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Regulation of Lactate Dehydrogenase and Change of Fermentation Products in Streptococci

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Steptococcus mutans JC 2 produced mainly lactate as a fermentation product when grown in nitrogen-limited continuous culture in the presence of an excess of glucose and produced formate, acetate, and ethanol, but no lactate, under glucose-limited conditions. The levels of lactate dehydrogenase (LDH) in these cultures were of the same order of magnitude, and the activity of LDH was completely dependent on fructose-1,6-diphosphate (FDP). The intracellular level of FDP was high and the level of phosphoenolpyruvate (PEP) was low under the glucose-excess conditions. In the glucose-limited cultures, all glycolytic intermediates studied, except PEP, were low. *S. mutans* FIL, which had an FDP-independent LDH and similar levels of glycolytic intermediates as *S. mutans* JC 2, produced mainly lactate under glucose-excess or under glucose-limited conditions. LDH of *Streptococcus bovis* ATCC 9809 was dependent on FDP for activity at a low concentration of pyruvate but had a significant activity without FDP at a high concentration of pyruvate. This strain also produced mainly lactate both under glucose-excess and glucose-limited conditions. The levels and characteristics of these LDHs were not changed by the culture conditions. These results indicate that changes in the intracellular level of FDP regulate LDH activity, which in turn influences the type of fermentation products produced by streptococci. PEP, adenosine 5'-monophosphate, adenosine 5'-diphosphate, and inorganic phosphate significantly inhibited LDH activity from *S. mutans* JC 2 and may also participate in the regulation of LDH activity in other streptococci.

Wolin (22) demonstrated that lactate dehydrogenase (LDH) from some streptococci has an absolute and specific requirement for fructose-1,6-diphosphate (FDP) for catalytic activity. Similar LDHs have also been found in other streptococci (3, 20), in all species of *Bifidobacterium* (11, 17, 19), in *Lactobacillus casei* (11, 18), and in *Lactobacillus xylosus* (16). The LDH of *L. casei* and *L. xylosus* also require manganese ions for catalytic activity. LDH from group N streptococci (10, 14) and *Acholeplasma laidlawii* (15) are also activated by FDP, although they may, under specific conditions, have a significant activity in the absence of FDP.

It was suggested (2, 21, 22) that this regulation of LDH activity by FDP could potentially control overall glycolysis in the homolactic acid bacteria. It has been shown that some of these bacteria also produce fermentation products other than lactate. In glucose-limited continuous culture, *L. casei* produced mainly lactate at a high dilution rate and produced formate, ethanol, and acetate at a low dilution rate (18). Carlsson and Griffith (7) found that *Streptococcus mutans* JC 2 and *Streptococcus sanguis* NCTC 10904 produced mainly lactate under nitrogen-limited conditions in the presence of excess glucose and produced formate, acetate and ethanol when glucose was limited. In *Streptococcus salivarius* ATCC 13419 and *Streptococcus bovis* ATCC 9809 the fermentation products were lactate, and formate, acetate, and ethanol under glucose-limited conditions. The yield of bacterial mass per mole of glucose utilized was significantly higher during glucose-limited than during nitrogen-limited growth. These findings suggest that the regulation of LDH activity by FDP controls only pyruvate conversion into fermentation products and not overall glycolysis (7, 18). This implies that the intracellular pool of FDP or the synthesis of enzymes involved in the conversion of pyruvate changes with the growth conditions of these organisms.

The purpose of this investigation was to determine the fermentation products, the intra-

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cellular pool of glycolytic intermediates, and the level and characteristics of LDH of *S. mutans* JC2 in continuous culture during glucose-limited and glucose-excess conditions. These parameters were also studied in *S. bovis* ATCC 9809 and in *S. mutans* FIL.

MATERIALS AND METHODS

Microorganisms. *S. mutans*, strain JC 2 (4) and strain FIL, and *S. bovis*, American Type Culture Collection (ATCC) strain 9809, were used. *S. mutans* FIL was from a culture designated E 49 (8) and had an FDP-independent LDH (Yamada, Endo, and Araya, unpublished data). Strain E 49, studied by Wittenberger et al. (21), and strain E 49, kindly supplied by D. Bratthall, University of Göteborg, did not have this FDP-independent LDH.

