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Glucose-6-Phosphate-Dependent Pyruvate Kinase in *Streptococcus mutans*

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Pyruvate kinase of *Streptococcus mutans* JC 2 had an absolute and specific requirement for glucose-6-phosphate. Inorganic phosphate was a strong inhibitor. The enzyme required K^+ or NH_4^+ and Mg^{2+} or Mn^{2+}. *S. mutans* FIL and E 49, *Streptococcus bovis* ATCC 9809, and *Streptococcus salivarius* ATCC 13419 had also glucose-6-phosphate-dependent pyruvate kinases, whereas *Streptococcus sanguis* NCTC 10904 had an enzyme activated by fructose-1,6-diphosphate.

During continuous culture of *Streptococcus mutans* the level of phosphoenolpyruvate (PEP), in constrast to other intermediates, was high during glucose-limited growth and low in nitrogen-limited growth in the presence of excess glucose (12). This finding indicates that the enzyme or enzymes catalyzing the breakdown of PEP are regulated.

We partially purified a pyruvate kinase from a cell-free extract of *S. mutans* JC 2 prepared as described previously (12). The cell-free extract was treated with protamine sulfate (0.2% wt/vol) (8) and chromatographed on diethylaminoethyl-cellulose with a linear gradient starting with 0.05 M tris(hydroxymethyl)aminomethane-maleate buffer, pH 6.0, and ending with 0.1 M NaCl in 0.1 M tris(hydroxymethyl)aminomethane-maleate buffer, pH 6.0. The enzyme was then precipitated with ammonium sulfate at 55 to 75% saturation (at 0 C). The purification was 15-fold. The enzyme preparation was stored in 40 mM potassium phosphate, pH 7.0. The pyruvate kinase activity was assayed (Fig. 1) in the presence of 0.4 mM glucose-6-phosphate (G6P) and 0.4 mM phosphate. The lack of reduced nicotinamide adenine dinucleotide oxidation in the absence of lactate dehydrogenase identified pyruvate as a product. No glucose phosphate isomerase (EC 5.3.1.9) activity was detected in the final enzyme preparation.

The partially purified enzyme had an absolute and specific requirement for G6P. Fructose-6-phosphate, glucose-1-phosphate, glucose, fructose-1,6-diphosphate, and adenosine 5'-monophosphate at a concentration of 0.4 mM could not replace G6P. The enzyme also required Mg^{2+} and NH_4^+ or K^+. The enzyme activity was 40% lower when 10 mM Mn^{2+} was used instead of 10 mM Mg^{2+}. Na^+ could not substitute for NH_4^+ or K^+. The enzyme was not inhibited by 10 mM sulfate. The optimal pH of the enzyme was 7.0. This combination of requirements for G6P and for NH_4^+ or K^+ is unique among bacterial pyruvate kinases so far studied (5).

Inorganic phosphate was a potent inhibitor of the pyruvate kinase from *S. mutans* JC 2 as is the case with pyruvate kinases from other microorganisms (3, 6, 7, 10). The enzyme activity was completely inhibited by 20 mM phosphate in the presence of 0.4 mM G6P. The effect of phosphate at concentrations lower than 1.5 mM was negligible.

The saturation curve for G6P was sigmoidal both in the presence of 0.4 and 4.4 mM phosphate (Fig. 1). At these concentrations of phosphate was hyperbolic, giving a K_m value of $1.9 \times$ was 5.3×10^{-5} and 2.6×10^{-4} M, respectively, and the Hill coefficient was 2.3 and 2.8.

The saturation curve for PEP in the presence of 0.4 mM G6P and 1 mM adenosine 5'-diphosphate was hyperbolic giving a K_m value of 1.9×10^{-4} M. In the presence of 0.4 mM G6P and 1 mM PEP, the saturation curve for adenosine 5'-diphosphate was also hyperbolic, and the K_m value was 3.8×10^{-4} M.

