Proc. Natl. Acad. Sci. USA
Vol. 77, No. 6, pp. 3351–3355, June 1980
Biochemistry

Regulation of acetyl-CoA carboxylase: Properties of CoA activation of acetyl-CoA carboxylase

(fatty acid synthesis/regulation of lipogenesis)

LI-AN YEH AND KI-HAN KIM

Department of Biochemistry, Purdue University, West Lafayette, Indiana 47907

Communicated by Edwin T. Mertz, March 24, 1980

ABSTRACT Acetyl-CoA carboxylase [acetyl-CoA:carbon-dioxide ligase (ADP-forming), EC 6.4.1.2] is activated by physiological concentrations of CoA. The CoA concentration dependency of this activation is sigmoidal; below 60 μM there is little or no activation, but the activation observed between 60 and 120 μM indicates that small changes in the concentration of CoA can cause significant changes in carboxylase activity. CoA activation of acetyl-CoA carboxylase accompanies polymerization of acetyl-CoA carboxylase. However, the binding site for CoA appears to be different from that of citrate. In contrast to citrate activation, which changes only the V_{max} of the reaction, CoA activation of carboxylase results in polymeric forms with a lower K_m for acetyl-CoA. The K_m for acetyl-CoA is 0.4 mM in the control enzyme, whereas that of the CoA-activated enzyme is as low as 4 μM. The K_m for ATP was not changed. Derivatives of CoA were not effective in activating the carboxylase, indicating that the CoA effect is specific. Arguments are presented that CoA could be a physiologically significant positive effector of the carboxylase.

Acetyl-CoA carboxylase [acetyl-CoA:carbon-dioxide ligase (ADP-forming), EC 6.4.1.2] catalyzes the rate-limiting step in the synthesis of long-chain fatty acids. The polymeric form of the enzyme is active whereas the protomeric form is inactive (1–4). Because various cellular metabolites are known to shift the equilibrium between the polymeric and protomeric states of the carboxylase *in vitro*, it has generally been assumed that the short-term control of the enzyme may be accomplished *in vivo* by cellular metabolites (5).

Because high concentrations of citrate cause aggregation and activation of the carboxylase *in vitro*, it has been proposed and widely accepted that citrate may be the positive allosteric effector of carboxylase *in vivo*, thus regulating the synthesis of long-chain fatty acids (5). However, there is no clear evidence for the occurrence of such a control mechanism *in vivo*. Increased fatty acid synthesis is frequently accompanied by no change or even a decrease in citrate concentration (6–10). Even in the chicken liver system, in which extensive studies on the mechanism of citrate activation of acetyl-CoA carboxylase have been carried out (5), inhibition of lipogenesis persisted under conditions of increased citrate levels (11). Similarly, when lipogenesis was inhibited in isolated rat hepatocytes with a hypolipidemic agent, this inhibition was accompanied by an increase in citrate concentration (12), again indicating the lack of a citrate effect on lipogenesis. There are other theoretical and experimental difficulties in the theory that citrate is a positive effector of carboxylase *in vivo*. These are discussed in detail in the *Discussion*.

In contrast to the lack of correlation between citrate and lipogenesis, it has been observed that the hepatic CoA concentration increases as the rate of fatty acid synthesis increases (8) and decreases under conditions such as lipogenesis inhibition (12) and gluconeogenesis (13–15). However, the physiological meaning of changes in the CoA concentration with respect to fatty acid synthesis has never been closely examined.

In this communication, we present evidence that CoA activates acetyl-CoA carboxylase in a manner consistent with CoA being a physiologically significant allosteric effector of the enzyme.

MATERIALS AND METHODS

Acetyl-CoA, CoA, Sepharose 2B, phenylmethylsulfonyl fluoride, and crystalline bovine serum albumin were purchased from Sigma. ATP was obtained from Schwarz/Mann; oxidized CoA, dephospho-CoA, and (1,N^6-etheno)-CoA were from P-L Biochemicals. $NaDodSO_4$ was a product of Schwarz/Mann and was recrystallized from ethanol.

