JOURNAL OF BACTERIOLOGY, Mar. 1982, p. 1034–1040
0021-9193/82/031034-07$02.00/0

Vol. 149, No. 3

Purification of Pyruvate Formate-Lyase from *Streptococcus mutans* and Its Regulatory Properties

SHOKO TAKAHASHI, KAZUHIKO ABBE, AND TADASHI YAMADA*

Laboratory of Oral Biochemistry, Tohoku University School of Dentistry, Sendai, 980 Japan

Received 19 August 1981/Accepted 9 November 1981

Pyruvate formate-lyase (EC 2.3.1.54) from *Streptococcus mutans* strain JC2 was purified in an anaerobic glove box, giving a single band on disk and sodium dodecyl sulfate electrophoresis. This enzyme was immediately inactivated by exposure to the air. Enzyme activity was unstable even when stored anaerobically, but the activity was restored by preincubating the inactivated crude enzyme with *S*-adenosyl-L-methionine, oxamate, and reduced ferredoxin or methylviologen. On the other hand, the purified enzyme was not reactivated. Either D-glyceraldehyde 3-phosphate or dihydroxyacetone phosphate strongly inhibited this enzyme. The inhibitory effects of these compounds were largely influenced by enzyme concentration. The inhibition by these triose phosphates in cooperation with the reactivating effect of ferredoxin and the fluctuations of both the enzyme and the triose phosphate levels may efficiently regulate the pyruvate formate-lyase activity in *S. mutans* in vivo.

When *Streptococcus mutans* is growing in an excess of glucose, the main fermentation product is lactate, but the organism produces only formate, acetate, and ethanol when growing under glucose limitation in continuous culture (4, 21). It has been suggested that the intracellular concentration of fructose 1,6-diphosphate regulates lactate dehydrogenase activity in vivo and, hence, regulates the balance of fermentation products (21). Since the first step to convert pyruvate into formate, acetate, and ethanol is catalyzed by pyruvate formate-lyase, the regulation of this enzyme is also expected to play an important role in this fermentation change in *S. mutans* (22). Recently the regulation of pyruvate formate-lyase and lactate dehydrogenase has also been suggested to be involved in the fermentation change of *Streptococcus lactis* (17) and *Lactobacillus bulgaricus* (15). Pyruvate formate-lyase catalyzes the reaction as follows: pyruvate + coenzyme A (CoA)→acetyl-CoA + formate.

The presence of pyruvate formate-lyase has been reported in several microorganisms, for example, *Escherichia coli* (9–11), *Streptococcus faecalis* (13), and clostridia (16, 20). But little information about the regulation of pyruvate formate-lyase is available because of the extreme instability of the enzyme to oxidation.

Knappe et al. (9–11) have purified the components of pyruvate formate-lyase from *E. coli* under aerobic conditions and have anaerobically reconstituted the enzyme and its activity. Lindmark et al. (13) have purified pyruvate formate-lyase from *S. faecalis* under an atmosphere of helium. Yamada and Carlsson have reported the inhibitory effect of glycolytic intermediates on pyruvate formate-lyase activity, but their studies were performed with crude extract rather than purified enzyme (22).

In the current study, we purified the pyruvate formate-lyase from *S. mutans* under anaerobic conditions and clarified the unique characteristics of this enzyme.

MATERIALS AND METHODS

Microorganism and growth conditions. *S. mutans* strain JC2 (3) was grown at 35°C for 15 h in an anaerobic glove box (21) in a medium containing 10 g of galactose, 2 g of NH_4HCO_3, 2 g of dried extract of yeast (Daigo Brand, Osaka, Japan), 100 mg of L-cysteine-hydrochloride, 200 mg of $MgSO_4 \cdot 7H_2O$, 10 mg of NaCl, 10 mg of $MnSO_4 \cdot 4H_2O$, and 10 mg of $FeSO_4 \cdot 7H_2O$ in 1 liter of 100 mM potassium phosphate buffer, pH 7.0. The cells were harvested at the logarithmic growth phase and washed three times with 40 mM potassium phosphate buffer, pH 7.0. The cells were then stored at −20°C. All these and the following procedures were carried out under strict anaerobic conditions, as described previously (21).

