercuribenzoic acid	33 μM	104

[a]The DEAE-II-A enzyme (0.25 units) was mixed with each of the various reagents (0.1 mL) and kept for 5 min at room temperature, at which time an aliquot of 0.1 mL of a 0.2M [^{14}C]sucrose (75 c.p.m./nmol) and of a 10mM Dextran T-10 solution were added. The reactions were carried out for 15 min at 37°, and terminated by heating for 3 min at 100°. All reagents were buffered at pH 6.0, and the final concentration refers to the concentration of the reagent after the addition of substrate. The analyses were carried out as described in Methods.

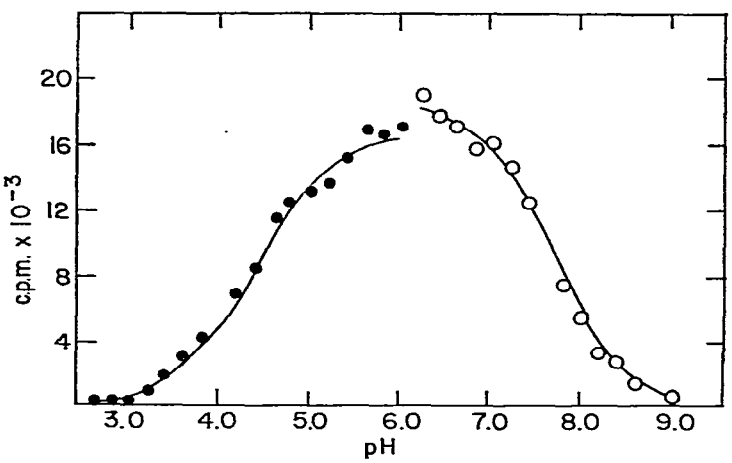

Fig. 5. pH–Rate profile. Dextransucrase (0.02 units) was mixed with 25 μL of the indicated buffer at various pHs and allowed to stand at 27° for 4 min. Fifty μL of substrate solution containing 0.2M [^{14}C]-sucrose (97 c.p.m./nmol) and mM Dextran T-10 was added to each tube. The reactions were carried out for 15 min at 37°, at which time the reaction was terminated by heating for 2 min at 100°. The analyses were carried out as described in Methods. Controls were run at selected pHs with heat-inactivated enzyme. Buffers used were ●----●, 0.3M citrate–phosphate; ○----○, 0.5M C-(*N*-morpholino)ethanesulfonic acid.

In the latter case, it appeared that the protein was precipitated by the heavy metal. The fact that *p*-mercuribenzoic acid had no effect, however, suggests that sulfhydryl groups do not play an important role in the activity of the enzyme.

Profile of the pH rate. — Fig. 5 shows the effect of pH on enzyme activity. The solid lines represent calculated curves, based on the assumption that only the monoprotonated form of the enzyme is active. The analysis of the curve was carried out by a curfit method[18], which employs a least-squares linearization of a fitting function. The pKs were calculated and found to have values of 4.4 and 7.7, with the optimum pH ranging from 5.8 to 6.2. These values are suggestive of a carboxyl group for the acid pK and an imidazole residue for the basic group.

Products of the enzyme-catalyzed reaction. — The dextran produced by *S. sanguis* ATCC 10558 has been shown to contain D-glucosyl residues linked by either α-(1→3) or α-(1→6) glycosidic bonds[19]. With the demonstration of two separate proteins having the ability to catalyze dextran formation, it became important to obtain some information about the structure of the products formed by each of these enzymes. It has been established[19,20] that the p.m.r. spectra of dextrans can be used to determine the proportion of (1→6)- and non-(1→6)-glycosidic bonds owing to the differences in the chemical shift of the anomeric protons involved in these linkages. The enzymes, at various stages of purification, were used to prepare polymers from sucrose as the only substrate. The reactions were carried out over an extended period of time in the presence of chloramphenicol or sodium azide to prevent bacterial growth. The progress of the reaction was followed by measuring the fructose concentrations

