THE OCCURRENCE OF MULTIPLE GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASES IN CARIOGENIC STREPTOCOCCI

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Summary: A novel metabolic situation has been found to exist in a number of strains of Streptococcus mutans. These organisms possess two separable glyceraldehyde-3-phosphate dehydrogenases which differ in their coenzyme specificities and molecular weights. One of these enzymes is NAD-linked and the other enzyme exhibits specificity for NADP. A possible physiological role for the NADP-linked enzyme in S. mutans is in the generation of NADPH, since this organism lacks both the oxidative portion of the Hexose Monophosphate Shunt pathway and transhydrogenase activity.

We have established previously (1) that a number of anaerobically grown, caries conducive strains of Streptococcus mutans (2,3,4) lack both glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconate dehydrogenase (6PGDH). Since recent evidence has indicated that perhaps the major function of the oxidative portion of the Hexose Monophosphate Shunt (HMS) pathway is in the generation of NADPH (5,6), the absence of at least the oxidative portion of this pathway imposes upon these organisms the necessity of utilizing alternative metabolic processes to generate the NADPH which is required for various reductive biosynthetic reactions. In this communication we report on the presence of multiple glyceraldehyde-3-phosphate dehydrogenases (GA3PDH) in S. mutans. One of these enzymes is NAD specific and is thought to function in glycolysis while the other is NADP specific and is thought to be responsible for the generation of NADPH in these organisms.

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MATERIALS AND METHODS

Chemicals. NAD, NADP, and glyceraldehyde-3-phosphate (GA3P) were purchased from Sigma. The GA3P was obtained as the barium salt of DL-glyceraldehyde-3-phosphate diethyl acetal and was converted to the free acid before being used as described in Sigma Bulletin No. 10. All other components of the enzyme assay systems were of the highest purity commercially available.

Growth of Cultures. All S. mutans strains and S. salivarius SS 2 were obtained from Dr. Harold V. Jordan. All other Streptococcus species were obtained from Dr. Jack London. The organisms were all grown anaerobically at 37°C in the complex medium described previously (1).

Preparation of Cell-Free Extracts. Cells in the late log phase of growth were harvested from 125 ml of medium by centrifugation, washed twice with 40 ml of 0.05 M potassium phosphate buffer, pH 6.2, and cell-free extracts were prepared in 10 ml of the washing buffer as described previously (1). Protein determinations were performed by the biuret method (7).

RESULTS AND DISCUSSION

Crude, cell-free extracts from seven different strains of S. mutans exhibited GA3PDH activity with either NAD or NADP, and all of the strains were virtually devoid of both G6PDH and 6PGDH activity (Table 1). The same results were obtained with another cariogenic organism S. salivarius (8) SS 2. The two GA3PDH activities appear to be constitutive in S. mutans since the specific activities were the same in cell-free extracts prepared from strain 6715-9 grown on sucrose, glucose, fructose, mannitol, and sorbitol.

In contrast to the results obtained with the cariogenic streptococci, four other non-cariogenic species of Streptococcus possessed both G6PDH and 6PGDH activity and exhibited GA3PDH activity only with NAD (Table 1). A direct correlation exists, therefore, among the cariogenic streptococci between the absence of the oxidative portion of the HMS pathway and the presence of GA3PDH activity with NADP. These results suggest that the physiological role of the NADP-GA3PDH activity in these organisms may be

Table I

GA3PDH Activity with NAD or NADP in Cell-Free Extracts of Cariogenic and Non-Cariogenic Streptococci

Organisms	Enzyme Activity (μmoles/minute/mgram protein)			
	GA3PDH (NADP)	GA3PDH (NAD)	G6PDH (NAD or NADP)	6PGDH (NAD or NADP)
Cariogenic Streptococci				
S. mutans 6715-9	0.128	0.332	< .001	< .001
S. mutans 10449	0.100	0.360	< .001	< .001
S. mutans 3720	0.095	0.425	< .001	< .001
S. mutans HS-6	0.120	0.372	< .001	< .001
S. mutans E-49	0.108	0.280	< .001	< .001
S. mutans AHT	0.101	0.290	< .001	< .001
S. mutans FA-1	0.120	0.342	< .001	< .001
S. salivarius SS 2	0.140	0.374	< .001	< .001
Non-Cariogenic Streptococci				
S. faecalis MR	< .001	0.490	0.081	0.071
S. faecalis 10CI	< .001	0.455	0.085	0.072
S. faecium N-55	< .001	0.375	0.070	0.065
S. sanguis 10558	< .001	0.370	0.095	0.091
S. lactis	< .001	0.410	0.410	0.075

