Carbohydrate Research, 158 (1986) 147–155
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

PURIFICATION AND CHARACTERISATION OF THE EXTRACELLULAR D-GLUCOSYLTRANSFERASE FROM SEROTYPE *c Streptococcus mutans*

TADASHI BABA*, TOMONARI OGAWA, NOBUO OKAHASHI, TSUYOSHI YAKUSHIJI**, TOSHIHIKO KOGA, MOTOI MORIMOTO***, AND SHIGEYUKI HAMADA

Department of Dental Research, National Institute of Health, Tokyo 141 (Japan)

(Received May 28th, 1986; accepted for publication, August 6th, 1986)

ABSTRACT

A simple method of purification for the extracellular D-glucosyltransferase (GTase) from a serotype *c* strain *Streptococcus mutans* was developed using chromatography on DEAE-Sephacel and CM-cellulose. The GTase had a molecular weight of 155,000 and an isoelectric point of 7.4. The enzyme converted sucrose, in the absence of dextran T-10, into a branched (1→6)-linked α-D-glucan having some α-(1→3)-linked D-glucosyl residues. The GTase was similar to GTases which have been isolated from other strains of serotype *c S. mutans* and which synthesise water-soluble glucans. In addition, the amino acid composition of the GTase protein was relatively similar to those of the GTases from serotype *g S. mutans* which synthesise water-soluble and water-insoluble glucans.

INTRODUCTION

Streptococcus mutans produces an extracellular D-glucosyltransferase (GTase, EC 2.4.1.5) which catalyses the formation of water-soluble (SG) and -insoluble (IG) α-D-glucans by the transfer of the D-glucosyl moiety from sucrose[1]. These glucans are important in the adherence and colonisation of *S. mutans* and the subsequent developement of dental caries in animals and humans[2–4].

S. mutans strains are divided into two sub-groups in terms of their ability to produce glucan[5,6]. Both SG and IG are synthesised by GTases from strains of serotypes *a*, *d*, and *g*, whereas those from strains of serotypes *b*, *c*, *e*, and *f* mainly produce SG. The enzymes from the former serotypes have been purified and characterised[7–10], but there are few reports on the purification of GTases from the latter serotypes of *S. mutans*.

Strains of *S. mutans* serotype *c* have been isolated most frequently from

*Present address: Institute of Applied Biochemistry, University of Tsukuba, Ibaraki 305, Japan.
**Present address: Department of Preventive Dentistry, Kagoshima University Dental School, Kagoshima 890, Japan.
***Present address: Department of Dental Public Health, Nihon University School of Dentistry at Matsudo, Chiba 271, Japan.

0008-6215/86/$ 03.50

human dental plaque[11], and it is important to characterise their GTases. The isolation of GTase is difficult because of its tendency to aggregate and because of contamination with D-fructosyltransferase (FTase, EC 2.4.1.9). Recently, chromatofocusing has been employed[6,12,13] for the purification of GTase from the serotype *c* strains. We now report a simple method of purification for the serotype *c* GTase that synthesises SG.

MATERIALS AND METHODS

Materials. — *S. mutans* MT8148, a typical strain of serotype *c*, was selected from the culture collection in the Department of Dental Research, The National Institute of Health, Tokyo. [U-^{14}C-Glucose]sucrose and [U-^{14}C-fructose]sucrose with specific activities of 0.31 and 0.32 mCi/mol, respectively, were purchased from New England Nuclear, and diluted with unlabelled sucrose before use. DEAE-Sephacel and CM-cellulose (CM-52) were products of Pharmacia Fine Chemicals and Whatman Chemical Separations Ltd., respectively. Brain heart infusion (BHI) was obtained from Difco Laboratories.

Preparation of crude enzyme. — *S. mutans* MT8148 was cultured for 18 h at 37° in a dialysate medium of BHI broth[14]. The organisms were collected by centrifugation at 9,000*g* for 10 min at 4°. Solid $(NH_4)_2SO_4$ was added to the centrifuged supernatant solution (5 L) to give 60% saturation. The mixture was stirred for 3 h and then centrifuged at 9,000*g* for 20 min. A solution of the pellet in a small volume of 50mM potassium phosphate buffer (pH 7.5) was extensively dialysed against the same buffer and then centrifuged at 3,000*g* for 10 min, and the supernatant fluid was used as the crude enzyme solution.

