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Comparative Study of Invertases of *Streptococcus mutans*

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Sucrase activity was studied in 13 strains of *Streptococcus mutans* representing the five Bratthall serotypes. Sucrose-adapted cells have sucrase activity in the 37,000 × *g*-soluble fraction of all strains. The enzyme was identified as invertase (β-D-fructofuranoside fructohydrolase; EC 3.2.1.26) because it hydrolyzed the β-fructofuranoside trisaccharide raffinose, giving fructose and melibiose as its products, and because it hydrolyzed the β-fructofuranoside dissaccharide sucrose, giving equimolar glucose and fructose as its products. Invertases of *c* and *e* strains exhibit two activity peaks by molecular exclusion chromatography with molecular weights of 45,000 to 50,000 and about 180,000; those of serotypes *a*, *b*, and *d* strains exhibit only a single component of 45,000 to 50,000 molecular weight. The electrophoretic mobility of invertases is different between the serotypes and the same within them. Inorganic orthophosphate (P_i) has a weak positive effect on the V_{max} of invertases of serotypes *c* and *e* cells but a strong positive effect on the invertases of serotype *b* cells; P_i has a strong positive effect on the apparent K_m of the invertases of serotype *d* cells, but has no effect on the V_{max}; P_i has a strong positive effect on both the apparent K_m and V_{max} of the invertases of serotype *a* cells. Thus, the invertases were different between all of the serotypes but similar within the serotypes. These findings support the taxonomic schemes of Coykendall and of Bratthall. It was additionally noted that 37,000 × *g*-soluble fractions of only serotypes *b* and *c* but not serotypes *a*, *d*, and *e* cells have melibiase activity, and it could be deduced that serotype *d* cells lack an intact raffinose permease system.

There have been several recent reports of sucrase activity in *Streptococcus mutans* (17, 18, 23, 26, 32, 33). This activity has been described both in the intracellular fluid of the cells (18, 26, 32) and in their culture liquor (17, 18), and some of these reports have identified the sucrase as invertase (EC 3.2.1.26; β-D-fructofuranoside fructohydrolase) (26, 32). Although certain common traits have been seen among the sucrases described of various *S. mutans* strains, some have been different. Perhaps the most striking trait has been that of apparent inorganic orthophosphate (P_i) regulation of enzyme activity of strain SL-1 (32). Other laboratories working with strains GS-5 (18, 23), HS-6 (17), 6715 (18), and K1R (26) have not observed such regulation. Two of these four strains (GS-5 and HS-6) are now recognized as belonging to different serological types of *S. mutans* than strain SL-1, although strains K1R and 6715 are felt to belong to the same type (1, 6-10, 15).

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Evidence that *S. mutans* is a relatively heterogeneous species has accumulated. Bratthall identified five serological types (1). Coykendall concluded that there are four groups based upon deoxyribonucleic acid (DNA)/DNA homologies and guanine-plus-cytosine contents (6-10), and Dunny et al. reached a similar conclusion based on DNA buoyant densities (15). Brown and Wittenberger (3), who studied lactate dehydrogenase kinetics, and Brown and Patterson (2), who studied polyolphosphate dehydrogenase, arrived at a similar conclusion. Others have described differing fermentation patterns among the serological groupings of *S. mutans* (7, 29), differing glucosyl transferase electrophoretic patterns (5), and differing cell wall compositions (21). It is remarkable that the groupings defined by each of these separate criteria correspond, and it thus appears that strains currently named *S. mutans* can be separated into at least four groups. Strains of all of these serotypes are known to be cariogenic.

Recently, Perch et al. (28) have proposed the further subdivision of Bratthall serotype *d* strains. For the sake of simplicity of terminology, we shall use the Bratthall *a* through *e* serotype designations in this paper, although

such a choice does not imply dispute with either the Coykendall (6–10) or Perch (28) schemes. In fact, Coykendall et al. have proposed a unification of their schemes (9) with that of Perch. Coykendall has now proposed (8) to assign species status to the five "genospecies" designated by Coykendall et al. (9).

The present paper reports a comparative study of the intracellular invertase activity of representative strains of the five Bratthall serotypes of *S. mutans*. Study has been restricted to the intracellular invertases to avoid the potentially confusing presence of extracellular glucosyl and fructosyl transferases. These latter enzymes can be readily misidentified as invertases because they produce, along with their glucan and fructan products, free fructose and glucose, respectively (31). In these studies, focus was given to the novel P_i regulation of invertase activity which has not been reported for other microorganisms (32). The data show that the invertases of the five Bratthall serotypes are distinct between them and similar within them. The differences in physical and regulatory properties among the invertases (β-D-fructofuranoside fructohydrolase; EC 3.2.1.26) of the five serotypes explain, in part, and extend the results reported by this and other laboratories.

