SYNTHESIS OF OLIGOSACCHARIDES BY DEXTRANSUCRASE FROM A LOCAL STRAIN OF *STREPTOCOCCUS MUTANS*

EM-ON BENJAVONGKULCHAI,[a] RUDEE SURARIT,[b,c] CHRISTOPHER BUCKE [d] AND M. R. JISNUSON SVASTI[c,e]

[a] *Department of Biochemistry, Faculty of Dentistry, Chulalongkorn University, Henri Dunant St., Bangkok 10330, Thailand.*

[b] *Department of Physiology and Biochemistry, Faculty of Dentistry, Mahidol University, Yothi St., Bangkok 10400, Thailand.*

[c] *Laboratory of Biochemistry, Chulabhorn Research Institute, Vibhavadee Rangsit Road, Bangkok 10210, Thailand.*

[d] *School of Biological and Health Science, Faculty of Engineering and Science, University of Westminster, 115 New Cavendish St., London W1M 8JS, U.K.*

[e] *Department of Biochemistry, Faculty of Science, Mahidol University, Rama VI Road, Bangkok 10400, Thailand.*

(Received April 30, 1996)

ABSTRACT

Dextransucrase was partially purified 100-fold from the growth media of a local strain of Streptococcus mutans *TPF-1. Incubation of the enzyme with sucrose followed by analysis of products by high performance ion chromatography (HPIC) showed that the enzyme could synthesize oligosaccharides such as nigerose. The inhibitory effect of various sugars on the activity of dextransucrase has been studied, and the capabilities of these sugars to act as glucosyl acceptors were also tested by HPIC. The results showed that among twenty sugars tested, panose, maltose and isomaltose were inhibitors of dextransucrase activity. These sugars were also good glucosyl acceptors whereas lactose, palatinose were poor acceptors and galactosucrose, melibiose, raffinose, stachyose, turanose and xylitol could not act as acceptors.*

INTRODUCTION

Dextransucrase or glucosyltransferase (EC 2.4.1.5) catalyzes the transfer of glucose moiety of sucrose to form dextran according to the following scheme:

$$\underset{\textit{sucrose}}{nG\text{-}F} \xrightarrow{\textit{dextransucrase}} \underset{\textit{dextran}}{Gn} + \underset{\textit{fructose}}{nF}$$

The enzyme can be produced by bacteria, such as the soil bacteria *Leuconostoc,*[1] and the oral bacteria *Streptococcus*.[2] Dextransucrases from *Streptococcus mutans* and the dextrans which they produce have been shown to play an important role in the development of dental plaque.[3,4] It has been suggested that the dextrans in the plaque serve as a matrix for adhesion, colonization and accumulation of bacteria,[5] or may be used as nutrients for producing acids, which cause tooth demineralization.[6] In addition, dextrans can modulate the diffusion of substances such as fluoride, sucrose and acids through plaque.[7]

Dextrans synthesized by bacteria are composed mainly of an α-1,6-glucosidic backbone with a highly branched structure due to α-1,3 glucosidic linkages.[2,8,9] Traces of α-1,2- and α-1,4 linkages[2,9,10] may also be found. The degree of branching depends on the bacterial strains, and the differences in the properties of the dextransucrases produced. The dextransucrase enzyme is highly specific for sucrose as the glucosyl donor substrate,[11] but can catalyze the transfer of glucose residues to a variety of different acceptor molecules ranging from monosaccharides to polysaccharides.[12-14] Synthesis of dextran and other oligosaccharides has been mainly studied in *Leuconostoc* species with rather few studies in *Streptococcus* species. In this paper, we have partially purified dextransucrase from local Thai strain of *Streptococcus mutans*, and studied its capability for synthesizing oligosaccharides.