Assay of pyruvate formate-lyase activity. The enzyme assay was performed by modification of the method described by Knappe et al. (9). The standard assay mixture contained in 2 ml: 100 mM potassium phosphate buffer (pH 7.2), 20 mM sodium pyruvate, 0.08 mM CoA, 1 mM NAD, 6 mM sodium DL-malate, 2 mM dithiothreitol, 26 μg (2.8 U) of citrate-synthase (pig heart, EC 4.1.3.7), and 46 μg (55 U) of malate dehydrogenase (pig heart, EC 1.1.1.37).

The mixture was prepared in a quartz cuvette with a side arm (Thunberg tube T-26; Nihon Sekiei Glass Co., Tokyo, Japan) in the anaerobic glove box. The

Thunberg tubes were then tightly stoppered before being taken out of the glove box. Sodium pyruvate or the pyruvate formate-lyase preparation in the side arm was then added, and reactions were run at 35°C. The activity was estimated spectrophotometrically by recording the rate of increase in absorbance at 340 nm.

One unit of pyruvate formate-lyase was defined as the amount of the enzyme which catalyzed the formation of 1 μmol of acetyl-CoA per min.

Purification of pyruvate formate-lyase. The frozen cell paste (10 g [wet weight]) was thawed and suspended in 47 ml of 40 mM potassium phosphate buffer (pH 6.8) plus 20 mM dithiothreitol. The cells were disrupted by sonic oscillation (200 W, 2 A) for 18 min at 0°C. The cell debris was spun down at 17,500 × *g* for 30 min at 4°C, and the supernatant fluid was centrifuged again at 95,000 × *g* for 60 min at 4°C. The supernatant fluid was dialyzed overnight against 40 mM potassium phosphate buffer (pH 6.8) at 4°C in the anaerobic glove box. The dialyzed sample, designated cell-free extract, was then concentrated with Lyphogel (Gelman Sciences, Inc., Ann Arbor, Mich.). Sodium pyruvate and potassium phosphate buffer were added to the extract to produce final concentrations of 50 mM sodium pyruvate and 100 mM potassium phosphate buffer, pH 6.6. The enzyme solution (17.7 ml) was then applied to a reactive blue 2-agarose (Sigma Chemical Co., St. Louis, Mo.) column (1.7 by 11 cm) equilibrated with 50 mM sodium pyruvate in 50 mM potassium phosphate buffer, pH 6.6. The column was successively washed with 50 ml of the same buffer, with 40 ml of 50 mM sodium pyruvate in 20 mM potassium phosphate buffer (pH 6.8), with 80 ml of 20 mM potassium phosphate buffer (pH 6.8), and then with 40 ml of 40 mM potassium phosphate buffer (pH 6.8)–1 mM dithiothreitol. Pyruvate formate-lyase was then eluted with a linear gradient created by mixing 200 ml of 40 mM potassium phosphate buffer (pH 6.8)–1 mM dithiothreitol and 200 ml of 150 mM potassium phosphate buffer (pH 7.2)–1 mM dithiothreitol (Fig. 1). The fractions which eluted from 45 to 60 mM potassium phosphate were pooled. After being concentrated with Lyphogel, the enzyme preparation was placed on a reactive blue 2-agarose column (1.5 by 6.0 cm) equilibrated with 50 mM potassium phosphate buffer (pH 7.1)–1 mM dithiothreitol. The column was then eluted with the same buffer. Fractions with high activity were collected and stored at 4°C. The purified enzyme preparation was used for the study of kinetics.

Gel filtration. The molecular weight of native enzyme was estimated by gel filtration on a Sephadex G-200 column equilibrated with 40 mM potassium phosphate buffer (pH 7.0) in the anaerobic glove box. The standard proteins used for calibration were cytochrome *c* (molecular weight, 12,500), hen egg albumin (molecular weight, 45,000), aldolase (molecular weight, 158,000), and catalase (molecular weight, 240,000).

Electrophoresis of pyruvate formate-lyase. Disk electrophoresis was carried out at 5 mA per tube for 1.5 h by the method described by Davis (5) with 10% polyacrylamide gel rods.

