PRELIMINARY NOTES

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Two types of pyruvate kinase in *Escherichia coli* K12

Pyruvate kinase (EC 2.7.1.40) catalyses the formation of pyruvate and ATP from phosphoenolpyruvate (PEP) and ADP, and is a key enzyme of glycolysis. Its cellular activity appears to be subject to several types of control. At low concentrations of PEP, the pyruvate kinase activity of biological material such as yeast[1–4], rat liver[5–7], rat epididymal adipose tissue[8], and *Escherichia coli* B (ref. 9), is greatly stimulated by fructose 1,6-diphosphate: the properties of this type of enzyme contrast with those of the pyruvate kinase of muscle, which is not activated by fructose diphosphate[1,10]. In some tissues, one form of pyruvate kinase may be converted to the other[8,11]; on the other hand, rat liver has been shown to contain both types of enzyme[7,10,12]. The hormonal or nutritional state of organisms affects also the amounts of pyruvate kinase: in rat liver[10,12,13] and yeasts[3,14], the pyruvate kinase contents of the tissues are lower during gluconeogenesis than under conditions requiring carbohydrate breakdown.

It is the purpose of this communication to report the presence in *E. coli*, K12, of two forms of pyruvate kinase: one of these (pyruvate kinase I) is activated by fructose diphosphate and is inducible, the other of these (pyruvate kinase II) is not significantly affected by fructose diphosphate and appears to be formed constitutively.

The organism used, *E. coli* K1-1, is a mutant of *E. coli* K12 devoid of phosphoenolpyruvate synthase activity[15]. Cultures were grown aerobically at 37° in a rotary shaker in media[16] containing essential salts, NH_4Cl and the appropriate carbon source at 25 or 50 mM concentration. Cultures were harvested late in the logarithmic phase, when they had reached cell densities of 0.5–0.7 mg dry wt./ml, and were washed with buffer containing 5 mM sodium potassium phosphate and 1 mM EDTA (pH 7.5). The cells, suspended in this buffer to approx. 30 mg dry wt./ml, were disrupted by being exposed at 0° to the output of a M.S.E. 100-W ultrasonic unit for 5 min. The material was centrifuged at 23 000 × *g* for 10 min at 0° and the supernatant solutions thus obtained were applied at 0° to a column of DEAE-cellulose (45 cm × 1.7 cm) equilibrated with the 5 mM phosphate–EDTA buffer. After collection of the void volume, the enzymes were eluted with a linear gradient of 0 to 0.5 M KCl in the same buffer. The pyruvate kinase activity of the eluted fractions was assayed spectrophotometrically at 22° as the rate of decrease in extinction at 340 nm in the presence of the enzyme, 10 mM 3,3-dimethylglutarate buffer (pH 6.5), 50 mM KCl, 5 mM $MgCl_2$, 2 mM ADP, 5 mM PEP, 0.08 mM NADH and 20 μg of crystalline lactate dehydrogenase (EC 1.1.1.28). One unit of pyruvate kinase is defined as the quantity of enzyme that catalyses the oxidation of 1 μmole of NADH per min under these conditions. No activity was observed in the absence of ADP, of PEP or of lactate dehydrogenase, or when crystalline malate dehydrogenase (EC 1.1.1.37) was substituted for lactate dehydrogenase.

As shown in Fig. 1, the pyruvate kinase activity of extracts of cells, which had been grown on acetate, malate or glucose as sole carbon source, was reproducibly

Abbreviation: PEP, phosphoenolpyruvate.