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MATERIALS AND METHODS

Microorganisms. Strains representing the five cell wall antigenic types described by Bratthall (1, 35) were studied: serotype *a*, E-49, AHT, OMZ-61; serotype *b*, FA-1, BHT; serotype *c*, NCTC-10449, GS-5, Ingbritt, identified as IB-1600 in our laboratory; serotype *d*, SL-1, 6715-11, OMZ-176; serotype *e*, LM7, B2. The cultures studied were from our own culture collection and cross-checked against strains of the same designation obtained from D. Bratthall (University of Göteborg, Göteborg, Sweden) and A. Coykendall (VA Hospital, Newington, Conn.). They were maintained by infrequent transfer in fluid thioglycolate medium (Baltimore Biological Laboratories, Baltimore, Md.) containing 20% (vol/vol) meat extract and excess $CaCO_3$, unless otherwise specified. Cells were grown in the complex medium of Jordan et al. (22) supplemented with 5 mg of Na_2CO_3 per 100 ml and 0.2% sucrose. The sugar was autoclaved separately and added aseptically to the basal medium. Cultures used for experiments were passed through at least two transfers in this broth before growth of cells for study.

Preparation of cell-free extracts. Cultures were grown in the sucrose-containing medium. After reaching early stationary phase, the cells were harvested by centrifugation and washed in 10 mM potassium phosphate buffer (pH 7.0). The cell yield from a 500-ml culture was suspended in 12 ml of buffer and disrupted for three 6-min intervals with a model W-185 sonic oscillator (Heat Systems-Ultra Sonics, Plainview, N.Y.) at maximum output, while being continuously cooled with circulating ice water. The broken cell suspension was then centrifuged at 37,000 × *g* at 4°C for 30 min. The enzyme activity in the 37,000 × *g*-soluble supernatant fluid was subsequently characterized, as will be described below.

Enzyme assays. Sucrase activity was routinely assayed by incubation of enzyme-containing materials with 100 mM sucrose and 100 mM potassium phosphate (pH 7.0), unless otherwise specified. After 30 min of incubation at 37°C, incubation mixtures were boiled for 10 min. The reaction rate was constant with time and proportional to the amount of enzyme in the reaction mixture. Boiled enzyme-containing material was similarly used as a control. The generation of free glucose was then detected with glucose oxidase as described below and was taken as an indication of sucrase activity; detection of reducing sugars (glucose plus fructose) led to identical conclusions. In assays of the activity of the sucrase using raffinose as substrate, the production of reducing sugar (fructose) was monitored as described below (32).

Sucrose phosphorylase activity was assayed by the method of Doudoroff (13, 32) by incubating crude, cell-free preparations with 100 mM sucrose and 16 mM P_i, monitoring the disappearance of P_i, as described below. The reaction was also carried out in the reverse direction by incubating the same enzyme preparation with 12 mM glucose-1-phosphate, 100 mM fructose, and 100 mM P_i, monitoring the appearance of sucrose, as described below. The reactions were stopped by boiling for 10 min. When the reaction was carried out in the forward direction, the limit of detection of P_i disappearance from the incubation mixture was less than 3 nmol/min per mg of protein; when the reaction was carried out in the reverse direction, the limit of detection of sucrose appearance in the incubation mixture was less than 0.8 nmol/min per mg of protein. A more sensitive assay for sucrose phosphorylase was also used. It measures the characteristic arsenolysis of glucose-1-phosphate in the presence of arsenate and sucrose phosphorylase (14, 32). Thus, 2 mM glucose-1-phosphate and 100 mM arsenate (pH 7.0) were incubated at 37°C with crude, cell-free enzyme preparations, and the appearance of free glucose was monitored after boiling for 10 min. By this method, the limit of detection of glucose production from glucose-1-phosphate was less than 0.3 nmol/min per mg of protein. To test for phosphatase activity, the enzyme preparations were incubated with 2 mM glucose-1-phosphate, and the appearance of free glucose was detected after boiling for 10 min.

To test whether the 37,000 × *g*-soluble fraction had either glucosyl and/or fructosyl transferase activity, this fraction was incubated with 100 mM sucrose to which [U-^{14}C]sucrose was added (New England Nuclear Corp., Boston). Two volumes of ice-cold ethanol were added and incorporation of ^{14}C into an ethanol-insoluble precipitate was monitored (19).