$NaH^{14}CO_3$ was purchased from Amersham/Searle (59.1 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels), purified as described (16), and then diluted with carrier to 80 mM $KH^{14}CO_3$ (0.4 mCi/mmol).

Wistar rats (230–280 g) from our departmental rat colony were maintained on a commercial rat diet fed ad lib.

Preparation of Acetyl-CoA Carboxylase. Acetyl-CoA carboxylase was purified by the procedure of Nakanishi and Numa (17), except that all buffers contained 0.2 mM phenylmethylsulfonyl fluoride. The purified enzyme had a specific activity of 12 units/mg of protein. Purity was examined by $NaDodSO_4$/acrylamide gel electrophoresis (18).

Partially purified enzyme was prepared as described (16). The specific activity of acetyl-CoA carboxylase in such preparations was between 70 and 90 milliunits/mg of protein in the presence of 10 mM citrate.

Assay of Acetyl-CoA Carboxylase. Acetyl-CoA carboxylase activity was measured as described (16). One unit of activity is defined as 1 μmol of malonyl-CoA formed per minute at 37°C.

Sucrose Density Gradient Centrifugation. A 0.2-ml enzyme sample was applied to a 5-ml linear gradient of 5–20% sucrose in 50 mM Tris·HCl, pH 7.5/1 mM EDTA/and 5 mM 2-mercaptoethanol. The citrate concentration used in the gradients is indicated in the appropriate figures. Samples were centrifuged for 90 min (or as specified) at 25°C in a SW 65 swinging bucket rotor at 45,000 rpm. Carboxylase activity in each fraction was assayed as described (16).

RESULTS

Effect of CoA on Acetyl-CoA Carboxylase. Like citrate activation of acetyl-CoA carboxylase, CoA activation of the enzyme was time dependent (Fig. 1). In the absence of CoA, 2 mM citrate activated carboxylase 5- to 6-fold in 30 min. Addition of 0.12 mM CoA to the activation mixture further activated the carboxylase. However, the time required for maximal

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

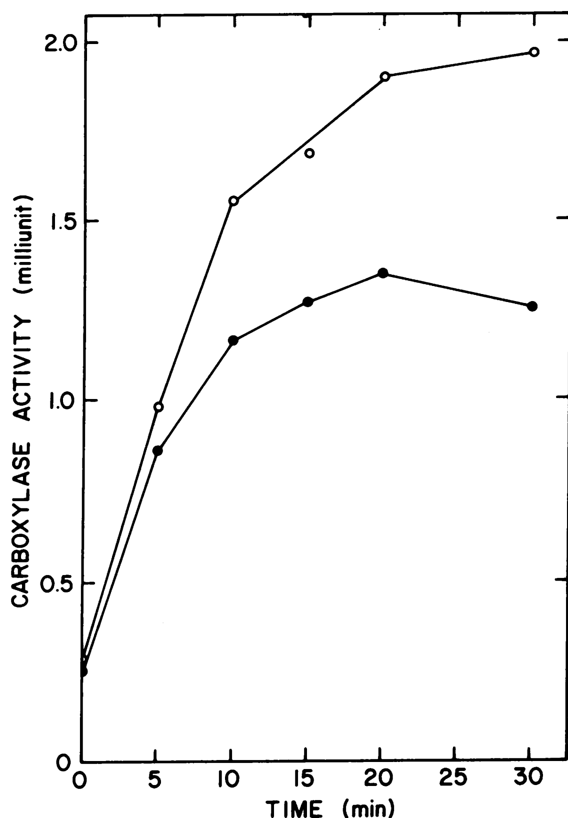

FIG. 1. Effect of CoA on acetyl-CoA carboxylase activation. Partially purified enzyme was incubated with or without 0.12 mM CoA in medium containing 2 mM citrate, 5 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM theophylline, and 0.2 mM phenylmethylsulfonyl fluoride at 37°C. Aliquots were withdrawn at the indicated times for assay of enzyme activity. ●, Control (no CoA); ○, 0.12 mM CoA.

activation did not change significantly. Although in this experiment, CoA was added to an enzyme preparation containing citrate, CoA activation of the carboxylase does not require citrate, as shown below.