Chemicals. Casamino Acids, tryptose, mitis salivarius agar, and yeast extract were obtained from Difco Laboratories, Detroit, Mich. FDP tetracyclohexylammonium salt, phosphoenolpyruvate (PEP) tricyclohexylammonium and potassium salts, disodium glucose-6-phosphate, adenosine 5-diphosphate (ADP), adenosine 5'-monophosphate (AMP), 3',5'-cyclic AMP, nicotinamide adenine dinucleotide (NAD), glycerate-2-phosphate, glycerate-3-phosphate, L-lactate, glucose-6-phosphate dehydrogenase (grade II, EC 1.1.1.49), glucosephosphate isomerase (EC 5.3.1.9), fructose-biphosphate aldolase (EC 4.1.2.13), glycerol-3-phosphate dehydrogenase (NAD^+, EC 1.1.1.8), triosephosphate isomerase (yeast, EC 5.3.1.1), pyruvate kinase (EC 2.7.1.40), and L-lactate dehydrogenase (rabbit muscle, EC 1.1.1.27) were obtained from Boehringer, Mannheim GmbH, Germany. Chloramphenicol, reduced NAD (NADH), NAD, adenosine 5'-triphosphate, and guanosine 5'-tetraphosphate tris(hydroxymethyl)aminomethane salt were from Sigma Chemical Co., St. Louis, Mo. Chromosorb 101, 80/100 mesh, was obtained from Johns-Manville, Denver, Colo. Polypropylene glycol 2025 was obtained from British Drug Houses Ltd., U.K.

Culture media. The media for the continuous cultures were prepared as described by Griffith and Carlsson (9). The glucose-limited medium contained 15 mM D-glucose and 900 ml of tryptose, yeast extract, and Casamino Acids solution/liter. The glucose-excess medium contained 55 mM D-glucose and 75 ml of the tryptose-yeast extract-Casamino Acids solution/liter. The medium for the batch culture was prepared as described for the glucose-limited medium but contained 0.1 M potassium phosphate buffer, pH 7.0, and 55 mM glucose.

Culture conditions. The conditions for continuous culture were similar to those described by Carlsson and Griffith (7). The working volumes of fermenters (FG-500, Biotec AB, Sweden) were 220 and 240 ml, respectively. The media were delivered by peristaltic pumps at a dilution rate of 0.120 ± 0.005 per h. The temperature was kept at 37 C, and the pH was continuously adjusted to 7.0 with 2 N KOH. Carbon dioxide gas, sterilized by filtration, was delivered through the main top joint of the fermenters at a rate of 50 ml/h. The medium for the batch culture was stored in an anaerobic glove box for more than 24 h before use. Batch cultures were carried out at 37 C in the anaerobic box. Cultures were checked for purity by growing them aerobically and anaerobically on blood agar and mitis salivarius agar plates.

Anaerobic glove box. An anaerobic glove box similar to that described by Aranki et al. (1) was made of welded sheet iron. The box had four glove ports, a perpex window, a cold (4 C) and warm (37 C) chamber, and a working space at room temperature. The atmosphere in the box contained 85% N_2, 10% H_2, and 5% CO_2 and was continuously circulated through a catalyst of palladium-coated aluminum pellets (type D, Englehard Industries, U.K.). The humidity was kept at 20 to 30% saturation by condensation of water vapor on the cooling plate in the cold chamber. A 0.1% (wt/vol) solution of benzyl viologen in 50 mM potassium phosphate buffer, pH 7.0, was reduced when stored in the box.

Anaerobic procedures. The bacterial cells and enzyme preparations were protected from oxygen during various phases of this work. Test solutions were prepared in the anaerobic box from water stored in the box for at least 24 h. Buffer solutions were prepared outside the box and stored in the box for more than 18 h before use. When cells were washed, they were suspended in buffer and placed in tubes with tight-fitting stoppers before being brought out of the box for centrifugation. The tubes were always opened in the box. For disintegration cells were suspended in buffer, together with glass beads, and brought out from the box in a tightly stoppered shaking bottle.

After disintegration the contents of the bottle were transferred into centrifuge tubes in the box. All reagents for photometry were mixed in a quartz cuvette with a side arm (Thunberg tube Hellma no. 191-OS, Beckman Instruments, Münich) in the box. The Thunberg tubes were then tightly stoppered before being taken out to the photometer.

Assay of glycolytic intermediates. The streptococci in 100 ml of continuous cultures were removed from the fermenter under CO_2 gas into a centrifuge tube cooled in an ice bath. The tube was then tightly capped and centrifuged at $20,000 \times g$ for 5 min at 4 C. Glycolytic intermediates in the cells were then extracted with 2.5 ml of 7.5% (wt/vol) perchloric acid for 30 min at 0 C. The residue of the cells was removed by centrifuging at $30,000 \times g$ for 10 min at 4 C. Then 0.5 ml of 1 M triethanolamine hydrochloride was added to the supernatant fluid, and the pH was adjusted at 0 C to 7.4 with 5 M K_2CO_3. Glycolytic intermediates in the extracts were measured within 1 h after neutralization according to Minakami et al. (13).