Purification of D-*glucosyltransferase.* — All purification procedures were carried out at 0–4°. Crude enzyme solution (50 mL) was applied to a column (2.7 × 8 cm) of DEAE-Sephacel previously equilibrated with 50mM potassium phosphate buffer (pH 7.5). The column was washed with the equilibrating buffer (250 mL), and proteins were eluted with a linear gradient of 0→M NaCl in the same buffer (400 mL of each). Fractions (15 mL) were collected at a flow rate of 50 mL/h. The GTase-containing fractions were combined, brought to 70% saturation with solid $(NH_4)_2SO_4$, and stirred overnight. After centrifugation at 35,000*g* for 20 min, the precipitate was dissolved with 25mM acetate buffer (pH 5.0) and the solution dialysed overnight against the same buffer (2 L). The dialysate was loaded onto a column (1.8 × 8 cm) of CM-cellulose which had been equilibrated with 25mM acetate buffer (pH 5.0). GTase was eluted at 35 mL/h with a linear gradient of 0→0.2M NaCl in the same buffer (200 mL of each). The fractions containing GTase activity were combined and dialysed against two changes of the same buffer (4 L). The final purification of GTase was accomplished by repeated chromatography on a CM-cellulose column (1.6 × 5 cm) with 25mM acetate buffer (pH 5.0) and a 0→0.15M NaCl gradient (100 mL of each). The fractions with GTase activity were combined, concentrated by a Mini-Module NM-3 ultrafiltration system (Asahi

Kasei Co. Ltd., Tokyo), and dialysed against 25mM potassium phosphate buffer (pH 7.0). The dialysed solution was used as a purified preparation of GTase.

Enzyme assays. — Assay procedures for measurements of GTase and FTase activities were essentially those reported[15], in which [U-^{14}C-glucose]sucrose and [U-^{14}C-fructose]sucrose were used as substrates, respectively. Each reaction mixture consisted of 0.1M potassium phosphate buffer (pH 6.0), 10mM [^{14}C]-labelled sucrose (70,000 c.p.m.), 0.02% of dextran T-10 (Pharmacia), and an appropriate amount of enzyme in a total volume of 20 μL. For measurement of FTase activity, the dextran T-10 was omitted. Each mixture was incubated for 30 min at 37°, spotted onto a piece of filter paper (1 × 2 cm; Toyo Roshi No. 514), and then soaked in methanol. After washing the paper with methanol, the incorporation of [^{14}C]glucose or [^{14}C]fructose into the methanol-insoluble material was measured with an Aloka liquid-scintillation counter. One unit (U) of GTase and FTase was defined as the amount of enzyme that transformed 1 μmol of [^{14}C-glucose]sucrose or [^{14}C-fructose]sucrose per min, respectively, into the methanol-insoluble material under the above conditions.

Electrophoresis. — Disc-PAGE (polyacrylamide gel electrophoresis) was carried out as described by Reisfeld *et al.*[16]. The purified enzyme (5 μg) was applied to a 6% polyacrylamide gel at pH 4.3. After electrophoresis at 2 mA/tube at 4°, the protein band was developed with Coomassie Brilliant Blue R-250 (Sigma). The band of enzyme activity was detected by incubating the gel for 24 h at 37° with 50mM potassium phosphate buffer (pH 6.0) containing 5% of sucrose and 0.02% of NaN_3, followed by staining with the periodic acid–Schiff base reagent[17]. SDS-PAGE of proteins was performed on a 7.5% polyacrylamide gel as described by Laemmli[18]. The purified GTase (8.4 μg) was reacted at 37° for 30 min in 1% of SDS (sodium dodecyl sulphate), 1% of 2-mercaptoethanol, 10mM Tris-HCl buffer (pH 6.8), and 20% of glycerol in a total volume of 0.2 mL. An aliquot (50 μL) of the mixture was then placed on each lane. After electrophoresis at 10 mA for 15 h at 4°, the bands of protein and enzyme activity were detected as described above. Ferritin (220k), phosphorylase b (94k), bovine serum albumin (67k), catalase (60k), ovalbumin (43k), and lactate dehydrogenase (36k) were used as the molecular weight standards.