The effect of different concentrations of CoA on the activation of acetyl-CoA carboxylase is shown in Fig. 2. In this experiment different concentrations of CoA were incubated with the enzyme for 30 min before activity was determined. In contrast to the citrate activation of carboxylase (5), CoA activation was sigmoidal with respect to CoA concentration. Up to 60 μM CoA there was little or no activation of the enzyme. A marked increase in activity was observed between 60 and 120 μM CoA. The hepatic concentration of CoA has been reported to be 0.36 mM (19). The exact concentration of CoA in the cytoplasm of normal liver cells is not clear; however, it varies between 5 μM (20) and 115 μM (21, 22) depending upon the methods of subcellular fractionation and the determination of subcellular water space. Therefore, the cellular concentration of CoA appears to be sufficient to affect the carboxylase activity. The most noteworthy aspect of the concentration dependency of activation is that small changes of CoA concentration in the range 60–120 μM can affect the activity drastically.

Effect of CoA on Sedimentation Behavior of Acetyl-CoA Carboxylase. To determine whether CoA activates the carboxylase by polymerization, the CoA-activated enzyme was subjected to sucrose density gradient centrifugation (Fig. 3). In the absence of either citrate or CoA, carboxylase sedimented with a sedimentation constant of 17 S, which is characteristic of the protomeric form. Preincubation of the enzyme with 120 μM CoA resulted in the formation of polymeric forms with a broad range of sedimentation constants (24–42 S). For comparison, the effect of 10 mM citrate on carboxylase aggregation is also shown in Fig. 3. This experiment indicates that CoA activation of the carboxylase accompanies enzyme aggregation.

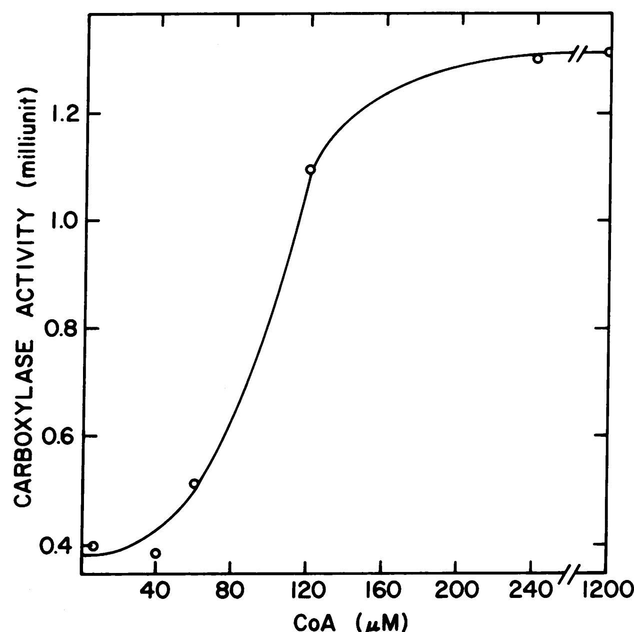

FIG. 2. Effect of different concentrations of CoA on the activation of acetyl-CoA carboxylase. Partially purified enzyme was preincubated with different concentrations of CoA as indicated for 30 min at 37°C in a medium containing 50 mM Tris·HCl (pH 7.5), 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, and 1 mM theophylline. The carboxylase activities were then assayed.