The assay mixture for glucose-6-phosphate and fructose-6-phosphate contained the extract and 0.5 mg of NAD phosphate in 1.0 ml. Extinction at 340 nm of the mixture was measured with a double beam spectrophotometer equipped with a recorder, 5 μl of 1 mg of glucose-6-phosphate dehydrogenase solution per ml was added, and the change in extinction at 340 nm was followed. After the termination of the reaction, 5 μl of 2 mg of glucosephosphate isomerase solution per ml was added. The quantities of glucose-6-phosphate and fructose-6-phosphate were calcu-

lated from the extinction attained at 340 nm, after the addition of the enzyme, using a millimolar extinction coefficient of 6.2 for NADH phosphate.

Dihydroacetone phosphate, glyceraldehyde-3-phosphate, and FDP were assayed in a reaction mixture containing the extract, 0.1 mg of NADH, and 5 μmol of Na_2 ethylenediaminetetraacetic acid, pH 7.4, in 1.0 ml. When less than 0.5 ml of the extract was used, 0.1 M triethanolamine hydrochloride buffer, pH 7.4, was added. Glycerol-3-phosphate dehydrogenase (NAD^+), triosephosphate isomerase, and fructose-biphosphate aldolase were added to the reaction mixture in that order. Oxidation of NADH was measured spectrophotometrically at 340 nm, and the amounts of the intermediates were calculated using a millimolar extinction coefficient of 6.2 for NADH at 340 nm.

Reaction mixture for assay of PEP contained the extract, 0.1 mg of NADH, 0.5 mg of ADP, and 50 μmol of $MgCl_2$ in 1.0 ml. L-Lactate dehydrogenase and pyruvate kinase were added in that order, and the amount of PEP was calculated.

Preparation of cell-free extract. The bacterial cells and cell-free extracts were protected from oxygen during all procedures. Streptococci in 100 ml of continuous culture were removed from the fermenter under CO_2 gas, harvested by centrifugation at 20,000 × *g* for 5 min at 4 C, washed twice with 0.04 M potassium phosphate buffer, pH 6.8, and suspended in 3 ml of the same buffer with one drop of polypropylene glycol. Then 2.5 ml of glass beads (0.10 to 0.11 mm) was added, and the cells were disintegrated for 3 min in a homogenizer (type MSK, B. Braun, Melsungen, Germany) under CO_2 cooling. Streptococci in 500 ml of exponentially growing culture were harvested by centrifugation. The cells were washed and then disintegrated in 10 ml of buffer with one drop of polypropylene glycol and 8 ml of glass beads. Cell debris was removed by centrifugation at 40,000 × *g* for 60 min at 4 C. The supernatant fluid was designated as the cell-free extract.

Assay of LDH activity. LDH of *S. mutans* JC 2 was partially purified during purification of pyruvate kinase (24). Since the purified enzyme was very unstable, LDH activity was studied in the cell-free extracts under anaerobic conditions. The enzyme in the extracts remained stable for at least 2 weeks when stored under anaerobic conditions at −80 C. The standard assay system contained cell-free extract: 200 μmol of tris(hydroxymethyl)aminomethane-hydrochloride buffer, pH 7.0; 0.33 μmol of NADH; 1 μmol of FDP tetracyclohexylammonium salt; and 100 μmol of sodium pyruvate in 3 ml. The reaction was started by adding cell-free extract from the side arm in the Thunberg tube and was run at 25 C. Oxidation of NADH was estimated from the decrease in extinction at 340 nm. One unit of LDH was defined as the amount of enzyme which oxidized 1 μmol of NADH/min. The level of LDH activity in the cell-free extract was determined by testing the activity of four different concentrations of the cell-free extract. The enzyme activity was always proportional to the concentration of enzyme.

Assay of alcohol dehydrogenase. The activity was estimated as described by Brown and Patterson (2). The reaction mixture contained 0.1 M glycine-NaOH buffer (pH 10.5), 2 mM NAD, 0.1 M ethanol, and cell-free extract. The reaction was run at 25 C under anaerobic conditions.

Glucose fermentation by resting cells. *S. mutans* JC 2 in 100 ml of glucose-limited continuous culture was harvested under CO_2 gas by centrifuging together with chloramphenicol (0.1 g/liter) at 20,000 × *g* for 10 min at 4 C. The cells were washed twice in 0.04 M potassium phosphate buffer, pH 6.8, which contained 0.1 g of chloramphenicol per liter, and resuspended in the same buffer. The resting cell suspension contained 500 μmol of potassium phosphate buffer (pH 7.0), 20 μmol of $MgCl_2$, 0.5 mg of chloramphenicol, and cells (5 mg dry weight) in 4.8 ml. After preincubation for 10 min at 37 C, the reaction was started by adding 0.2 ml of 0.2 M D-glucose. In the control, 0.2 ml of water was added instead of D-glucose. The reaction was run at 37 C for 15 and 30 min under constant stirring and was stopped by adding 0.6 ml of freshly prepared 25% (wt/vol) metaphosphoric acid. All these procedures were carried out under anaerobic conditions. After centrifugation the supernatant fluid was analyzed for fermentation products.

