INFECTION AND IMMUNITY, Sept. 1985, p. 674–678
0019-9567/85/090674-05$02.00/0
Copyright © 1985, American Society for Microbiology
Vol. 49, No. 3

Pyruvate Dehydrogenase Activity in *Streptococcus mutans*

JAN CARLSSON,* ULLA KUJALA, AND MAJ-BRITT K. EDLUND
Department of Oral Microbiology, University of Umeå, S-90187 Umeå, Sweden

Received 5 March 1985/Accepted 13 June 1985

***Streptococcus mutans* NCTC 10449 and *Escherichia coli* K-12 strain 37 were grown under aerobic and anaerobic conditions. In cell extracts of both strains, pyruvate dehydrogenase activity dependent on thiamine pyrophosphate, coenzyme A, and NAD was shown. The enzyme was induced by pyruvate in the growth medium, and there was higher activity in aerobically grown cells than in anaerobically grown cells. Acetyl phosphate was a potent inhibitor of the activity. This inhibition was partly overcome by inorganic phosphate.**

Oral streptococci require sugars as carbon and energy sources (9). In the oral cavity, this requirement may be satisfied by the glucose of the salivary secretions (12), although the glucose level of these secretions is only 5 to 40 μmol/liter (19). *Streptococcus mutans* has a high-affinity transport system for glucose that works with a half-maximum rate already at 5 μM glucose (14, 16), and in an anaerobic environment glycolysis of *S. mutans* is terminated by an ATP-yielding conversion of pyruvate into the fermentation products formate, acetate, and ethanol (10, 28, 30, 31). Although the organism has a constitutive lactate dehydrogenase, almost no lactate is formed. The reason is that this enzyme is absolutely dependent on fructose 1,6-bisphosphate for activity, and at low extracellular levels of glucose the intracellular pool of fructose 1,6-bisphosphate is too low to activate the enzyme (30). However, on exposure to high concentrations of glucose, the intracellular level of this intermediate increases and lactate will be the main fermentation product. In an aerobic environment, pyruvate formate-lyase is inactivated by oxygen (1), and the fructose bisphosphate-dependent lactate dehydroganse pathway is the only pathway of pyruvate conversion described thus far under these conditions.

In a study on the effect of an aerobic environment on the energy metabolism of oral streptococci, it was found that *Streptococcus sanguis* and *S. mutans* consumed oxygen in the presence of pyruvate. The oxygen uptake by *S. sanguis* could be explained by a pyruvate oxidase, which decarboxylated pyruvate in the presence of oxygen with the formation of acetyl phosphate and hydrogen peroxide (13). This enzyme could not be shown in *S. mutans*, but low activity of a pyruvate dehydrogenase was found in permeabilized cells.

In aerobic and facultatively anaerobic bacteria, pyruvate dehydrogenase is a multienzyme complex composed of multiple copies of three enzymes (23) which catalyze the overall reaction: pyruvate + NAD^+ + coenzyme A (CoA) → acetyl-CoA + NADH + H^+ + CO_2. With this enzyme, *S. mutans* has an alternative pathway to the fructose bisphosphate-dependent lactate dehydrogenase in converting pyruvate into fermentation products under aerobic conditions. The present paper describes the conditions for induction of pyruvate dehydrogenase in *S. mutans* and in *Escherichia coli* and some characteristics of this enzyme activity in cell extracts.

* Corresponding author.

