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

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1. Introduction

All pyruvate carboxylases purified thus far from mammalian and avian species have been found to be inactive in the absence of an acyl-CoA. In contrast, the pyruvate carboxylase purified from *Pseudomonas citronellolis* and *Aspergillus niger* show maximal activity in the absence of an acyl-CoA. In yeast, this enzyme is active in the absence of acetyl-CoA but either CoA or its acetyl derivative is capable of inducing a 2-fold stimulation of enzymic activity [1]. The reaction catalysed by pyruvate carboxylase from pigeon kidney apparently requires "acetyl coenzyme A" as an added cofactor:

$$\text{pyruvate} + \text{ATP} + HCO_3^- \xrightleftharpoons{\text{Acetyl-CoA; } Mg^{2+}} \text{oxalacetate} + \text{ADP} + P_i \quad (1)$$

Although the pyruvate carboxylase from chicken liver has been reported to exhibit classical Michaelis–Menten kinetics [2], it has been suggested from kinetic data that acetyl-CoA either exerts an allosteric effect or induces a conformational change in the enzyme. The requirement for activation by acetyl-CoA is engendered in the formation of enzyme–biotin–CO_2 from ATP and HCO_3^- (eq. 2)

$$\text{E–biotin} + \text{ATP} + HCO_3^- \rightleftharpoons \text{E–biotin–}CO_2 + \text{ADP} + P_i \quad (2)$$

$$\text{E–biotin–}CO_2 + \text{pyruvate} \rightleftharpoons \text{E–biotin} + \text{oxalacetate} \quad (3)$$

We report that studies with the enzyme from pigeon kidney suggest that the actual kinetics observed with acetyl-CoA are considerably more complex than previously indicated. This enzyme exhibits a non-linear relationship in a double reciprocal plot of activity versus acetyl-CoA concentration, characteristic of a co-operative effect.

2. Methods and materials

Pigeon kidney pyruvate carboxylase was purified and enzymic activity was measured by an optical assay of the rate of NADH-oxidation (eq. 4 and 5). The reaction mixture contained in 2.0 ml: 100 μmoles Tris-HCl buffer, pH 7.7; 80 μmoles $KHCO_3$; 16 μmoles $MgCl_2$; 0.4 μmoles NADH; 3 μmoles ATP; 6 μmoles sodium pyruvate; 10.5 U malate dehydrogenase; 0.5 mg serum albumin and acetyl-CoA as shown (fig. 1). Units (U) of pyruvate carboxylase were added to start the reaction. It was followed by the NADH oxidation at 340 nm (30°). In order to compensate for NADH oxidation due to contamination of the enzyme preparation by lactate dehydrogenase (eq. 6) all assays were carried out against a control without ATP and acetyl-CoA.

$$\text{pyruvate} + HCO_3^- + \text{ATP} \xrightarrow{PC} \text{oxalacetate} + \text{ADP} + P_i \quad (4)$$

$$\text{oxalacetate} + \text{NADH} + H^+ \xrightarrow{MDH} \text{malate} + NAD^+ \quad (5)$$

$$\text{pyruvate} + \text{NADH} + H^+ \xrightarrow{LDH} \text{lactate} + NAD^+ \quad (6)$$

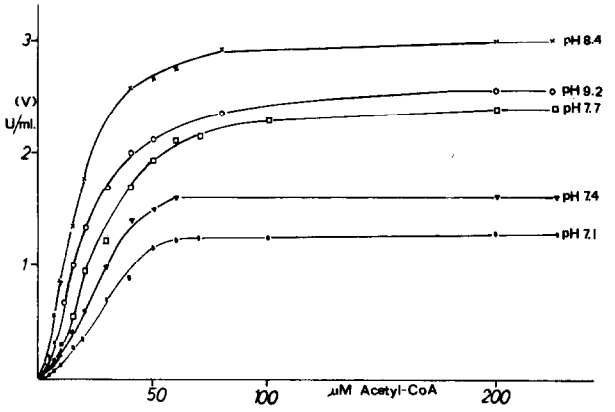

Fig. 1. Activation of pyruvate carboxylase by acetyl-CoA at different pH's in the optical assay. Synthetic acetyl-CoA and pH of the test solution as shown in the figure.