Analysis of fermentation products. To the supernatant fluid of culture used for dry weight determination, one-ninth volume of freshly prepared 25% (wt/vol) metaphosphoric acid was added. The fermentation products, except formic acid, were determined by injecting 0.5 μl of this solution into a gas chromatograph (model 5751 G, Hewlett-Packard GmbH, Wintemburg, Germany) with a hydrogen flame detector, a 1-mV span recorder, and a digital integrator (3373 B, Hewlett-Packard). A coiled glass column (approximately 1.8 m by 2 mm inner diameter) packed with a porous polymer (Chromosorb 101) was run isothermally at 200 C, with the inlet at 200 C and the detector at 240 C (6). Isovaleric acid was the internal standard. For analysis of formic acid, 3 μl of the acidified supernatant fluid was injected into a gas chromatograph (model 5710 A, Hewlett-Packard) with thermal conductivity detectors. Coiled glass columns (approximately 1.9 m by 3 mm inner diameter) packed with Chromosorb 101 were run isothermally at 165 C, with the inlet at 165 C and the detector at 250 C. No internal standard was used. Standard curves relating peak area to concentration were obtained by injecting 0.5- and 3-μl portions of standard solutions in metaphosphoric acid.

Other analytical methods. The protein concentration of the cell-free extract was measured by the method of Lowry et al. (12). Dry weight of the cells was determined as described by Carlsson (5).

RESULTS

Intracellular level of glycolytic intermediates and LDH activity in S. mutans JC 2 grown in continuous culture. *S. mutans* JC 2 produced mainly lactate during steady-state growth in the presence of an excess of glucose. FDP was predominant among the glycolytic intermediates studied (Fig. 1). Under glucose limitation formate, acetate, and ethanol, but no lactate, were produced (Fig. 1). The intracellular level of PEP was higher and the level of all

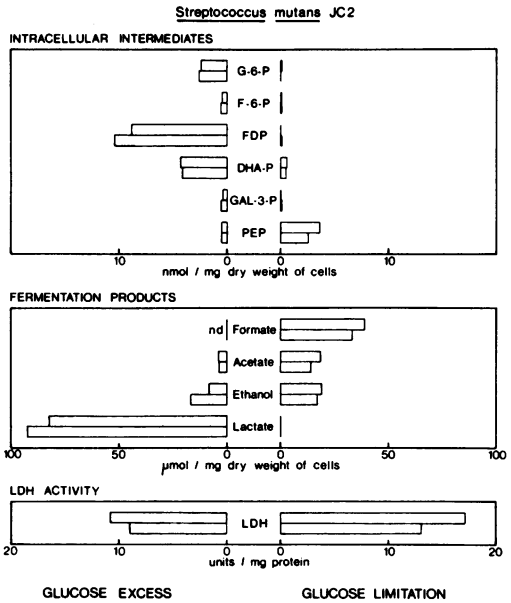

FIG. 1. *Intracellular glycolytic intermediates, fermentation products, and LDH activity of S. mutans JC2 grown under glucose excess and glucose limitation. Abbreviations: G-6-P, glucose-6-phosphate; F-6-P, fructose-6-phosphate; DHA-P, dihydroxyacetone phosphate; GAL-3-P, glyceraldehyde-3-phosphate; nd, not determined. Data from two independent experiments are shown. LDH activity was assayed by the standard assay system.*

other intermediates was lower under glucose limitation than under glucose excess. Determination of the intracellular level of pyruvate was not possible because pyruvate was present as a fermentation product in the medium. However, the amount of pyruvate was less than 1% of the other fermentation products.

The level of LDH activity in *S. mutans* JC 2 was about the same in cells grown under glucose limitation and under glucose excess (Fig. 1).

Fermentation products from resting cells of S. mutans JC 2. Cells washed after growth in glucose-limited continuous culture and incubated in a buffered glucose solution in the presence of chloramphenicol produced mainly lactate (Fig. 2). Lactate was also the main extracellular product of glucose fermentation when chloramphenicol was omitted. When glucose was omitted from the cell suspension, no fermentation products were formed.