MATERIALS AND METHODS

Microorganisms and growth conditions. *S. mutans* NCTC 10449 and *E. coli* K-12 strain 37, an Str^r mutant of W3102 (2), were studied. They were kept on blood agar plates (18) both aerobically and anaerobically. The anaerobic atmosphere was 10% hydrogen in nitrogen in a glove box. The strains were grown in a broth containing the following (per liter): 17 g of Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 3 g of Phytone (BBL), 2.5 g of NaCl, 10 g of glucose, 5 g of yeast extract (Difco Laboratories, Detroit, Mich.), 0.1 mol of potassium phosphate (pH 7.0), 0.01 mol of ammonium bicarbonate, and various amounts of sodium pyruvate. Ammonium bicarbonate and sodium pyruvate solutions were sterilized by filtration. The other ingredients of the medium were autoclaved. Phosphate and glucose solutions were autoclaved separately. The medium used in anaerobic experiments was prepared aerobically and then stored for a week in the anaerobic glove box. Anaerobic cultures (500 ml) were incubated at 37°C in the anaerobic box. Aerobic cultures (500 ml) were incubated at 37°C in the air in 1-liter Erlenmeyer flasks on a shaker having a circular orbital motion (100 rpm). The turbidity of the cultures was followed at 600 nm. The cells were harvested in the exponential phase of growth and washed four times by centrifugation in a solution containing 40 mM potassium phosphate buffer (pH 7.0), 66 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, and 0.5 mM $MgCl_2$. The cells were then frozen at −80°C in this solution supplemented with 1 mM EDTA and 3 mM dithiothreitol.

Cell extract. The cell extract was prepared under anaerobic conditions as previously described (11). The aerobically grown washed intact cells were brought into the anaerobic box and washed twice in 40 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA and 3 mM dithiothreitol. The cells were disintegrated by a homogenizer (type MSK; B. Braun, Melsungen, Federal Republic of Germany) as previously described (11). A cell extract was obtained after unbroken cells and cell debris had been removed by centrifugation at 40,000 × *g* for 20 min in a refrigerated centrifuge. The cell extract (5 ml) was dialyzed under aerobic conditions overnight at 4°C in membrane tubing (Spectrapor 1, 10 mm; Spectrum Medical Industries, Inc., Los Angeles, Calif.) against 2 liters of 10 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 3 mM $MgCl_2$, and 5 mM 2-mercaptoethanol. The activity of the pyruvate dehydrogenase complex did not decrease by this treatment. The extract was stored at −80°C with no loss

