Coenzyme A Activation of Acetyl-CoA Carboxylase*

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Acetyl-CoA carboxylase is activated by physiological concentrations of CoA. Activation of partially purified enzyme by CoA is accompanied by a decrease in the $K_m$ for acetyl-CoA from 0.2 mM to about 4 $\mu$M, which is the physiological concentration of acetyl-CoA in the cytosol. CoA activation of the purified enzyme is accompanied by an increase in the $V_{max}$, without changing the $K_m$ for acetyl-CoA. The $K_m$ for acetyl-CoA of the purified enzyme is about 10 to 40 $\mu$M. The purification procedure results in a decrease in the $K_m$ for acetyl-CoA; under these conditions, CoA activation does not cause further lowering of the $K_m$. CoA activation is accompanied by polymerization of the enzyme. However, CoA activation is not causally related to polymerization.

There is one CoA binding site/subunit of acetyl-CoA carboxylase. CoA binding at that site is not affected by the presence of citrate, but palmityl-CoA inhibits CoA binding. CoA alone cannot reverse palmityl-CoA inhibition of the carboxylase. Bovine serum albumin and CoA together can activate the palmityl-CoA-inhibited enzyme. This indicates that the involvement of bovine serum albumin-like protein, CoA, and palmityl-CoA may play a physiologically significant role in the control of acetyl-CoA carboxylase.

However, in spite of the amplification effect of covalent modification with respect to the effects of allosteric metabolites, the $K_m$ for acetyl-CoA is still relatively high (0.2 mM) compared to the cytosolic acetyl-CoA concentration. This suggests that additional factors are necessary for the functioning of carboxylase under in vivo conditions. Neither activation by dephosphorylation (6), nor by citrate (1), can lower the $K_m$ for acetyl-CoA sufficiently to reach the cytosolic acetyl-CoA concentration.

Recently we reported that physiological concentrations of CoA can activate the partially purified acetyl-CoA carboxylase. In this case the $K_m$ for acetyl-CoA is decreased to as little as 4 $\mu$M (7). These preliminary experiments suggested a possible physiological role for CoA in the activity of carboxylase. However, when the effect of CoA was examined with purified enzyme, the activation effect was minimal, indicating differences in the properties of partially purified and highly purified enzyme preparations (7).

In this communication, we show that the purified enzyme does respond to CoA activation when it is treated with Dowex 1-X8, a step which is used to remove tightly bound citrate (8). There is one CoA binding site on the carboxylase. Binding of CoA to this site activates the enzyme by changing either the $V_{max}$ or the $K_m$ for acetyl-CoA, depending on the purity of the enzyme preparation. Binding of CoA is inhibited by palmityl-CoA but not by citrate. Additional characteristics of the CoA activation of carboxylase are presented.

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, and oxidized CoA, dephospho-CoA, and (1,1'-etheno)-CoA from P-L Biochemicals. Sodium dodecyl sulfate was a product of Schwarz/Mann and was recrystallized from ethanol. NaH$^{14}$CO$_3$ was purchased from Amersham/Searle (59.1 mCi/mmol), purified as previously described (2), and then diluted with carrier to make 80 mM KH$^{14}$CO$_3$ (0.4 mCi/mmol). [G-$^{3}$H]CoA was purchased from New England Nuclear Corp. (15 mCi/mmol).

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

Purification of Acetyl-CoA Carboxylase—Three different procedures have been used in the purification of acetyl-CoA carboxylase. Each procedure yielded enzyme with somewhat different properties presumably due to proteolytic activities during the purification. First, acetyl-CoA carboxylase was purified by the method of Nakanishi and Numa (9), except that all buffers contained 0.2 mM phenylmethylsulfonyl fluoride. This procedure routinely gave a preparation with a specific activity of 12 units/mg of protein and a subunit molecular weight of 220,000, as well as proteolytic products of molecular weights 120,000 and 118,000.

