REGULATORY PROPERTIES OF PYRUVATE DEHYDROGENASE FROM ESCHERICHIA COLI*

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The pyruvate dehydrogenase complex (PDC), which catalyzes overall Reaction 1, occupies an important branch point in metabolism and is, therefore, a likely candidate for metabolic regulation. PDC consists of three enzymes (1,2), pyruvate dehydrogenase (PDH), dihydrolipoyl transacetylase (LTA), and a flavoprotein, dihydrolipoyl dehydrogenase, which act sequentially in that order. Previous studies (3,4) indicate that the activity of PDC from pig heart and Escherichia coli is inhibited by the products of pyruvate oxidation, acetyl CoA and DPNH, and these inhibitions are reversed by CoA and DPN, respectively. The site of DPNH inhibition is the flavoprotein component of PDC (4,5,6). The site of acetyl CoA inhibition has not been established.

$$CH_3COCO_2H + CoA + DPN^+ \longrightarrow \text{acetyl CoA} + CO_2 + DPNH + H^+ \qquad (1)$$

Since PDH catalyzes the first and, apparently, irreversible step in pyruvate oxidation, it seemed likely that its activity is subject to regulation. This is indeed the case. The present communication shows that the activity of E. coli PDH is stimulated by phosphoenolpyruvate (PEP) and is subject to feedback inhibition by acetyl CoA. Acetyl CoA is competitive with pyruvate. Results reported elsewhere (7) indicate that certain nucleoside monophosphates and GDP are also positive effectors for E. coli PDH.

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METHODS

PDC was isolated from sonic extracts of E. coli (Crookes strain) and was resolved into PDH, LTA, and flavoprotein by procedures described previously (1,8). The assay of PDH activity is based on Reaction 2. The routine assay

$$CH_3COCO_2H + 2Fe(CN)_6^{-3} + H_2O \xrightarrow{(TPP)} CH_3CO_2H + CO_2 + 2Fe(CN)_6^{-4} + 2H^+ \quad (2)$$

mixture contained (final concentration) 20 mM N-tris(hydroxymethyl)methylglycine (Tricine) buffer (pH 7.5), 5 mM potassium pyruvate, 10 mM $MgCl_2$, 0.1 mM thiamine pyrophosphate (TPP), 1.88 mM potassium ferricyanide, and a minimum of 30 µg of PDH or 60 µg of PDC in a total volume of 1.0 ml. Reduction of ferricyanide was monitored at 420 mµ with a Gilford recording spectrophotometer at 25°. Modifications of the assay conditions are noted in the text. Enzyme activity is expressed as micromoles of ferricyanide reduced per minute and is based on the initial rate. PDC activity, based on Reaction 1, was determined by monitoring DPNH formation at 340 mµ and 25°. The assay mixture contained 50 mM potassium phosphate buffer (pH 8.0), 0.2 mM TPP, 1 mM $MgCl_2$, 2.5 mM DPN, 0.13 mM CoA, 2.4 mM cysteine.HCl, 1 mM pyruvate, and about 2 µg of PDC in a total volume of 1.0 ml. Activity is expressed as micromoles of DPNH formed per minute and is based on the initial rate. Protein was determined by the biuret method (9). PEP (sodium salt) was purchased from Calbiochem, and acetyl CoA (lithium salt) was obtained from Nutritional Biochemicals and P-L Biochemicals.

RESULTS

In its physiological role PDH utilizes as oxidant the lipoyl moiety ($LipS_2$) which is covalently bound to LTA (Eq. 3). The lipoyl moiety is re-

$$CH_3COCO_2H + LipS_2 \xrightarrow{(TPP)} CH_3CO\text{-}SLipSH + CO_2 \quad (3)$$

ductively acetylated in this reaction. Ferricyanide can replace the protein-bound lipoyl moiety as oxidant, producing acetate (Eq. 2) rather than a thioester. Use of ferricyanide permits study of the PDH-catalyzed step in pyruvate oxidation (1,10). The activity of PDH in the ferricyanide-linked model reaction is stimulated by PEP and is inhibited by acetyl CoA (Fig. 1).