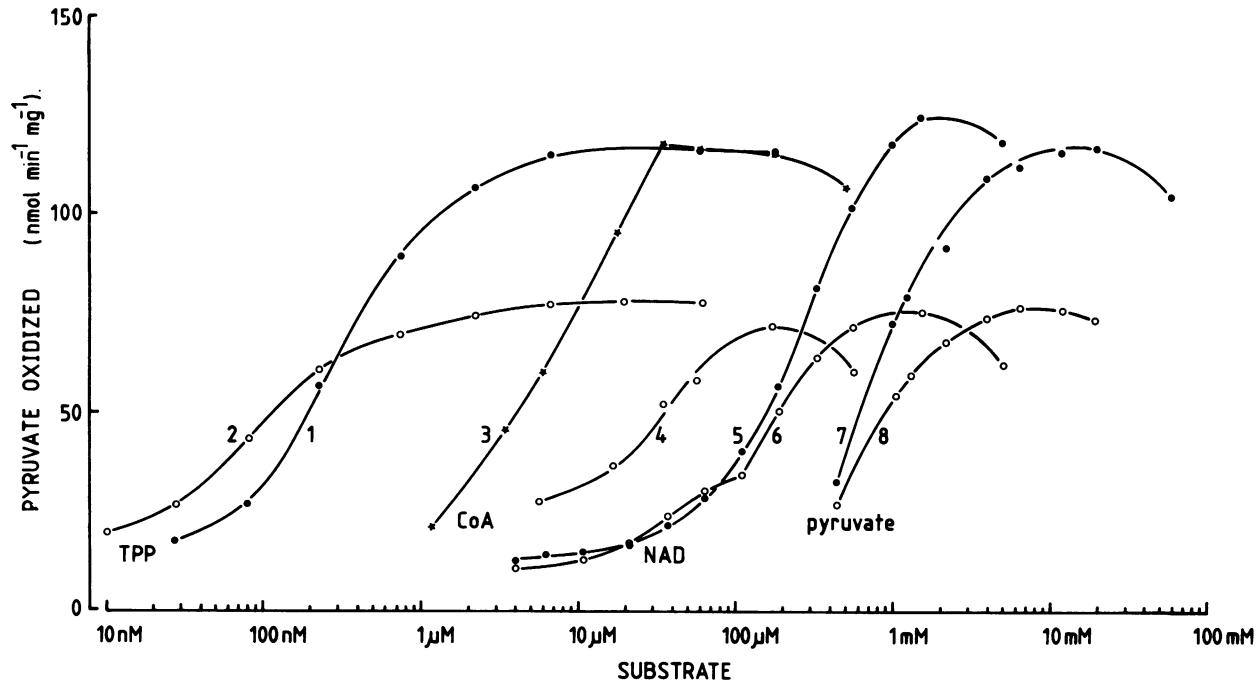

FIG. 1. Overall activity of the pyruvate dehydrogenase complex in cell extract of aerobically grown *S. mutans* measured by the ferricyanide method under aerobic conditions. The concentration of individual components was varied in a reaction mixture containing 100 mM Tricine-KOH (pH 7.0), 0.18 mM TPP, 0.175 mM CoA, 1 mM NAD, 6 mM $MgCl_2$, 12 mM sodium pyruvate, and 1 mM potassium ferricyanide. Closed and open symbols indicate reaction mixtures supplemented with 100 mM potassium phosphate (pH 7.0) and 5 mM potassium phosphate, respectively. In experiments 1 and 2, TPP was varied; in experiments 3 and 4, CoA was varied; in experiments 5 and 6, NAD was varied; and in experiments 7 and 8, pyruvate was varied.

of activity for at least 3 months. The protein concentration of the cell extract was measured by the biuret method (20).

Enzyme activity in cell extract. The overall activity of the pyruvate dehydrogenase complex was measured at 25°C in a standard reaction mixture containing 100 mM potassium phosphate (pH 7.0), 100 mM Tricine-KOH (pH 7.0), 12 mM sodium pyruvate, 6 mM $MgCl_2$, 0.18 mM thiamine pyrophosphate (TPP), 0.175 mM CoA, 2 mM NAD, 1 mM potassium ferricyanide, and the cell extract. The reaction was initiated by the addition of pyruvate, and the initial rate was monitored spectrophotometrically by following the reduction of ferricyanide into ferrocyanide at 430 nm (ϵ = 1,030 M^{-1} cm^{-1}; 6). The overall activity was also measured by following the reduction of NAD into $NADH_2$ at 340 nm under anaerobic conditions. The standard reaction mixture without ferricyanide was then prepared in the anaerobic box in a quartz cuvette fitted with a Thunberg-type side bulb containing pyruvate. The reaction was initiated by the addition of pyruvate, and the change in extinction at 340 nm was followed. The actual concentration of CoA in reagents was determined by the phosphotransacetylase method (3), the concentration of acetyl-CoA was determined by the citrate synthase method (3), and the concentration of acetyl phosphate was determined by the phosphotransacetylase method (4).

The activity of NADH-ferricyanide oxidoreductase was measured in a reaction mixture containing 100 mM potassium phosphate (pH 7.0), 100 mM Tricine-KOH (pH 7.0), 0.5 mM $NADH_2$, 1 mM potassium ferricyanide, and the cell extract. The reaction was initiated by the addition of the cell extract, and the initial rate was monitored by following the reduction of ferricyanide.

Products of the pyruvate dehydrogenase reaction. Acetyl phosphate was determined by the method of Lipman and Tuttle (21) in standard reaction mixtures with 3 mM pyruvate and 20 U of phosphotransacetylase, but no ferricyanide. Acetate was determined in standard reaction mixtures containing 0.5, 1, 2, or 3 mM pyruvate, 20 U of phosphotransacetylase, and 4 U of acetate kinase, but no ferricyanide. The reaction mixtures were incubated at 37°C overnight, the proteins were precipitated with 0.4 M perchloric acid, and the supernatant fluid was adjusted to pH 7.4 with 5 M potassium carbonate before acetate was determined with the acetate kinase method (4).

Chemicals. All enzymes and biochemicals were from Boehringer GmbH, Mannheim, Federal Republic of Germany.

