THE PARTICIPATION OF ACETYL-CoA IN PYRUVATE CARBOXYLASE

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INTRODUCTION

Pyruvate carboxylase catalyzes the formation of oxaloacetate from pyruvate, bicarbonate, ATP and magnesium ions. This reaction in pigeon liver has been shown to absolutely require catalytic amounts of acetyl-CoA (Utter and Keech, 1960); the reaction in photosynthetic bacteria (Fuller and Kornberg, 1961), rat liver (Henning and Seubert, 1964) and baker's yeast (Benedict, 1964) (Ruiz et al., 1965) is stimulated by acetyl-CoA; whereas the reaction in Aspergillus niger (Bloom and Johnson, 1962) and Pseudomonas citronellolis (Seubert and Remberger, 1961) is not affected by the additions of acetyl-CoA.

Utter and Keech (1960) have shown that C^{14}-acetyl-CoA does not enter oxaloacetate formed during the carboxylase reaction. Nor is acetyl-CoA effective in this reaction by reacting with bicarbonate to form malonyl-CoA. Acetyl-CoA is required only for the formation of the CO_2-Biot-Enz complex from bicarbonate, ATP and Biot-Enz (Scrutton and Utter, 1964). The transfer of the beta carboxyl group of oxaloacetate to pyruvate is not affected by acetyl-CoA (Scrutton and Utter, 1964) (Gailiusis et al., 1964). No data has been presented which will explain how acetyl-CoA participates with ATP or bicarbonate

to form the CO_2-Biot-Enz complex whereas acetyl-CoA is not functional in the formation of this complex from oxaloacetate in the oxaloacetate-pyruvate exchange reaction. We would like to present data which offers a partial explanation of this phenomena. Acetyl-CoA accelerates yeast pyruvate carboxylase independent of ATP and pyruvate concentrations but causes a significant change in the Km for bicarbonate.

METHODS AND MATERIALS

The enzyme used in these experiments was isolated from autolyzates of baker's yeast by the procedure of Gailiusis et al. (1964). The 35-45% $(NH_4)_2SO_4$ fraction was dialyzed for 3 hours at 0°C against 7 liters of 0.005 M phosphate buffer pH 7.0. The dialyzate was adsorbed onto C-gamma alumina gel at a ratio of 1 mg protein : 3 mg gel. The gel had previously been batch equilibrated with 0.05 M phosphate buffer pH 7.0. The protein was eluted from the gel with increasing concentrations of phosphate buffer pH 7.0. The purification scheme is shown in Table I.

Table I. Purification Scheme of Yeast Pyruvate Carboxylase

Fraction	Total Protein mg	Total Activity cpm x 10^{-6}	Specific Activity cpm x 10^{-3} / mg protein	Enzyme Yield %
Crude	2921	34.5	11.8	100
Soluble	2215	34.6	15.6	100
35-45% $(NH_4)_2SO_4$	487	13.9	28.7	38.2
Dialyzate	--	--	44.6	--
0.1 M eluate C-γ gel	13.2	3.8	290.3	11.1

The purity of the enzyme at the gel stage was 25 fold over the crude extracts and was used immediately for assays. The enzyme at this stage of purification was stable for 6-10 hours. Storage of the enzyme at -20°C resulted in 50% loss in activity in 1-2 days.

Purification of the enzyme beyond this stage by calcium phosphate gel adsorption and elution resulted in a 80-100 fold purification but the enzyme was extremely unstable. The addition of sucrose to the enzyme solutions did not afford stability to further purification. This purification scheme differs from that described by Ruiz-Amil et al. (1965) where a 50 fold purification was obtained at the $(NH_4)_2SO_4$ stage and 140 fold upon DEAE chromatography.

Pyruvate carboxylase was assayed for activity be the procedure of Gailiusis et al. (1964). The reaction mixtures contained in micromoles/ml: 100, phosphate buffer pH 7.0; 5, GSH; 10, ATP; 10, $MgCl_2$; 10, potassium pyruvate; 3, potassium oxaloacetate (trapping amounts); 50, potassium bicarbonate (sp. act. 0.4 μc/μmole) and 370 μg of protein. The reactions were incubated for 15 minutes at 37°C and the reactions were stopped by the addition of 2,4-dinitrophenylhydrazine·HCl. The amount of C^{14}-bicarbonate which was incorporated into the isolated C^{14}-2,4-dinitrophenylhydrazone of oxaloacetate was used as a measure of enzyme activity.

RESULTS

The ATP and pyruvate velocity curves of pyruvate carboxylase in the presence and absence of acetyl-CoA are shown in Fig. 1. All of these curves are typical Michaelis-Menton substrate velocity curves. The addition of 7.7×10^{-5} M acetyl-CoA to reaction tubes containing increasing amounts of ATP or pyruvate results in about 60% stimulation in enzyme activity. Lineweaver-Burke reciprocal plots of this data are shown in Fig. 2. The addition of acetyl-CoA to these reaction mixtures results in a plot which intersects the ordinate at points differing from those of the ATP or pyruvate controls. Both of these plots show non-competitive acceleration. The addition of acetyl-CoA to these mixtures in the presence of