To minimize enzyme degradation, the purification procedure of Witters and Vogt was utilized (10). In the absence of NaF, this procedure yielded a preparation with a specific activity of 3.4 units/mg of protein, a subunit molecular weight of 240,000, and essentially no proteolytic products. The low specific activity of this enzyme preparation has been explained on the basis of its high phosphate
content (about 5 mol of phosphate/mol of enzyme). The molecular weight of the enzyme is significantly higher than that obtained by the method of Nakanishi and Numa. The high specific activity of the first preparation could thus be due to partial proteolysis since it is well known that such proteolytic action activates the carboxylase enzyme.

Finally, to obtain carboxylase with minimal modification of its native state, an affinity chromatography column was used as follows. The partially purified enzyme preparation, at the 30% ammonium native state, an affinity chromatography column was used as follows. The carboxylase was eluted by the CoA treatment. Carboxylase prepared by this method was not completely pure since it showed traces of lower molecular weight contaminants. The specific activity of enzyme prepared by this method was 1 unit/mg of protein.

**Assay of Acetyl-CoA Carboxylase**—Acetyl-CoA carboxylase was assayed by measuring the formation of [1-14C]malonyl-CoA as described by Majerus et al. (13). The standard reaction mixture contained the following components in a final volume of 500 µl: 50 mM Tris-HCl pH 7.5, BSA, 150 µg, 10 mM citrate, 10 mM MgCl2, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 4.1 mM ATP, 0.13 mM acetyl-CoA, and 13.3 mM KH2CO3 (1.5 × 105 cpm). The reaction was started by the addition of an appropriate amount of enzyme which had been preincubated at 37°C for 30 min. The reaction was carried out for 2 to 3 min and terminated by the addition of 25 µl of 6 N HCl. One hundred µl of the reaction mixture was plated on a disk of glass fibre filter, dried under an infrared lamp, and the radioactivity was measured. One unit of activity was defined as 1 mol of malonyl-CoA formed/min at 37°C. In those experiments where CoA activation was examined, citrate was omitted from the reaction mixture unless otherwise specified.

**Preparation of Citrate-free Enzyme**—Dissociation of tightly bound citrate was carried out by the method of Hashimoto et al. (8). Dowex 1×2 was washed extensively and equilibrated with a large excess of 50 mM Tris-HCl, pH 7.5, containing 0.25 mM sucrose, 1 mM EDTA, and 5 mM β-mercaptoethanol. Three-tenths ml of the resin was packed into a small column (a disposable Pasteur pipette). One hundred µl of the purified enzyme (100 µg) in the same buffer was then passed through the column. The column was washed with the same buffer and 100 µl fractions were collected. The recovery of the enzyme was quantitative and the specific activity was unchanged before and after the column treatment (12 to 14 units/mg) when the activity was measured in the presence of 10 mM citrate. This treatment removes 99% of the residual citrate (6).

**HCoA Binding to the Carboxylase**—One hundred forty µl of the Dowex-treated enzyme (0.1 mg/ml) was incubated with different concentrations of [1-14C]HCoA (32 × 105 cpm/µmol) in a total volume of 200 µl for 20 min at 37°C. Following incubation, 170 µl was placed on a Millipore filter (HAWP02400 pore size 0.45 µm), which had been prewashed in ice-cold 50 mM Tris-HCl, pH 7.5, containing 0.25 mM sucrose, 1 mM EDTA, and 5 mM β-mercaptoethanol. The CoA and citrate concentrations used in the gradients is indicated in the appropriate figures. Samples were centrifuged for 90 min (or as specified) at 25°C in an SW 65 swinging bucket rotator at 45,000 rpm.