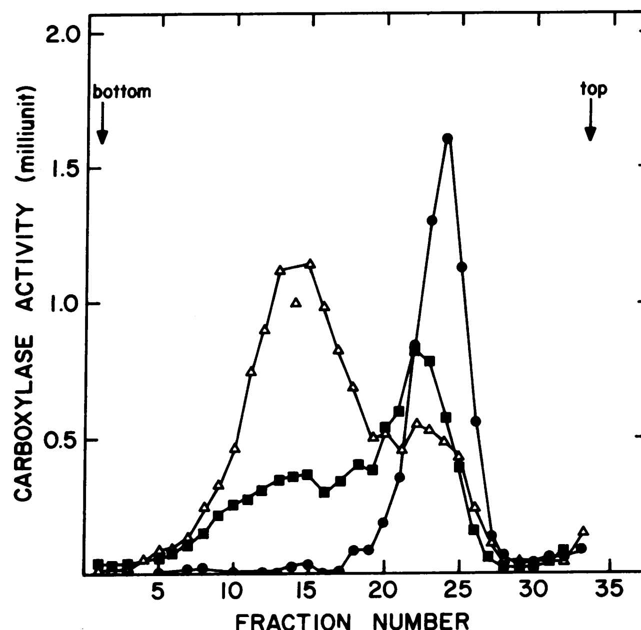

FIG. 3. Effect of CoA activation on the sedimentation pattern of acetyl-CoA carboxylase. Partially purified enzyme was preincubated with no addition (●), 10 mM citrate (△), or 0.12 mM CoA (■) in a medium containing 50 mM Tris·HCl (pH 7.5), 1 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride at 37°C for 30 min. Samples were placed on a 5–20% sucrose gradient in 50 mM Tris/1 mM EDTA/5 mM 2-mercaptoethanol. Centrifugation was carried out at 45,000 rpm for 90 min. Acetyl-CoA carboxylase activity in each fraction was assayed after further activation by incubation in the presence of 2 mM sodium citrate at 37°C for 30 min.

Relationship Between Citrate and CoA Activation of Acetyl-CoA Carboxylase. In contrast to citrate activation of carboxylase (see *Discussion*), CoA activation of the enzyme was observed in the presence of a physiological concentration of CoA. However, because both citrate and CoA activation of the carboxylase accompany enzyme aggregation, it was of interest to compare their modes of action.

Addition of CoA to the maximally activated carboxylase at a given citrate concentration further activated the enzyme (Fig. 4). Even at a saturating citrate concentration (10 mM), CoA activated the enzyme still further, indicating that the binding site(s) of CoA may be different from that of citrate. Because CoA acted even at saturating concentrations of citrate, we examined the sedimentation profile of the carboxylase under these conditions. Addition of CoA to enzyme that had been activated in the presence of 10 mM citrate resulted in the formation of an even faster sedimenting species, 50–55 S (Fig. 5). This experiment, supports the view that CoA activation of the carboxylase may be due to the formation of polymeric forms of the enzyme.

Effect of CoA on the K_ms of Acetyl-CoA Carboxylase for Substrates. Citrate is known to increase V_{max} without changing K_m for any of the substrates of the enzyme (5), although a slight decrease in K_m for acetyl-CoA by citrate has been reported (23).

The CoA-activated enzyme showed a marked decrease in the K_m for acetyl-CoA (Fig. 6). The calculated K_m for acetyl-CoA of the control enzyme in the absence of CoA was about 0.4 mM, which is similar to the values reported previously (24). When the carboxylase was activated by preincubation with 0.12 mM CoA, new species of the carboxylase with a drastically decreased K_m for acetyl-CoA were observed. The K_ms for acetyl-CoA for the carboxylase incubated with CoA were between 0.004 and 0.4 mM. The new species of carboxylase formed in the presence of CoA had a K_m value as low as 4 μM. The cytosolic acetyl-CoA concentration in rat liver has been reported to be 5 μM (20).

Although ATP-citrate lyase, which is responsible for generating the cytosolic acetyl-CoA for fatty acid synthesis, increases under physiological conditions of stimulated fatty acid synthesis, such increases in enzyme activity require a matter of days (25) and cannot be a factor in the short-term regulation of the car-

