REGULATION OF PYRUVATE KINASES BY SUCCINYL COENZYME A*

E. Bruce Waygood† and B. D. Sanwal

Department of Cell Biology, Medical Sciences Building
University of Toronto, Toronto 181, Canada

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SUMMARY - The fructose-1,6-diphosphate (FDP) activated pyruvate kinases from Escherichia coli, rat liver and yeast are inhibited by succinyl-CoA. In the case of the enzyme from E. coli, the inhibition by succinyl-CoA only occurs in the presence of the activator, FDP. Succinyl-CoA and ATP in combination show cooperative inhibition.

INTRODUCTION - Pyruvate kinases from diverse sources, notably from rat liver (1), yeast (2), and E. coli (3) are known to be susceptible to allosteric regulation. While the positive effector for the enzyme from all of these sources is FDP (2,4), a number of different negative effectors have been found for the liver and the yeast enzyme. The liver pyruvate kinase is inhibited in an allosteric manner by L-alanine (5,6) and ATP (7) while the yeast enzyme is inhibited by ATP and several other nucleoside mono- and triphosphates (8). Physiological experiments dealing with the effect of glucocorticoids on the intermediary metabolism of liver have shown (5) that yet other negative effectors may exist for the liver enzyme. In case of E. coli pyruvate kinase negative effectors have not been discovered so far. In a recent paper, Lowry et al (9) discussed the need for a negative feed back inhibition of pyruvate kinase in this organism. Their findings (9) demonstrated that phosphoenolpyruvate (PEP) accumulated in

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succinate grown E. coli, while the FDP concentration did not change significantly from the levels found in glucose grown cells. This situation did not occur in acetate grown cells where the level of FDP was low. From these findings in E. coli and physiological findings in rat liver alluded to earlier (5) it occurred to us that some metabolite intimately connected with succinate metabolism may inhibit the FDP-activated pyruvate kinases. In the following account we show that succinyl-CoA inhibits, possibly allosterically, pyruvate kinases from rat liver, E. coli and yeast.

EXPERIMENTAL - The FDP-activated pyruvate kinase from E. coli was obtained as a homogeneous preparation (unpublished method) free from the AMP-activated pyruvate kinase (3). Rat liver enzyme was purified according to a published procedure (1). A crude extract (2) was used as the source of pyruvate kinase from baker's yeast. The pyruvate kinases from all sources were assayed spectrophotometrically by coupling with lactate dehydrogenase (3). Control experiments showed that this enzyme was unaffected by the various positive and negative effectors of the pyruvate kinases discussed later.

RESULTS AND DISCUSSION - The inhibition of pyruvate kinase from E. coli by succinyl-CoA is shown in Fig. 1. There are two noteworthy features of this inhibition. First, there is a very narrow range of succinyl-CoA concentration over which a steep inhibition of enzyme activity occurs, and, second, FDP counteracts succinyl-CoA inhibition. Suprisingly, the enzyme is not inhibited by succinyl-CoA in the absence of FDP. This is shown in Fig. 2A. It is thus clear that the presence of the allosteric activator on the enzyme surface is required before significant inhibition is caused. ATP, a well known inhibitor of liver

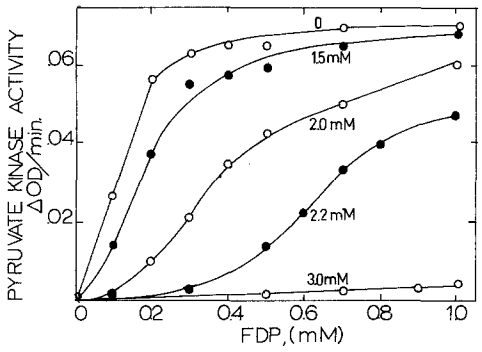

Fig. 1.

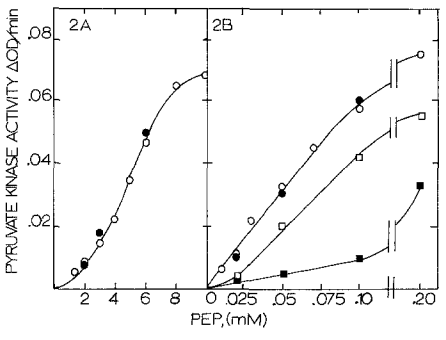

Fig. 2.