**RESULTS**

**Activation of Purified Acetyl-CoA Carboxylase by CoA**—In contrast to the partially purified enzyme, the purified acetyl-CoA carboxylase does not respond to CoA activation (7). This difference between purified and crude enzyme has been investigated in terms of the following hypotheses: (a) Conventional purification procedures expose the carboxylase to high citrate concentrations for an extended period of time in order to facilitate the purification. Such treatment causes significant changes in the enzyme structure due to the presence of tightly bound citrate on the enzyme. When citrate is tightly bound to the enzyme, activation by CoA may be insignificant. (b) It is known that the carboxylase is very sensitive to partial proteolysis (11, 12). Partial proteolysis may make the purified carboxylase unresponsive to CoA activation; or (c) in vivo the carboxylase normally exists in an inactive state as a result of association with some inhibitor which is dissociated during purification. CoA may function in the removal of this hypothetical inhibitor.

Previously, Hashimoto et al. (8) used Dowex 1 to remove most of the tightly bound citrate from the purified carboxylase. When the purified carboxylase was treated with Dowex 1 according to the procedure of Hashimoto et al. (8), the treated enzyme became very sensitive to CoA activation (Fig. 1). As shown in Fig. 1, CoA activation is a time-dependent process, as is also the case with citrate activation; maximum activation is obtained at 20 min at 37°C with CoA. Although Dowex treatment is known to remove the tightly bound citrate, it is uncertain whether anything else is removed.

Our search for the presence of palmitoyl-CoA in the purified enzyme according to the procedure of Ogihara et al. (17) yielded negative results.

Recently, Witters and Vogt (10) introduced a simplified purification procedure for acetyl-CoA carboxylase using polyethylene glycol. This procedure also exposes the carboxylase to high citrate, but only for a brief period. Carboxylase purified by this method has a higher molecular weight (240,000) than that of enzyme prepared by Nakanishi and Numa's procedure (220,000). As reported by Witters and Vogt (10), no degraded enzyme species (M, = 118,000 and 120,000) were found following sodium dodecyl sulfate gel electrophoresis.

![Fig. 1. Activation of acetyl-CoA carboxylase by CoA. Acetyl-CoA carboxylase purified by the method of Nakanishi and Numa (9) and treated with Dowex 1-X8 to remove citrate was incubated with or without 0.15 mM CoA in medium containing 1 mM dithiothreitol, 1 mM theophylline, and 100 µg of BSA at 37°C. Omission of theophylline from the incubation has no effect on CoA action. Aliquots were withdrawn at the indicated times for the enzyme assay. Control: no CoA. ○—○, 0.15 mM CoA, □—□.](image)
When this enzyme preparation was treated with CoA, it was activated as in the case with the partially purified enzyme preparations (7), without Dowex treatment (Fig. 2).

As will be shown later, the nature of CoA activation of these two purified enzyme preparations is different from that of the partially purified enzyme preparations. Although these experiments do not illuminate the reasons for the unresponsiveness of purified enzyme to CoA activation, they do show that the purified enzymes do respond to CoA activation under appropriate conditions. Also, these experiments indicate that purification procedure causes enzyme modification. The enzyme prepared by the method of Witters and Vogt (10) has about one-third of the specific activity of the enzyme prepared by the method of Nakamish and Numa (9). Although it has been suggested that the low specific activity might be due to the presence of the highly phosphorylated form of the carboxylase, the experiments presented here suggest the possibility that the enzyme was tightly bound to the enzyme and could not be eluted from it possibly due to the presence of excess phosphate groups on the enzyme.

The previous studies with partially purified enzyme showed that CoA activation of the carboxylase was accompanied by enzyme aggregation (7). Since enzyme aggregation is a function of protein concentration, the effect of CoA on different enzyme concentrations was examined, as shown in Fig. 3. The maximum activation of carboxylase was observed at 140 μM CoA and higher concentrations of CoA slightly decreased the degree of activation. The concentration of CoA required for the half-maximum activation with three different enzyme concentrations is the same (15 μM CoA) and the effect was independent of the enzyme concentration. These concentrations of the carboxylase undergo polymerization in the presence of CoA as will be seen later (Figs. 5 and 6). This observation suggests that the observed aggregation is due to the enzyme activation. This interpretation is further supported by results which will be discussed later.