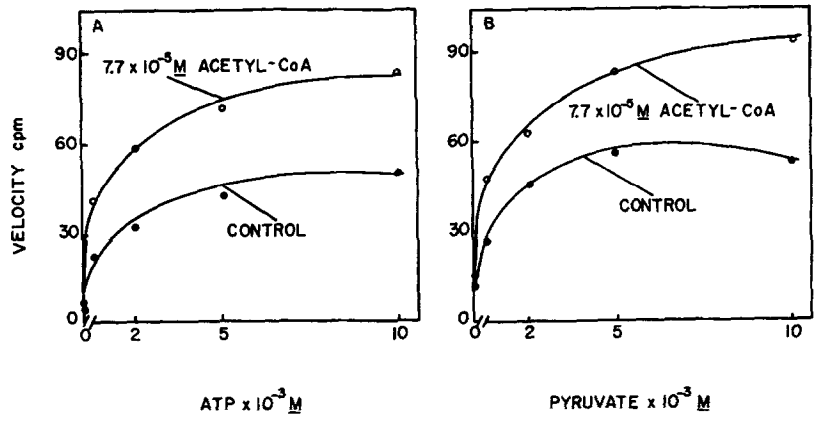

Fig. 1. Pyruvate carboxylase velocity curves (A) ATP, (B) pyruvate.

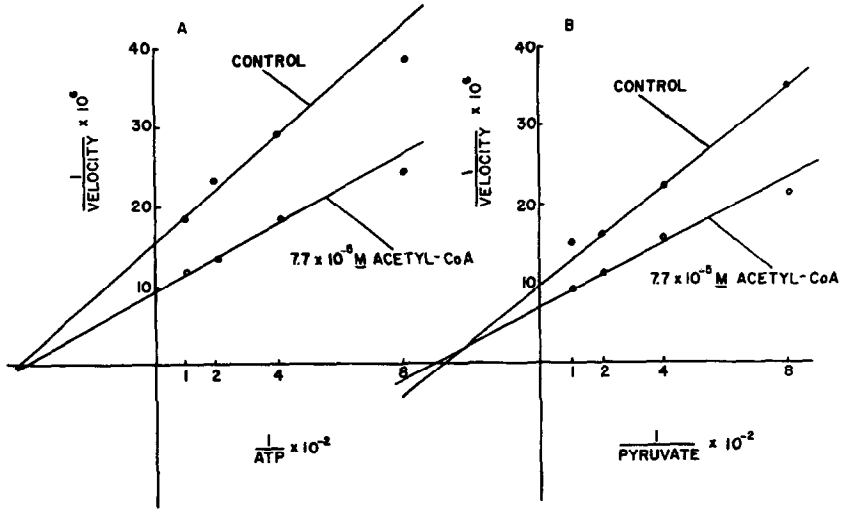

Fig. 2. Lineweaver-Burke reciprocal plots of velocity curves (A) ATP, (B) pyruvate.

5.0×10^{-2} M bicarbonate does not appreciably alter the Km values for ATP or pyruvate but does result in increased V_{max} values.

Lineweaver-Burke reciprocal plots for bicarbonate velocity curves in the presence and absence of acetyl-CoA are shown in Fig. 3. The plots in the presence and absence of acetyl-CoA do not intersect the ordinate at the same point showing non-competi-

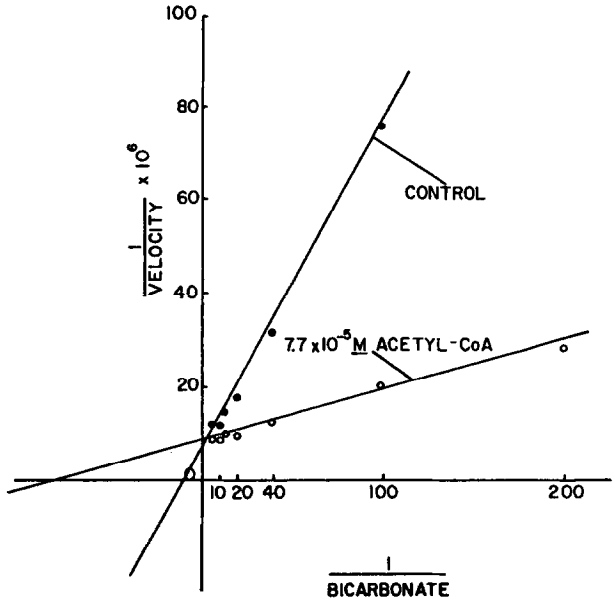

Fig. 3. Lineweaver-Burke reciprocal plot of the bicarbonate velocity curves. The reaction mixtures contained 270 μg of protein.

tive acceleration. This point is verified by the fact that high concentrations of bicarbonate do not overcome the acetyl-CoA stimulation. The addition of acetyl-CoA to these mixtures results in a 7 fold decrease in the Km value for bicarbonate.

The results presented in this paper support the conclusion that the addition of acetyl-CoA to yeast pyruvate carboxylase results in a change in the tertiary structure of the protein and causes a lower Km value for bicarbonate. Acetyl-CoA seems specific in this response for the addition of acetyl -CoA causes no significant change in the Km values for the other substrates of pyruvate carboxylase. This response may offer an explanation of why acetyl-CoA is only functional in the formation of the CO_2-Biot-Enz complex from bicarbonate and not from the beta carboxyl group of oxaloacetate.

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