Preparation of cell-free extracts have been described in the text (Materials and Methods). The enzyme assays were as follows: NAD-linked GA3PDH. The reaction mixture contained: Glyceraldehyde-3-phosphate (GA3P), 1.0 μmole; NAD, 1.0 μmole; sodium arsenate, 1.0 μmole; cysteine-HCl, 5.0 μmoles; Tris-HCl buffer, pH 8.5, 100 μmoles; and cell-free extract in a final volume of 1.0 ml. NADP-linked GA3PDH. The reaction mixture contained: GA3P, 1.0 μmole; NADP, 1.0 μmole; mercaptoethanol, 5.0 μmoles; Tris-HCl buffer, pH 8.5, 100 μmoles; and cell-free extract in a final volume of 1.0 ml. In both cases, pyridine nucleotide reduction was followed at 340 nm in a Beckman DB-G equipped with a Sargeant SRLG recorder. The reactions were run at 25°C. The assay procedure for G6PDH and 6PGDH have been described in another publication (1).

as a metabolic alternative to the oxidative portion of the HMS pathway for the generation of NADPH. This interpretation of the results was reinforced

by the fact that cell-free extracts of S. mutans do not contain detectable levels of transhydrogenase activity when assayed according to the procedure described for "Reaction 1" by Keister and San Pietro (9).

In order to determine whether the NAD- and NADP-linked GA3PDH activities in S. mutans were due to a single enzyme which is catalytically active with both NAD and NADP, or to distinct NAD- and NADP-specific enzymes an attempt was made to separate these two activities. As shown in Fig. 1A partial resolution of the NAD and NADP activities was achieved when a cell-free extract of S. mutans 6715-9 was passed through a Sephadex G-200 column. Complete separation of the two activities was achieved by chromatography of the cell-free extract on a DEAE cellulose column (Fig. 1B). It thus seems clear that the GA3PDH activity observed with NAD and NADP in cell-free extracts of S. mutans strain 6715-9 is in fact due to distinct enzymes which differ in coenzyme specificity.

The emergence of the NADP-linked GA3PDH from the Sephadex G-200 column before the NAD-linked enzyme (Fig. 1A) indicates that the two enzymes differ from one another in molecular weight. Other distinguishing features of the two GA3PDHs were found in their differential responses to cysteine and arsenate. The NAD-linked enzyme exhibited a marked dependence upon cysteine for catalytic activity whereas the NADP-linked enzyme did not (Fig. 1B). Furthermore, the NAD-linked enzyme was stimulated by 1.0 mM arsenate while the activity of the NADP-specific enzyme was inhibited 60% by this concentration of arsenate. The observed effects of both cysteine and arsenate on the two enzymes from S. mutans 6715-9 suggest that the NAD-linked enzyme is similar to the previously described D-glyceraldehyde-3-phosphate: NAD oxidoreductase (phosphorylating), EC 1.2.1.12 (10), whereas the NADP-linked enzyme is similar to the D-glyceraldehyde-3-phosphate:NADP oxidoreductase, EC 1.2.1.9 (10). Detailed studies dealing with the chemical and physical properties of these two enzymes are currently in progress.

Attempts to separate the NADP- and NAD-linked enzymes from S. mutans

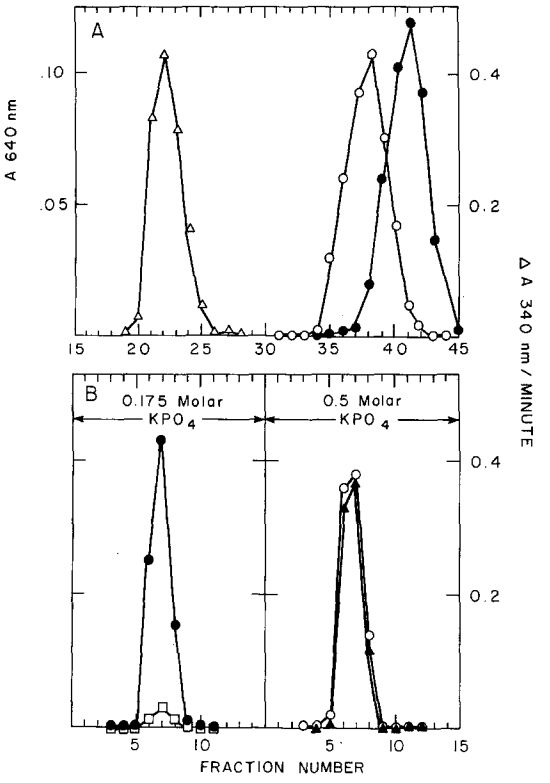

Figure 1. Chromatographic separation of NAD- and NADP-linked GA3PHDs. Cell-free extracts were prepared as described previously (Materials and Methods) and all column procedures were carried out at 4°C.

A. Gel filtration chromatography: A Sephadex G-200 column (1.9 X 100 cm) was equilibrated with 0.05M potassium phosphate buffer. 2.5 ml of the cell-free extract (10 mg protein/ml) from S. mutans 6715-9 was applied to the column and eluted with the equilibrating buffer. 4.0 ml fractions were collected and assayed for both NAD- and NADP-linked GA3PDH activity as described in Table I. The blue dextran marker was measured at 640 nm. Blue dextran marker, Δ - Δ; NADP-linked activity, 0 - 0; NAD-linked activity ● - ●.