Fig. 1 E. coli enzyme. This graph shows the activation of pyruvate kinase by FDP, and the inhibition of that activation by succinyl-CoA at the given concentrations. The velocity in the absence of FDP is 0.001. A coupled spectrophotometric assay method was used in which pyruvate formed is reduced by NADH in the presence of excess lactate dehydrogenase. The assay mixture contained 0.15 mM NADH, 2 mM ADP, 0.4 mM PEP, 10 mM $MgCl_2$ and 20 µg of crystalline beef heart lactate dehydrogenase (sigma, type III) in a 0.03 M N-2-hydroxyethylpiperazine-N^1-2-ethanesulfonic acid buffer, pH 7.0, to a total volume of 1 ml. The reaction was started by the addition of pyruvate kinase and was followed at 21°C at 340 nanometers with a Gilford model 2400 recording spectrophotometer. Activity is given as the change in absorbance at 340 mµ.

Fig. 2 E. coli enzyme. A. Velocity versus PEP concentration in the absence of FDP; o-o, without succinyl-CoA; •-•, with 2.0 mM succinyl-CoA.
B. Velocity versus PEP concentration in the presence of 1 mM FDP; o-o, without succinyl-CoA and ATP; •-•, without succinyl-CoA and with 2.5 mM ATP; □-□, with 2 mM succinyl-CoA and without ATP; ■-■, with 2 mM succinyl-CoA and with 2.5 mM ATP. The assay system was as in Fig. 1 except ADP was at 1 mM concentration.

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pyruvate kinase (5) does not affect the E. coli enzyme at a concentration of 2.5 mM in the presence of FDP (Fig. 2B). However, when present together with succinyl-CoA (in the presence of FDP), ATP brings about cooperative inhibition. Thus, as can be seen from Fig. 2B, at a concentration of 0.1 mM PEP, 2.0 mM succinyl-CoA causes about 44% inhibition and 2.5 mM ATP causes none; in the presence of both of these together the

enzyme is inhibited to the extent of about 90%. Cooperative inhibition is a well documented mechanism of control (10) in some biochemical pathways.

Like the E. coli enzyme, pyruvate kinase from rat liver is also inhibited by succinyl-CoA. At a concentration of 0.2 mM PEP and 1 μM FDP, 1.5 mM succinyl-CoA causes about 65% inhibition. Similarly, the yeast enzyme (assayed in the presence of 0.1 mM FDP, 0.1 mM PEP, 100 mM KCl and 2 mM ADP at pH 7.0) is inhibited to the extent of 75% by 2 mM succinyl-CoA. There are two questions that arise with regard to succinyl-CoA inhibition. First, it may be asked whether this compound inhibits the pyruvate kinases merely by competing at the nucleotide (ADP) binding site and, second, if the inhibition is to have any physiological meaning, it may be asked whether other CoA derivatives cause effects similar to succinyl-CoA. With both the rat liver and E. coli pyruvate kinases, inhibition by succinyl-CoA can be shown to be non-competitive with ADP. For the rat liver enzyme this is shown in Fig. 3. High concentrations of CoA also inhibit the enzyme (K_i = 5 mM) but this inhibition in contrast to that caused by succinyl-CoA is competitive with ADP (Fig. 3). The E. coli enzyme is similar to the rat liver enzyme in this regard (results not shown).

The inhibition by succinyl-CoA seems to be quite specific. Malonyl-CoA (2 mM), acetyl-CoA (5 mM) and succinate (6 mM) do not inhibit the E. coli pyruvate kinase. It is already known (4,5) that these compounds are without effect on the rat liver enzyme.

At present it is hard to assess the physiological significance of succinyl-CoA inhibition of the pyruvate kinases from various sources, especially in view of the fact that know-

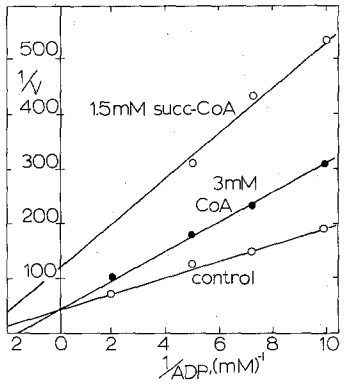

Fig. 3 Rat liver enzyme. Double reciprocal plot of velocity versus ADP concentration with CoA and succinyl-CoA present as shown. The assay was the same as in Fig. 1 with 0.2 mM PEP, 1 μM FDP and 100 mM KCl.

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ledge regarding changes in levels of succinyl-CoA in different metabolic states is almost non-existent. It is well known, however, that treatment of animals with glucocorticoids leads to an accelerated gluconeogenesis (5) and under such conditions levels of several tricarboxylic acid cycle intermediates increase. In E. coli, similarly, gluconeogenesis predominates when succinate is the carbon source and when levels of succinyl-CoA are liable to increase due to the induction of succinate thiokinase (11). In animals, bacteria, and possibly yeast, therefore, succinyl-CoA inhibition may provide the mechanism whereby the supply of PEP needed for gluconeogenesis may not be depleted by the uninhibited operation of pyruvate kinase.

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