Sodium dodecyl sulfate (SDS) electrophoresis was performed by the method of Weber and Osborn (18) with 5% polyacrylamide gel rods. The purified enzyme and standard proteins were incubated with 1% SDS and 1% 2-mercaptoethanol, at 100°C for 5 min, and they were subjected to SDS-polyacrylamide gels at 8 mA per tube for 4 h. The gel rods were stained with Coomassie brilliant blue and calibrated with molecular weight standards, trypsin inhibitor (molecular weight, 21,500), bovine serum albumin (molecular weight, 68,000), RNA-polymerase α-subunit (molecular weight, 39,000), β-subunit (molecular weight, 155,000), and β′-subunit (molecular weight, 165,000).

Homogenous preparation of pyruvate formate-lyase was cross-linked with 0.35% glutaraldehyde at room temperature for 2 h by the method of Griffith (7). The reaction was terminated by adding $NaHSO_3$ solution (final 0.5%, wt/vol), and the cross-linked enzyme was subjected to the SDS treatment as described above.

Optimum pH. The following buffers were utilized to determine the optimum pH for pyruvate formate-lyase: 100 mM potassium phosphate buffer (pH 6.6 to 7.2), 100 mM glycine-NaOH buffer (pH 6.5 to 8.3), 50 mM MOPS [3-(*N*-morpholino)propanesulfonic acid]–NaOH buffer (pH 6.1 to 7.2), and 50 mM Tris-maleate buffer (pH 5.5 to 7.3). The pH of the reaction mixture was examined immediately after the assay of the enzyme activity.

Assay of acetyl-CoA and formate formation. Formation of acetyl-CoA was estimated by the method of Decker (6), using the standard assay mixture.

The reaction mixture for the assay of formate formation was similar to the standard assay mixture, but citrate-synthase, malate dehydrogenase, and NAD were omitted, and 2 μg (2 U) of phosphate acetyltransferase (*Clostridium kluyveri*, EC 2.3.1.8) was added. The reactions were run at 35°C for 16, 26, and 36 min, respectively, and then stopped by heating to 100°C for 5 min. The formate production was determined as described by Quayle (14) with the following mixture: 40 mM potassium phosphate buffer (pH 7.2), 1 mM lithium NAD (formate free), 0.2 U of formate dehydrogenase (yeast, EC 1.2.1.2), and sample solution containing 0.05 to 0.15 mM formate.

Analysis for the formations of acetyl-CoA and for-

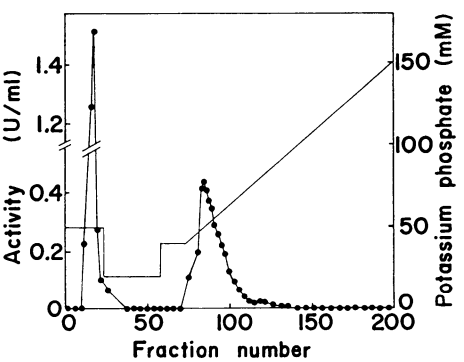

FIG. 1. Elution profile of pyruvate formate-lyase on the first reactive blue 2-agarose column chromatography. Fractions of 3.0 ml of eluate were collected. Elution was carried out as described in the text. Fractions eluted from 45 to 60 mM potassium phosphate were pooled. Symbols: ●, enzyme activity; ——, potassium phosphate buffer concentration.

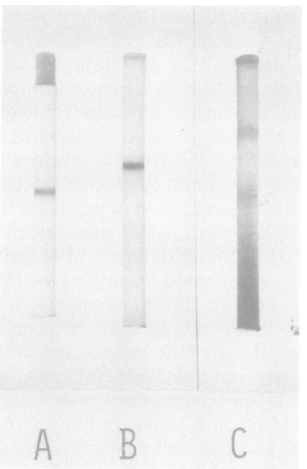

FIG. 2. Lane A, Polyacrylamide gel electrophoresis of the pyruvate formate-lyase. The small band is the running dye. Lane B, SDS-polyacrylamide gel electrophoresis of the pyruvate formate-lyase. Lane C, SDS-polyacrylamide gel electrophoresis of pyruvate formate-lyase cross-linked with 0.35% glutaraldehyde. The two bands from the top to the bottom represent dimer and monomer of the cross-linked pyruvate formate-lyase. Experimental details were given in the text.

mate was carried out simultaneously at the same temperature (35°C) and with the same concentration (0.7 μg/ml) of the purified pyruvate formate-lyase.