Analytical procedures. — Protein was determined by the method of Hartree[19], using bovine serum albumin as the standard.

For determination of the amino acid composition, the enzyme protein was hydrolysed with 6M hydrochloric acid containing 4% of thioglycolic acid at 110° for 24, 48, 72, and 96 h in evacuated sealed tubes. The amino acids in the hydrolysates were analysed by using a Shimadzu liquid chromatograph LC-3A fitted with a column (4 mm × 15 cm) of Shim-pack ISC-07/S1504 (Shimadzu). Cysteine was determined[20] from the amount of cysteic acid in performic acid-oxidised protein.

Methylation analysis of polysaccharides formed by purified GTase was performed according to the method of Lindberg[21], as described previously[5]. Alditol acetate derivatives of partially methylated D-glucose were analysed by gas–liquid chromatography with a GC-4CPF instrument (Shimadzu).

RESULTS AND DISCUSSION

Fig. 1 shows that most GTase activity passed through the DEAE-Sephacel column. A small amount of GTase was also eluted with 0.3M NaCl together with FTase activity. The non-adsorbed fractions of GTase were combined and subjected to column chromatography on CM-cellulose. As shown in Fig. 2, a major peak of GTase activity was observed at the 0.1M NaCl eluate, and small peaks of enzyme activity were found in the eluate of 0.05 and 0.13M NaCl. In the present experiment, only GTase-containing fractions from the major peak were further purified by repeated column chromatography on CM-cellulose (Fig. 3). The fractions with GTase activity were combined, concentrated, and dialysed against 25mM potassium phosphate buffer (pH 7.0). The dialysate was used as a purified GTase. When 10 μL of the purified preparation with 2.21 U of GTase/mL was incubated with [^{14}C-

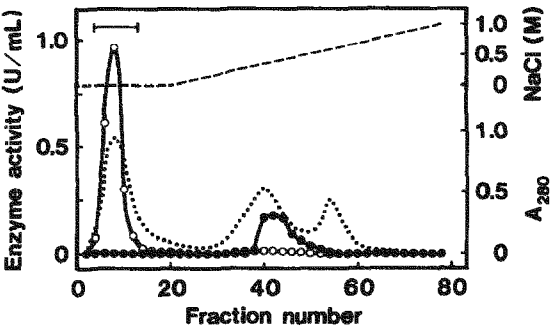

Fig. 1. Chromatography of crude GTase on a column (2.7 × 8 cm) of DEAE-Sephacel. Crude enzyme solution (50 mL) from culture liquor (5 L) was applied to the column equilibrated with 50mM potassium phosphate buffer (pH 7.5). Proteins were eluted with a linear gradient of 0→M NaCl (----). Fractions (15 mL) were collected at 50 mL/h and assayed for GTase (○) and FTase (●). Protein (····) was followed by A_{280}.

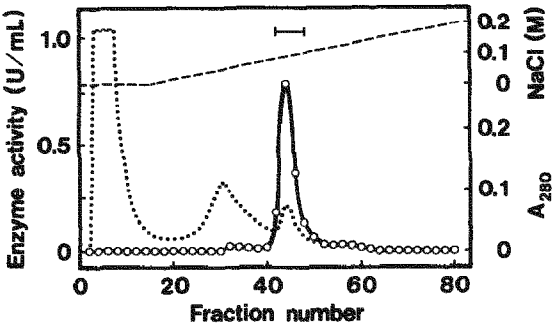

Fig. 2. Chromatography of GTase on a column (1.8 × 8 cm) of CM-cellulose. The enzyme solution from Fig. 1 was loaded on the column equilibrated with 25mM acetate buffer (pH 5.0) and eluted at 35 mL/h with a linear gradient (----) of 0→0.2M NaCl. GTase activity (○) was measured in each fraction (6 mL). Protein (····) was followed by A_{280}.