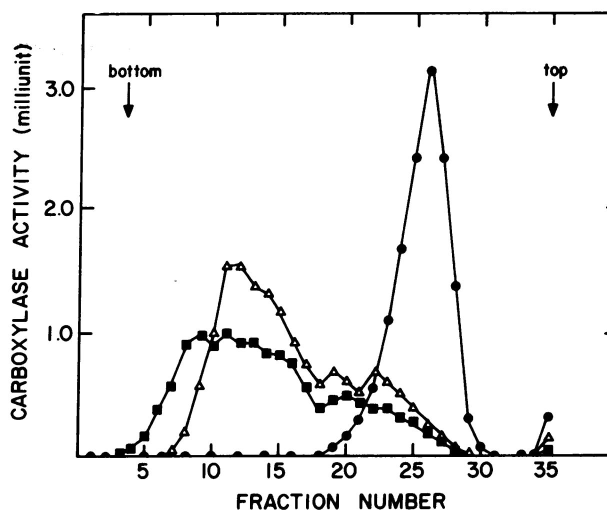

FIG. 5. Effect of CoA on the sedimentation pattern of citrate-activated acetyl-CoA carboxylase. Partially purified enzyme was preincubated with or without 2 mM citrate in a medium containing 50 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, and 0.2 mM phenylmethylsulfonyl fluoride at 37°C for 30 min. The sample containing 2 mM citrate was divided in half after the preincubation, and CoA was added to a final concentration of 0.12 mM for further incubation (5 min). Samples were placed on sucrose gradients and subjected to centrifugation. Acetyl-CoA carboxylase was assayed according to the standard assay procedure. ●, No addition; △, 2 mM citrate; ■, 2 mM citrate and then 0.12 mM CoA for 5 min.

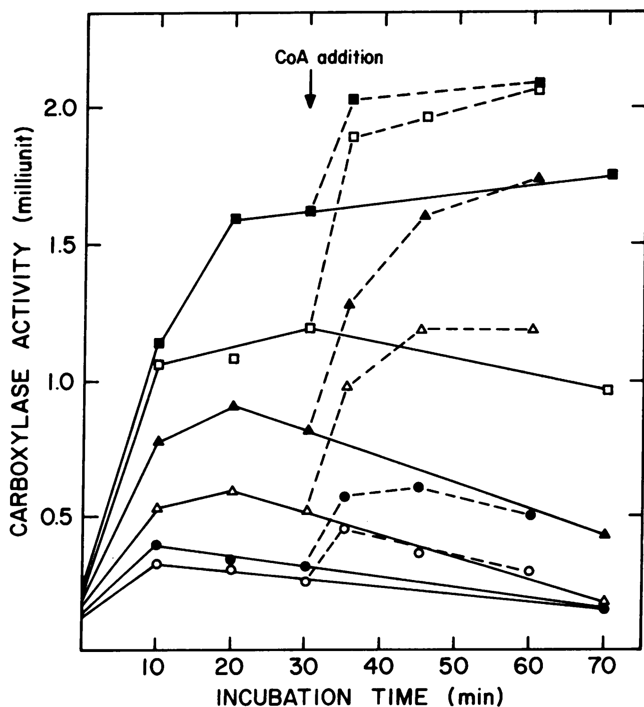

FIG. 4. Effect of CoA on citrate-activated acetyl-CoA carboxylase. Partially purified enzyme was incubated in a medium containing 50 mM Tris (pH 7.5), 5 mM $MgCl_2$, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, and different concentrations of citrate at 37°C for 30 min. Each sample was divided into two portions after 30-min preincubation, and CoA was added to one portion for further incubation. At the indicated times aliquots were withdrawn for assay of enzyme activity. ○, No citrate; ●, 0.1 mM; △, 0.5 mM; ▲, 1.0 mM; □, 2.0 mM; ■, 10 mM. Broken lines represent the activity after addition of CoA (0.12 mM).