When *S. mutans* JC 2 was grown in continuous culture under glucose limitation or under nitrogen limitation in an excess of glucose (12), no significant differences in the levels of pyruvate kinase in cell-free extracts were found. The pyruvate kinase in these extracts had an absolute requirement for G6P.

The intracellular level of PEP is high and the level of G6P is low when this organism is kept in glucose-limited continuous culture, and the level of PEP is low and the level of G6P is high when it is grown under nitrogen limitation in an

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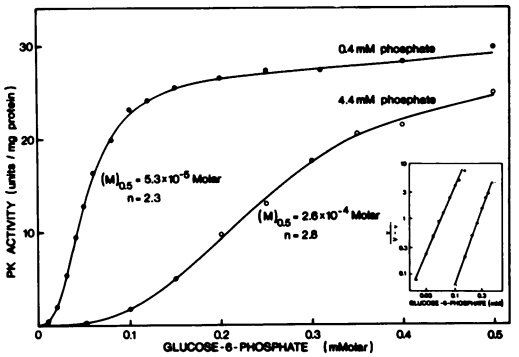

FIG. 1. *Effect of G6P concentration on the activity of pyruvate kinase from S. mutans JC 2 in the presence of low (0.4 mM) and high (4.4 mM) concentrations of phosphate. Pyruvate kinase activity due to the oxidation of reduced nicotinamide adenine dinucleotide was estimated at 25 C from the decrease in extinction at 340 nm using a double-beam spectrophotometer (DB-G; Beckman Instruments Ltd.) with recorder. The reaction mixture contained 0.05 M tris-(hydroxymethyl)aminomethane-hydrochloride buffer (pH 7.0), 0.1 M NH_4Cl, 0.01 M $MgSO_4$, 0.12 mM reduced nicotinamide adenine dinucleotide, 1 mM adenosine 5'-diphosphate, 1 mM PEP tricyclohexylammonium salt, various concentration of G6P, and 8.3 μg of L-lactate dehydrogenase per ml. Reactions were initiated by the addition of the partially purified enzyme giving a final concentration of 1.26 μg of protein/ml and 0.4 mM potassium phosphate, pH 7.0, in the reaction mixture. Another series of reaction mixtures contained 4.4 mM potassium phosphate, pH 7.0.*

excess of glucose (12). The present results imply that G6P could serve as a precursor activator of glycolysis. Under glucose limitation, the intracellular level of G6P is low and pyruvate kinase is only slightly activated. PEP accumulates in the cell and may be used to transport glucose into the cell by the PEP-dependent phosphotransferase system (9).

Cell-free extracts of batch cultures of *Streptococcus bovis* ATCC 9809, *S. mutans* FIL (12), *S. mutans* E 49 (4), and *Streptococcus salivarius* ATCC 13419 contained pyruvate kinases, which required G6P for activity. Pyruvate kinase in cell-free extracts from *Streptococcus sanguis* NCTC 10904 (strain 804; 2), on the other hand, was activated by fructose-1,6-diphosphate and was in this respect similar to pyruvate kinase of *Streptococcus lactis* (3).

S. mutans, strains JC 2 and E 49, have neither glucose-6-phosphate dehydrogenase (EC 1.1.1.49) nor 6-phosphogluconate dehydrogenase (EC 1.1.1.44) of the hexose monophosphate pathway (1, 11). *S. sanguis* NCTC 10904, which has both of these enzymes (11), has a pyruvate kinase activated by fructose-1,6-diphosphate as do most other bacterial pyruvate kinases with a catabolic function (5, 6). G6P is the branchpoint of the Embden-Meyerhof pathway to the hexose monophosphate pathway. Organisms with both pathways may have their catabolic pyruvate kinase regulated by fructose-1,6-diphosphate, whereas those streptococci without the oxidative portion of the hexose monophosphate pathway have an advantage in regulating the activity of their pyruvate kinase by G6P.

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