RESULTS

The overall activity of a pyruvate dehydrogenase complex in *S. mutans* could not be shown under aerobic conditions by following the reduction of NAD, because the cell extract contained NADH oxidase activity. The use of ferricyanide reduction as a monitor of the activity was possible, however, because the cell extract contained an NADH-ferricyanide oxidoreductase. The extract thus catalyzed the reduction of ferricyanide into ferrocyanide in the presence of pyruvate, TPP, CoA, and NAD (Fig. 1; Table 1). When tested under anaerobic conditions without ferricyanide in the standard reaction mixture, NAD was reduced to $NADH_2$. When samples were taken at regular intervals from the standard reaction mixture containing 3 mM pyruvate and phosphotransacetylase but no ferricyanide, it was found that the concentration of acetyl phosphate increased to 1.4 mM and then decreased. When this reaction mixture was supplemented also with acetate kinase and incubated overnight,

TABLE 1. Overall activity of pyruvate dehydrogenase complex measured by the ferricyanide method under aerobic conditions in cell extracts[a]

Reaction mixture	Enzyme activity (nmol of pyruvate oxidized min^{-1} mg of $protein^{-1}$) in	
	S. mutans	*E. coli*
Standard	223 ± 1	604 ± 37
− TPP	29 ± 1	32 ± 8
− NAD	17 ± 1	89 ± 14
− CoA	8 ± 1	46 ± 4
− Pyruvate	0	0
− Phosphate[b]	133 ± 19	325 ± 24
+ 10 mM iodoacetate[c]	8 ± 4	552 ± 44
+ 1 mM arsenite[c]	5 ± 1	38 ± 11

[a] Cell extracts were prepared from *S. mutans* and *E. coli* grown in the presence of 80 mM pyruvate under aerobic conditions. Means and standard deviations (n = 3) are given.
[b] The phosphate concentration was decreased from 100 to 0.2 mM.
[c] Activity was determined 3 min after the initiation of the reaction.

1.03 ± 0.08 (standard deviation) mol of acetate was formed per mol of pyruvate. The addition of 1 mM arsenite to the reaction mixture abolished the enzyme activity (Table 1). These characteristics of the enzyme activity of *S. mutans* indicated that the enzyme was a pyruvate dehydrogenase, and the activity was similar to that of *E. coli* K-12 (Table 1). There was one difference between the enzymes of *S. mutans* and *E. coli*. The enzyme of *S. mutans*, but not that of *E. coli*, was inhibited by 10 mM iodoacetate (Table 1). The pH optimum of the pyruvate dehydrogenase of *S. mutans* was around 7.

The overall activity of the pyruvate dehydrogenase complex was higher in aerobically grown cells of *S. mutans* than in anaerobically grown cells (Table 2). The addition of pyruvate to the growth medium increased the activity of cells grown under both aerobic and anaerobic conditions (Table 2).

The use of ferricyanide reduction as a monitor of pyruvate dehydrogenase activity was possible because the NADH-ferricyanide oxidoreductase activity of the cell extracts was at least 6 times higher than the pyruvate dehydrogenase activity in extracts of aerobically grown cells and at least 25 times higher in anaerobically grown cells. The rate of ferricyanide reduction in the pyruvate dehydrogenase assay or in the presence of $NADH_2$ (0.5 mM) was not influenced by oxygen. The oxidation of 1 mol of pyruvate in the standard reaction mixture resulted in the reduction of 1.97 ± 0.03 (standard deviation) mol of ferricyanide under aerobic conditions and 1.84 ± 0.07 (standard deviation) mol of ferricyanide under anaerobic conditions. The rate of ferricyanide reduction in the presence of $NADH_2$ (0.5 mM) was not influenced by acetaldehyde (1 mM), and the rate was decreased only by about 10% in the presence of 1 mM acetyl-CoA or 12 mM pyruvate. The activity of NADH-ferricyanide oxidoreductase was not dependent on phosphate, and it was not influenced by 1 mM acetyl phosphate, 10 mM iodoacetate, or 1 mM arsenite.