Intracellular levels of glycolytic intermediates and LDH activity in S. bovis ATCC 9809 and S. mutans FIL. *S. bovis* 9809 formed mainly lactate when grown under glucose limitation or glucose excess. However, the changes in the intracellular pool of the glycolytic intermediates under these conditions were similar to those found in *S. mutans* JC 2 (Fig. 3). The level of LDH activity in *S. bovis* grown under glucose excess and glucose limitation was of the same order of magnitude (Fig. 3).

S. mutans FIL, which has an FDP-independent LDH, produced mainly lactate under conditions of glucose excess or glucose limitation. The changes in levels of glycolytic intermediates and levels of LDH activity were similar to those in *S. mutans* JC 2 and *S. bovis* ATCC 9809 (Fig. 4).

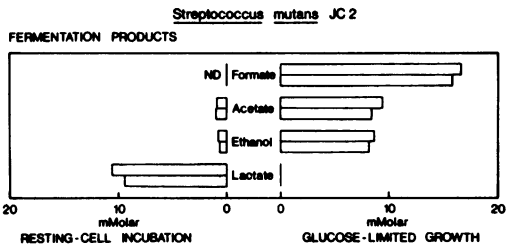

FIG. 2. *Fermentation products of S. mutans JC 2 when grown in continuous culture under glucose limitation (glucose-limited growth) and of washed cells from such a culture when incubated in buffered glucose solution (resting-cell incubation). Data from two independent experiments are shown. The figure shows the concentration of fermentation products in the resting-cell suspension after 30 min of incubation. ND, Not determined.*

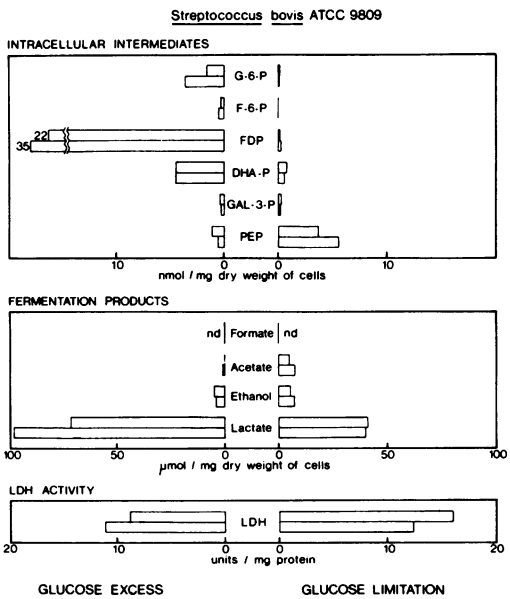

FIG. 3. *Intracellular glycolytic intermediates fermentation products and LDH activity of S. bovis 9809 grown under glucose excess and glucose limitation. Symbols are the same as those in Fig. 1. LDH activity was assayed by the standard assay system.*

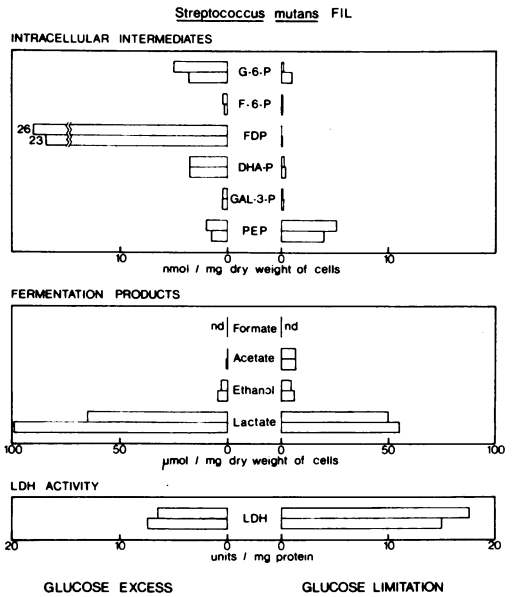

FIG. 4. *Intracellular glycolytic intermediates, fermentation products, and LDH activity of S. mutans FIL grown under glucose excess and glucose limitation. Symbols are the same as those in Fig. 1. LDH activity was assayed by the standard assay system without FDP.*

Characteristics of LDH. *S. mutans* JC 2 had an LDH dependent on FDP. At a high concentration (66.8 mM) of phosphate (Fig. 5), the concentration for half saturation for FDP was 1.5×10^{-3} M and was similar to that reported for LDH of other strains of *S. mutans* tested under similar conditions (2, 21). The concentration for half saturation for FDP has previously only been estimated in the presence of a high concentration of phosphate (2). In the presence of a low concentration (0.3 mM) of phosphate (Fig. 6), the LDH of *S. mutans* JC 2 had a half-saturation concentration value for FDP of 6.7×10^{-5} M.

