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

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Received 12 January 1973

1. Introduction

It has been well established that gluconeogenesis is a process which takes place only in the liver and the kidney. Nevertheless until now there has been no comparative studies of the allosteric behaviour of regulatory enzymes from kidney and liver in the same avian species. In order to make this comparative study, pyruvate carboxylase (PC) of pigeon liver [1] and kidney [2] were studied simultaneously. In all cases where pyruvate carboxylases from mammalian and avian liver were purified, it has been shown that they are inactive in the absence of acetyl-CoA. However, the pyruvate carboxylases from *Pseudomonas citronellolis* and *Aspergilus niger* are exceptional in that they show a maximal activity even in the absence of an acyl-CoA. The yeast enzyme is active in the absence of acetyl-CoA [3] but this activity can also be approximately doubled either with CoA-SH or with acetyl derivatives alone. The reaction which is catalysed by the PC from pigeon liver requires acetyl-CoA as a cofactor (eq. 1):

$$\text{Pyruvate} + \text{ATP} + \text{HCO}_3^- \xrightleftharpoons{\text{Acetyl-CoA};\,Mg^{2+}} \text{Oxalacetate} + \text{ADP} + \text{P}_i \qquad (1)$$

The activation through acetyl-CoA is probably necessary only for the formation of enzyme–biotin–CO_2 complex from ATP and HCO_3^- (eq. 2):

$$\text{E–biotin} + \text{ATP} + \text{HCO}_3^- \rightleftharpoons \text{E–biotin–CO}_2 + \text{ADP} + \text{P}_i \qquad (2)$$

* The author wishes to dedicate this communication to his teacher Prof. Dr. Th. Wieland on his 60th birthday.

$$\text{E–biotin–CO}_2 + \text{Pyruvate} \rightleftharpoons \text{E–biotin} + \text{Oxalacetate} \qquad (3)$$

In this communication we report that the acetyl-CoA kinetics in pigeon liver PC is probably more complex than it has been shown previously. A parabola is observed in a double reciprocal plot of the activity of pigeon liver PC as a function of acetyl-CoA concentration. The results lead to the conclusion that either acetyl-CoA produces an allosteric effect or induces a conformational change in the enzyme.

2. Methods and materials

Pigeon liver pyruvate carboxylase was purified [1] (specific activity 16.6) and the enzymatic activity was measured optically by utilizing the NADH oxidation. The reaction mixture (final vol 2 ml) contained: 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). 0.025 Units of PC were added to start the reaction. The oxidation of NADH was followed at 340 nm (30°). All the measurements were carried out using a control to which ATP and acetyl-CoA were not added.

$$\text{Pyruvate} + \text{HCO}_3^- + \text{ATP} \xrightarrow{\text{PC}} \text{Oxalacetate} + \text{ADP} + \text{P}_i \qquad (4)$$

$$\text{Oxalacetate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}^+ \qquad (5)$$

Coenzymes and enzymes were purchased from Boehringer (W. Germany). All the other reagents were of the highest purity commercially obtainable. Acetyl-

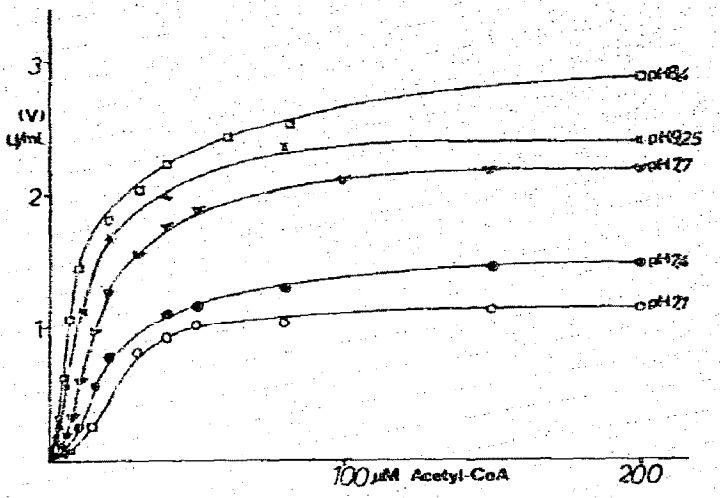

Fig. 1. Activation of pyruvate carboxylase by acetyl-CoA at different pH's in the optical assay. Synthetic acetyl-CoA and pH of the test solution as shown in the figure. One unit is the amount of enzyme catalyzing the conversion of 1 μmole pyruvate to oxalacetate per min at 30°.

CoA was prepared according to Lynen and Wieland [4]. All solutions were freshly prepared prior to the experiments.