When the overall activity of the pyruvate dehydrogenase

TABLE 2. Overall activity of pyruvate dehydrogenase complex of cell extracts grown under aerobic and anaerobic conditions in the presence and absence of pyruvate

Pyruvate (80 mM) in growth medium	Enzyme activity (nmol of pyruvate oxidized min^{-1} mg of $protein^{-1}$)[a] in			
	S. mutans		*E. coli*	
	Aerobic cells	Anaerobic cells	Aerobic cells	Anaerobic cells
Absent	131 ± 15	9 ± 2	199 ± 19	162 ± 8
Present	223 ± 5	79 ± 16	604 ± 37	431 ± 114

[a] Enzyme activity was determined under aerobic conditions in the standard reaction mixture by the ferricyanide method. Means and standard deviations (n = 3) are given.

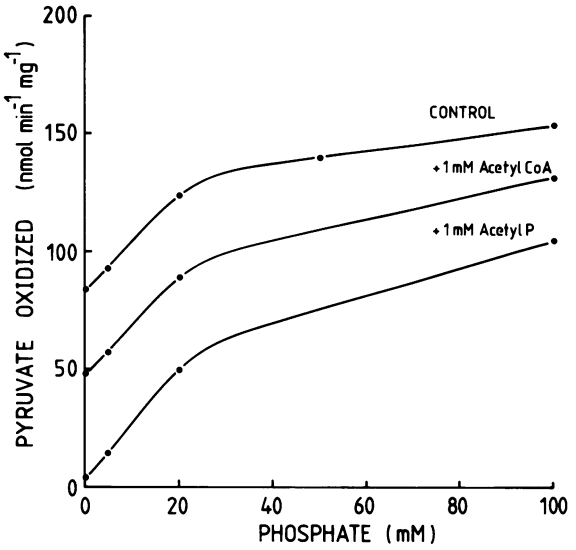

FIG. 2. Effect of 1 mM acetyl phosphate or 1 mM acetyl-CoA on the overall activity of the pyruvate dehydrogenase complex in cell extract of aerobically grown *S. mutans* measured by the ferricyanide method at various concentrations of phosphate in a reaction mixture containing 5 mM potassium phosphate (pH 7.0), 100 mM Tricine-KOH (pH 7.0), 0.18 mM TPP, 0.175 mM CoA, 2 mM NAD, 6 mM $MgCl_2$, 12 mM sodium pyruvate, and 1 mM potassium ferricyanide.

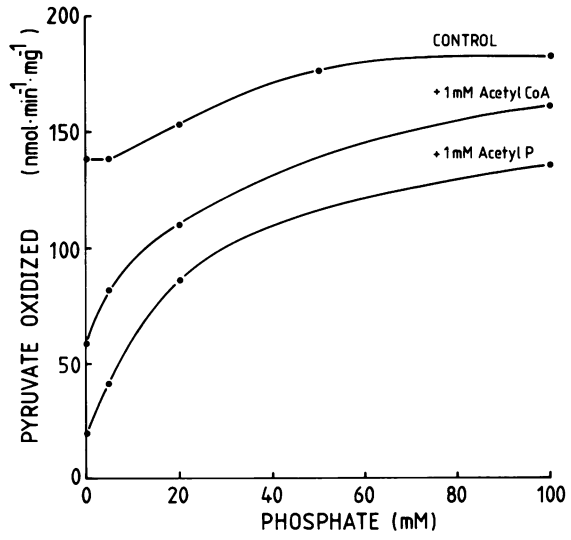

FIG. 3. Effect of 1 mM acetyl phosphate or 1 mM acetyl-CoA on the overall activity of the pyruvate dehydrogenase complex in cell extract of aerobically grown *E. coli* measured by the ferricyanide method at various concentrations of phosphate in the reaction mixture described in the legend to Fig. 2.