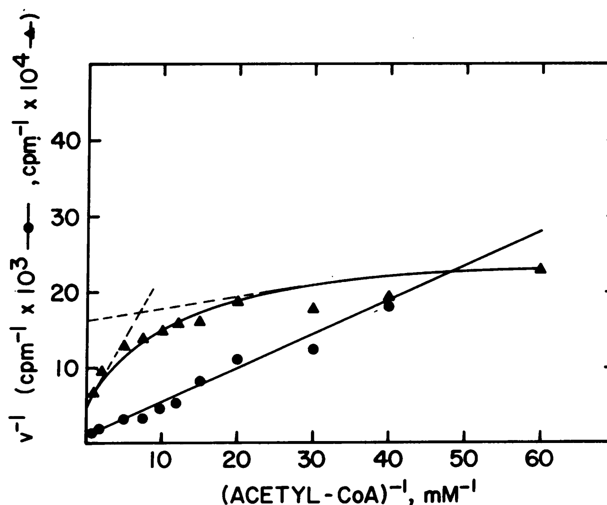

FIG. 6. Double-reciprocal plots of velocity and acetyl-CoA of CoA-activated enzyme. Partially purified enzyme in the absence of citrate was preincubated in the presence or absence of 0.12 mM CoA in a medium containing 50 mM Tris-HCl (pH 7.5). 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM theophylline at 37°C for 30 min. The effect of different concentrations of acetyl-CoA on the velocity of acetyl-CoA carboxylase was determined. ●, Control enzyme; ▲, CoA-activated enzyme.

boxylase by increasing acetyl-CoA concentrations. Thus, changes in the K_m for acetyl-CoA in the CoA-activated enzyme have a significant physiological meaning in the control of acetyl-CoA carboxylase and lipogenesis. On the other hand, the K_m for ATP of the CoA-activated carboxylase does not change (data not shown).

Specificity of CoA Activation of Acetyl-CoA Carboxylase. Because CoA activation of the carboxylase is a time-dependent process, the effect of different CoA derivatives [dephospho-CoA, (1,N^6-etheno)CoA, and oxidized CoA] during the preincubation period was examined. Oxidized CoA exhibited about 20% of the effectiveness of CoA, whereas dephospho-CoA and (1,N^6-etheno)CoA were inactive. A separate experiment using [^{3}H]CoA indicated that during the preincubation of the carboxylase preparations with CoA, there was no degradation of the CoA molecules.

DISCUSSION

Although citrate activates acetyl-CoA carboxylase *in vitro*, there are many difficulties with the theory that citrate alone is the physiologically positive effector of carboxylase *in vivo*. One of the most serious difficulties associated with the citrate activator theory is that the cellular concentration of citrate does not reflect the rate of fatty acid synthesis *in vivo*. Many studies carried out to examine the relationship between cellular concentrations of citrate and the rate of fatty acid synthesis indicate that the citrate concentration either does not change or that it changes in the direction opposite to that predicted for a regulatory role in fatty acid synthesis in rat liver (6, 12).

Studies of the citrate activation of purified carboxylase indicate that citrate is required at two steps (5, 26). For enzyme activation, citrate is required only in the micromolar range (3 μM), but higher concentrations of citrate (up to 6 mM) are required for keeping the carboxylated species of the enzyme in its polymeric state during the enzyme reaction. However, the total cellular concentration of citrate is only about 0.1–0.3 mM (9, 27), and a good portion of this metabolite is intramitochondrial. On the other hand, acetyl-CoA carboxylase activation by CoA occurs in the presence of physiological concentrations. The cytosolic concentration of CoA in normal cells has been reported to be 5 μM when liver cells were fractionated by the method of lyophilization and homogenization in an organic solvent followed by density gradient centrifugation (20). However, when cells from fasted animals were fractionated by the modified digitonin method (21), the cytosolic concentration of CoA was about 23 μM. Because the cells from fasted animals contain only about one-fifth of the normal concentration (20) it follows that the cytosolic concentration is about 115 μM. The cytosolic water content was taken to be 2 ml/g dry weight of cells (13). Direct homogenization of liver tissue yielded about 130 μM in the cytosol (22). In this case, the breakage of mitochondria was assessed by mitochondrial marker enzymes. It is also interesting to note that small changes in the CoA concentration around 60 μM affect the activity profoundly, as shown in this report.

Citrate's role as a negative effector of the reactions of phosphofructokinase (28–30) and pyruvate dehydrogenase (31) is well known. These are two of the most important reactions involved in the conversion of carbohydrate into fatty acids. If citrate is the positive effector of acetyl-CoA carboxylase, and thus of the process of fatty acid synthesis in the liver, it is difficult to imagine how citrate can act at the phosphofructokinase and pyruvate dehydrogenase steps in one way and at the acetyl-CoA carboxylase step in another so that citrate could stimulate fatty acid synthesis.