Reactivation of pyruvate formate-lyase. Inactivated pyruvate formate-lyase was previously incubated with the following standard reactivating mixture: 0.15 mM *S*-adenosyl-L-methionine, 10 mM sodium oxamate, 4 mM dithiothreitol, 0.4 mM $Fe(SO_4)_2(NH_4)_2$, 1 mM methylviologen, or 0.064 mg of ferredoxin per ml (*Clostridium pasteurianum*; Sigma Chemical Co.), and 100 mM potassium phosphate buffer (pH 7.2) at 30°C for 60 min in the anaerobic glove box. The pyruvate formate-lyase activity was then measured with the standard assay mixture.

Other analytical methods. The protein concentration of the crude cell-free extract was measured by the biuret method (12). The protein concentration of the purified enzyme preparation was estimated by a dye-binding method (2) with Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, Calif.). Triose phosphate isomerase activity was assayed as described by Beisenherz (1).

Chemicals. D-Glyceraldehyde 3-phosphate diethylacetal and dihydroxyacetone phosphate dimethylketal were purchased from Boehringer Mannheim GmbH, (Mannheim, West Germany). D-Glyceraldehyde 3-phosphate and dihydroxyacetone phosphate were prepared from D-glyceraldehyde 3-phosphate diethylacetal and dihydroxyacetone phosphate dimethylketal by instructions of the manufacturer. The analysis with glycerol 3-phosphate dehydrogenase (rabbit muscle, EC 1.1.1.8) and triose phosphate isomerase (rabbit muscle, EC 5.3.1.1) confirmed that no glyceraldehyde 3-phosphate was contained in the dihydroxyacetone phosphate reagent. Other reagents were obtained commercially.

RESULTS

Purification and identification of pyruvate formate-lyase. Pyruvate formate-lyase from *S. mutans* strain JC2 was purified with reactive blue 2-agarose affinity chromatography in an anaerobic glove box. During the purification procedure the enzyme activity was always separated into two peak fractions, one in the void fraction (Fig. 1). But a similar elution profile was obtained when either one of the two fractions was again subjected to the same chromatography. Thus, the enzymes in these two fractions did not seem to be isozymes.

The purified enzyme preparation gave a single band on disk and SDS electrophoresis in polyacrylamide gels (Fig. 2). The enzyme was purified overall by 25-fold, and the specific activity of the purified enzyme was 12 U/mg (Table 1). The purified pyruvate formate-lyase from *S. mutans* strain JC2 was unstable. The storage of the purified enzyme at 4°C for 2 weeks in 50 mM potassium phosphate buffer (pH 7.2) containing 1 mM dithiothreitol in the anaerobic glove box reduced the activity by 50%.

When pyruvate, CoA, malate dehydrogenase, or citrate-synthase was excluded from the standard assay mixture with the purified pyruvate formate-lyase, no NADH was produced. The activity was lost immediately by exposure to the air for a few seconds. Almost the same amount of acetyl-CoA and formate were produced

TABLE 1. Summary of the purification procedure of pyruvate formate-lyase from *S. mutans* strain JC2

Purification step	Total activity (U)	Total protein (mg)	Sp act (U/mg)	Relative purity
Cell-free extract	90.1	144.0	0.48	1
First reactive blue 2-agarose chromatography	12.0	2.8	4.29	8.9
Second reactive blue 2-agarose chromatography	6.0	0.5	12.0	25.0

through the reaction catalyzed by the purified enzyme.