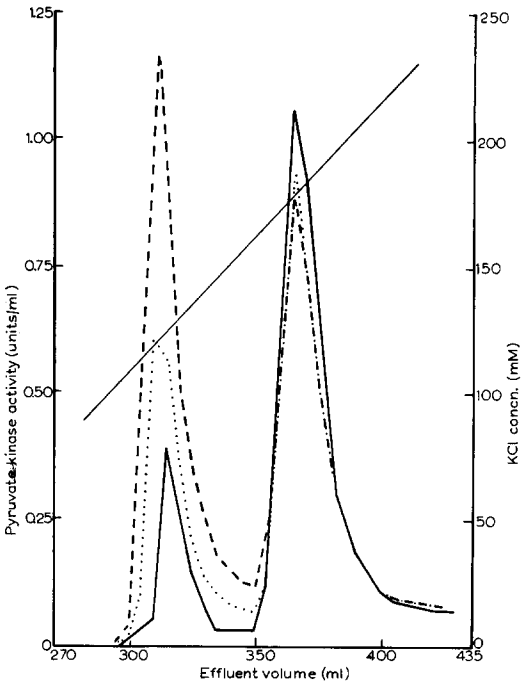
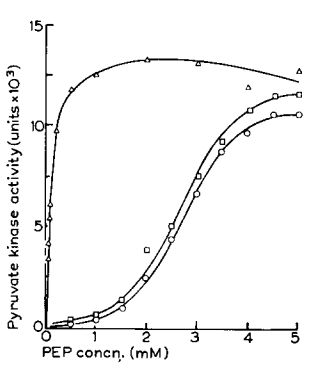


Fig. 1. Chromatography on DEAE-cellulose of the pyruvate kinase activity of extracts of *E. coli* K1-1 (containing 80 mg of protein), grown on 25 mM glucose (— — —), 25 mM L-malate (· · · · · ·) and 50 mM acetate (———) as sole carbon source. The diagonal line indicates the KCl gradient applied to the column. Pyruvate kinase activity was assayed in the absence of fructose diphosphate, as described in the text.



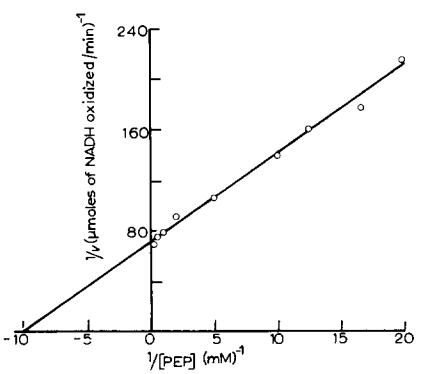


Fig. 2. Effect of PEP concentration on the activity of pyruvate kinase I alone (○), in the presence of fructose diphosphate (△), and in the presence of 0.4 mM AMP (□). Each assay was performed with 14 μg of pyruvate kinase I obtained from *E. coli* K1-1 grown on L-malate.

Fig. 3. Effect of PEP concentration on the activity of pyruvate kinase II in the absence of fructose diphosphate. Each assay was performed with 36 μg of pyruvate kinase II obtained from *E. coli* K1-1 grown on glucose.

resolved into two peaks. The quantity of the first of these enzymes (pyruvate kinase I), eluted by approx. 0.12 M KCl, varied markedly with the nature of the growth substrate, whereas that of the second (pyruvate kinase II), eluted by approx. 0.18 M KCl, appeared to be independent of the growth conditions. The amount of pyruvate kinase I found in acetate-grown cells was less than 20%, and that in malate-grown cells was less than 35%, of that present in extracts of the organism grown on glucose.

Not only the quantities but also the kinetic properties of pyruvate kinase I differed from those of pyruvate kinase II. As shown in Fig. 2, pyruvate kinase I exhibited sigmoid kinetics when the enzyme activity was measured at various concentrations of PEP, but this curve was transformed to a normal Michaelis–Menten type when 1 mM FDP was added; under these conditions, the K_m for PEP was 0.13 mM. In contrast to the pyruvate kinase of *E. coli* B (ref. 9), 0.5 mM AMP did not significantly alter the sigmoid shape of the curve obtained in the absence of fructose diphosphate. Michaelis–Menten-type kinetics were observed when the ADP concentration was varied, in the absence or presence of fructose diphosphate.