TABLE III

P.M.R. ANALYSIS OF ENZYMICALLY FORMED DEXTRANS[a]

	Bonds	
State of purification of the enzyme	*(1→6)* (%)	*Non-(1→6)* (%)
Crude	72	28
Hydroxylapatite	79	21
DEAE I		
A	77	23
B	82	18
Bio-Gel		
A	72	28
B	82	19
DEAE II		
A[b]	73	27
B[b]	80	20

[a]The polysaccharides were produced in mixtures containing 30 to 50 units of enzyme from each of the indicated stages of purification in 0.2M sucrose (100 mL), at pH 6.0, 37° in the presence of 0.01M NaN_3. The isolation of the polysaccharides and preparation for p.m.r. analysis are described in the Methods section. [b] The spectra were recorded in the presence of 5M KSCN.

by reducing-sugar analysis. The reactions were terminated when about 50% of the substrate had been consumed. The polymeric products were isolated and purified by ethanol precipitation and exhaustive dialysis. The p.m.r. spectra of the products were recorded on solutions in deuterium oxide in the presence of ~5M potassium thiocyanate with a Fourier-transform p.m.r. spectrometer. The resonances at 4.96 and 5.33 p.p.m. have been assigned to the protons of the anomeric carbon atoms of the residues involved in (1→6)- and non-(1→6)-glycosidic bonds[19,20], respectively. By integration of the peaks the proportion of (1→6)- and non-(1→6)-linkages was calculated (see Table III). Both enzyme fractions A and B catalyzed the formation of polymers that contain (1→6) and non-(1→6) bonds. As chemical analysis[19] had shown that the only non-(1→6) bond present was α-(1→3), we have assumed that the non-(1→6) peak is due solely to (1→3) bonding. Both enzyme preparations A and B produce polymers with (1→6) and (1→3) linkages, the product of the A enzyme consistently having a greater proportion of (1→3) bonds than that of the B preparation.

The observation that the enzyme fractions could catalyze both α-(1→6)- and α-(1→3)-bond formation raises the question as to the nature of the activity. One possibility is the involvement of a transglycosylase activity, such that a rearrangement

TABLE IV

P.M.R. ANALYSIS OF DEXTRAN AND OLIGOSACCHARIDES TREATED WITH ENZYME FRACTIONS[a]

	Substrate					
	Dextran			*Oligosaccharides*		
Enzyme fraction	*Units of enzyme*	*(1→6) (%)*	*non-(1→6) (%)*	*Units of enzyme*	*(1→6) (%)*	*non-(1→6) (%)*
None		92.4	7.6		90.8	9.2
Crude	25.3	92.6	7.4	10.6	89.1	10.9
Hydroxylapatite				31.2	91.6	8.4
DEAE I						
A	25.0	94.9	5.1	14.2	92.7	7.3
B	26.3	95.2	4.8	23.8	92.5	7.5
Bio-Gel						
A	33.8	93.0	7.0	16.7	88.2	11.8
B	48.0	92.5	7.5	14.8	90.3	9.7
DEAE II						
A				7.2	89.5	10.5
B				3.9	90.6	9.4

[a]Dextran T-10 (d.p. 60) or a mixture of oligosaccharides (d.p. 10) were incubated for 24 h at 37°, in the presence of 0.01M NaN_3, with the indicated units of dextran sucrase obtained at different stages of purification. The products were lyophilized, and the p.m.r. spectra were recorded, with a Bruker HX 90 F.T. spectrometer, on solutions in D_2O in the presence of 5M KSCN for dextran, or of 0.5M KSCN for the oligosaccharides.

from an α-(1→6) to an α (1→3) bond might occur, even though this is thermodynamically unfavorable. To examine this possibility, experiments were done in which enzyme preparations at various stages of purification were added to substrates that contained preponderantly (1→6) bonds. Following the reaction, the products were examined for increase in the proportion of (1→3) bonds by p.m.r. Two types of substrates were employed, a polymer having an average chain-length of 60 D-glucosyl residues, and an oligosaccharide mixture having an average chain-length of 10 D-glucosyl residues. The data presented in Table IV indicate that no increase in the proportion of (1→3) bonding was observed when the enzyme from any step in the purification was utilized in the reactions. This appears to be true regardless of the size of the substrate.