Additional experiments are required to establish conclusively that CoA is the physiologically significant positive effector for fatty acid synthesis instead of or in combination with citrate. However, the properties of CoA activation of acetyl-CoA carboxylase, as reported here, suggest that CoA activation of acetyl-CoA carboxylase might be physiologically important. In support of these *in vitro* studies, one can list a number of physiological experiments in which CoA concentrations change depending upon the status of fatty acid synthesis. For example, conditions favoring gluconeogenesis result in a decrease in free CoA (13–15) whereas CoA concentrations increase under conditions favoring lipogenesis (8). In this connection, it should be mentioned that Linn *et al.* (32) reported a CoA requirement for fatty acid synthetase activity. It is interesting to note that, although citrate is constantly degraded in the process of acetyl-CoA production during lipogenesis, CoA only serves as an acyl group carrier and is constantly regenerated. Also, it should be noted that CoA is not a competitive inhibitor of acetyl-CoA carboxylase with respect to acetyl-CoA, although CoA is competitive with respect to palmityl-CoA inhibition of the carboxylase. However, it should be emphasized that these compounds are not substrates or inhibitors for the catalytic activity of the carboxylase but affect the quaternary structure of the enzyme. Therefore, simple competition as revealed by kinetic studies might be misleading.

The relationship between covalent modification of acetyl-CoA carboxylase (31, 33–35) and CoA activation of the enzyme should be mentioned. Our preliminary studies indicate that the phosphorylated form of acetyl-CoA carboxylase does not respond to CoA activation at all at physiological concentrations. Such observations support the theory that covalent modification of acetyl-CoA carboxylase is an amplification mechanism for allosteric control by cellular metabolites (36, 37).

Elucidation of the exact mechanism of CoA action requires the use of a homogeneous enzyme preparation that has not been exposed to citrate. Unfortunately, all the existing purification procedures utilize high citrate concentrations (20 mM) for the stabilization of carboxylase during purification. It is difficult to remove bound citrate from such enzyme preparation. Thus, highly purified enzyme shows only a minimal effect of CoA. However, extensive dialysis against citrate-free buffer and treatment of the dialyzed enzyme with Dowex in the chloride form result in a decrease in the citrate content (38). Such an enzyme preparation shows increased sensitivity toward CoA activation, indicating that the minimal effect of CoA with conventionally purified enzyme preparations is due to the presence of citrate. The results of these studies will be published elsewhere. Highly purified preparations have been shown to contain no fatty acyl-CoA (39).

We thank Dr. G. Kohlhaw for generous gifts of various CoA analogues and [^{3}H]CoA. This is Journal Paper No. 7702 from Purdue University Agricultural Experimental Station. This research was supported by Grant AM-12865 from the National Institutes of Health.

1. Vagelos, P. R., Alberts, A. W. & Martin, D. B. (1963) *J. Biol. Chem.* **238**, 533–540.
2. Numa, S., Ringelmann, E. & Lynen, F. (1965) *Biochem. J.* **343**, 243–257.
3. Gregolin, C., Ryder, E., Kleinschmidt, A. K., Warner, R. C. & Lane, M. D. (1966) *Proc. Natl. Acad. Sci. USA* **56**, 148–155.
4. Gregolin, C., Ryder, E., Warner, R. C., Kleinschmidt, A. K. & Lane, M. D. (1966) *Proc. Natl. Acad. Sci. USA* **56**, 1751–1758.
5. Lane, M. D., Moss, J. & Polakis, S. E. (1974) *Curr. Top. Cell. Regul.* **8**, 139.
6. Halestrap, A. P. & Denton, R. M. (1974) *Biochem. J.* **142**, 365–377.