Unlike pyruvate kinase I, pyruvate kinase II exhibited normal Michaelis–Menten kinetics in the absence of fructose diphosphate, and the addition of fructose diphosphate did not significantly stimulate enzymic activity. The K_m for PEP of pyruvate kinase II (0.1 mM; Fig. 3) was closely similar to that of pyruvate kinase I when that enzyme was tested in the presence of fructose diphosphate.

The properties of the two types of pyruvate kinase suggest their physiological roles. The catabolism of glucose through glycolysis necessitates the formation of pyruvate from PEP: the activation of pyruvate kinase I by fructose diphosphate makes it likely that in *E. coli* K12, as in other organisms, the rate of glycolysis is, in part, controlled by the intracellular levels of this activator. In contrast, growth on malate or acetate would not require this enzyme to play a major role in energy provision: the apparent repression of pyruvate kinase I synthesis during these growth conditions would also reflect this diminished need for pyruvate formation from PEP. On the other hand, the synthesis of a number of cell components, such as CoA, alanine, valine, leucine, isoleucine, and other materials derived wholly or in part from pyruvate, is a constant requirement of cells however grown: it is thus suggestive that the amounts of one type of pyruvate kinase (pyruvate kinase II) appear to be independent of the nature of the growth substrate.

Although there is as yet no definite evidence that pyruvate kinase I is a different species of enzyme protein from pyruvate kinase II, rather than a different form of the same enzyme, the observation that the fractionation behaviour and the properties of the two types of pyruvate kinase in *E. coli* K12 are identical in the presence or absence of EDTA or of ATP, argues against the interconvertibility of two forms of the same enzyme, as has been reported for the pyruvate kinase of rat epididymal adipose tissue[11].

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Interconvertible forms of muscle phosphorylase phosphatase

Phosphorylase *a* phosphatase (phosphorylase phosphohydrolase, EC 3.1.3.17) catalyzes the conversion of phosphorylase *a* (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1) to phosphorylase *b* (refs. 1 and 2). The present paper reports preliminary evidence that skeletal muscle phosphorylase phosphatase exists in two interconvertible forms.

Phosphorylase *a* phosphatase was obtained from pigeon breast muscle. The tissue was homogenized with two volumes of 250 mM sucrose containing 50 mM glycylglycine buffer (pH 7.2) and adjusted to pH 7.0. Aliquots of the homogenate (2 ml) were incubated for different periods of time at 37° without any addition. The samples were then passed through Sephadex G-25 columns (1 cm × 20 cm) equilibrated with a solution containing 250 mM sucrose and 50 mM glycylglycine buffer (pH 7.2), and the first 1.5 ml of the colored effluent were collected. Reactivation or further inactivation of the phosphatase was performed at 37° by incubating 0.25-ml aliquots of the eluate with the indicated additions in a total volume of 0.3 ml. The final concentrations of the additions were as follows: mercaptoethanol, 10 mM; theophylline, 20 mM; ATP–$MgCl_2$, 2.5 mM; phosphocreatine–$MgCl_2$, 5 mM; $MgCl_2$, 5 mM; cyclic 3′,5′-AMP, 0.017 mM; ATP, 2.5 mM and phosphocreatine, 5 mM. Reactions were stopped by the addition of 2.7 ml of a cold solution containing 10 mM mercaptoethanol, 5 mM EDTA and 40 mM glycerophosphate buffer (pH 6.8). The diluted samples were assayed for phosphatase activity. The assay mixture containing 0.02 ml of the enzyme sample and 0.01 ml of ^{32}P-labeled phosphorylase *a* (48 Cori units; 1000–3000 counts/min per Cori unit) was incubated at 30° for 5 min, and the radioactive phosphate liberated was determined as previously indicated[3]. Phosphatase activity was expressed as the rate of consumption of phosphorylase *a*. ^{32}P-labeled