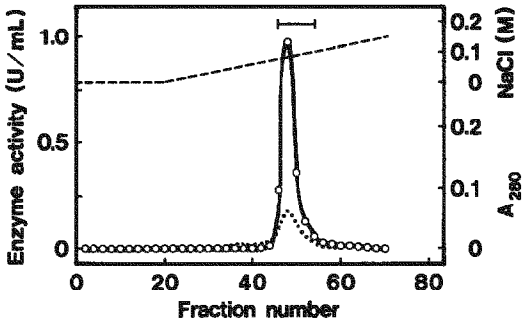

Fig. 3. Re-chromatography of GTase on a column (1.6 × 5 cm) of CM-cellulose. GTase fractions from Fig. 2 were loaded on the column equilibrated with 25mM acetate buffer (pH 5.0) and eluted at 35 mL/h with a linear gradient (----) of 0→0.15M NaCl. GTase activity (○) was measured in each fraction (4 mL). Protein (····) was followed by A_{280}.

fructose]sucrose under the standard assay conditions, no incorporation of [^{14}C]fructose into methanol-insoluble material was found. This result demonstrates the absence of FTase activity in the final GTase preparation. There was no significant loss of GTase activity during storage at −20° for at least 6 months.

Table I shows the result of a typical purification. The present purification procedure achieved a 27% yield of GTase and ~66-fold purification from the crude enzyme solution. When the purification of GTase from the same crude preparation was performed by the chromatofocusing method, using a column of Polybuffer exchanger PBE94 (Pharmacia), followed by column chromatography on hydroxyapatite, as described previously[6,9], the specific activity of GTase increased to 1.75 U/mg of protein (7.8 U/mg of protein in ref. 6) with a 4% yield. The DEAE- and CM-cellulose ion-exchangers are relatively inexpensive, and can be easily regenerated and used repeatedly. In addition, the present procedure does not involve the addition of Triton X-100[13] or SDS[22], the removal of which can be difficult.

Fig. 4 shows that the purified GTase was detected as a single protein band on disc-PAGE (pH 4.3). The electrophoretic mobility of the protein band coincided with that of the enzyme activity. Five separate experiments on SDS-PAGE of the purified enzyme revealed a single protein band with a molecular weight of 155,000 ±2,000 (Fig. 5). The same result was obtained in SDS-PAGE after heating the

TABLE I

PURIFICATION OF EXTRACELLULAR D-GLUCOSYLTRANSFERASE FROM SEROTYPE *c Streptococcus mutans*

Purification step	*Total protein (mg)*	*Total activity (U)*	*Specific activity (U/mg of protein)*	*Recovery (%)*	*Purification factor*
Crude[a]	213	56.3	0.27	100	1
DEAE-Sephacel	86.1	28.1	0.33	50	1.2
1st CM-cellulose	2.66	17.0	6.39	30	23.7
2nd CM-cellulose	0.84	15.0	17.9	27	66.3

[a]Ammonium sulfate fraction (0–60% saturation) from culture supernatant of *S. mutans* MT8148 (5 L).

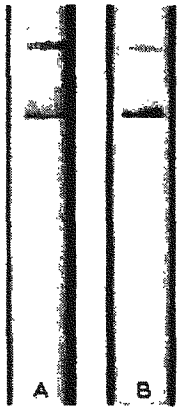

Fig. 4. Disc-PAGE of purified GTase. The purified enzyme (5 μg) was applied to a 6% polyacrylamide gel at pH 4.3. The bands of protein (A) and enzyme activity (B) were detected as described in the Materials and Methods.

sample either at 100° for 5 min, or at 37° for 30 min. When the enzyme was treated at 100° for 5 min without 2-mercaptoethanol, SDS-PAGE gave the same molecular weight. These findings indicate that the purified GTase is a monomeric protein. The GTase purified by the chromatofocusing method also gave a protein or an activity band in SDS-PAGE, the position of which was identical to that of the GTase isolated by the present procedure. Furthermore, the activity of the two