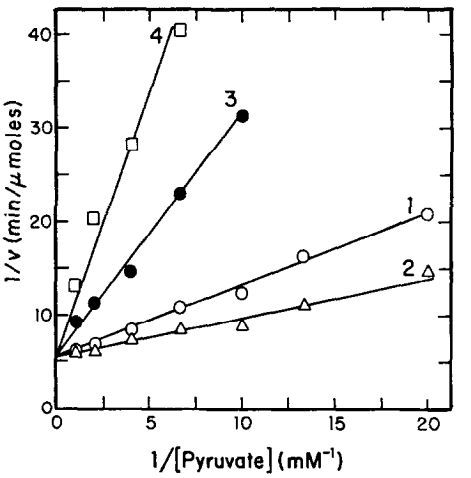

Figure 1. Effect of PEP and acetyl CoA on PDH activity with ferricyanide as electron acceptor. The assay mixture (pH 7.8) contained 77 μg of highly purified PDH, and the reaction was initiated by the addition of pyruvate (in 2 to 10 ul). Other components and conditions were as described under Methods. Curve 1, no additions; curve 2, 0.1 mM PEP; curve 3, 0.01 mM acetyl CoA; curve 4, 0.03 mM acetyl CoA.

Acetyl CoA is a powerful inhibitor and is competitive with pyruvate; K_i (apparent) = 3.3×10^{-6} M. Similar results were obtained with PDC, which contains PDH bound noncovalently to LTA (1). The activity of the bound PDH

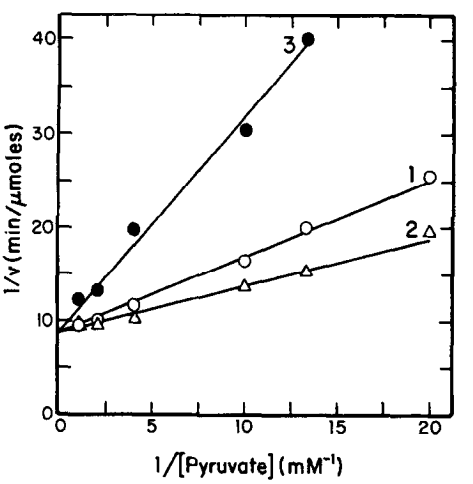

Figure 2. Effect of PEP and acetyl CoA on PDC activity with ferricyanide as electron acceptor. The assay mixture (pH 7.5) contained 230 μg of PDC. Other conditions were as in Fig. 1. Curve 1, no additions; curve 2, 0.1 mM PEP; curve 3, 0.01 mM acetyl CoA.

is stimulated by PEP and is inhibited by acetyl CoA (Fig. 2). Acetyl CoA is competitive with pyruvate; K_i (apparent) = 6×10^{-6} M. Identical values of K_m for pyruvate (1.3×10^{-4} M) were obtained with the free and bound PDH. PEP lowers the K_m (apparent) for pyruvate to 6.5×10^{-5} M. Previous studies (7) revealed that AMP, CMP, GMP, UMP and GDP also stimulate the activity of both free and bound PDH. In contrast to the effect observed with PEP, these nucleotides do not alter the apparent K_m value for pyruvate.

Hill plots (log $\frac{v}{V-v}$ versus log [Pyruvate]) of data obtained at pH 7.5 with several preparations of PDH gave n values which ranged from 1.4 to 1.9. These results suggest that PDH possesses two substrate binding sites. The n value is decreased to 1 when either acetyl CoA or PEP is present (Fig. 3). Nucleotides which act as positive effectors for PDH do not affect the value of n (7). Binding studies have been undertaken to confirm and extend these observations.