Polyacrylamide gel electrophoresis. Crude enzyme extracts (37,000 × *g*-soluble) were concentrated approximately 10-fold by membrane filtration. Amicon PM-30 filters were used. From 10 to 50 μl of the concentrated 37,000 × *g* fraction was applied to the top of polyacrylamide gels prepared in glass tubes (6 by 60 mm) at pH 8.3 using the method of Davis (11). After electrophoresis at 4°C for approximately 3 h, gels were removed from the tubes and incubated in 100 mM sucrose and 100 mM potassium phosphate buffer, pH 6.2, at 37°C for 30 min. The enzyme activity was disclosed by incubation for 30 min in the dark at room temperature with the following enzyme-dye couple: yeast hexokinase (100 times higher affinity for glucose than for fructose) [5 U/ml]); adenosine 5'-triphosphate (1 mM); $MgCl_2$ (1 mM); yeast glucose-6-phosphate dehydrogenase (5 U/ml); oxidized nicotinamide adenine diphosphate (1 mM); tris(hydroxymethyl)aminomethane-hydrochloride (100 mM, pH 7.5); phenazine methosulfate (0.15 mM); nitroblue tetrazolium chloride (0.8 mM) (32). Bromophenol blue was used as tracking dye. The gels were fixed by immersion in 7% acetic acid.

Chromatography and dialysis. Sephadex G-150 column chromotography was used to estimate the molecular weight of the enzyme in the crude, cell-free extract. Molecular weight standards of thyroglobulin (approximately 6×10^5 molecular weight), yeast alcohol dehydrogenase (approximately 1.4×10^5 molecular weight), bovine serum albumin (approximately 6.8×10^4 molecular weight), ovalbumin (approximately 4.0×10^4 molecular weight), α-chymotrypsinogen (approximately 2.5×10^4 molecular weight), and ribonuclease (approximately 1.37×10^4 molecular weight) were used to calibrate the column, as previously detailed (32). The elution profiles of the standards were detected by measuring absorbance at 280 nm.

To identify the products of enzymatic reaction with sucrose, incubation mixtures were chromatographed and detected on paper, as previously detailed (32).

In order to remove P_i from the 37,000 × *g*-soluble fraction, this fraction was also chromatographed on small Sephadex G-25 columns, collecting only the void volume as identified by the peak effluent volume of blue dextran. As an alternative method of removing P_i, crude enzyme extracts were exhaustively dialyzed against water.

Chemicals. All chemicals used for the enzyme couple detection of glucose were obtained from Sigma Chemical Co. (St. Louis, Mo.). Sephadex G-150 and G-25 and blue dextran were purchased from Pharmacia Fine Chemicals, Piscataway, N.J. Sugars used for testing the specificity of the enzyme were of the highest commercially available purity: glucose, fructose, sucrose, and raffinose (J. T. Baker, Philipsburg, N.J.); melezitose, α-methyl glucoside, β-methyl glucoside, melibiose (Pfanstiehl Laboratories, Waukegan, Ill.); lactose and cellobiose (Pfanstiehl and Sigma).

Chemical analyses. Glucose was analyzed by the glucose oxidase method (Worthington Biochemicals Corp., Freehold, N.J.) using reagents dissolved in 175 mm potassium phosphate buffer (pH 7.0). Reducing sugar was analyzed by the colorimetric method of Nelson (27). Protein was analyzed by the biuret method (20). P_i was analyzed by the method of Burnham and Hageage (4) or by the method of Fiske and SubbaRow (16). Sucrose was analyzed by the method of van Handel (36).

RESULTS

Enzyme identification and location. Essentially all of the sucrase activity found in the sonified suspension of washed cells could be recovered in the 37,000 × *g*-soluble cell fraction, consistent with earlier observations of strains SL-1, NCTC 10449, E-49, and FA-1 (32). This 37,000 × *g* fraction will be subsequently called the crude, cell-free extract. The specificity of the sucrase was tested against various di- and trisaccharides. Incubation of the crude, cell-free extract from the 13 strains with these substrates indicated that the enzyme was highly specific for sucrose (Table 1). The enzyme did not show α-glucosidase activity because neither melezitose nor α-methyl glucoside was hydrolyzed. (Strain BHT was the sole exception, showing activity for melezitose, although not for α-methyl glucoside.) There was no β-glucosidase activity as neither β-methyl glucoside nor cellobiose was hydrolyzed; there was no β-galactosidase activity as lactose was not hydrolyzed. However, raffinose, an unsubstituted β-fructofuranosyl-containing trisaccharide, was hydrolyzed. Previously published work with strain SL-1, both with similar crude enzyme preparations and with diethylaminoethyl-cellulose partially purified preparations, gave the same results, and these preparations were shown to yield equimolar glucose and fructose from sucrose (32).

The products of raffinose degradation by the enzyme were seen chromatographically to be fructose and the α-galactoside melibiose, as expected of invertase and previously detailed for strain SL-1 (32). (Sometimes melibiose could not be detected; see below.) Thus, the sucrase of all the strains tested could be identified as invertase (EC 3.2.1.26; β-D-fructofuranoside fructohydrolase).

Melibiose, an α-galactoside, was cleaved by the crude enzyme preparations of the serotypes *b* and *c* strains tested, but not by any of the strains of serotypes *a*, *d*, or *e*. Further work will be required to establish whether the apparent melibiase activity is due to a separate enzyme or due to the invertase of serotypes *b* and *c* strains.