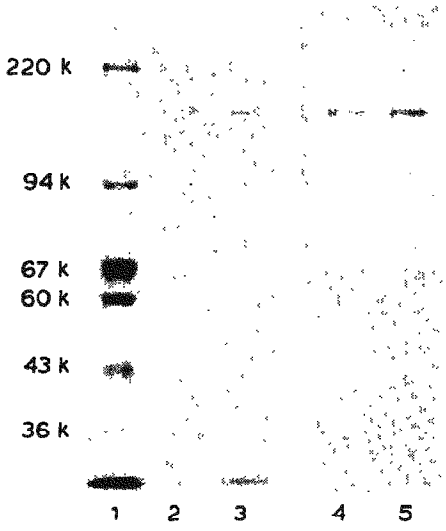

Fig. 5. SDS-PAGE of GTase on 7.5% slab gel. The bands of protein (lanes 2 and 3) and enzyme activity (lanes 4 and 5) were detected as described in the Materials and Methods. Lane 1, standard proteins; lanes 2 and 4, 2.1 μg of GTase purified by the present procedure; lanes 3 and 5, 27.5 μg of GTase with specific activity of 0.33 U/mg of protein obtained[6] by chromatofocusing on a column of PBE94.

GTases was enhanced 2- to 4-fold by addition of dextran T-10. Only SG was synthesised from sucrose in the absence of the dextran. These results confirmed the identity of the GTases.

In some experiments, *S. mutans* MT8148 was cultured in a chemically defined medium of Terleckyj *et al.*[23] with or without 0.1mM phenylmethylsulfonyl fluoride[24,25]. After centrifugation, the cell-free culture supernatant was subjected to SDS-PAGE and then incubated with sucrose. The bands possessing FTase activity were detected in positions of molecular weight of 95,000 and below. It was also found that the 155k band synthesising SG was clearly detected (results not shown). Therefore, it is considered that GTase obtained by the present method is not modified during cultivation of the organism due to the possible presence of proteases.

As shown in Table II, the purified GTase from *S. mutans* MT8148 was characterised, and its properties were compared with those of GTases prepared from other serotype *c* strains[6,12,13,26]. *S. mutans* MT8148 GTase was relatively sensitive in the temperature range between 40 and 50°. Although the GTase activity was not affected by heating at 40° for 30 min, the enzyme was almost inactivated by incuba-

TABLE II

CHARACTERISATION OF EXTRACELLULAR D-GLUCOSYLTRANSFERASES FROM SEROTYPE *c Streptococcus mutans*

Property	*Serotype* c *strain of* Streptococcus mutans[a]			
	MT8148	*GS-5*	*Ingbritt*	*Ingbritt*
Molecular weight	155,000	45,000 (140,000)[b]	151,000	158,000 and ~173,000
Isoelectric point (pI)	7.4	6.2 (7.5)[b]	8.1–8.4	7.4
Temperature optimum (degrees)	~37	37	n.d.[c]	n.d.
pH optimum	5.5–6.5	6.0	6.5	5.5–6.0
Thermal stability	40° for 30 min	n.d.	n.d.	n.d.
K_m value for sucrose (mM)	17.3	2.1	17.2	11.0
Inhibition	SDS, urea, $HgCl_2$, guanidine-HCl	n.d.	n.d.	n.d.
Activation by dextran T-10 (fold)	2–4	~12	2.4	~10
Effect of concentrated $(NH_4)_2SO_4$	+[d]	+	n.d.	n.d.
Product from sucrose	Water-soluble α-1,3- 8.3% α-1,6- 53.6% α-1,3,6- 7.9% terminal 30.3%	Water-soluble α-1,3- 30.7% α-1,6- 69.3%	Water-soluble α-1,6- with 17.7% of α-1,3,6- branching structure	Water-soluble α-1,3- 10.6% α-1,6- 41.3% α-1,3,6- 6.2% α-1,3,4- 14% terminal 21.3%
Reference	Present study	ref. 26	ref. 13	ref. 6

[a]Culture media of *Streptococcus mutans* are as follows: MT8148, dialysed BHI; GS-5, 1% glucose–0.05% dextran T-10-Todd-Hewitt (chemically defined medium[23] for ref. 12); Ingbritt[13], minimal defined medium of Fujiwara *et al.*[27]; Ingbritt[6], dialysed BHI. [b]Data are cited from ref. 12. [c]Not determined. [d]Formation of an insoluble glucan.