MATERIALS AND METHODS

Materials

A local Thai strain of *Streptococcus mutans* TPF-1 was obtained from Professor D. Bratthall, Lund University, Sweden. Todd-Hewitt media was from Difco Laboratories, Detroit, MI, U.S.A. U[^{14}C]-sucrose was purchased from New England Nuclear, Wilmington, DE, U.S.A. Other chemicals were of reagent grade and were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Culture conditions

Streptococcus mutans TPF-1 was grown in two 2.5 L flasks containing Todd-Hewitt medium supplemented with 1% (w/v) sucrose and incubated for 15-18 h at 37 °C in a CO_2 chamber.

Enzyme purification

The cell suspension was centrifuged at 3,000 *g* for 15 min. The enzyme in the supernatant was precipitated with 75% saturated ammonium sulfate. The precipitate was redissolved in 0.1 M potassium phosphate buffer, pH 6.0 and applied to a Sepharose 6B-CL column (1.6 x 87 cm), equilibrated and eluted with the same buffer.

Enzyme assay

Dextransucrase activity was determined by a modification of the radioisotope methods.[15] The 0.2 ml reaction mixture contained 2.5 mM U[^{14}C]-sucrose (0.037 $\mu C_i/\mu$mol) in 0.1 M potassium phosphate buffer, pH 6.0 and was incubated at 37 °C for 30 min. The reaction was terminated by addition of 0.8 ml of cold ethanol and left at 4 °C. The precipitated polysaccharide was collected on a 2.4 cm glass-fibre filter and washed twice with 5 ml of cold ethanol. The filter paper was counted for radioactivity using toluene-2,5-diphenyloxazole-1,4-bis-2-(5-phenyloxazolyl)benzene scintillation fluid. One unit of dextransucrase is defined as the amount of enzyme required to incorporate 1 μmole of glucose into glucan polysaccharide per minute under standard assay conditions.

Protein assays

The protein content of solutions was measured by the method of Bradford[16] with bovine serum albumin as standard. The protein content of column effluents was followed by measurement of A_{280}.

Effect of sugars on enzyme activity

Partially purified enzyme (4 mU) was assayed with U[^{14}C]-sucrose as described, but in the presence and absence of 0.1% (w/v) of tested sugar.

Time course study

Partially purified dextransucrase (36 mU) was incubated at 37 °C with 4% (w/v) sucrose in 0.1 M potassium phosphate buffer, pH 6.0 for 100, 290 and 480 min. Synthesized oligosaccharide products were analyzed on a Dionex High Performance Ion Chromatograph (HPIC) equipped with a pulsed amperometric detector. Products were separated on a Dionex CarboPac PA-1 pellicular anion exchange column, eluted with a linear gradient of 0.1 M NaOH, and 0.05 M NaOH plus 0.2 M sodium acetate.

Test of capability of sugars to act as substrates and glucosyl acceptors

Partially purified enzyme (5 mU) was incubated in 0.1 M potassium phosphate buffer, pH 6.0 at 37 °C with 0.2% (w/v) of the tested sugars (e.g., maltose, isomaltose, panose, lactose, palatinose, galactosucrose, melibiose, raffinose, stachyose, turanose and xylitol) in the presence or absence of 0.2% (w/v) sucrose. Reaction products were analyzed by Dionex HPIC as described above.

RESULTS AND DISCUSSION

Most studies on dextransucrase have been carried out on enzyme isolated from *Leuconostoc* species. However, the present studies have been performed on dextransucrase from the oral bacteria *Streptococcus mutans* TPF-1, because of our interest in this enzyme in terms of its role in dental plaque formation. Moreover, we have used an isolate from a local Thai strain of this bacteria, since detailed studies on its enzymology have not been performed. Accordingly, dextransucrase from the crude extract of *S. mutans* TPF-1 were partially purified by precipitation with 75% saturated ammonium sulfate, followed by Sepharose 6B-CL chromatography fractionation. As can be seen from the column elution profile (Fig. 1), the major peak of dextransucrase enzyme had a high molecular weight, and can be separated from many contaminating proteins by this gel

filtration step. The two purification steps combined (Table 1) gave more than 100-fold purification with a very good yield of over 40%. The enzyme is still not pure at this stage, but was considered sufficiently pure for studies of oligosaccharide synthesizing activity, since further purification inevitably lowers the yield, which will decrease the potential for scaling up the synthesis reaction.