DISCUSSION

A procedure for the purification of dextransucrase from *S. sanguis* ATCC 10558 is reported. The purification scheme employs separations based on differences in charges and mol. wt., as well as in the adsorptive behavior on hydroxylapatite. The procedure yielded two protein fractions that are capable of catalyzing dextran formation. One of the proteins (B) appears to be homogeneous, whereas the second (A) is ~90% pure. The overall yield for the two proteins was 13%. Recent reports[15] have described a similar preparation from *S. sanguis* OMZ 9, which employs ion-exchange and gel-filtration steps. The authors isolated a single protein from the culture fluids in pure form with a recovery of ~13%. McCabe and Smith[16] have recently described a procedure for the isolation of dextran sucrase from *S. mutans* with a recovery of 65%; they employed affinity chromatography and gel filtration on a hydrophobic support. Ciardi *et al.*[21] have also achieved high recovery of enzyme from *S. mutans* 6715 with the utilization of ultrafiltration, however, the homogeneity of these preparations is uncertain, and the specific activities are relatively low. The individual steps in the purification described in this report provide reasonable recoveries; however, steps involving concentration routinely resulted in substantial losses of activity. A major feature of the purification is the separation of two enzymes that catalyze dextran formation from sucrose. The specific activities of these enzymes are higher than those reported for dextran sucrase purified from other organisms[8,10,12,13,16,17].

The mol. wts. of the two enzymes were found to be ~100 000. These values are comparable to similar data obtained on dextran sucrase isolated from other sources[6,8,10,11], which range from 69 000 to 175 000. However, other preparations were observed[13] to have a much higher mol. wt. (800 000). The basis for these differences is not understood, but may be of importance. It is possible that the enzymes are bound to endogeneous polysaccharide. The fact that endogeneous carbohydrate has been detected in our preparations and in the preparations described by Scales *et al.*[13] and by Ciardi and associates[7,8,21] would be consistent with this idea. It is also possible that the enzyme is capable of forming aggregates of some fundamental subunit[14].

Two forms of dextransucrase were observed in our studies. Other investigators have also observed multiple forms of the enzyme from other organisms, either directly by purification[10,13,14] or indirectly[11,22]. In an attempt to better understand the function of the two enzymes, we have investigated the structure of the two polysaccharides produced. Both are D-glucans and show very little difference in structural characteristics as determined by p.m.r. Both spectra contain resonances of anomeric protons corresponding to α-(1→6)-glycosidic bonds and non-α-(1→6)-glycosidic bonds. As previous studies[19] on the native polysaccharide indicate that only (1→6) and (1→3) bonds were present, we attribute the non-(1→6) bonds entirely to (1→3) bonds. This was confirmed by methylation analysis on the polysaccharide produced by each purified enzyme[23]. The proportion of (1→3) bonding in the polysaccharide produced by enzyme A is slightly greater than that found in the polymer produced by enzyme B. However, the differences are probably too small to be significant. Robyt and Taniguchi[24] have suggested that non-(1→6) bonds and branches are formed by transfer of nascent D-dextran chains, which are bonded to the enzyme, to acceptor molecules. Such a process could explain the ability of the purified enzyme to catalyze both α-(1→6) and α-(1→3) linkages. Alternative proposals for the mechanism of branch formation have been made, including the participation of a branching enzyme[25] as in the case of glycogen and starch[26]. However, the inability to observe such a rearrangement by p.m.r. tends to argue against this suggestion. These observations are in agreement with the report by Sidebotham[27].