B. DEAE ion exchange chromatograpy. DE 52 from Reeve Angel was washed and equilibrated with 0.05 M potassium phosphate buffer, pH 6.2 and a column (2.0 X 20 cm) was packed under pressure. After application of 7.0 ml (12 mg protein/ml) of crude, cell-free extract from S. mutans 6715-9, the following step-wise elution procedure was used employing 250 ml of each eluting buffer: 0.05 M KPO_4, pH 6.2; 0.175 M KPO_4, pH 6.2; 0.5 M KPO_4, pH 6.2. Fractions of 10 ml each were collected and assayed for GA3PDH activity as described in Table I except for the omission of cysteine as indicated below. NAD-linked activity + cysteine, ● - ●; NAD-linked activity minus cysteine, □ - □; NADP-linked activity + cysteine, 0 - 0; NADP-linked activity minus cysteine ▲ - ▲.

6715-9 by disc gel electrophoresis were unsuccessful because the cysteine requirement of the NAD-linked enzyme in this organism interfered with the

activity stains employed. However, the GA3PDH activity with NAD in another cariogenic Streptococcus, S. salivarius SS 2, was found to be stimulated by cysteine but did not exhibit an absolute requirement for the sulfhydryl compound. When a cell-free extract from this organism was subjected to disc gel electrophoresis, a distinct separation of NADP-linked from the NAD-linked GA3PDH was achieved. Thus the cariogenic S. salivarius strain SS 2, like S. mutans 6715-9, appears to possess distinct GA3PDHs, which are specific for NAD and NADP respectively. This strain of S. salivarius also shows two electrophoretically distinct GA3PDH activity bands with NAD alone (Fig. 2). The physiological significance and characterization

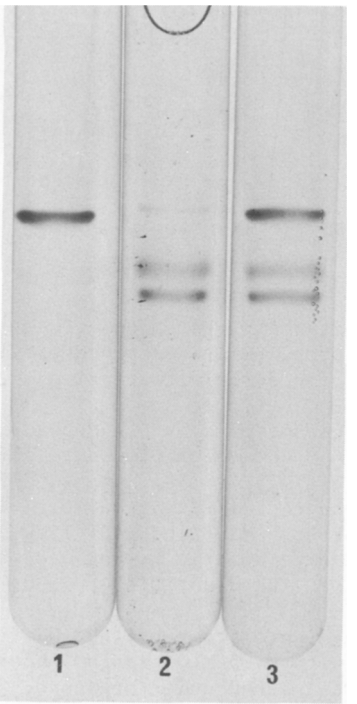

Figure 2. Separation of NAD- and NADP-linked GA3PDHs by polyacrylamide gel electrophoresis. A crude, cell-free extract from S. salivarius SS 2 (200 μg protein) was applied to the top of polyacrylamide gels prepared in 6 X 100 mm glass tubes according to the pH 9.3 system of Ornstein and Davis (20) as described in the Buchler Instruments Polyanalyst Manual. After electrophoresis was completed, the gels were stained for GA3PDH activity with the following system: Tris-HCl buffer, pH 8.5, 100 μmoles/ml; NAD and/or NADP, 2.0 μmoles/ml; GA3P 2.0 μmoles/ml; nitroblue tetrazolium chloride, 0.8 μmole/ml; phenazine methosulfate, 0.15 μmoles/ml. The gels were incubated in the staining solution at 25° C for 30 min. The pyridine nucleotide content of the reaction mixtures as numbered in the Figure above were as follows: 1, NADP; 2, NAD; 3 NAD + NADP.

of the two NAD-linked activities is currently under investigation.

The observation that cariogenic streptococci possess both an NAD- and an NADP-linked GA3PDH are significant since this is the first report of two separable and constitutive GA3PDHs with different coenzyme specificities from a bacterial source. The occurrence of multiple GA3PDHs with different coenzyme specificities has previously been reported in higher plants (10) and in certain photosynthetic microorganisms such as Euglena (11,12,13) and green algae (14). However, photosynthetic bacteria have been shown to possess only an NAD-linked enzyme (12,15). In addition, GA3PDHs which are active with both NAD and NADP have been found in blue-green algae (16), Alicaligenes faecalis (17), and Acetobacter xylinum (18), but these activities, where critically studied, have been found to be due to a single enzyme.

The existence of both NAD- and NADP-linked GA3PDHs in S. mutans 6715-9 and in S. salivarius SS 2 raises an important question concerning metabolic control. Under anaerobic growth conditions, these organisms are homofermentative and convert about 90% of the glucose carbon to lactic acid (19). Since the lactate dehydrogenase in these organisms is NAD specific (A. T. Brown, unpublished observation) and no transhydrogenase is present, the percentage of glucose carbon which is utlimately oxidized by each of the two GA3PDHs must be under stringent cellular control. The presence of an NADP-linked GA3PDH in all of the cariogenic streptococci examined thus far might also prove to be a biochemical characteristic useful in distinguishing caries-active from noncaries-active oral streptococci.

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