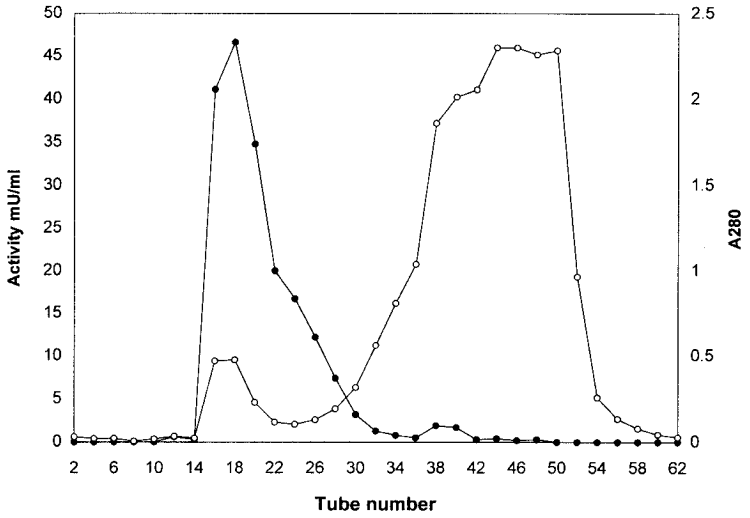

Fig. 1 Gel filtration of 75% ammonium sulphate fraction of *S. mutans* on a Sepharose 6B-CL column equilibrated with 0.1 M potassium phosphate buffer, pH 6.0. ●—● dextransucrase activity (mU/ml); ○—○ A_{280}.

TABLE 1 Partial purification of dextransucrase from *S. mutans* TPF-1

Fraction	Total activity (mU)	Total protein (mg)	Specific activity (mU/mg)	Purification fold	Yield (%)
Culture media	4,953	750.6	6.6	1	100
75% Ammonium sulfate	2,561	137.1	18.7	2.8	51.7
Sepharose 6B-CL column	2,106	3.1	679.3	102.9	42.5

The partially purified dextransucrase showed optimum pH at pH 6.0 and optimum temperature at 40 °C. The enzyme could be stored at 4 °C and at -20 °C, but lost activity when lyophilized. Our preliminary studies show that this enzyme did not require the exogenous dextran acceptor for detection of activity. From the inhibition studies, isomaltose, maltose and panose were found to effectively inhibit about 40-70% of enzyme activity, while other sugars had little or no effect (Table 2). These results were similar to the inhibition of glucan synthesis by dextransucrase from different strains of *S. mutans* observed by others.[17-19] However, the effect of panose (6-α-glucosylmaltose) on dextransucrase activity has never been tested by other workers, while our studies showed that panose was the most effective inhibitor of the carbohydrates tested. Conversely, palatinose has been found to inhibit dextransucrase from other strains of *S. mutans*,[20,21] but this compound did not appear to inhibit dextransucrase from the local Thai strain. It should also be noted that the concentrations of carbohydrates used in this study (0.1% w/v or 2-6 mM) were lower than those reported in earlier studies (11-100 mM), so the inhibition observed here by panose is significant. Moreover, our data correlate with the inhibitory effect of these sugars on adhesion of *S. mutans* to glass, where panose showed strongest inhibition, followed by maltose and isomaltose.[22]