No sucrose phosphorylase activity could be detected in any of the crude, cell-free enzyme preparations by the method of Doudoroff (13) carried out both in the forward and reverse directions. The more sensitive arsenolysis of

glucose-1-phosphate by sucrose phosphorylase in the presence of arsenate (14) could also not be detected (Table 2). Furthermore, glucose phosphatase activity could not be detected in the enzyme preparations. However, invertase activity was apparent in the presence of sucrose, enzyme preparation, and either 100 mM P_i or 100 mM arsenate. With enzyme preparations of

TABLE 1. *Specificity of the sucrases from crude, cell-free extracts of 13 strains of S. mutans representing five serotypes* [a]

Serotype	Strain	Relative enzyme activity of substrates (%)							
		Sucrose	Lactose	β-Methylglucoside	Cellobiose	α-Methylglucoside	Melibiose	Melezitose	Raffinose
a	AHT	100	0	0	0	–	0	0	11
	OMZ-61	100	0	0	0	0	0	0	12
	E49	100	0	0	0	0	0	0	8
b	BHT	100	0	0	0	0	4	260	34
	FA-1	100	0	0	0	0	19	0	18
c	NCTC 10449	100	0	0	0	0	8	0	11
	Ingbritt	100	0	0	0	0	5	0	7
	GS-5	100	0	0	0	0	6	0	6
d	SL-1	100	0	0	0	0	0	0	7
	6715-11	100	0	0	0	0	0	0	5
	OMZ-176	100	0	0	0	0	0	0	11
e	B2	100	0	0	0	0	0	0	6
	LM-7	100	0	0	0	0	0	0	6

[a] All substrates were incubated with 37,000 × *g*-soluble extracts of sucrose-grown cells in the presence of 100 mM potassium phosphate buffer at the optimum pH of the sucrases for the respective strains. Enzyme activity was monitored as detailed in Materials and Methods.

TABLE 2. *Inability of crude, cell-free extracts of 13 strains of S. mutans representing five serotypes to catalyze arsenate-dependent hydrolysis of glucose-1-phosphate* [a]

Serotype	Strain	Enzyme activity of incubation mixtures (U/mg of protein)			
		Sucrose + P_i + enzyme	Sucrose + arsenate + enzyme	Glucose-1-P + P_i + enzyme	Glucose-1-P + arsenate + enzyme
a	AHT	0.367	0.353	0	0
	OMZ-61	0.195	0.275	0	0
	E49	0.062	0.061	0	0
b	BHT	0.023	0.032	0	0
	FA-1	0.009	0.013	0	0
c	NCTC 10449	0.098	0.116	0	0
	Ingbritt	1.153	0.759	0	0
	GS-5	0.003	0.004	0	0
d	SL-1	0.039	0.037	0	0
	6715-11	0.733	0.711	0	0
	OMZ-176	0.328	0.035	0	0
e	B2	0.050	0.041	0	0
	LM-7	0.757	0.686	0	0

[a] Glucose production was monitored by the glucose oxidase procedure described in Materials and Methods. The assay mixtures contained the following components in a final volume of 1.0 ml: sucrose or glucose-1-phosphate, 2 μmol; potassium phosphate, pH 7.0, or sodium arsenate, pH 7.0, 100 μmol; enzyme, generally 150 μg of protein; and distilled water to volume.

most strains, the effects of arsenate and P_i were similar, as previously shown (32).

Because no ^{14}C was detected in ethanol-precipitated incubation mixtures of [U-^{14}C]sucrose with crude, all-free enzyme preparations, there was no evidence of glucosyl or fructosyl transferase activity.

pH optima. The pH optima of the invertases of all the strains were broad (pH 5.5 to 7.0) and were not clearly different between the serotypes.

Molecular weight estimation. Strains of serotypes *a*, *b*, and *d* showed only one molecular weight component of their investases. In all cases, the molecular weight was approximately 45,000 to 50,000 as determined by chromatography of the crude, cell-free extracts on Sephadex G-150 (Fig. 1). However, crude, cell-free extracts of serotypes *c* and *e* strains showed two peaks by such chromatography. It was of interest that the higher-molecular-weight component behaved as though it had a molecular weight about four times that of the lower-molecular-weight component. It is thus possible that there exists a tetrameric form of the enzyme. This characteristic thus distinguished serotypes *c* and *e* from the other serotypes but did not distinguish them from each other.

Electrophoretic mobility. The electrophoretic mobility of the invertases was consistent within serotypes but different between them (Fig. 2). In most cases, only a single activity band was seen upon electrophoresis of the crude, cell-free extracts from all the strains examined. However, a very weak satellite band could be detected for strains GS-5 and B-2. For the various serotypes, the major components of invertase activity could be ordered from greatest mobility to least mobility as $b > d > a > c > e$. Repetitive observations of electrophoretic mobility of invertases of any strain showed that the ratio of mobility of the enzyme to that of the tracking dye had a coefficient of variation of less than 1.3%. The average mobilities of invertase relative to tracking dye are given in Table 3.