The regulatory characteristics of PDH exhibited in the ferricyanide-linked model reaction (Eq. 2) are also exhibited in the physiological reaction (Eq. 1). Thus, the activity of PDC in the DPN-linked reaction is inhibited

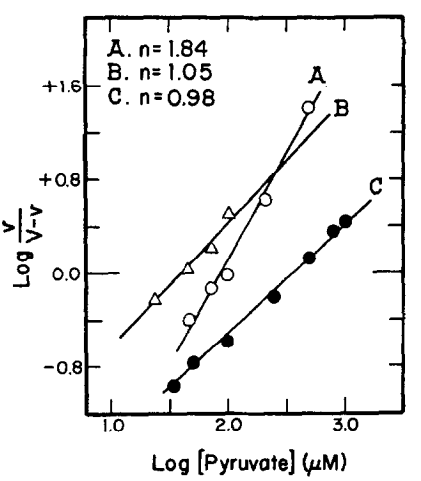

Figure 3. Hill plots showing the order with respect to pyruvate of the reaction catalyzed by PDH with ferricyanide as electron acceptor. Curve A, no additions; curve B, 0.1 mM PEP; curve C, 0.01 mM acetyl CoA.

by acetyl CoA, and this inhibition is competitive with respect to pyruvate (Fig. 4). Under the assay conditions used K_m (apparent) for pyruvate is 5.1×10^{-4} M, and K_i (apparent) for acetyl CoA is 2.5×10^{-4} M. The latter value is markedly higher than the K_i (apparent) value for acetyl CoA (6×10^{-6} M) found in the ferricyanide-linked reaction. Preliminary experiments indicate that CoA, acting on the PDH component of PDC, decreases the inhibition caused by acetyl CoA in the DPN-linked reaction. Analysis of the CoA effect is complicated by the fact that CoA is a required cofactor in the DPN-linked reaction and by the fact that CoA is rapidly oxidized in the presence of ferricyanide.

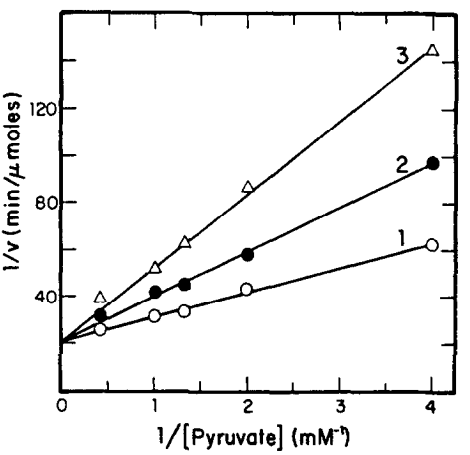

Figure 4. Effect of acetyl CoA on PDC activity with DPN as electron acceptor. Curve 1, no acetyl CoA added; curve 2, 0.15 mM acetyl CoA; curve 3, 0.5 mM acetyl CoA. The reaction was initiated by the simultaneous addition of pyruvate and CoA (0.065 mM) in 0.05 ml. Other components and conditions were as described under Methods.

DISCUSSION

Several investigators have suggested (3,4,11-14) that the oxidation of pyruvate under physiological conditions may be regulated by acetyl CoA/CoA and DPNH/DPN ratios. This suggestion is based on the following observations. Firstly, oxidation of long-chain fatty acids or intermediates of the tricarboxylic acid cycle by mitochondrial preparations inhibits pyruvate oxi-

dation, and this inhibition is prevented by uncoupling agents (11-14). Secondly, the activity of purified preparations of the pyruvate dehydrogenase complex (PDC) from pig heart and E. coli is inhibited by acetyl CoA and by DPNH, and these inhibitions are reversed by CoA and DPN, respectively (3,4). It should also be noted that the synthesis of PDC in E. coli is induced by its substrate, pyruvate (15).

The present investigation directs attention to the remarkable regulatory characteristics of the pyruvate dehydrogenase (PDH) component of PDC from E. coli. The activity of PDH is stimulated by PEP and by certain nucleotides (7) and is subject to feedback inhibition by acetyl CoA. It seems likely that this regulatory enzyme plays a major role in the control of pyruvate oxidation in E. coli. Studies are in progress to determine whether similar regulatory mechanisms govern the activity of pyruvate dehydrogenases from mammalian tissues.

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