TABLE 2 Effect of some sugars and derivatives on dextransucrase activity

Compounds	Activity (%)	Structure*
Sucrose	100.0	glu(α1-2)fru
Cellobiose	86.6	glu(β1-4)glu
Fructose	78.9	
Galactosucrose	98.9	gal(α1-2)fru
Glucose	88.8	
Inositol	86.7	
Isomaltose	53.9	glu(α1-6)glu
Lactose	88.6	gal(β1-4)glu
Maltitol	90.3	
Maltose	51.5	glu(α1-4)glu
Maltotriose	74.4	glu(α1-4)glu(α1-4)glu
Mannitol	88.1	
Melibiose	87.2	gal(α1-6)glu
Nigerose	85.6	glu(α1-3)glu
Palatinose	97.2	glu(α1-6)fru
Panose	29.2	glu(α1-6)glu(α1-4)glu
Raffinose	89.8	gal(α1-6)glu(α1-2)fru
Sorbitol	96.3	
Stachyose	94.4	gal(α1-6)gal(α1-6)glu(α1-2)fru
Turanose	90.4	glu(α1-3)fru
Xylitol	96.4	

*Structures are shown for di-, tri- and tetrasaccharides, where gal = galactose, glu = glucose, fru = fructose

When partially purified dextransucrase was incubated with 4% (w/v) sucrose at 37 °C for various times (Fig. 2, Table 3), sucrose was gradually broken down to fructose and glucose. In addition, synthesis products arising from transfer reactions also appear with time, including the major products nigerose (gluα1-3glu) and an unknown product (U). In addition, other minor products were also detectable (data not shown). Interestingly, glucan polymers containing α1-3 linkages are known to have low solubility and to play an important role in the adhesion of *S. mutans* to teeth.

TABLE 3 Relative amount of compounds after incubation of *S. mutans* dextransucrase with sucrose for various times

Compound	% Compounds at		
	100 min	290 min	480 min
Glucose	6.86	13.4	15.3
Fructose	14.6	23.1	29.0
Sucrose	77.7	60.3	51.3
Nigerose	0.76	1.69	1.65
Unknown	–	1.12	2.33

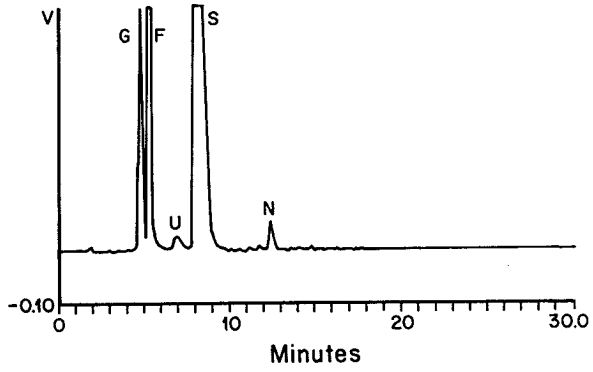

Fig. 2 HPIC of synthetic products obtained by incubating dextransucrase with 4% (w/v) sucrose in 0.1 M potassium phosphate buffer, pH 6.0 for 480 min. Reaction products were analyzed on a Dionex CarboPac PA-1 column, eluted with a linear gradient of 0.1 M NaOH and 0.05 M NaOH + 0.2 M sodium acetate. S = sucrose; G = glucose; F = fructose; N = nigerose; U = unknown peak. Samples were diluted 50-fold.

When dextransucrase was incubated with the above disaccharides and trisaccharides in the absence of sucrose, no peaks of synthesis products were detectable by HPIC, indicating that none of them could be used as substrates for the enzyme. This result suggests that our dextransucrase enzyme is also highly specific to sucrose as found earlier.[11] On the other hand, when enzyme was incubated with isomaltose, maltose, panose, palatinose or lactose in the presence of sucrose, new products were detectable by HPIC (Fig. 3), indicating that these sugars could act as glucosyl acceptors. However, transfer products could not be detected when galactosucrose, melibiose, raffinose, stachyose, turanose or xylitol were incubated with enzyme in the presence of sucrose (Fig. 4).