P_i regulation of invertase. Striking differences were noted between the effects of P_i on invertase of the various serotypes. The V_{max} of the invertases of serotypes *c* and *e* strains was only weakly affected by P_i (Fig. 3 and 4) and the apparent K_m was unaltered. Differences could not be noted either within or between these serotypes. However, P_i was a potent positive effector of the enzyme of serotypes *a*, *b*, and *d* strains, affecting each serotype differently. Thus, for serotype *d* strains, P_i strongly affected the apparent K_m but not V_{max} (Fig. 5); for serotype *b* strains, P_i strongly affected V_{max} but not the apparent K_m (Fig. 6); for serotype *a* strains, both apparent K_m and V_{max} were strongly affected (Fig. 7).

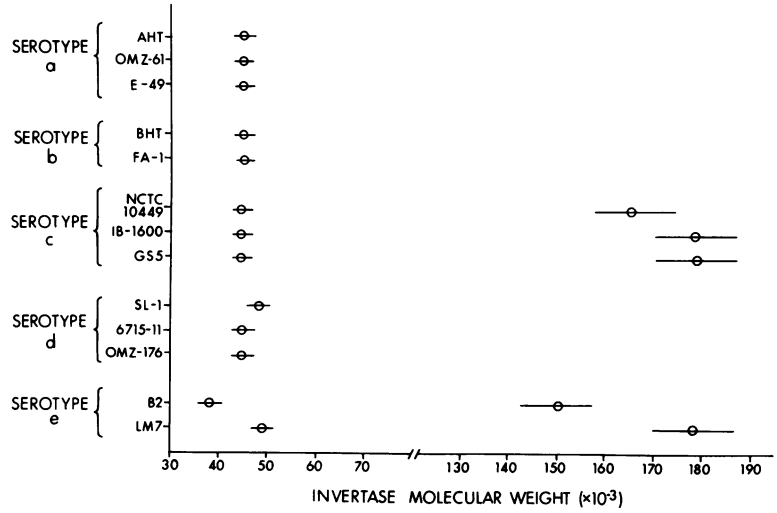

FIG. 1. *Plot of molecular weight estimates of invertases of 13 strains of S. mutans representing five serotypes. A 1.5-ml amount of a 37,000 × g-soluble fraction or of various molecular weight standards was chromatographed on a column (2 by 60 cm) of Sephadex G-150 previously equilibrated with 10 mM potassium phosphate buffer (pH 7.0). The gel was eluted at approximately 15 ml/h with the equilibrating buffer using upward flow. Peak effluent volumes of invertases and molecular weight standards were detected as detailed in Materials and Methods. The molecular weight of the invertases was estimated by comparison of the K_{avg} of invertases and the molecular weight standards on a semilog plot (32).*

