APPARENT CO-OPERATIVE EFFECT OF ACETYL-CoA ON SHEEP KIDNEY PYRUVATE CARBOXYLASE*

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The enzyme pyruvate carboxylase (pyruvate : CO_2 ligase (ADP), EC 6.4.1.1.) has been isolated from four different sources, viz, avian liver mitochondria (Utter and Keech, 1963), sheep kidney cortical mitochondria (Ling and Keech, 1966), bakers' yeast (Ruiz-Amil et. al., 1965) and Pseudomonas citronellolis (Seubert and Remberger, 1961). The liver and kidney enzymes both exhibit an absolute requirement for acetyl-CoA which has been reported to exhibit classical Michaelis-Menten kinetics (Keech and Utter, 1963). The yeast enzyme is active in the absence of acetyl-CoA but either CoA or its acetyl derivative are capable of inducing a two-fold stimulation of enzymic activity (Ruiz-Amil et. al., 1965). It has been suggested from kinetic data that acetyl-CoA exerts either an allosteric effect or induces a conformational change in the enzyme. A change in the apparent Michaelis constant for bicarbonate in the presence of acetyl-CoA (Cooper and Benedict, 1966) has been attributed to a change in the tertiary structure of the protein. No acetyl-CoA requirement has been demonstrated for the bacterial enzyme.

Studies with the enzyme reported here as well as studies carried out with

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the enzyme isolated from chicken liver[1]. suggest that the actual kinetics observed with acetyl-CoA are considerably more complex than previously indicated. The enzyme from kidney will be shown here to exhibit a non-linear relationship in a double reciprocal plot of activity versus acetyl-CoA concentration characteristic of a co-operative effect. The enzyme from chicken liver shows pseudo-linearity at relatively high concentrations of acetyl-CoA as previously reported but also shows a non-linear relationship at lower concentrations[1].

EXPERIMENTAL

Pyruvate carboxylase was purified from freeze-dried kidney cortical mitochondria according to the method described by Ling and Keech (1966). Enzymic activity was measured by a modification of the method described by Gailiusis et. al. (1964). Each assay mixture (total volume, 0.50 ml) contained in micromoles/ml: 5,tris HCl buffer pH 8.4; 5, sodium pyruvate; 1.25, ATP; 3.3, $MgCl_2$; 5, C^{14} sodium bicarbonate (1.7×10^5 c.p.m. per μ mole); acetyl-CoA as shown and 0.27 units of pyruvate carboxylase. The reactions were incubated at 30° for ten minutes and stopped by the addition of saturated 2,4-dinitrophenylhydrazine.HCl. After centrifuging, aliquots of the supernatant were dried on Whatman 3MM paper discs and counted in a Tricarb Scintillation Counter. To avoid any possible confusion arising from impurities in the acetyl-CoA solution (Stadtman, 1957), the solution was treated chromatographically (Zetterstrom and Ljunggren, 1951), the purified compound eluted with 10^{-4}M-EDTA, pH 7.6, and its concentration determined by measuring the extinction at 259 mμ.

RESULTS AND DISCUSSION

Reaction velocities were measured at varying acetyl-CoA concentrations and

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1. M.C. Scrutton and M.F. Utter, personal communication.

graphs prepared by plotting $\frac{1}{v}$ as a function of $\frac{1}{(\text{acetyl-CoA})}$ were found to be non-linear (Fig. 1). However, when $\frac{1}{v}$ was plotted against $\frac{1}{(\text{acetyl-CoA})^2}$ a

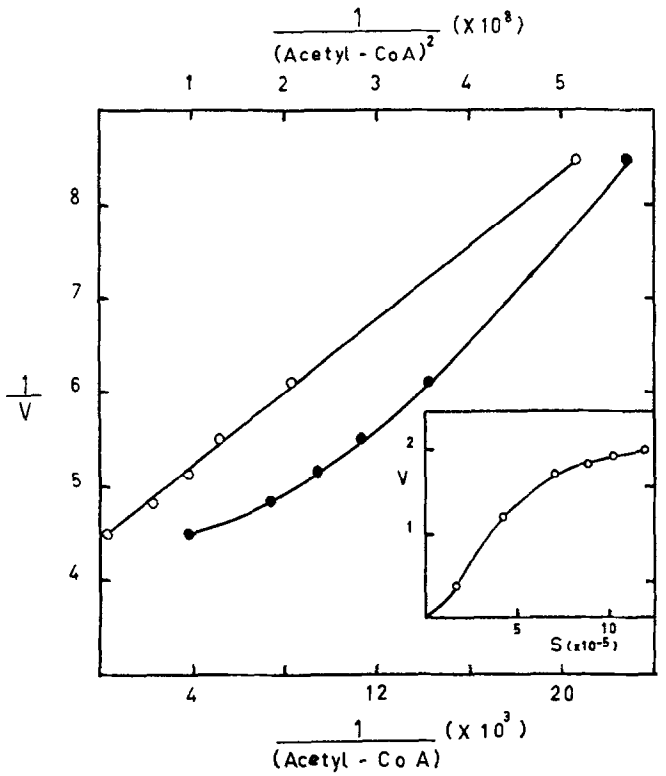

Fig. 1. ●—● a plot of the reciprocal of the velocity against the reciprocal of the molar concentration of acetyl-CoA. O—O a plot of the reciprocal of the velocity against the reciprocal of the square of the molar concentration of acetyl-CoA. The slope of the line was determined by the method of least squares. The insert shows a plot of velocity as a function of acetyl-CoA concentration.