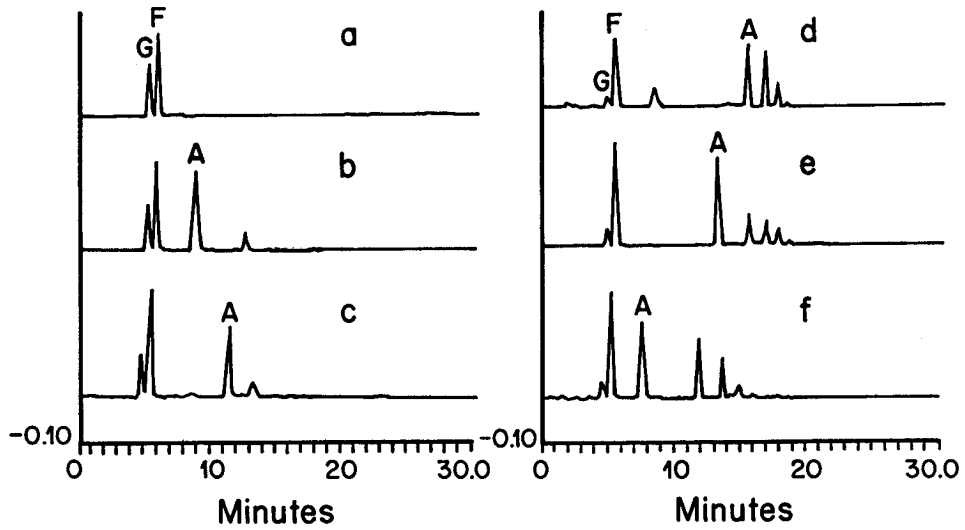

Fig. 3 HPIC of synthetic products obtained by incubating dextransucrase with tested sugars, followed by analysis on a Dionex CarboPac PA-1 column, as described in Fig. 2. G = glucose; F = fructose; A = acceptor. *a.* sucrose only; *b.* sucrose + lactose; *c.* sucrose + palatinose; *d.* sucrose + panose; *e.* sucrose + maltose; *f.* sucrose + isomaltose. Samples were diluted 40-fold.

The finding that isomaltose, maltose and panose not only acted as inhibitors of dextransucrase, but were also good glucosyl acceptors indicates that the inhibition of glucan synthesis may be due to the acceptor action of these carbohydrates.[19,23] Interestingly, one of the products obtained in the transfer reaction when maltose was used as an acceptor appears to be panose, as earlier suggested by paper chromatographic techniques.[24] This would indicate that the glucosyl group of sucrose appears to have been transferred to C_6 of maltose. These observations are of interest since inhibition of bacterial adhesion to teeth offers a possible approach to the prevention of dental caries, and may be promoted by compounds that inhibit dextransucrase activity or that give products which show lowered capability for adhesion. Moreover, the present studies

indicate that dextransucrase from a local strain of *S. mutans* may potentially be used for synthesis of oligosaccharides.

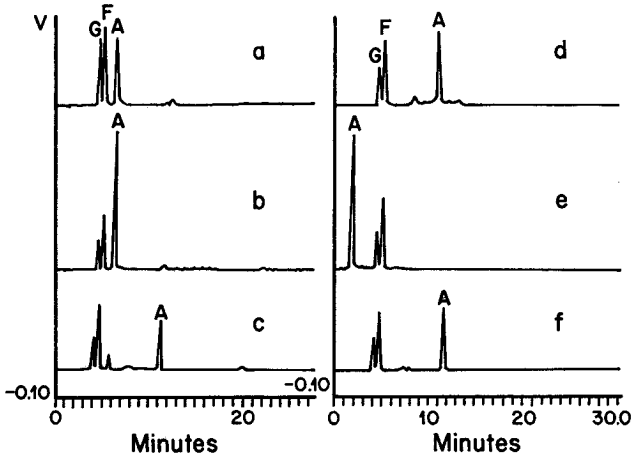

Fig. 4 HPIC of products obtained by incubating dextransucrase with tested sugars, followed by analysis on a Dionex CarboPac PA-1 column, as described in Fig. 2. G = glucose; F = fructose; A = acceptor. *a*. sucrose + galactosucrose; *b*.sucrose + melibiose; *c*. sucrose + raffinose; *d*. sucrose + turanose; *e*. sucrose + xylitol; *f*. sucrose + stachyose. Samples were diluted 40-fold.