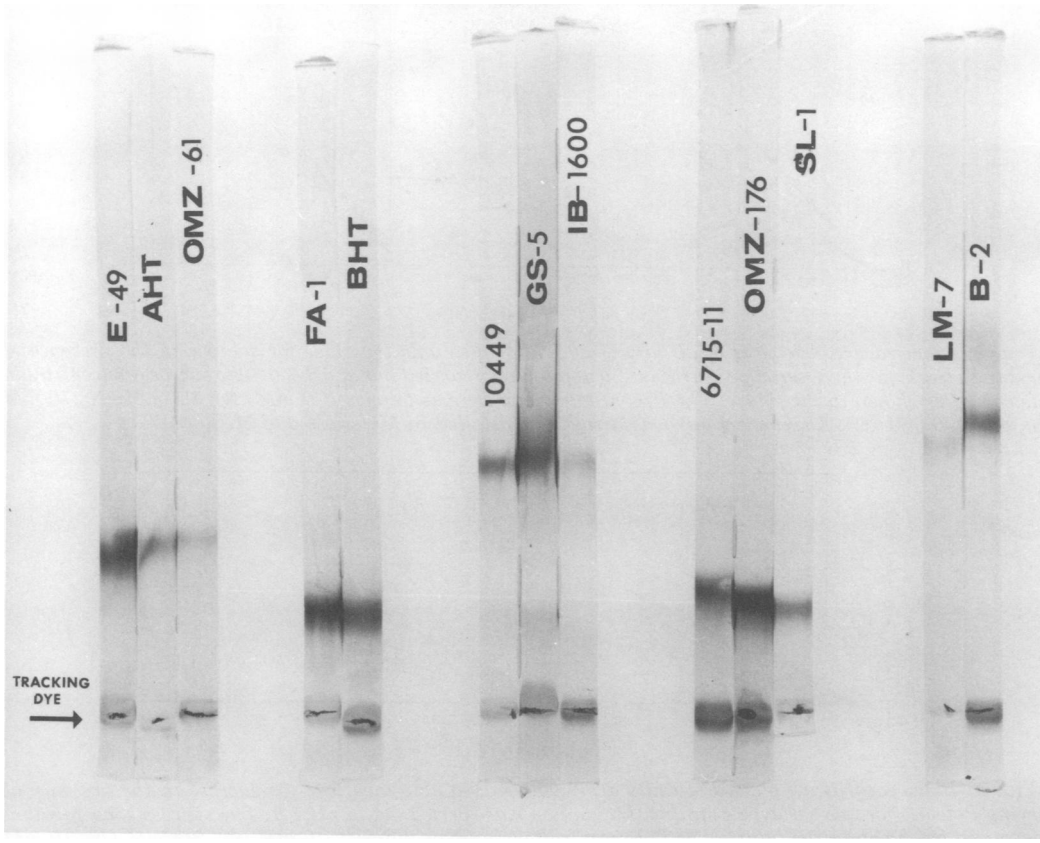

FIG. 2. *Acrylamide gels of 37,000 × g-soluble fractions with invertase activities detected as detailed in Materials and Methods.*

TABLE 3. *Summary of characteristics of intracellular invertases of Streptococcus mutans serotypes*

Serotypes	Characteristics			
	No. of molecular components[a]	"Melibiase"	P_i effector	Acrylamide gel electrophoresis (invertase/ tracking dye)
a	1	–	Strong; both K_m and V_{max} effects	0.740
b	1	+	Strong; V_{max} only	0.833
c	2	+	Weak	0.628
d	1	–	Strong; K_m only	0.818
e	2	–	Weak	0.598

[a] Sephedex G-150 column chromatography was used to estimate the molecular weight.

It was possible that the invertases of the 13 strains are incapable of activity in the absence of P_i. To test this question, sonified suspensions of the various strains were freed of P_i either by passage through columns of Sephadex G-25 (24 by 0.4 mm) or by exhaustive dialysis against water. Enzyme activity was assayed before the chromatography or the dialysis procedures, after them, and after P_i was readded to the chromatographed or dialyzed crude enzyme preparations. Such chromatography or dialysis reduced invertase activity from 40 to 87% for the various strains by comparison with the enzyme activity without such chromatography or dialysis. The nonchromatographed or nondialyzed crude extracts were assayed in the presence of 1 mM P_i, pH 7.0. Readdition of P_i to a concentration of 1 mM returned enzyme activ-

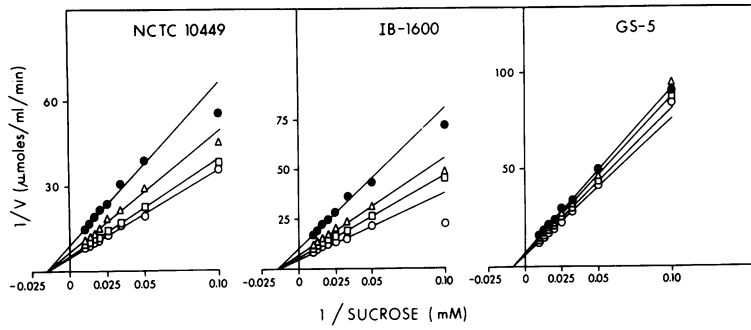

FIG. 3. *Lineweaver-Burk plots of affinity of 37,000 × g-soluble invertase for sucrose in the presence of varying P_i levels for three serotype c strains. Incubation was carried out at pH 7.0. The sucrose concentration was varied as shown in the figure. The P_i concentrations were as follows: 1.0 mM (●), 3.3 mM (△), 10 mM (□), and 100 mM (○). Enzyme activity was assayed as detailed in Materials and Methods.*

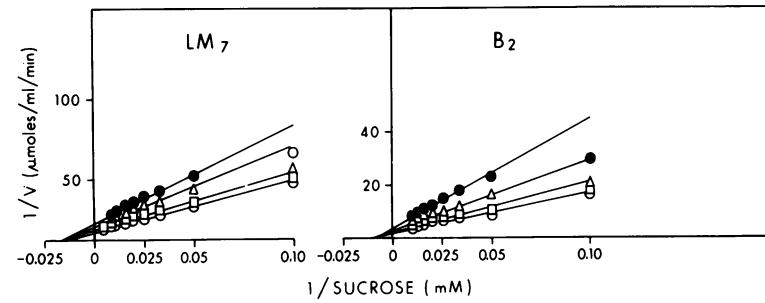

FIG. 4. *Lineweaver-Burk plots of affinity of 37,000 × g-soluble invertase for sucrose in the presence of varying P_i levels for two serotype e strains. Incubation was carried out at pH 7.0. The sucrose concentration was varied as shown in the figure. The P_i concentrations were as follows: 1.0 mM (●), 3.3 mM (△), 10 mM (□), and 100 mM (○). Enzyme activity was assayed as detailed in Materials and Methods.*

ity to 45 to 98% of prechromatography/predialysis levels. There was no clear distinction between the serotypes with respect to this phenomenon. Analysis of P_i in the chromatographed or dialyzed materials by the Fiske and SubbaRow method (16) showed residual phosphate to range from 0.00 to 0.09 μmol/ml, with most samples having undetectable P_i. The P_i detection limit was less than 0.005 μmol/ml.