The effect of FDP on LDH activity was also a function of pyruvate concentration. The half-saturating concentration for pyruvate was 6.7×10^{-3} M in the presence of 0.33 mM FDP and 1.4×10^{-2} M in the presence of 0.03 mM FDP. The effect of some glycolytic intermediates and phosphate compounds on the LDH activity is shown in Table 1. PEP, AMP, and ADP were strong inhibitors of LDH activity. The inhibition by PEP was especially effective at a low concentration of FDP (Fig. 6). The optimal pH of LDH from *S. mutans* JC 2 was about 6.2. At pH 7.0 the activity was reduced about 10%.

To clarify the differences among LDH in *S. mutans* JC 2, *S. mutans* FIL, and *S. bovis* 9809,

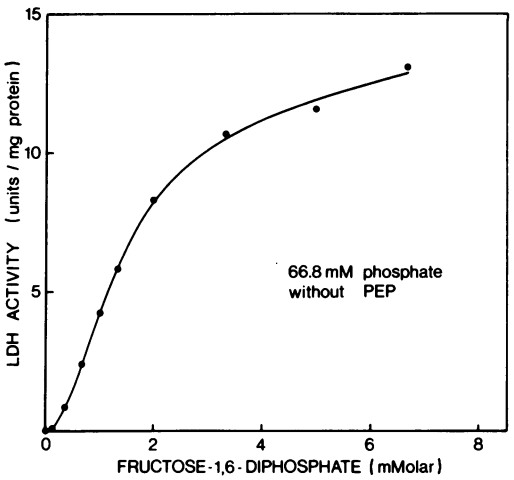

FIG. 5. *Effect of FDP concentration on the activity of LDH from S. mutans JC 2 at a high concentration of phosphate. The standard assay system was used and tris(hydroxymethyl)aminomethane-hydrochloride buffer was replaced by 200 µmol of potassium phosphate buffer, pH 7.0.*

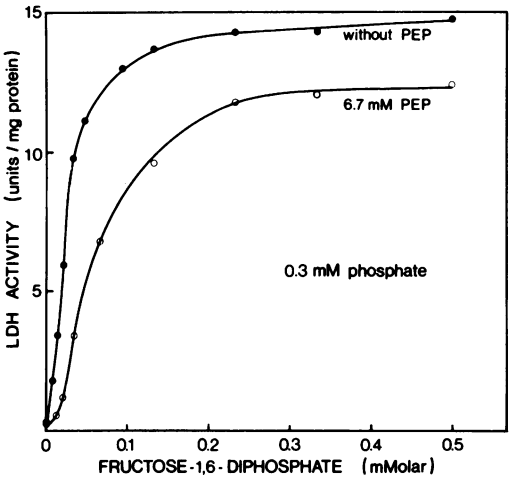

FIG. 6 *Effect of FDP concentration on the activity of LDH from S. mutans JC 2 at a low concentration of phosphate. The standard assay system was used. PEP was present in one series of experiments as indicated.*

the LDH activity was assayed under four different conditions (Table 2). The LDH of *S. mutans* JC 2 had no activity without FDP (Lo and Ho, Table 2). The LDH of *S. bovis* ATCC 9809 had a significant activity in the absence of FDP at a high (33 mM) concentration of pyruvate (Ho, Table 2). At this concentration of pyruvate, the LDH of *S. bovis* was maximally activated by 1.3 µM FDP. At a low concentration of pyruvate, the LDH of *S. bovis* was highly dependent on FDP for catalytic activity (Lf and Lo, Table

TABLE 1. *Effects of various substances on the activity of LDH from S. mutans JC 2*[a]

Additions	Activity (%)
None	100
Potassium phosphate	80
Glucose-6-phosphate	86
Fructose-6-phosphate	61
Glycerate-3-phosphate	79
Glycerate-2-phosphate	45
PEP tricyclohexylammonium salt	30
PEP potassium salt	30
Glycerol-2-phosphate	50
ATP	55
ADP	27
AMP	13
3',5'-cyclic AMP	51
Guanosine tetraphosphate	84

[a] The concentration of added substances was 6.7 mM. The standard assay system was used, but the concentration of FDP was 0.033 mM. After assaying the activity, the pH of the reaction mixture was measured. The range of pH values was 6.95 to 7.05. ATP, Adenosine 5'-triphosphate.

2). LDH of *S. mutans* FIL was active in the absence of FDP even at low pyruvate concentration (Lo). FDP did not activate this enzyme. The characteristics of LDH from these three strains were not influenced by the growth conditions (Table 2).

Alcohol dehydrogenase activity. Little alcohol dehydrogenase (EC 1.1.1.1) activity was found in *S. mutans* JC 2 grown both under glucose-limited and glucose-excess conditions, although the cells produced a lot of ethanol during their glucose-limited growth.