EXPERIMENTAL

Materials. — *Streptococcus sanguis* ATCC 10558 was obtained from Dr. Samuel Rosen of The Ohio State University School of Dentistry. Stock cultures were stored at −20° in 50% glycerol suspension. Dextran T-10, which has an average mol. wt. of 10 000, is a product of Pharmacia Fine Chemical (Piscataway, NJ 08854). This material was extensively dialyzed and lyophilized to a dry powder prior to use. A mixture of oligosaccharides with a degree of polymerization (d.p.) of 10 was prepared from Dextran T-10 by partial acid hydrolysis with 0.1M HCl for 75 min at 100°. Commercial enzymes were obtained from Sigma Chemical Co. (St. Louis, MO 13178), as well as nucleotides, and D_2O. All other chemicals were of reagent grade, and derived from commercial sources. [U-^{14}C]Sucrose and D-[^{14}C]glucose were obtained from New England Nuclear (Boston, MA 02118).

Methods. — Two procedures for enzyme activity measurements were utilized. Method A based on the measurement of D-fructose released, and method B based on the incorporation of radioactive D-glucose into polymeric form.

Method A. This procedure, which was utilized throughout the purification, is a modification of the procedure described by Carlsson *et al.*[28], in which the D-fructose that is formed is measured enzymically in a coupled assay. The reaction mixtures contained 5mM Dextran T-10, 0.1M sucrose, 0.01M potassium phosphate buffer (pH 6.0), and enzyme in a total volume of 0.2 ml. Although the reaction is not

dependent on Dextran T-10, the rate was stimulated about 4-fold in its presence. The controls were identical with the exception that the enzyme had been kept at 100° for 3 min prior to the reaction. After 15 min at 37°, the reaction was stopped by heating in a 100° bath for 2 min. Aliquots were withdrawn and added to reaction mixtures containing 0.2 units each of hexokinase, D-glucose phosphate isomerase, and D-glucose 6-phosphate dehydrogenase, $NADP^+$ (0.2 μmol), ATP (0.2 μmol), Tris-hydrochloride (10 μmol) (pH 7), and $MgCl_2$ (0.4 μmol) per mL. The reaction mixtures were maintained at 37° for 20 min, and the absorbance at 340 nm was measured. The absorbance of each control was subtracted from the respective experimental reaction mixture. One unit of enzyme is defined as that amount of enzyme catalyzing the release of 1 μmol of D-fructose per min.

The results obtained with this assay when crude enzyme preparations were used were erroneously high due to the presence of an invertase in the culture fluid. However, the level of invertase was no greater than 10% of the dextran sucrase levels, and the impurity was removed in large part in the initial steps of purification.

Method B. The alternative assay used in some experiments was based upon the measurement of the incorporation of D-[^{14}C]glucose into a polysaccharide. The reaction mixtures consisted of 0.1M [U-^{14}C]sucrose (4.84 10^5 c.p.m.), 5mM Dextran T-10, 0.01M potassium phosphate buffer (pH 6.0), and enzyme in a volume of 0.1 mL. The reactions mixtures were maintained at 37°. Aliquots were withdrawn at 0 and 15 min, immediately spotted on Whatman 1 MM chromatography paper, and dried with a stream of hot air to halt the reaction. After development of the chromatogram in 2:2:1 (v/v) butanol–pyridine–0.01M sodium borate, the radioactive spots at the origin were counted in a liquid scintillation counter in 20 mL of a scintillation fluid consisting of 3.8 g of 2,5-diphenyloxazole and 45 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene per L of toluene. The difference in radioactivity between 0 and 15 min was used to calculate the μmol of D-glucose incorporated. The assay was also performed with boiled enzyme-controls, incubated at 37°, in place of zero-time controls.

P.m.r. analysis. — The polysaccharides were synthesized by cell-free reactions employing enzyme preparations at various stages of purification. The reaction mixtures contained 0.2M sucrose (buffered at pH 6.0), 0.01M NaN_3, and enzyme. The reactions were carried out for up to 4 days, and the polysaccharides were isolated by ethanol precipitation and exhaustive dialysis. Analysis of the polysaccharide structures by p.m.r. was based on the assignments of Pasika and Cragg[20]. The p.m.r. spectra were recorded with a Bruker HX-90, Fourier-transform spectrometer on polysaccharide solutions (~100 mg/mL) in D_2O and sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. Some spectra were recorded in the presence of up to 5M KSCN. Up to 2500 scans were required for good spectra. Prior to introduction into the spectrometer, all samples were treated by repeated freeze-drying and dissolution in D_2O, to exchange the labile protons with deuterons.