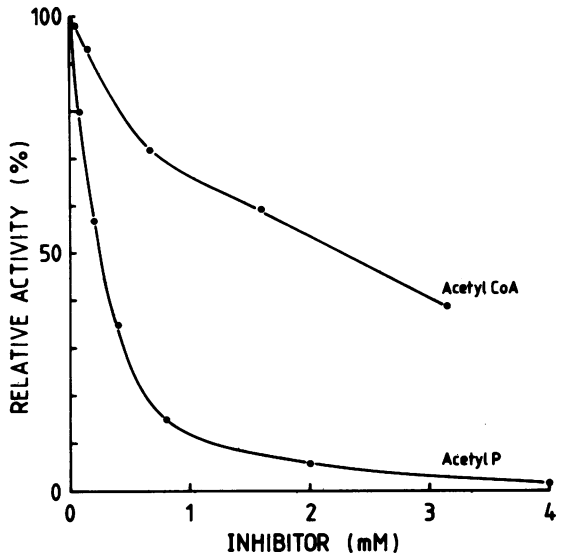

FIG. 4. Effect of various concentrations of acetyl phosphate or acetyl-CoA on the overall activity of the pyruvate dehydrogenase complex in cell extract of aerobically grown *S. mutans* measured by the ferricyanide method under aerobic conditions in the reaction mixture described in the legend to Fig 2.

complex was monitored by following the reduction of NAD under anaerobic conditions in reaction mixtures without ferricyanide, it was found that the activity was almost completely inhibited within 2 to 3 min. In reaction mixtures with ferricyanide under aerobic or anaerobic conditions, the rate of ferricyanide reduction was only slightly decreased within 10 min.

The activity of the pyruvate dehydrogenase complex was stimulated by inorganic phosphate in both *S. mutans* and *E. coli* (Fig. 1, 2, and 3; Table 1). Acetyl phosphate decreased the activity, and this inhibition could partly be overcome by phosphate (Fig. 2 and 3). Acetyl-CoA was much less efficient in inhibiting the activity of the pyruvate dehydrogenase complex than was acetyl phosphate (Fig. 2, 3, and 4).

In the presence of 5 mM inorganic phosphate, the activity of the pyruvate dehydrogenase complex of *S. mutans* was slightly decreased (5 to 15%) by 1 mM glucose 6-phosphate, fructose 6-phosphate, 2-phosphoglycerate, 3-phosphoglycerate, phosphoenolpyruvate, AMP, or ATP. The activity was increased about 20% by 1 mM ADP. The effect of fructose bisphosphate, glyceraldehyde 3-phosphate, and dihydroxyacetone phosphate could not be evaluated, because the extract contained aldolase and NAD-dependent glyceraldehyde 3-phosphate dehydrogenase.

DISCUSSION

S. mutans has many valuable requisites for a successful life in the oral cavity, and it is an important organism in the pathogenesis of dental caries (15). Pyruvate occupies a key position in the metabolism of this organism, and the pathways of pyruvate conversion into fermentation products are subjected to a strict metabolic control. Thus, the synthesis of pyruvate formate-lyase is induced when the sugar supply limits the growth of the organism under anaerobic conditions, and the activity of this enzyme is inhibited by the triose phosphates, glyceraldehyde 3-phosphate and dihydroxyacetone phosphate, when the sugar is supplied in excess (28, 31). Pyruvate formate-lyase is rapidly inactivated if the organism is exposed to oxygen (1). The lactate dehydrogenase of *S. mutans* is constitutive, but it is absolutely dependent on fructose 1,6-bisphosphate for activity, and the enzyme works only when sugar is supplied in excess (30).

The present demonstration of pyruvate dehydrogenase activity in *S. mutans* complements the current picture of this versatile organism. There was more activity in aerobically grown cells than in anaerobically grown cells, and the enzyme was induced by pyruvate in the growth medium. This enzyme could thus be formed in cells in which pyruvate formate-lyase has been inactivated by oxygen (1), and it will then be a valuable alternative to lactate dehydrogenase in the conversion of pyruvate into fermentation products.