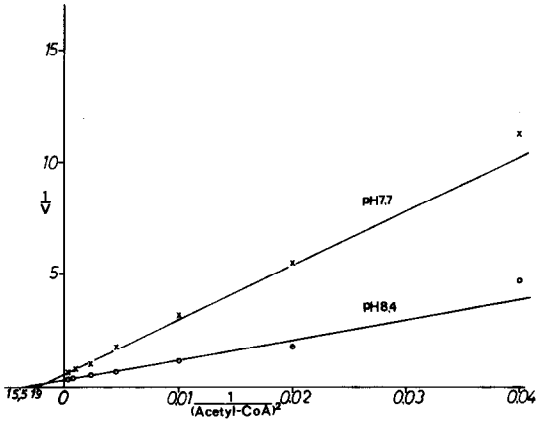

Fig. 3. A plot of the reciprocal of the velocity against the reciprocal of the square of the µmolar concentration of acetyl-CoA.

Coenzymes and enzymes were purchased from Boehringer (W. Germany). All the other reagents were of the highest purity commercially obtainable. Acetyl-CoA was prepared according to Lynen and Wieland [3]. All solutions were freshly prepared prior to the experiments.

3. Results and discussion

Reaction velocities were measured at varying acetyl-CoA concentrations and graphs derived by plotting V as a function of acetyl-CoA at different pH values (fig. 1); $1/V$ versus 1/[acetyl-CoA] (fig. 2) and $1/V$ against $1/[\text{acetyl-CoA}]^2$ (fig. 3). When $1/V$ was plotted against $1/[\text{acetyl-CoA}]^2$, a straight line was obtained (fig. 3). This type of plot is indicative of a mechanism involving more than one molecule activator. The activation of pigeon kidney pyruvate carboxylase is strongly influenced by the pH of the solution (fig. 1). At the saturation concentration of acetyl-CoA the curve of V_{max} against pH shows a maximum at pH 8.4 (figs. 1 and 6). As in the case of chicken liver [4] the homotropic cooperativity expressed by the Hill coefficients

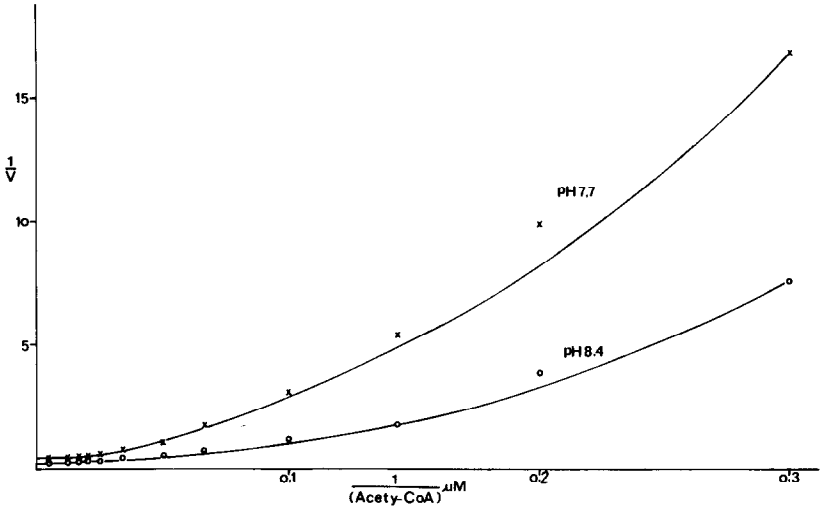

Fig. 2. A plot of the reciprocal of the velocity against the reciprocal of the µmolar concentration of acetyl-CoA.

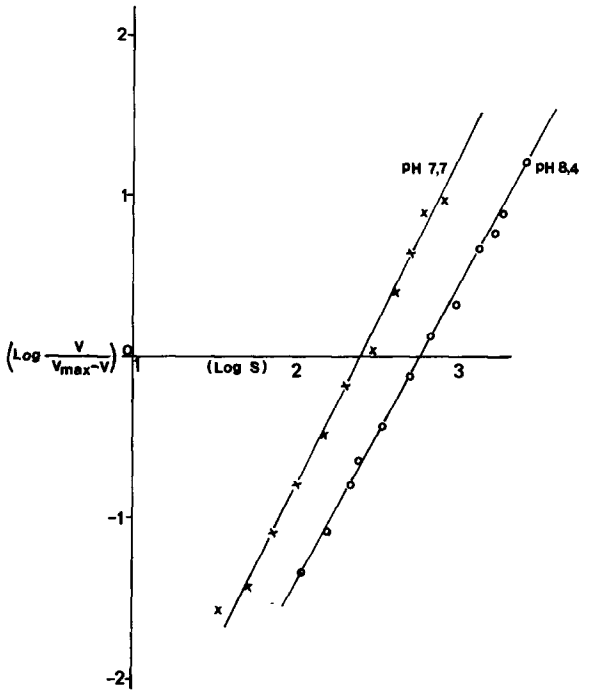

Fig. 4. The data of fig. 1 fitted to the emperical Hill equation (only pH 7.7 and 8.4 are shown here).