Other analytical procedures. — Protein was estimated by measurement of the absorbance at 280 nm or by a modification of the procedure described by Miller[29]. Estimates of mol. wt. were obtained by gel filtration on Bio-Gel P-200 according to

the procedure described by Andrews[17]. Reducing sugars were measured by the procedure of Nelson[30], and hexoses by the anthrone procedure[31].

Cell growth. — The cells were grown, at 37° with light stirring, on Brain-Heart Infusion broth (Difco Laboratories, Detroit, MI 48232) supplemented with 1% of D-glucose. Cultures of 10 L were inoculated with a 10% inoculum in order to minimize contamination. The acid production was monitored, and the pH was maintained between 6.5 and 7 by the regular addition of 10% NaOH. When acid production and cell growth (as measured by absorbance at 660 nm) had stopped, the culture was chilled and the cells were removed by centrifugation in a Szent-Györgi continuous flow centrifuge at 4°, or batchwise at 10 000*g*.

Enzyme purification. — All operations in the purification were carried out at 4° except where indicated.

On hydroxylapatite. Sodium azide was added to the cell-free culture fluid to a concentration of 0.01M. The fluid was then concentrated in an Amicon hollow-fiber concentrator to 1/10th of the original volume. After dilution to the original volume, the sample was reconcentrated. This process was repeated until the concentrated sample had a conductivity of less than 4 kA/Vm, at which time hydroxylapatite was added at a rate of ~100 g/2 L of fluid. The pH was adjusted to 5.5, and the mixture was stirred overnight at 4°. After allowing the gel to settle, the major portion of the supernatant fluid was withdrawn, and the precipitate was collected by centrifugation at 4 000*g* for 5 min. The precipitate was then washed sequentially with 200-mL batches of 0.01M, 0.1M, 0.2M potassium phosphate buffer at pH 6.0, and then with 0.5M buffer at pH 6.8 and occasionally with M buffer at the same pH. The major portion of the activity was eluted in the 0.5M fraction, with lesser amounts in the 0.2M and M fractions. The active fractions were pooled, and dialyzed and concentrated on the hollow-fiber concentrator, until the conductivity of the concentrated sample was ~1 kA/Vm.

On DEAE-cellulose (step I, pH 6.0). The dialyzed sample was placed on a 40×2.5 cm column of DEAE-cellulose that had been equilibrated with 0.01M potassium phosphate (pH 6.0). The column was washed with 280 mL of the same buffer, and then batchwise with 0.05M NaCl (415 mL), 0.1M NaCl (480 mL), and 0.2M NaCl (330 mL) in 0.01M phosphate buffer at pH 6.0, 8-mL fractions being collected. The enzyme was eluted with 0.01M phosphate and 0.05M NaCl (see Fig. 1). The individual peaks were pooled, concentrated in a rotary evaporator under reduced pressure at a bath temperature of 25–30°, and dialyzed against distilled water.

Bio-Gel P-100. Each of the two enzyme fractions obtained in the DEAE-cellulose purification step were lyophilized, redissolved in a small volume (1–2 mL) and applied to a 51×1.1 cm column of Bio-Gel P-100. Elution was carried out with 0.01M KCl buffered with 0.01M potassium phosphate, pH 6.0, at a flow rate of approximately 10 mL/h. The elution profiles of enzyme activity and protein were similar for the two enzyme fractions separated on DEAE-cellulose (Fig. 2). The enzyme activities in both fractions were eluted from the columns at the void volumes.

DEAE-cellulose (step II, pH 6.8). The active fractions from each of the Bio-Gel P-100 columns were pooled and dialyzed against de-ionized water until the con-

ductivity was < 2 kA/Vm. The samples were then applied to a column (1.1×20 cm) DEAE-cellulose equilibrated with 0.01M potassium phosphate buffer, pH 6.8, and elution was carried out with a linear NaCl gradient (600 mL) up to 0.1M in the same buffer. Fractions were collected, and protein and enzyme activity estimated. The active fractions were pooled, as shown in Fig. 3.