tion at 50° for 30 min. There was no significant inhibition and activation of the GTase by various metal ions (Li^+, Zn^{2+}, Ba^{2+}, Co^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}, and Fe^{3+}; 5mM each), EDTA (5mM), Tween 80 (1%), and Triton X-100 (1%). However, the enzyme activity was markedly inhibited by $HgCl_2$ (5mM), guanidine hydrochloride (1.25M), SDS (mM), and urea (2M), As described by Kuramitsu and Wondrack[12], addition of high concentrations of $(NH_4)_2SO_4$ to our GTase led to the formation of insoluble glucan. The maximum formation occurred at 1.0–1.5M $(NH_4)_2SO_4$. The other properties of our purified GTase were consistent with those of GTases from *S. mutans* GS-5 and Ingbritt. Only SG was synthesised from sucrose by the present GTase, and was found to consist of α-1,6- (54%), α-1,3- (8%), and α-1,3,6-linked (8%) D-glucosyl residues. These results confirm that this GTase is essentially an SG-synthesising enzyme (GTase-S) that forms a branched (1→6)-linked α-D-glucan with α-(1→3)-linked D-glucosyl residues. However, crude enzymes of *S. mutans* MT8148 produced a small proportion of IG [consisting mainly of α-(1→3)-linked D-glucosyl residues] from sucrose, as well as SG. An IG-synthesising enzyme (GTase-I) may be removed by the column chromatography in the present experiment.

Table III demonstrates that the amino acid composition of purified GTase is similar to those of GTase-S and GTase-I from *S. mutans* 6715 (serotype *g*)[14]. The amounts of Glx and Asx (25.8, 22.8, and 21.1%) for the serotype *c* GTase, the

TABLE III

AMINO ACID COMPOSITION OF D-GLUCOSYLTRANSFERASE FROM SEROTYPE *c Streptococcus mutans*

Amino acid	*Composition (mol%)*		
	Serotype c *GTase*	*Serotype* g *GTase-S*[a]	*Serotype* g *GTase-I*[b]
Cys	2.6 (39)[c]	0.3	0.2
Asx[d]	12.0 (182)	10.1	9.0
Thr	5.1 (77)	5.8	5.4
Ser	9.4 (142)	12.5	14.7
Glx[d]	13.8 (209)	12.7	12.1
Pro	2.4 (36)	4.7	4.1
Gly	18.7 (283)	13.6	15.2
Ala	8.3 (126)	11.6	8.8
Val	3.8 (57)	4.0	3.8
Met	1.1 (16)	1.0	0.9
Ile	2.9 (44)	2.7	2.3
Leu	4.6 (70)	4.3	4.4
Phe	2.1 (32)	2.5	2.8
Try	3.2 (49)	3.2	2.7
His	2.2 (34)	1.9	2.4
Lys	4.6 (69)	7.0	8.7
Trp	0.3 (5)	0.1	0.2
Arg	2.9 (44)	2.1	2.3

[a]Water-soluble-glucan-synthesising D-glucosyltransferase from *S. mutans* 6715 (serotype *g*). The data are cited from ref. 14. [b]Water-insoluble-glucan-synthesising D-glucosyltransferase from *S. mutans* 6715 (serotype *g*). The data are cited from ref. 14. [c]Residues per molecule. The calculations were based on a molecular weight of 155,000. [d]Asx, aspartic acid or asparagine; Glx, glutamic acid or glutamine.

serotype *g* GTase-S, and the serotype *g* GTase-I were greater than those of the basic amino acids His, Lys, and Arg (9.7, 11.0, and 13.4%, respectively). This result explains the low pI values (4.3 and 4.9) of the GTase-S and GTase-I from *S. mutans* 6715 (*g*) because of the large proportion of the acidic amino acids. Shimamura *et al.*[8] also found a preponderance of acidic amino acids for GTase-S, with a pI of 4.1, from the same serotype *g* strain. However, our purified GTase had a pI of 7.4 (Table II). The reason for the basic pI value of the serotype *c* GTase may be due to the possibility that some of the glutamyl and aspartyl groups in the native protein are present as amides.