DISCUSSION

S. mutans JC 2 produced different fermentation products when grown under glucose limitation or glucose excess. The difference in culture conditions may influence the synthesis or activity of the enzymes involved in the conversion of pyruvate to fermentation products. A similar level of LDH activity was found in steady-state cultures of *S. mutans* JC 2 grown under glucose limitation and glucose excess (Fig. 1) but no difference in the characteristics of LDH from these cultures could be detected (Table 2). In addition, washed cells from glucose-limited cultures produced mainly lactate when incubated in a buffer containing a high concentration of glucose (Fig. 2). These results rule out the possibility that the change of fermentation products results merely from a regulation of the synthesis of enzymes involved in the conversion of pyruvate into fermentation products.

The difference in intracellular levels of FDP in glucose excess and in glucose limitation (Fig. 1) appears to be the main reason for the change of fermentation products in *S. mutans* JC 2. LDH of *S. mutans* JC 2 was absolutely dependent on FDP for activity (Fig. 5 and 6), and the intracellular level of FDP was very low under glucose limitation. *S. mutans* FIL, which has a LDH independent of FDP, had similar levels of glycolytic intermediates as *S. mutans* JC 2 and produced lactate under glucose excess or under glucose limitation.

The effect of FDP as a modulator of LDH activity from *S. mutans* JC 2 is also influenced by other intracellular constituents. At a high concentration of phosphate, a much higher concentration of FDP was required to activate this LDH than at a low concentration of phosphate (Fig. 5 and 6). The half-saturation concentration of FDP was almost 100 times higher at high (66.8 mM) than at low (0.3 mM) concentrations of phosphate. In addition pyruvate modified the effect of FDP. At a low concentration of pyruvate, FDP was much less effective in activating the LDH of *S. mutans* JC 2 than at a high concentration of pyruvate.

In contrast to other intermediates studied, the intracellular level of PEP was higher under glucose limitation than under glucose excess

TABLE 2. *Relative activity of LDH in cell-free extracts of streptococci grown in continuous culture under nitrogen limitation in an excess of glucose and under glucose limitation*

Growth conditions	Assay systems[a]	Relative activity (%) of: *S. mutans* JC 2		*S. bovis* ATCC 9809		*S. mutans* FIL	
		Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
Glucose excess	Hf	100	100	100	100	100	100
	Lf	45	40	86	75	76	75
	Lo	0	0	11	7	78	76
	Ho	0	0	71	59	120	125
Glucose limitation	Hf	100	100	100	100	100	100
	Lf	52	47	94	87	79	77
	Lo	0	0	4	4	82	81
	Ho	0	0	69	69	122	126

[a] Other components of the assay systems were the same as in the standard assay system. Hf, High pyruvate concentration (33 mM) with optimal concentration of FDP (0.33 mM); Lf, low concentration of pyruvate (3.3 mM) with optimal concentration of FDP (0.33 mM); Lo, low pyruvate concentration (3.3 mM) with no FDP; Ho, high pyruvate concentration (33 mM) with no FDP.

(Fig. 1), and PEP was a relatively strong inhibitor of the LDH activity in *S. mutans* JC 2 (Table 1). The inhibition of LDH activity by PEP was especially pronounced at low concentration of FDP (Fig. 6). This could mean that PEP has a significant effect on the LDH activity in glucose-limited cultures. AMP and ADP inhibited LDH activity (Table 1) and this may also be important for regulating pyruvate conversion in streptococci. A change in the conversion of pyruvate to formate, acetate, and ethanol instead of lactate may provide the organism with more adenosine 5'-triphosphate (7). Thus, the activity of the FDP-dependent LDH of *S. mutans* JC 2 seems to be regulated by changing the intracellular level of FDP. The effect of FDP may also be significantly modified by the intracellular levels of PEP, pyruvate, AMP, ADP, and inorganic phosphate.

The presence of two types of LDH in *S. bovis* ATCC 9809, one FDP-dependent and the other FDP-independent, cannot be excluded. However, the characteristics of LDH in cell-free extracts of *S. bovis* are similar to those of a partially purified LDH from *A. laidlawii* (15). Both LDHs are activated by FDP at high pyruvate concentrations and are highly dependent on FDP for activity at low pyruvate concentrations (Table 2).

The characteristics of LDH activity in *S. mutans* FIL and *S. bovis* ATCC 9809 are unique among those streptococci studied, whereas LDHs which are dependent on FDP for activity as LDH of *S. mutans* JC 2 have been found in many streptococci (2, 20–22). The conversion of pyruvate into fermentation products is probably regulated in the same way in these latter streptococci as in *S. mutans* JC 2.