3. Results and discussion

Reaction velocities were measured at different 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

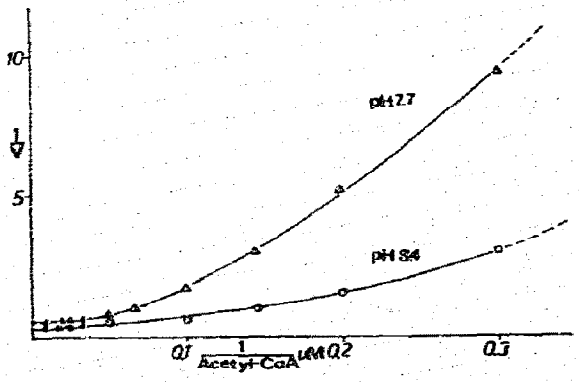

Fig. 2. A plot of the reciprocal of the velocity against the reciprocal of the micromolar concentration of acetyl-CoA.

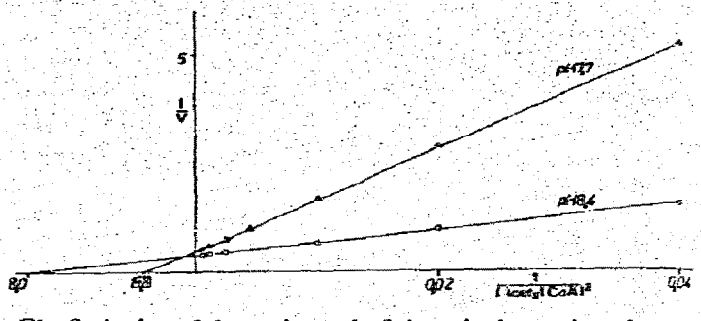

Fig. 3. A plot of the reciprocal of the velocity against the reciprocal of the square of the micromolar concentration of acetyl-CoA.

$1/V$ against $1/[\text{acetyl-CoA}]^2$. In the latter case a straight line was obtained (fig. 3). This type of plot is indicative of a mechanism involving more than one molecule activator. This activation of pigeon liver pyruvate carboxylase is strongly influenced by the pH of the solution (fig. 1). At supra maximal concentrations of acetyl-CoA, the curve of V_{max} against pH gives a maximum at pH 8.4 (figs. 1 and 6). The homotropic cooperativity expressed by the Hill coefficients (figs. 4 and 5) with respect to the binding of acetyl-CoA increases with decreasing pH, indicating that an ionizing group participates in the allosteric control by acetyl-CoA. An analysis of the relationship between $pS_{0.5}$ and pH, according to Dixon and Webb [5], gives a pK value of 8.4 for this group (fig. 6).

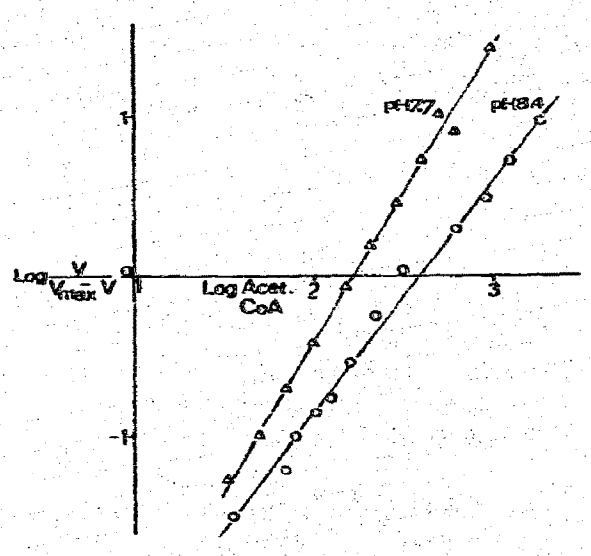

Fig. 4. The data of fig. 1 fitted to the emperical Hill equation (only pH 7.7 and 8.4 are shown here).

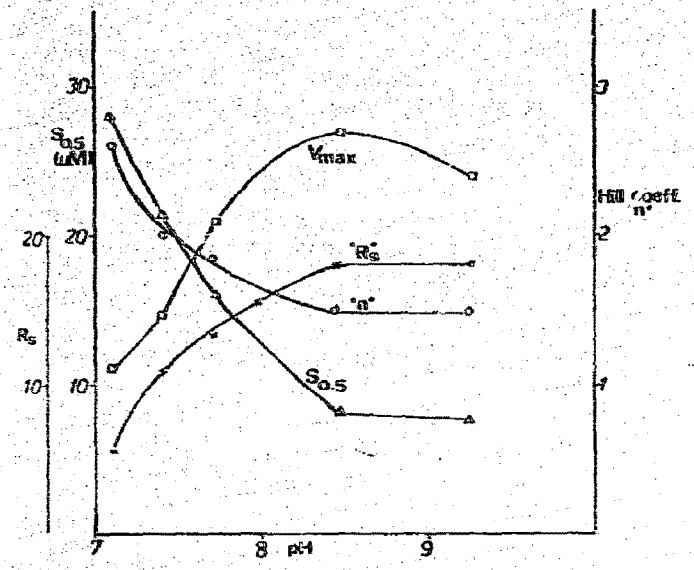

Fig. 5. Variation of $S_{0.5}$ values, V_{max}, the Hill coefficients n and the R_S values for acetyl-CoA as a function of pH.