The activity of pyruvate dehydrogenase is strictly regulated both in mammalian tissues and in bacteria. The activity of the mammalian enzyme complex is mainly regulated by phosphorylation and dephosphorylation. Phosphorylation is accompanied by a decrease in activity, and dephosphorylation restores the activity (29). In addition to this covalent modification of the enzyme, the enzyme activity is subjected to feedback control. It is inhibited by acetyl-CoA and $NADH_2$, and this inhibition is reversed by CoA and NAD, respectively (29). Among streptococci, the pyruvate dehydrogenase complex is known to be present in *Streptococcus faecalis* (22, 24) and in group N streptococci (8). The regulation of bacterial enzyme activity has, however, been studied in detail only in *E. coli* (23) and *Azotobacter vinelandii* (5). The activity is inhibited by acetyl-CoA, and this inhibition is overcome by pyruvate. $NADH_2$ is a strong competitive inhibitor with respect to NAD (5, 23). Positive effectors are phosphate (26), phosphoenolpyruvate (25), and a low energy charge of the adenylate nucleotides (27). Although a crude enzyme preparation is not suitable for evaluating the regulatory properties of an enzyme, the present study indicated also that the pyruvate dehydrogenase activity of *S. mutans* is inhibited by $NADH_2$. Acetyl phosphate was a more potent inhibitor than acetyl-CoA both in *S. mutans* and in *E. coli*. This has not previously been reported for any pyruvate dehydrogenase complex. Phosphotransacetylase can, however, be closely associated with the bacterial pyruvate dehydrogenase complex (6), and a study of the role of acetyl phosphate in the regulation of pyruvate dehydrogenase has to wait until this enzyme complex of *S. mutans* has been purified.

Pyruvate dehydrogenase may be useful for *S. mutans* in providing the cell with carbon dioxide. This is important, because *S. mutans* lacks the oxidative portion of the hexose monophosphate pathway (7), the usual carbon dioxide generator of the cell. Pyruvate dehydrogenase of *S. mutans* may also be important in energy metabolism, because acetyl-CoA can be converted into acetyl phosphate and this can be used to generate ATP. It was recently reported that *S. mutans* strains with the capacity to induce high levels of NADH oxidase and superoxide dismutase on exposure to an aerobic environment grew faster under aerobic conditions than strains that lacked NADH oxidase (17). It is probable that pyruvate dehydrogenase is also important for the growth of *S. mutans* in the presence of oxygen.

ACKNOWLEDGMENT

This study was supported by the Swedish Medical Research Council (project 4977).

LITERATURE CITED

1. **Abbe, K., S. Takahashi, and T. Yamada.** 1982. Involvement of oxygen-sensitive pyruvate formate-lyase in mixed-acid fermentation by *Streptococcus mutans* under strictly anaerobic condi-