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REFERENCES

1 H. W. SCHERP, *Science*, 173 (1971) 1199–1205.
2 M. PASCAL AND R. DEDONDER, *Carbohydr. Res.*, 24 (1972) 365–377.
3 R. DEDONDER, in R. PIRAS AND H. G. PONTIS (Eds.), *Biochemistry of the Glycosidic Linkage*, Academic Press, New York, 1972, pp. 21–78.
4 R. CHAMBERT, G. TREBOUL, AND R. DEDONDER, *Eur. J. Biochem.*, 41 (1974) 285–300.
5 E. NEWBRUN AND J. CARLSSON, *Arch. Oral Biol.*, 14 (1969) 461–468.
6 A. M. CHLUDZINSKI, G. R. GERMAINE, AND C. F. SCHACTELE, *J. Bacteriol.*, 118 (1974) 1–7.
7 J. E. CIARDI AND C. L. WITTENBERGER, *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, 33 (1974) 1435.
8 J. E. CIARDI, in W. H. BOWEN, R. J. GENCO, AND T. C. O'BRIEN (Eds.), *Immunologic Aspects of Dental Caries*, Information Retrieval, Washington, 1976, p. 101.
9 K. FUKUI, Y. FUKUI, AND M. TAKAFUMI, *J. Bacteriol.*, 118 (1974) 796–804.
10 M. KOBAYASHI AND K. MATSUDA, *Biochim. Biophys. Acta*, 370 (1974) 441–449.
11 H. KURAMITSU, *Infect. Immun.*, 10 (1974) 227–235.
12 M. NIINOBE AND T. KOBAYASHI, *Angew Chem.*, 46 (1972) 81–88.
13 W. R. SCALES, L. W. LONG, AND J. R. EDWARDS, *Carbohydr. Res.*, 42 (1975) 325–338.
14 M. KOBAYASHI AND K. MATSUDA, *J. Biochem. (Tokyo)*, 79 (1976) 1301–1308.
15 J. P. KLEIN, M. SCHOELLER, AND R. FRANK, *Biochimie*, 58 (1976) 1047–1056.
16 M. M. MCCABE AND E. E. SMITH, *Infect. Immun.* 16 (1977) 760–765.
17 P. ANDREWS, *Biochem. J.*, 96 (1965) 595–605.
18 P. R. BEVINGTON, *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill, New York, 1969, p. 237.
19 A. T. ARNETT AND R. M. MAYER, *Carbohydr. Res.*, 42 (1975) 339–345.
20 W. M. PASIKA AND L. H. CRAGG, *Can. J. Chem.*, 41 (1963) 293–299.
21 J. E. CIARDI, A. J. BEAMAN, AND C. L. WITTENBERGER, *Infect. Immun.*, 18 (1977) 237–246.
22 M. CESKA, B. GRANETH, B. NORRMAN, AND B. GUGGENHEIM, *Acta Chem. Scand., Ser. A*, 26 (1972) 2223–2230.
23 S. KRAUS, unpublished data.
24 J. ROBYT AND H. TANIGUCHI, *Arch. Biochem. Biophys.*, 174 (1976) 129–135.
25 F. A. BOVEY, *J. Polym. Sci.*, 35 (1959) 167–182.
26 J. LARNER, *J. Biol. Chem.*, 202 (1953) 491–502.
27 R. L. SIDEBOTHAM, *Adv. Carbohydr. Chem. Biochem.*, 30 (1974) 371–444.
28 J. CARLSSON, E. NEWBRUN, AND B. KRASSE, *Arch. Oral Biol.*, 14 (1969) 469–475.
29 G. L. MILLER, *Anal. Chem.*, 31 (1959) 964.
30 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375–380.
31 S. SEIFTER, S. DAYTON, B. NOVIC, AND E. MUNTWYLER, *Arch. Biochem.*, 25 (1960) 191–200.