The value of "n" (Hill coefficients) were calculated from the slopes of the graphs (fig. 4) at different pH values (table 1). Although "n" is not elementary kinetic parameter of an enzyme, but is a complex function of both the number of interaction binding sites per enzyme molecule and the strength of the interaction [6], it has been suggested that a value greater than 1 indicates that the binding of acetyl-CoA to the enzyme involves cooperative interaction requiring more than one molecule of acetyl-CoA. The "n" value changes from 2.6 at pH 7.1 to 1.7 at pH 9.2 and this change in "n" value is related to the change of K_m from 28 μM at pH 7.1 to 7.9 μM at pH 9.2 (table 1). This pH effect on $S_{0.5}$ (or on the "n" value) of pigeon liver enzyme

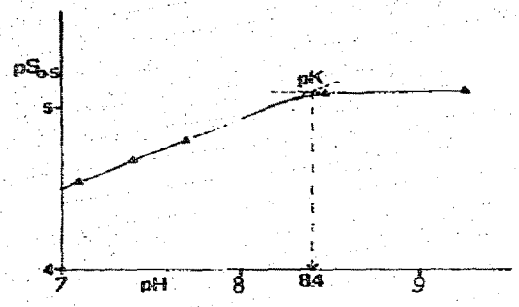

Fig. 6. Variation of the $pS_{0.5}$ values for acetyl-CoA as a function of pH.

is stronger than on the kidney pyruvate carboxylase [2] as is also shown in the table. The increase of pH decreases the "n" value but increases the enzyme–activator affinity. This effect is 2-fold lower in the case of kidney PC where the value decreases from 26.7 μM at pH 7.1 to 14.9 at pH 9.2. This can be explained by the assumption that the OH^- ions work as the heterotropic effector in relation to acetyl-CoA, which suggests that a heterotropic effector (OH^- ions) removes the homotropic cooperativity of acetyl-CoA. This would mean that OH^- ions decrease the cooperativity (fig. 1) by changing the balance (through subunit interaction; from T-form to R-form) to a form with greater affinity (R-form) for acetyl-CoA according to the concerted mechanism (all or nothing) of Monod et al. [7]. The acetyl-CoA can now be attached directly to the resulting higher affinity state without first having to change the subunit form. This effect of pH value on acetyl-CoA interaction with pigeon liver PC becomes more complex if one tries to explain it by the Koshland theory [8].

From the vertical intersect in the double reciprocal

Table 1

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

pH	Liver					Kidney				
	n	R_S	V_{max} [U]	$S_{0.5}$ [μM]	$pS_{0.5}$	n	R_S	V_{max} [U]	$S_{0.5}$ [μM]	$pS_{0.5}$
7.1	2.6	5.5	1.1	28.0	4.55	2.25	5.00	1.24	26.7	4.57
7.4	2.0	11.0	1.44	21.3	4.67	2.1	6.25	1.61	22.3	4.65
7.7	1.9	12.7	2.1	15.8	4.8	2.0	7.7	2.38	19.0	4.72
8.4	1.8	18.0	2.7	8.0	5.1	1.8	9.0	3.05	15.5	4.81
9.2	1.7	18.0	2.4	7.9	5.1	1.6	10.5	2.54	14.9	4.83

plot (fig. 2), the plot of V against acetyl-CoA concentration (fig. 1) and $1/V$ against $1/[\text{acetyl-CoA}]^2$ (fig. 3) gives the same (K_m) $S_{0.5}$ values. The K_m values were also calculated from the Hill equation. When log $V/(V_{max} - V) = 0$. Cooper and Benedict [9] showed that the binding of acetyl-CoA was correlated with the change in the tertiary structure of yeast pyruvate carboxylase. The results in this communication show that the activation of PC from pigeon liver by acetyl-CoA is an allosteric effect, showing the homotropic cooperativity [7, 10] as is also found for PC from pigeon kidney [2]. These cooperative effects have also been shown for other enzymes [11–13].

In all preparations, and under all conditions studied, the activation of pigeon liver pyruvate carboxylase is dependent on the acetyl-CoA concentration. The results in figs. 1 and 2 show a sigmoid curve. This suggests that more than one molecule of acetyl-CoA per active site is necessary for the enzyme activation or that cooperative interactions between the bound activator molecules occur. These findings are consistent with the proposal that acetyl-CoA acts as allosteric effector for pyruvate carboxylase from pigeon liver.

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