Molecular weight and subunit structure. The molecular weight of the native enzyme was estimated at 170,000 to 180,000 by molecular sieving through a Sephadex G-200 column. The mobility of the dissociated protein in SDS-polyacrylamide gels corresponded to a subunit molecular weight of 90,000 (Fig. 2). Thus the pyruvate formate-lyase in a native form was considered to consist of two identical subunits. In 5% SDS-polyacrylamide gels, samples of pyruvate formate-lyase previously cross-linked with 0.35% glutaraldehyde showed two bands corresponding to monomer and dimer. The mobility of monomer thus obtained appeared to be greater than that achieved without glutaraldehyde. Griffith (7) has reported that the reduced polypeptide oligomers formed by reaction with glutaraldehyde are generally found to migrate at a rate significantly faster than is expected from their calculated molecular weight.

Effect of pH and temperature on pyruvate formate-lyase activity. The optimum pH of the activity was 7.5 in 100 mM glycine-NaOH buffer. Eighty percent of the activity was observed at pH 7.0 and 8.3, and 40% of the activity was observed at pH 6.5. It was impossible to maintain the pH of the assay mixture above 7.2 with buffers, except glycine-NaOH buffer, because of the presence of 10% carbon dioxide in the atmosphere of the anaerobic glove box. But the activity was higher in 100 mM potassium phosphate buffer (pH 7.2) than in 100 mM glycine-NaOH buffer, pH 7.5.

The pyruvate formate-lyase activity at 35°C was about 78 times that at 22°C.

Effect of substrate concentration on pyruvate formate-lyase activity. The substrate saturation curve at various pyruvate concentrations was hyperbolic. The apparent K_m for pyruvate was 5.4 mM when the reaction was initiated by the addition of pyruvate and 2.6 mM when started by the addition of the enzyme. With varying CoA, the kinetic response was also hyperbolic. The apparent K_m value (0.024 mM) for CoA was also higher when the reaction was initiated by the addition of pyruvate than it was when started by the addition of the enzyme (0.0091 mM). The maximum velocity was the same in all of these cases.

Inhibitory effect of D-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. A 1 mM concentration each of glucose 6-phosphate, glucose 1-phosphate, fructose 6-phosphate, fructose 1-phosphate, fructose 1,6-diphosphate, ribose 5-phosphate, glycerate 3-phosphate, glycerate 2-phosphate, and phosphoenolpyruvate had no significant effect on the activity of the purified enzyme. Nor did 1 mM ATP, ADP, AMP, GTP, GDP, or GMP affect the activity. The addition of 1 mM thiamine pyrophosphate did not stimulate the enzyme activity. Ten millimolar each of EDTA, KCl, NaCl, NaF, KF, $MgSO_4$, $MgCl_2$, and KBr or 1 mM $MnCl_2$ exerted no effect.

Both D-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate strongly inhibited the pyruvate formate-lyase. The concentration of D-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate required for 50% inhibition varied with the concentration of the pyruvate formate-lyase in the reaction mixtures (Fig. 3). The lower the concentration of the enzyme, the more potent was the inhibition of either D-glyceraldehyde 3-phosphate or dihydroxyacetone phosphate. The enzyme at high concentration overcame the inhibitory effect of these intermediates. The concentration of D-glyceraldehyde 3-phosphate required for 50% inhibition was about one third of that of dihydroxyacetone phosphate. Even when double amounts of malate dehydrogenase and citrate-synthase were added to the standard assay mixture in the