DISCUSSION

The intracellular sucrase from all of the *S. mutans* Bratthall serotype representatives can be identified as invertase (EC 3.2.1.26; β-D-fructofuranoside fructohydrolase) based on substrate specificity studies and identification of equimolar glucose and fructose as its products (32). Only strain BHT showed evidence of additional α-glucosidase activity, and none of the strains showed evidence of sucrose phosphorylase activity. In all cases, the invertase was found in the 37,000 × *g*-soluble cell fraction and was not associated with particulate fractions. It cannot be unequivocally established that the invertases were not disassociated from particulate fractions as a result of sonication. However, this possibility seems unlikely because no appreciable particulate-associated enzyme activity was detected. No glucosyl or fructosyl transferase activity was detected in the 37,000 × *g*-soluble cell fractions.

It had been previously reported (32) that strain SL-1 had intracellular invertase capable of cleaving raffinose. Similarly, cell-free preparations of the other two Bratthall serotype *d* strains studied here, 6715-11 and OMZ-176, were also capable of raffinose cleavage. However, intact serotype *d* cells are reported to be incapable of raffinose fermentation (29, 35). Thus, one must conclude that serotype *d* strains lack an intact raffinose permease system and are thus cryptic for raffinose.

The apparent differences between the intracellular invertases of representative strains of the five Bratthall serotypes of *S. mutans* are summarized in Table 3. Serotypes *c* and *e* strains are distinguished from serotypes *a*, *b*, and *d* strains by the presence of two molecular weight components of the enzyme, the higher-molecular-weight one behaving, by molecular

exclusion chromatography on Sephadex G-150, consistent with being at least a tetramer of the lower-molecular-weight component.

Invertases of *c* and *e* strains were also indistinguishable from each other on the basis of P_i effects. P_i functions as a weak positive effector, altering V_{max} but not apparent K_m. By contrast, P_i has no effect on V_{max} but a strong effect on the apparent K_m of serotype *d* strains. This behavior is consistent with the sequential binding of the enzyme to activator and then substrate (12). For serotype *b* strains, P_i has no effect on the apparent K_m but a strong positive effect on V_{max}, consistent with binding of enzyme randomly to either substrate or activator (12). With respect to serotype *a* strains, both apparent K_m and V_{max} are altered. Of course, these preliminary kinetic data do not establish a mechanism of action. They nonetheless allow discrimination between serotypes. Thus, serotypes *a*, *b*, and *d* strains can be distinguished from one another and from serotypes *c* and *e* strains by the characteristics of the P_i activation of their invertase.

The retention of invertase activity in the absence of P_i indicates that the invertases of the various strains are not strictly dependent upon the presence of P_i for their activity, but only regulated by it. However, it remains possible that trace binding of P_i to the invertases could not be eliminated by the column chromatographic or dialysis procedures, even though trace P_i presence usually could not be detected.

The apparent "melibiase" activity of the crude, cell-free extracts distinguished serotypes *b* and *c* strains from those of the other serotypes. Aside from the electrophoretic mobility of the enzyme on acrylamide gel, this was the only trait which distinguished strains of serotypes *c* from *e*. Indeed, Shklair and Keene (29)

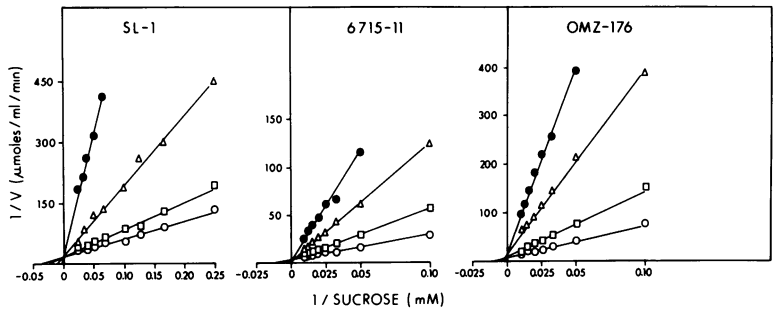

FIG. 5. *Lineweaver-Burk plots of affinity of 37,000 × g-soluble invertase for sucrose in the presence of varying P_i levels for three serotype d strains. Incubation was carried out at pH 7.0. The sucrose concentration was varied as shown in the figure. The P_i concentrations were as follows: 1.0 mM (●), 3.3 mM (△), 10 mM (□), and 100 mM (○). Enzyme activity was assayed as detailed in Materials and Methods.*