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LITERATURE CITED

1. Aranki, A., S. A. Syed, E. B. Kenney, and R. Freter. 1969. Isolation of anaerobic bacteria from human gingiva and mouse cecum by means of a simplified glove box procedure. Appl. Microbiol. **17**:568–576.
2. Brown, A. T., and C. E. Patterson. 1973. Ethanol production and alcohol dehydrogenase activity in *Streptococcus mutans*. Arch. Oral Biol. **18**:127–131.
3. Brown, A. T., and C. L. Wittenberger. 1972. Fructose-1,6-diphosphate-dependent lactate dehydrogenase from a cariogenic streptococcus: purification and regulatory properties. J. Bacteriol. **110**:604–615.
4. Carlsson, J. 1968. A numerical taxonomic study of human oral streptococci. Odontol. Revy **19**:137–160.
5. Carlsson, J. 1971. Nutritional requirements of *Streptococcus salivarius*. J. Gen. Microbiol. **67**:69–76.
6. Carlsson, J. 1973. Simplified gas chromatographic procedure for identification of bacterial metabolic products. Appl. Microbiol. **25**:287–289.
7. Carlsson, J., and C. J. Griffith. 1974. Fermentation products and bacterial yields in glucose-limited and nitrogen-limited cultures of streptococci. Arch. Oral Biol. **19**:1105–1109.
8. Fitzgerald, R. J., and P. H. Keyes. 1960. Demonstration of the etiologic role of streptococci in experimental caries in the hamster. J. Am. Dent. Assoc. **61**:9–13.
9. Griffith, C. J., and J. Carlsson. 1974. Mechanism of ammonia assimilation in streptococci. J. Gen. Microbiol. **82**:253–260.
10. Jonas, H. A., R. F. Anders, and G. R. Jago. 1972. Factors affecting the activity of the lactate dehydrogenase of *Streptococcus cremoris*. J. Bacteriol. **111**:397–403.
11. Kandler, O., and E. Lauer. 1974. Neuere Vorstellungen zur Taxonomie der Bifidobakteren. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. I Orig. Reihe A **228**:29–45.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
13. Minakami, S., C. Suzuki, T. Saito, and H. Yoshikawa. 1965. Studies on erythrocyte glycolysis. I. Determination of the glycolytic intermediates in human erythrocytes. J. Biochem. **58**:543–550.
14. Mou, L., D. P. Mulvena, H. A. Jonas, and G. R. Jago. 1972. Purification and properties of nicotinamide adenine dinucleotide-dependent D- and L-lactate dehydrogenase in a group N streptococcus. J. Bacteriol. **111**:392–396.
15. Neimark, H., and M. C. Tung. 1973. Properties of a fructose-1,6-diphosphate-activated lactate dehydrogenase from *Acholeplasma laidlawii* type A. J. Bacteriol. **114**:1025–1033.
16. Rogosa, M. 1974. *Lactobacillus*, p. 576–593. *In* R. E. Buchanan and N. E. Gibbons (ed.), Bergey's manual of determinative bacteriology, 8th ed. The Williams & Wilkins Company, Baltimore.
17. de Vries, W., S. J. Gergrandy, and A. H. Stouthamer. 1967. Carbohydrate metabolism in *Bifidobacterium bifidum*. Biochim. Biophys. Acta **136**:415–425.
18. de Vries, W., W. M. C. Kapteijn, E. G. van der Beek, and A. H. Stouthamer. 1970. Molar growth yields and fermentation balances of *Lactobacillus casei* L3 in batch cultures and in continuous cultures. J. Gen. Microbiol. **63**:333–345.
19. de Vries, W., and A. H. Stouthamer. 1968. Fermentation of glucose, lactose, galactose, mannitol, and xylose by bifidobacteria. J. Bacteriol. **96**:472–478.
20. Wittenberger, C. L., and N. Angelo. 1970. Purification and properties of a fructose-1,6-diphosphate-activated lactate dehydrogenase from *Streptococcus faecalis*. J. Bacteriol. **101**:717–724.
21. Wittenberger, C. L., M. P. Palumbo, R. B. Bridges, and A. T. Brown. 1971. Mechanisms for regulating the activity of constitutive glucose degradative pathways in *Streptococcus fecalis*. J. Dent. Res. **50**:1094–1103.
22. Wolin, M. J. 1964. Fructose-1,6-diphosphate requirement of streptococcal lactic dehydrogenase. Science **146**:775–777.
23. Yamada, T., and J. Carlsson. 1973. Phosphoenolpyruvate carboxylase and ammonium metabolism in oral streptococci. Arch. Oral Biol. **18**:799–812.
24. Yamada, T., and J. Carlsson. 1975. Glucose-6-phosphate-dependent pyruvate kinase in *Streptococcus mutans*. J. Bacteriol. **124**:562–563.