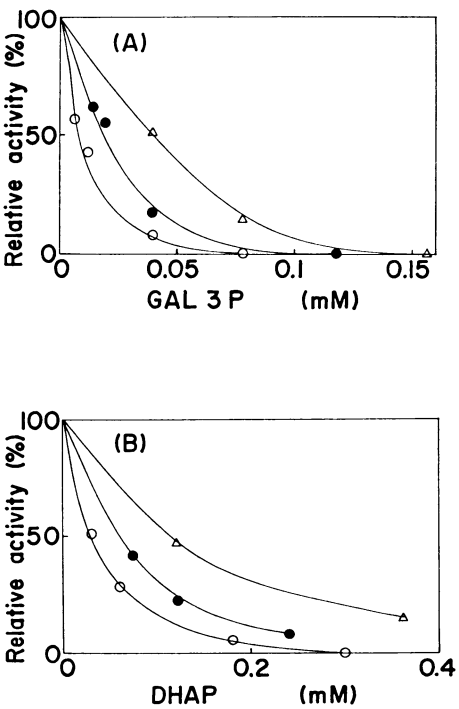

FIG. 3. (A) Inhibitory effect of D-glyceraldehyde 3-phosphate on pyruvate formate-lyase activity at different concentrations of enzyme. Symbols: ○, 0.35 μg/ml; ●, 0.7 μg/ml; and △, 1.4 μg/ml. (B) Inhibitory effect of dihydroxyacetone phosphate (DHAP) at different concentrations of enzyme. Symbols: ○, 0.35 μg/ml; ●, 0.7 μg/ml; and △, 1.4 μg/ml.

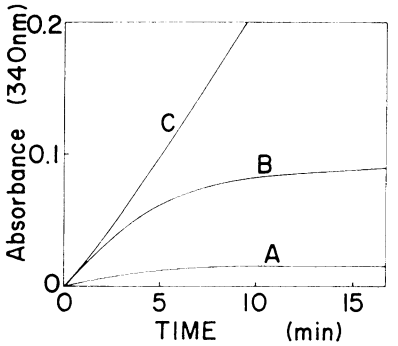

FIG. 4. Progress curves for the reactions catalyzed by pyruvate formate-lyase. Curve A, Reaction was started by the addition of pyruvate after 10 min of preincubation of the enzyme with 0.2 mM D-glyceraldehyde 3-phosphate at 35°C. Curve B, Reaction was initiated by the addition of enzyme in the mixture containing both substrates and 0.2 mM D-glyceraldehyde 3-phosphate. Curve C, The standard reaction without D-glyceraldehyde 3-phosphate. The assay mixture was the same as described in the text and contained 1.4 μg of the purified pyruvate formate-lyase.

presence of either D-glyceraldehyde 3-phosphate or dihydroxyacetone phosphate, the same magnitude of inhibitory effect was observed. The absence of triose phosphate isomerase activity in the purified enzyme preparation was confirmed.

The shape of the progress curve varied according to whether or not the inhibitor was preincubated with the enzyme (Fig. 4). Preincubation of the enzyme with D-glyceraldehyde 3-phosphate made the inhibitory effect remarkable. Without preincubation of the enzyme with D-glyceraldehyde 3-phosphate, the inhibition was initially negligible, but the enzyme activity gradually decreased. The rate at the steady state was essentially identical whether or not the enzyme was preincubated with D-glyceraldehyde 3-phosphate.

Reactivation of pyruvate formate-lyase. Even when stored in the anaerobic glove box, both the crude enzyme preparation and the purified pyruvate formate-lyase were unstable. However, part of the enzymatic activity was recovered when the inactivated crude enzyme preparation had been incubated with 0.15 mM *S*-adenosyl-L-methionine and 10 mM oxamate at 30°C for 60 min. More activity was recovered with the concomitant addition of reduced ferrodoxin (0.064 mg/ml) or methylviologen (1 mM) to the reactivating system (Table 2). However, the addition of neither ferredoxin nor methylviologen to the standard assay mixture stimulated the pyruvate formate-lyase activity. In contrast, once it had been inactivated, the purified enzyme could not be reactivated by the reactivating system described above.

DISCUSSION

Pyruvate formate-lyase from *S. mutans* strain JC2 was purified in an anaerobic glove box, giving a single band on disk and SDS-polyacrylamide gels (Fig. 2). The molecular weight of native enzyme was 170,000 to 180,000, and the enzyme was considered to consist of two identical subunits. Similar to pyruvate formate-lyase from *E. coli* (9, 10), clostridia (16, 20), and *S. faecalis* (13), the enzyme was immediately inactivated by exposure to the air.