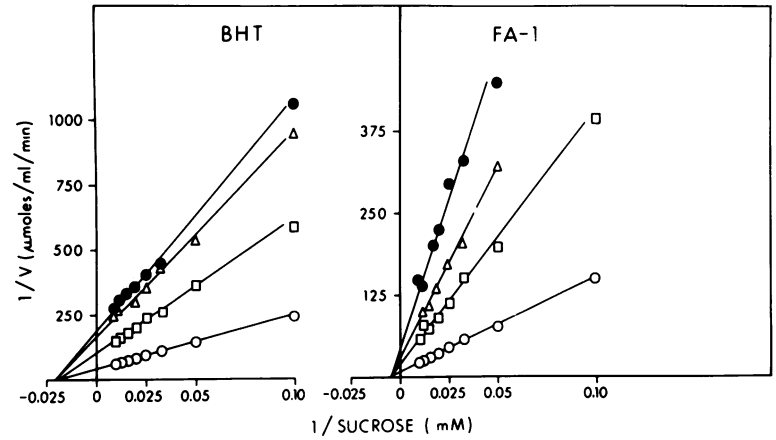

FIG. 6. *Lineweaver-Burk plots of affinity of 37,000 × g-soluble invertase for sucrose in the presence of varying P_i levels for two serotype b strains. Incubation was carried out at pH 7.0. The sucrose concentration was varied as shown in the figure. The P_i concentrations were as follows: 1.0 mM (●), 3.3 mM (△), 10 mM (□), and 100 mM (○). Enzyme activity was assayed as detailed in Materials and Methods.*

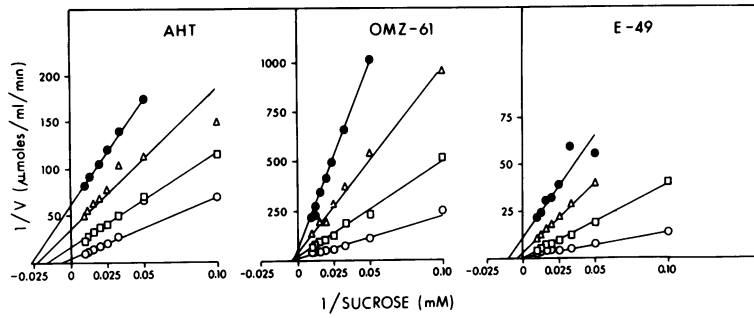

FIG. 7. *Lineweaver-Burk plots of affinity of 37,000 × g-soluble invertase for sucrose in the presence of varying P_i levels for three serotype a strains. Incubation was carried out at pH 7.0. The sucrose concentration was varied as shown in the figure. The P_i concentrations were as follows: 1.0 mM (●), 3.3 mM (△), 10 mM (□), and 100 mM (○). Enzyme activity was assayed as detailed in Materials and Methods.*

have reported that only serotype *e* strains fail to ferment melibiose. This additionally suggests that melibiose fermentation by intact cells of serotypes *a* and *d* strains (29, 35) may depend upon enzymes not found in the 37,000 × *g*-soluble fraction of sucrose-adapted cells.

Previously, Coykendall et al. (6–10) had found by DNA/DNA homologies and guanine-plus-cytosine content analyses that he could distinguish only four groups of *S. mutans* and that he could not distinguish between Bratthall serotypes *c* and *e*. Based on DNA buoyant densities (15), enzymological evidence (2, 3, 5, 7), and cell wall analyses (21), others have reached similar conclusions. The present data provide biochemical evidence consistent with these groupings and additionally supply data consistent with the antigenic distinction between *c* and *e* (1, 28) and with the biochemical differences between *c* and *e* reported by others (5, 7, 29, 35).

When this work was near completion, Perch et al. (28) published antigenic data which suggested the existence of two additional serotypes among strains biochemically identical to serotype *d* strains of *S. mutans*. No data were observed in the present study that support or exclude this distinction.

It appears reasonable to suggest, in view of the considerable diversity of this one enzyme among these serotypes of *S. mutans* and its consistency with other observed characteristics, that the species *S. mutans* may possibly have diverse evolutionary origins. Such an idea was presented by London et al. based upon study of immunological relationships among the aldolases of streptococci and other gram-positive nonsporeforming bacteria (24, 25). The diversity of modes and extents of P_i regulation of these invertases suggest its possible significance in the regulation of glycolysis, sucrose carbon flow to glycolytic and various synthetic products, and subsequent manifestation of dental caries (31).

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