straight line was obtained (Fig. 1). This type of plot is indicative of a mechanism involving more than one molecule of activator (Lineweaver and Burk, 1934). Other explanations for this departure from classical Michaelis-Menten kinetics are possible (Morrison, 1965). The activation of kidney pyruvate carboxylase by acetyl-CoA is similar to other allosteric activations and inhibitions which do not yield linear Lineweaver-Burk plots (Okazaki and

Kornberg, 1964; Sanwal et. al., 1963; Umbarger and Brown, 1958). Therefore, kinetic data were fitted to the empirical Hill equation.

$$\log \frac{v}{V - v} = n \log A - \log K$$

where V, v, A, n and K are maximal velocity, initial reaction velocity, acetyl-CoA concentration, the interation coefficient and apparent Michaelis constant respectively, and plotted (Fig. 2). The value of n (1.9) was calculated from the slope of the graph. Although n is not an elementary kinetic

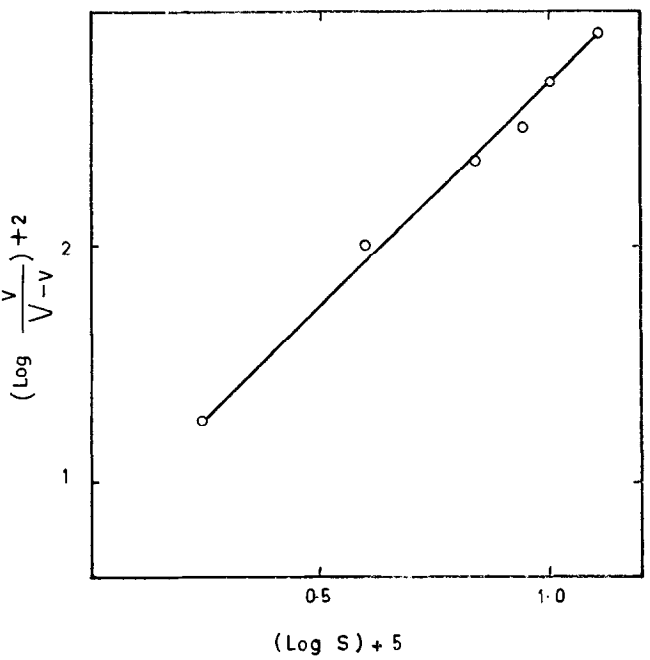

Fig. 2. The data of Fig. 1 fitted to the empirical Hill equation. The slope of the line was determined by the method of least squares.

parameter of an enzyme, but is a complex function of both the number of interacting binding sites per enzyme molecule and the strength of the interaction (Atkinson et. al., 1965), a value greater than 1.0 has been suggested to indicate that the binding of acetyl-CoA to the enzyme involves co-operative interaction requiring more than one molecule of acetyl-CoA. The

enzyme from chicken liver has an n value of approximately 3 under somewhat similar conditions[1].

The co-operative effect of acetyl-CoA in the activation of the kidney enzyme is not pH dependent since it has been shown to occur over the pH range from 7.3 to 9.3. Also, the enzyme is reversibly inactivated by freezing (Ling and Keech, 1966). However, the co-operative phenomenon is apparently not affected by the reversible cold inactivation since the same kinetics were observed immediately after thawing the enzyme as were obtained after the enzyme had been stored for five hours at room temperature. During the same period the maximum velocity doubled.

From the vertical intercept of the double reciprocal plot and the plot of velocity versus acetyl-CoA concentration (Fig. 1) it is possible to calculate the apparent Michaelis constant, i.e., the concentration of acetyl-CoA which yields half maximum velocity. By these methods a value of 4.1×10^{-5}M is obtained. This value is almost double the molarity of 2.2×10^{-5}M reported previously (Ling and Keech, 1966) from the pseudo-linearity at relatively high concentrations of acetyl-CoA.

Cooper and Benedict (1966) have presented evidence which suggests that a change in tertiary structure occurs on the addition of acetyl-CoA to yeast pyruvate carboxylase. The results presented in this communication indicate that the activation of kidney pyruvate carboxylase by acetyl-CoA is an allosteric effect, involving homotropic co-operative interaction between at least two activating sites (Monod, et. al., 1963; Monod, et. al., 1965). This co-operative effect is similar to that observed for ATP in deoxythymidine kinase (Okazaki and Kornberg, 1964), for substrates of the enzymes NAD-specific isocitrate dehydrogenase (Sanwal, et. al., 1963) and L-threonine deaminase (Umbarger and Brown, 1958) and for the inhibition of phosphorylase 'b' by ATP (Madsen, 1964). These enzymes, including kidney pyruvate carboxylase, have been shown to exhibit a co-operative interaction between homologous binding sites involving more than one molecule of substrate, inhibitor or activator.

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