The enzyme activity of both the crude and the purified preparation was unstable even when the preparations were stored in the anaerobic glove box. But, like pyruvate formate-lyase from *E. coli* (9–11), the enzyme activity was restored by preincubating the inactivated enzyme with *S*-adenosyl-L-methionine and oxamate. Addition of either reduced ferredoxin or methylviologen

TABLE 2. Reactivation of pyruvate formate-lyase in crude cell-free extract[a] of *S. mutans*

Treatment	Relative activity[b] (%) after storage for (days):	
	13	33
Without reactivation	6.2	0.8
After 60-min of incubation with		
Standard reactivating[c]	103.2	60.8
Methylviologen omitted	11.4	3.4
S-Adenosyl-L-methionine omitted	NT[d]	1.0
Oxamate omitted	NT	4.8
Methylviologen omitted and ferredoxin added[e]	NT	32.5

[a] The cells were disrupted by sonic oscillation for 18 min at 0°C, and the cell debris was spun down at 17,500 × *g* for 30 min. The supernatant was designated crude cell-free extract.

[b] Percentage of activity, taking the activity immediately after the preparation of the crude cell-free extract as 100. The activity was estimated with the standard assay mixture containing 40 μg of crude cell-free extract as described in the text.

[c] The standard reactivating mixture was described in the text and contained 2 mg of crude cell-free extract per ml.

[d] NT, Not tested.

[e] Methylviologen was omitted, and 0.064 mg of ferredoxin per ml was added to the reactivating mixture.

stimulated the reactivation, but the purified enzyme could not be reactivated at all. Thus *S. mutans* seems to have a reactivation system as *E. coli* has. Since the presence of ferredoxin in *S. mutans* has been reported (8), ferredoxin may be implicated in the activation of pyruvate formate-lyase in vivo.

The pyruvate formate-lyase from *S. mutans* was strongly inhibited by either D-glyceraldehyde 3-phosphate or dihydroxyacetone phosphate. Preincubation of the enzyme with D-glyceraldehyde 3-phosphate made the inhibition more prominent (Fig. 4). When the enzyme was not incubated previously with the inhibitor, the inhibition was negligible at the initial stage. But the inhibition became potent with time. The inhibitory effects of both D-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate on the pyruvate formate-lyase activity were largely influenced by the concentration of the enzyme. The higher the concentration of the enzyme, the fewer inhibitory effects were observed (Fig. 3). These findings suggest that the formation and dissociation of the enzyme-inhibitor complex might be markedly slower than the reaction between the enzyme and substrate and that these triose phosphates might be slow tight-binding inhibitors (19). But it is difficult to rule out the possibility of irreversible inhibition. Further intensive research is required to clarify the precise mechanism of this inhibition.

The inhibition of the dihydroxyacetone phosphate with the crude enzyme preparation may have been overlooked in the previous report (22), because the assay system in the previous work required a high concentration of the enzyme.

D-Glyceraldehyde 3-phosphate was a much more potent inhibitor than dihydroxyacetone phosphate. But since the intracellular concentration of dihydroxyacetone phosphate was higher than that of D-glyceraldehyde 3-phosphate (21), both inhibitors may take part in the regulation of pyruvate formate-lyase in vivo.

The level of pyruvate formate-lyase was high when *S. mutans* was grown under glucose limitation (22) or grown on galactose (Table 1). The cells produce acids other than lactic acid under these growing conditions. The inhibition by the triose phosphates may amplify the effect of the induction of pyruvate formate-lyase and the effect of the shift of the intracellular concentration of triose phosphates (21, 22), since the large amount of the enzyme diminishes the inhibitory effect of the triose phosphates and vice versa.

Thus, the inhibition by both D-glyceraldehyde 3-phosphate and dihydroxyacetone phosphate in cooperation with the reactivating effect of ferredoxin and the fluctuation of both the enzyme and the triose phosphate levels may efficiently regulate the pyruvate formate-lyase activity in *S. mutans* in vivo, and so *S. mutans* produces exclusively acids other than lactic acid under glucose limitation.

ACKNOWLEDGMENTS

This study was supported in part by a grant-in-aid for scientific research (B) no. 00548306 (1980, 1981) and a grant-in-aid for developmental scientific research (2) no. 56870097 (1981) from the Japanese Ministry of Education.