tions. J. Bacteriol. **152**:175–182.
2. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36**:525–557.
3. **Bergmeyer, H.-U. (ed.)** 1970. Methoden der enzymatischen Analyse, 2. Auflage. Verlag-Chemie, Weinheim, Federal Republic of Germany.
4. **Bergmeyer, H.-U. (ed.)** 1984. Methods of enzymatic analysis, vol. 6. Verlag-Chemie, Weinheim, Federal Republic of Germany.
5. **Bosma, H. J., A. C. de Graaf-Hess, A. de Kok, C. Veeger, A. J. W. G. Visser, and G. Voordouw.** 1982. Pyruvate dehydrogenase complex from *Azotobacter vinelandii*: structure, function and inter-enzyme catalysis. Ann. N.Y. Acad. Sci. **378**:265–286.
6. **Bresters, T. W., J. Krul, P. C. Scheepens, and C. Veeger.** 1972. Phosphotransacetylase associated with the pyruvate dehydrogenase complex from the nitrogen fixing *Azotobacter vinelandii*. FEBS Lett. **22**:305–309.
7. **Bridges, R. B.** 1977. Ribose biosynthesis in *Streptococcus mutans*. Arch. Oral Biol. **22**:139–145.
8. **Broome, M. C., M. P. Thomas, A. J. Hillier, and G. R. Jago.** 1980. Pyruvate dehydrogenase activity in group N streptococci. Aust. J. Biol. Sci. **33**:15–25.
9. **Carlsson, J.** 1972. Nutritional requirements of *Streptococcus sanguis*. Arch. Oral Biol. **17**:1327–1332.
10. **Carlsson, J., and C. G. Griffith.** 1974. Fermentation products and bacterial yields in glucose-limited and nitrogen-limited cultures of streptococci. Arch. Oral Biol. **19**:1105–1109.
11. **Carlsson, J., Y. Iwami, and T. Yamada.** 1983. Hydrogen peroxide excretion by oral streptococci and effect of lactoperoxidase-thiocyanate-hydrogen peroxide. Infect. Immun. **40**:70–80.
12. **Carlsson, J., and T. Johansson.** 1973. Sugar and the production of bacteria in the human mouth. Caries Res. **7**:273–282.
13. **Carlsson, J., and U. Kujala.** 1985. Pyruvate oxidase activity dependent on thiamine pyrophosphate, flavin adenine dinucleotide and orthophosphate in *Streptococcus sanguis*. FEMS Microbiol. Lett. **25**:53–56.
14. **Ellwood, D. C., P. J. Phipps, and I. R. Hamilton.** 1979. Effect of growth rate and glucose concentration on the activity of the phosphoenolpyruvate phosphotransferase system in *Streptococcus mutans* Ingbritt grown in continuous culture. Infect. Immun. **23**:224–231.
15. **Hamada, S., and H. D. Slade.** 1980. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol. Rev. **44**: 331–384.
16. **Hamilton, I. R., and E. J. St. Martin.** 1982. Evidence for the involvement of proton motive force in the transport of glucose by a mutant of *Streptococcus mutans* strain DR0001 defective in glucose-phosphoenolpyruvate phosphotransferase activity. Infect. Immun. **36**:567–575.
17. **Higuchi, M.** 1984. The effect of oxygen on the growth and mannitol fermentation of *Streptococcus mutans*. J. Gen. Microbiol. **130**:1819–1826.
18. **Holdeman, L. V., E. P. Cato, and W. E. C. Moore (ed.).** 1977. Anaerobe laboratory manual, 4th ed. Virginia Polytechnic Institute and State University, Blacksburg.
19. **Kelsay, J. L., K. E. McCague, and J. M. Holden.** 1972. Variations in flow rate, metabolites and enzyme activities of fasting human parotid saliva. Arch. Oral Biol. **17**:439–445.
20. **Layne, E.** 1957. Spectrophotometric and turbimetric methods for measuring proteins. Methods Enzymol. **3**:447–454.
21. **Lipman, F., and L. C. Tuttle.** 1945. A specific micromethod for the determination of acyl phosphates. J. Biol. Chem. **159**:21–28.
22. **O'Kane, D. J., and I. C. Gunsalus.** 1948. Pyruvic acid metabolism. A factor required for oxidation by *Streptococcus faecalis*. J. Bacteriol. **56**:499–506.
23. **Reed, L. J.** 1974. Multienzyme complexes. Acc. Chem. Res. **7**:40–46.
24. **Reed, L. J., F. R. Leach, and M. Koike.** 1958. Studies on a lipoic acid-activating system. J. Biol. Chem. **232**:123–142.
25. **Schwartz, E. R., L. O. Old, and L. J. Reed.** 1968. Regulatory properties of pyruvate dehydrogenase from *Escherichia coli*. Biochem. Biophys. Res. Commun. **31**:495–500.
26. **Schwartz, E. R., and L. J. Reed.** 1970. Regulation of the activity of the pyruvate dehydrogenase complex of *Escherichia coli*. Biochemistry **9**:1434–1439.
27. **Shen, L. C., L. Fall, G. M. Walton, and D. E. Atkinson.** 1968. Interaction between energy charge and metabolite modulation in the regulation of enzymes of amphibolic sequences. Phosphofructokinase and pyruvate dehydrogenase. Biochemistry **7**:4041–4045.
28. **Takahashi, S., K. Abbe, and T. Yamada.** 1982. Purification of pyruvate formate-lyase from *Streptococcus mutans* and its regulatory properties. J. Bacteriol. **149**:1034–1040.
29. **Wieland, O. H.** 1983. The mammalian pyruvate dehydrogenase complex: structure and regulation. Rev. Physiol. Biochem. Pharmacol. **96**:123–170.
30. **Yamada, T., and J. Carlsson.** 1975. Regulation of lactate dehydrogenase and change of fermentation products in streptococci. J. Bacteriol. **124**:55–61.
31. **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 (ed.), Proceedings: microbial aspects of dental caries (a special supplement to Microbiology Abstracts). Information Retrieval Inc., Washington, D.C.