The chromatofocusing method has been established in the purification of GTases from *S. mutans* serotype *a, d,* and *g*[9,14] in addition to serotype *c* GTase[6]. The procedure reported here for the isolation of the serotype *c* GTase-S is also simple and rapid, and permits large quantities of crude GTase samples to be purified up to homogeneous protein.

More recently, the presence of the extracellular GTase-I from serotype *c S. mutans* has been reported[28,29]. The purification and characterisation of both serotype *c* GTase-S and -I is essential to further study of the initial mechanism of dental caries. The isolation of the GTase-I is now under way in our laboratory.

REFERENCES

1 D. G. Wenham, T. D. Hennessey, and J. A. Cole, *J. Gen. Microbiol.*, 114 (1979) 117–124.
2 H. Mukasa and H. D. Slade, *Infect. Immun.*, 10 (1974) 1135–1145.
3 R. J. Gibbons and J. van Houte, *Annu. Rev. Med.*, 26 (1975) 121–136.
4 S. Hamada and H. D. Slade, *Microbiol. Rev.*, 44 (1980) 331–384.
5 T. Yakushiji, M. Inoue, and T. Koga, *Carbohydr. Res.*, 127 (1984) 253–266.
6 S. Sato, T. Koga, and M. Inoue, *Carbohydr. Res.*, 134 (1984) 293–304.
7 K. Fukui, Y. Fukui, and T. Moriyama, *J. Bacteriol.*, 118 (1974) 796–804.
8 A. Shimamura, H. Tsumori, and H. Mukasa, *Biochim. Biophys. Acta*, 702 (1982) 72–80.
9 T. Koga, S. Sato, T. Yakushiji, and M. Inoue, *FEMS Microbiol.*, 16 (1983) 127–130.
10 H. Tsumori, A. Shimamura, and H. Mukasa, *J. Gen. Microbiol.*, 131 (1985) 553–559.
11 S. Hamada, N. Masuda, T. Ooshima, S. Sobue, and S. Kotani, *Jpn. J. Microbiol.*, 20 (1976) 33–44.
12 H. K. Kuramitsu and L. Wondrack, *Infect. Immun.*, 42 (1983) 763–770.
13 H. Mukasa, A. Shimamura, and H. Tsumori, *Biochim. Biophys. Acta*, 719 (1982) 81–89.
14 T. Furuta, T. Koga, T. Nisizawa, N. Okahashi, and S. Hamada, *J. Gen. Microbiol.*, 131 (1985) 285–293.
15 T. Koga and M. Inoue, *Carbohydr. Res.*, 93 (1981) 125–133.
16 R. A. Reisfeld, U. J. Lewis, and D. E. Williams, *Nature (London)*, 195 (1962) 281–283.
17 R. M. Zacharius, T. E. Zell, J. H. Morrison, and J. J. Woodlock, *Anal. Biochem.*, 30 (1969) 148–152.
18 U. K. Laemmli, *Nature (London)*, 227 (1970) 680–685.
19 E. F. Hartree, *Anal. Biochem.*, 48 (1972) 422–427.
20 E. Schram, S. Moore, and E. J. Bigwood, *Biochem. J.*, 57 (1954) 33–37.
21 B. Lindberg, *Methods Enzymol.*, 28 (1972) 178–195.
22 R. R. B. Russell, *Microbios*, 23 (1979) 135–146.
23 B. Terleckyj, N. P. Willett, and G. D. Shockman, *Infect. Immun.*, 11 (1975) 649–655.
24 D. E. Fahrney and A. M. Gold, *J. Am. Chem. Soc.*, 85 (1963) 997–1000.
25 G. Mooser, D. Shur, M. Lyou, and C. Watanabe, *J. Biol. Chem.*, 260 (1985) 6907–6915.
26 H. K. Kuramitsu, *Infect. Immun.*, 12 (1975) 738–749.
27 S. Fujiwara, S. Kobayashi, and H. Nakayama, *Arch. Oral Biol.*, 23 (1978) 601–602.
28 A. C. Kenney and J. A. Cole, *FEMS Lett.*, 16 (1983) 159–162.
29 H. Mukasa, H. Tsumori, and A. Shimamura, *Infect. Immun.*, 49 (1985) 790–796.