ACKNOWLEDGEMENTS

This study was supported by the Chulabhorn Research Institute and the Thai-UK Biotechnology Programme. The technical assistance of Miss Sirinun Nilwarangkoon is appreciated.

REFERENCES

1. Hehre, E. J. (1951) ***Adv. Enzymol.*** **11**, 297.
2. Montville, T. J., Cooney, C. L. and Sinskey, A. J. (1978) ***Adv. Appl. Microbiol.*** **24**, 55.
3. Hamada, S. and Slade, H. D. (1980) ***Microbiol. Rev.*** **44**, 331.
4. Rölla, G., Scheie, A. A. and Ciardi, J. E. (1985) ***Scand. J. Dent. Res.*** **93**, 105.
5. Gibbons, R. J., in ***Glucosyltransferases, Glucans, Sucrose and Dental Caries***, Doyle, R. J. and Ciardi, J. E., eds. (1983), pp. 11, IRL Press, Oxford.
6. Van Houte, J., Russo, J. and Prostak, K. S. (1989) ***J. Dent. Res.*** **68**, 451.
7. McNee, S. G., Geddes, D. A. M., Weetman, D. A., Sweeney, D. and Beeley, J. A. (1982) ***Arch. Oral Biol.*** **27**, 981.
8. Robyt, J. F. and Taniguchi, H. (1976) ***Arch. Biochem. Biophys.*** **174**, 129.
9. Jeanes, A., Haynes, W. C., Wilham, C. A., Rankin, J. C., Melvin, E. H., Austin, M. J., Cluskey, J. E., Fisher, B. E., Tsuchiya, H. M. and Rist, C. E. (1954) ***J. Am. Chem. Soc.*** **76**, 5041.
10. Monsan, P. and Paul, F. (1995) ***FEMS. Microbiol. Rev.*** **16**, 187.
11. Grier, T. J. and Mayer, R. M. (1981) ***Arch. Biochem. Biophys.*** **212**, 651.
12. Robyt, J. F. and Eklund, S. H. (1983) ***Carbohydr. Res.*** **121**, 279.
13. Mayer, R. M., Matthews, M. M., Futerman, C. L., Parnaik, V. K. and Jung, S. M. (1981) ***Arch. Biochem. Biophys.*** **208**, 278.
14. Bhattacharjee, M. K. and Mayer, R. M. (1993) ***Carbohydr. Res.*** **242**, 191.
15. Kuramitsu, H. K. (1975) ***Infect. Immun.*** **12**, 738.
16. Bradford, M. M. (1976) ***Anal. Biochem.*** **72**, 248.
17. Sharma, M., Holbrook, S. and Newbrun, E. (1976) ***J. Dent. Res.*** **54A**, 110.
18. Chludzinski, A. M., Germaine, G. R. and Schachtele, C. F. (1976) ***J. Dent. Res.*** **55**, c75.
19. Nisizawa, T., Imai, S., Takeuchi, K., Shibata, K., Araya, S., Yoshikawa, S. and Okada, S. (1980) ***J. Dent. Res.*** **59B**, Abstr.319.
20. Kobayashi, M., Funane, K. and Oguma, T. (1995) ***Biosci. Biotech. Biochem.*** **59**, 1861.
21. Minami, T, Fujiwara, T., Ooshima, T., Nakajima, Y. and Hamada, S. (1990) ***Oral. Microbiol. Immunol.*** **5**, 189.
22. Koga, T., Horikoshi, T., Fujiwara, T. and Hamada, S. (1988) ***Microbiol. Immunol.*** **32**, 25.
23. Koepsell, H. J., Tsuchiya, H. M., Hellman, N. N., Kazenko, A., Hoffman, C. A., Sharpe, E. S. and Jackson, R. W. (1953) ***J. Biol. Chem.*** **200**, 793.
24. Fukui, K. and Moriyama, T. (1983) ***Microbiol. Immunol.*** **27**, 917.