7. Cook, G. A., Nielsen, R. C., Hawkins, R. A., Mehlman, M. A., Lakshmanan, M. R. & Veech, R. L. (1977) *J. Biol. Chem.* **252**, 4421–4424.
8. Goodridge, A. G. (1973) *J. Biol. Chem.* **248**, 1939–1945.
9. Spencer, A. F. & Lowenstein, J. M. (1967) *Biochem. J.* **103**, 342–348.
10. Guynn, R. W., Veloso, D. & Veech, R. L. (1972) *J. Biol. Chem.* **247**, 7325–7331.
11. Clarke, S. D., Watkins, P. A. & Lane, M. D. (1979) *J. Lipid Res.* **20**, 974–985.
12. McGune, S. A. & Harris, R. A. (1979) *J. Biol. Chem.* **254**, 10095–10101.
13. Williamson, J. R., Browning, E. T. & Scholz, R. (1969) *J. Biol. Chem.* **244**, 4607–4616.
14. Yeh, Y.-Y. & Leveille, G. A. (1971) *J. Nutr.* **101**, 911–918.
15. Nakamura, T., Nomoto, N., Yagi, R. & Oya, N. (1970) *J. Vitaminol.* **16**, 89–98.
16. Lee, K.-H. & Kim, K.-H. (1977) *J. Biol. Chem.* **252**, 1748–1751.
17. Nakanishi, S. & Numa, S. (1970) *Eur. J. Biochem.* **16**, 161–173.
18. Inoue, H. & Lowenstein, J. M. (1972) *J. Biol. Chem.* **247**, 4825–4832.
19. Abiko, Y. (1975) in *Metabolism of Sulfur Compounds*, ed. Greenberg, V. M. (Academic, New York), pp. 1–29.
20. Soboll, S., Scholz, R., Freisl, M., Elbers, R. & Heldt, H. W. (1976) in *Use of Isolated Liver Cells and Kidney Tubules in Metabolic Studies*, eds. Tager, J. M., Soling, H. D. & Williamson, J. R. (North-Holland, Amsterdam), pp. 29–40.
21. Siess, E. A., Brocks, D. G. & Wieland, O. H. (1976) *FEBS Lett.* **69**, 265–271.
22. Oram, J. F., Wenger, J. I. & Neely, J. R. (1975) *J. Biol. Chem.* **250**, 73–78.
23. Hashimoto, T. & Numa, S. (1971) *Eur. J. Biochem.* **18**, 319–331.
24. Carlson, C. A. & Kim, K.-H. (1974) *Arch. Biochem. Biophys.* **164**, 490–501.
25. Lowenstein, J. M. (1968) in *The Metabolic Roles of Citrate*, ed. Goodwin, T. W. (Academic, New York), pp. 61–86.
26. Numa, S., Hashimoto, T., Nakanishi, S. & Okasaki (1972) in *Current Trends in the Biochemistry of Lipids*, eds. Ganguly, J. & Smellie, R. M. S. (Academic, New York), pp. 27–39.
27. Greenbaum, A. L., Gumaa, K. A. & McLean, P. (1971) *Arch. Biochem. Biophys.* **143**, 617–663.
28. Passanneau, J. V. & Lowry, O. H. (1963) *Biochem. Biophys. Res. Commun.* **13**, 372–379.
29. Underwood, A. H. & Newsholme, E. A. (1965) *Biochem. J.* **95**, 868–875.
30. Underwood, A. H. & Newsholme, E. A. (1967) *Biochem. J.* **104**, 296–299.
31. Carlson, C. A. & Kim, K.-H. (1973) *J. Biol. Chem.* **248**, 378–380.
32. Linn, T. C., Stark, M. J. & Srere, P. A. (1980) *J. Biol. Chem.* **255**, 1388–1392.
33. Lee, K.-H. & Kim, K.-H. (1977) *J. Biol. Chem.* **252**, 1748–1751.
34. Hardie, D. G. & Cohen, P. (1978) *FEBS Lett.* **91**, 1–7.
35. Witters, L. A., Kowaloff, E. M. & Avruch, J. (1979) *J. Biol. Chem.* **254**, 245–248.
36. Carlson, C. A. & Kim, K.-H. (1974) *Arch. Biochem. Biophys.* **164**, 478–489.
37. Witters, L. A., Moriarity, D. & Martin, D. B. (1979) *J. Biol. Chem.* **254**, 6644–6649.
38. Numa, S., Goto, T., Rigelmann, E. & Riedel, B. (1967) *Eur. J. Biochem.* **3**, 124–128.
39. Ogiwara, H., Tanahl, T., Nikawa, J. & Numa, S. (1978) *Eur. J. Biochem.* **89**, 33–41.