(figs. 4 and 5) with respect to the binding of acetyl-CoA increases with decreasing pH, indicating the participation of an ionizing group in the allosteric control

Table 1
Influence of pH on the Hill coefficients and on the activation by the acetyl-CoA of pyruvate carboxylase from pigeon kidney.

pH	n	R_S	V_{max} (U/ml)	$S_{0.5}$	$pS_{0.5}$
7.1	2.25	5.0	1.24	26.7	4.5735
7.4	2.1	6.25	1.61	22.3	4.6514
7.7	2.0	7.7	2.38	19.0	4.7212
8.4	1.8	9.0	3.05	15.5	4.8097
9.25	1.6	10.5	2.54	14.9	4.8268

by acetyl-CoA. An analysis of the relationship between $pS_{0.5}$ and pH according to Dixon and Webb [5] points to a pH of 8.08 for this group (probably sulfhydryl or ammonium). The values of "n" were calculated from the slopes of the graphs (fig. 6) at different pH values (table 1). Although n is not an elementary kinetic 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 [6], it has been suggested that a value greater than one indicates that the binding of acetyl-CoA to the enzyme involves cooperative interaction requiring more than one molecule of acetyl-CoA. Analysis of the effect of pH on the values has also been undertaken (table 1 and fig. 5). This value decreases from 2.25 at pH 7.1 to 1.6 at pH 9.25, and this decrease is associated with a decrease in the activator constant from 26.7 μM at pH 7.1 to 14.9 at pH 9.25. The vertical intercept of the

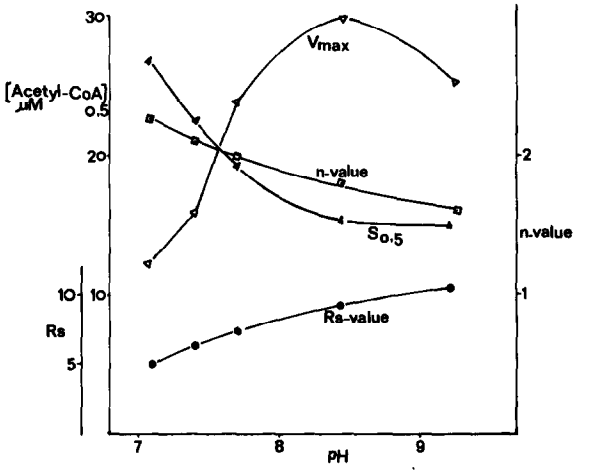

Fig. 5. Variation of $S_{0.5}$ values, V_{max}, the Hill coefficients n and the R_S values for acetyl-CoA as a function of pH.

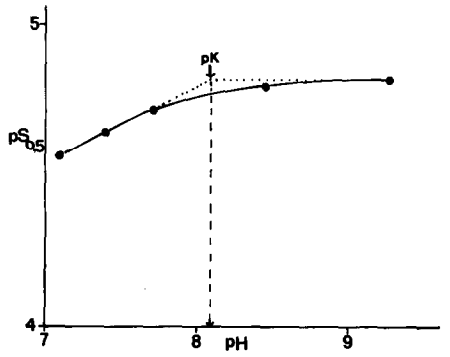

Fig. 6. Variation of the $pS_{0.5}$ values for acetyl-CoA as a function of pH.

double reciprocal plot (fig. 2), the plot of velocity versus acetyl-CoA concentration (fig. 1) and the plot $1/V$ against $1/[\text{acetyl-CoA}]^2$ yielded the apparent K_m ($S_{0.5}$) values shown in table 1. Cooper and Benedict [7] 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 cooperativity. This cooperative effect is similar to that observed for ATP in deoxythymidine kinase, and for substrates of the enzymes NAD-specific isocitrate dehydrogenase and L-threonine deaminase, which have been shown to exhibit a cooperative interaction between homologous binding sites involving more than one molecule of substrate or activator.

Pyruvate carboxylase from pigeon kidney shows absolute requirement for activation by acetyl coenzyme A in all preparations and under all conditions tested thus far, though other CoA compounds have different effects on the enzyme from chicken liver [4]. Examination of the initial reaction rate as a function of activator concentration reveals a sigmoid curve, suggesting that more than 1 molecule of activator per active site is required for the activation process or that cooperative interaction between the bound activator molecules occur. It thus seems that acetyl-CoA acts as an allosteric effector for pyruvate carboxylase from pigeon kidney.

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