LITERATURE CITED

1. **Beisenherz, G.** 1955. Triosephosphate isomerase from calf muscle. Methods Enzymol. **1**:387–391.
2. **Bradford, M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
3. **Carlsson, J.** 1968. A numerical taxonomic study of human oral streptococci. Odontol. Revy **19**:137–160.
4. **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.
5. **Davis, B. J.** 1964. Disc electrophoresis. II. Method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**:404–427.
6. **Decker, Karl.** 1971. Acetyl-coenzyme A UV-spectrophotometric assay, p. 1988–1993. *In* Hans Ulrich Bergmeyer (ed.), Methods of enzymatic analysis, 2nd ed. Academic Press, Inc., New York.
7. **Griffith, I. P.** 1972. The effect of cross-links on the mobility of proteins in dodecyl sulphate-polyacrylamide gels. Biochem. J. **126**:553–560.
8. **Higuchi, M., M. Higuchi, and S. Araya.** 1978. Possible contribution of transferable exogenous DNA in prokaryotic evolution, p. 447–454. *In* Haruhiko Noda (ed.), Origin of life. Center for Academic Publications Japan, Tokyo.
9. **Knappe, J., H. P. Blaschkowski, P. Gröbner, and T. Schmitt.** 1974. Pyruvate formate-lyase of *Escherichia coli*: the acetyl-enzyme intermediate. Eur. J. Biochem. **50**:253–263.
10. **Knappe, J., J. Schacht, W. Möckel, Th. Höpner, H. Vetter, Jr., and R. Edenharder.** 1969. Pyruvate formate-lyase reaction in *Escherichia coli*: the enzymatic system converting an inactive form of the lyase into the catalytically active enzyme. Eur. J. Biochem. **11**:316–327.
11. **Knappe, J., and T. Schmitt.** 1976. A novel reaction of *S*-adenosyl-L-methionine correlated with the activation of pyruvate formate-lyase. Biochem. Biophys. Res. Commun. **71**:1110–1117.
12. **Layne, E.** 1957. Spectrophotometric and turbidimetric methods for measuring proteins. Methods Enzymol. **3**:447–454.
13. **Lindmark, D. G., P. Paolella, and N. P. Wood.** 1969. The pyruvate formate-lyase system of *Streptococcus faecalis*: purification and properties of the formate-pyruvate exchange enzyme. J. Biol. Chem. **244**:3605–3612.
14. **Quayle, J. R.** 1966. Formate dehydrogenase. Methods Enzymol. **9**:360–364.
15. **Rhee, S. K., and M. Y. Pack.** 1980. Effect of environmental pH on fermentation balance of *Lactobacillus bulgaricus*. J. Bacteriol. **144**:217–221.
16. **Thauer, R. K., F. H. Kirchniawy, and K. A. Jungermann.** 1972. Properties and function of the pyruvate-formate-lyase reaction in clostridiae. Eur. J. Biochem. **27**:282–290.
17. **Thomas, T. D., K. W. Turner, and V. L. Crow.** 1980. Galactose fermentation by *Streptococcus lactis* and *Streptococcus cremoris*: pathways, products, and regulation. J. Bacteriol. **144**:672–682.
18. **Weber, K., and M. Osborn.** 1969. The reliability of molecular weight determination by dodecyl sulfate-poly-

acrylamide gel elecrophoresis. J. Biol. Chem. **244**:4406–4412.
19. **Wiliams, J. W., and J. F. Morrison.** 1979. The kinetics of reversible tight-binding inhibition. Methods Enzymol. **63**:437–467.
20. **Wood, N. P., and K. Jungermann.** 1972. Inactivation of the pyruvate formate-lyase of *Clostridium butyricum*. FEBS Lett. **27**:49–52.
21. **Yamada, T., and J. Carlsson.** 1975. Regulation of lactate dehydrogenase and change of fermentation products in streptococci. J. Bacteriol. **124**:55–61.
22. **Yamada, T., and J. Carlsson.** 1976. The role of pyruvate formate-lyase in glucose metabolism of *Streptococcus mutans*, p. 809–819. *In* H. M. Stiles, W. J. Loesche, and T. C. O'Brien (ed.), Proceedings: Microbial Aspects of Dental Caries (a special supplement to Microbiology Abstracts), vol. 3. Information Retrieval, Inc., Washington, D.C.