ACTIVATION OF ACETYL-CoA CARBOXYLASE AND ASSOCIATED ALTERATION OF SEDIMENTATION CHARACTERISTICS OF THE ENZYME

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Acetyl-CoA carboxylase, the biotin-enzyme that catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA (Wakil 1958)

$$CH_3COSCoA + ATP + CO_2 \xrightarrow{Mn^{++}} COOHCH_2COSCoA + ADP + Pi$$

has been reported to be the rate-limiting reaction of long chain fatty acid synthesis (Ganguly 1960, Numa, Matsuhashi and Lynen, 1961). It has recently been reported that stimulation of fatty acid synthesis in a rat adipose enzyme system by tricarboxylic acid cycle intermediates, most notably citrate, occurs through activation of acetyl-CoA carboxylase (Martin and Vagelos 1962). Evidence was presented that the carboxylase itself is activated when it is incubated with citrate and that no free, activating intermediate is formed from citrate.

The carboxylase from rat adipose tissue has now been purified approximately 70-fold by ammonium sulfate fractionation, dialysis, ultracentrifugation to remove inactive protein, and calcium phosphate gel adsorption and elution. This further purification has permitted more definitive studies on the mechanism of activation of the enzyme by citrate.

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The possibility that citrate, a known chelating agent, activates the enzyme by removal of an inhibitor from the enzyme has been tested by substituting other effective chelating agents such as versene, Perma Kleer, or α,α'-dipyridyl for citrate. These agents, when tested at concentrations at which citrate is stimulatory, did not activate the carboxylase. Therefore the activation by citrate can not be explained solely by its chelator properties.

The second possibility considered was that citrate might give rise to an intermediate in catalytic amounts that remains firmly attached to the carboxylase and acts in a transcarboxylating role. Two potent inhibitors of the Krebs cycle, trans-aconitate and fluorocitrate (generously provided by Dr. E.M. Gal), were tested to see if blocking the metabolism of citrate at the aconitase reaction would also block citrate activation of the carboxylase. Trans-aconitate, when incubated with the enzyme and citrate, did not block activation by citrate. Similarly, fluorocitrate did not block the activation by citrate. However, fluorocitrate, when incubated alone with the enzyme, was found to stimulate the carboxylase to the same extent as the stimulation observed with equivalent concentrations of citrate. Such equivalent activation by citrate and fluorocitrate ruled out the possibility that these compounds activate the carboxylase by giving rise to Krebs cycle intermediates.

The last possibility tested was that citrate alters the enzyme configuration or structure in a fashion analogous to the recently reported hormone

effects of glutamic dehydrogenase (Yielding and Tomkins 1960, Wolff 1962). Because the enzyme was not pure enough for optical study of sedimentation characteristics, the sucrose gradient method (Martin and Ames 1961) was used. The enzyme sedimentation was followed by enzymatic activity alone. As shown in Fig. 1 the control carboxylase sediments with a sharp peak after 3 hours of centrifugation at 3°. Using bovine liver catalase as a standard, the calculated $s_{20,w}$ is 18.5 S. If the enzyme is preincubated under the conditions for maximal activation ($5X10^{-3}$ M citrate at 30° for 30 minutes) and then centrifuged in a similar sucrose gradient system except for the presence of citrate, $5X10^{-3}$ M, a broader peak is observed which sediments much faster. The calculated $s_{20,w}$ is approximately 38 S. When enzyme (preincubated in the absence of citrate) is centrifuged in a sucrose gradient with citrate present, the majority of the enzyme is present in the position of the 18.5 S enzyme although there is some faster sedimenting enzyme. If one assumes no change in shape or hydration of the protein, approximate molecular weights of 500,000 and 1,500,000 can be estimated for the control and citrate-preincubated enzyme from the sedimentation constants. Although the data are consistent with aggregation of the enzyme when it is incubated with citrate, a change in shape or hydration would also explain the more rapid sedimentation. If the increased rate of sedimentation is due to aggregation, the control and activated enzymes are related approximately as monomer and trimer.

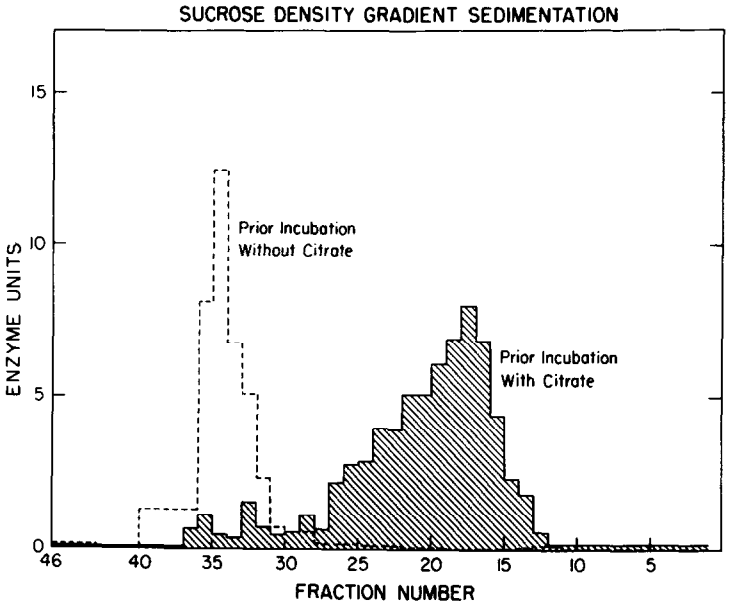

Fig. 1. Sucrose gradient sedimentation of control and citrate-preincubated acetyl-CoA carboxylase. Carboxylase (.3 mg) was preincubated in 0.13 M imidazole·HCl, pH 6.5, $5x10^{-3}$ M citrate, and $6.6x10^{-3}$ M 2-mercaptoethanol for 30 minutes at 30°. A control contained no citrate in the preincubation. Control enzyme, 0.1 ml containing 0.15 mg of protein, was layered on a sucrose gradient containing 0.01 M 2-mercaptoethanol and 0.01 M imidazole·HCl, pH 6.5. Citrate-preincubated enzyme, 0.1 ml containing 0.2 mg of protein, was layered on a similar gradient except for the addition of $5x10^{-3}$ M citrate, pH 6.5. After 3 hours of centrifugation at 38,000 r.p.m., 3°, the gradients were fractionated and analyzed. Total acetyl-CoA carboxylase was assayed in both control and citrate preincubated experiments by subjecting all fractions to preincubation for 30 minutes at 30° with citrate, $5x10^{-3}$ M. The fully activated enzyme was assayed for ability to catalyze the fixation of $C^{14}O_2$ in the presence of acetyl-CoA, ATP and manganese, as previously reported (Martin and Vagelos 1962) except that the reactions were incubated for 30 minutes.

Evidence that citrate activation of the carboxylase is closely associated with the formation of the rapidly sedimenting enzyme includes the following: both activation and change in sedimentation characteristics require the same preincubation conditions; those Krebs cycle intermediates which activate the enzyme

cause the change in sedimentation of the enzyme; finally both processes are reversible upon dilution of the citrate. These experiments suggest that citrate activation of the carboxylase is associated with a physical change in the enzyme. The nature of this change is under further investigation.

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