As in the case of the partially purified enzyme, CoA activation is profoundly affected by the presence of Mg²⁺ as shown in Fig. 4. Inclusion of 5 mM Mg²⁺ in the activation mixture not only completely abolished the activation effect of CoA, but the addition of Mg²⁺ to the activated enzyme also results in its prompt deactivation. The exact mechanism of Mg²⁺ action has yet to be established. However, it has been reported that divalent cations such as Mn²⁺ do cause gross structural changes of the CoA molecule (14). This effect of Mg²⁺ on CoA action could alternatively be due to its binding...
to the enzyme rather than CoA. As will be shown later, Mg\(^{2+}\) inhibits CoA binding to the carboxylase.

**Relationship between CoA-mediated Aggregation and Activation of the Carboxylase**—It has long been established that the proteomes of the carboxylase are inactive and only the polymers are active (see Ref. 1). Although CoA activation of the partially purified enzyme was accompanied by enzyme aggregation and it has been suggested that these two phenomena are related (7), the experiments described in the previous section suggest that CoA activation is independent of carboxylase concentration. Therefore, the relationship between the two events was investigated further.

When the sedimentation behavior of enzyme purified by the method of Nakanishi and Numa (9) was examined in the absence of free citrate (citrate was removed by dialysis), the carboxylase sedimented as a protomer. As shown in Fig. 5, when 0.15 mM CoA was added to the purified enzyme, about two-thirds of the enzyme was polymerized. However, the effect of CoA in the activation of this type of carboxylase was less than 20%, showing virtually no activation effect in spite of the polymerization. On the other hand, when the same dialyzed enzyme was subjected to Dowex treatment, and the sedimentation pattern was examined, the carboxylase sedimented as a protomer in the absence of CoA activation. When CoA was added to the Dowex-treated enzyme, two-thirds of the enzyme was again polymerized as in the case of the dialyzed enzyme (Fig. 6). When the effect of CoA was examined with respect to enzyme activation, the Dowex-treated enzyme was activated 5-fold. In this experiment, the carboxylase assay of the fractions were assayed in the presence of 10 mM citrate and the effect of CoA in the figure is not apparent.

These experiments suggest that aggregation of carboxylase occurs in the presence of CoA once the enzyme is exposed to citrate irrespective of the tightly bound citrate. However, the CoA activation of the carboxylase occurs only when the enzyme is treated with Dowex. Further evidence that the two events, enzyme activation and polymerization, are not causally related comes from the following experiments. When the partially purified carboxylase (35% ammonium sulfate stage in the procedure of Nakanishi and Numa (9)) was prepared in buffer without citrate, the enzyme preparation sedimented as proteomes, as shown in Fig. 7. When this enzyme preparation was activated by CoA, the activation was about 2-fold, without the formation of polymers. This experiment indicates that in enzyme preparations which have not been exposed to citrate, CoA activation can occur without enzyme aggregation. Under the same conditions, the addition of citrate causes enzyme polymerization (data not shown).

**CoA Binding Site on Acetyl-CoA Carboxylase**—The experiments discussed above indicate that CoA activation is not directly related to CoA-mediated polymerization of the carboxylase. To elucidate the relationship between CoA and citrate action, we have examined the binding site of CoA on the carboxylase and the effect of various ligands on CoA binding to the enzyme.
is supported by studies of the effects of various ligands on the mode of carboxylase activation by citrate and citrate. This conclusion is in accordance with our findings concerning the mode of carboxylase activation by citrate and citrate. The enzyme solution is subjected to high citrate concentrations and is activated by CoA, the presence of which is much lower than that of the partially purified enzyme (0.2 mM). When these highly purified enzyme preparations are activated by CoA, the K_m values for acetyl-CoA remain unchanged, as shown in Table II. In these experiments, the Nakanishi-Numa enzyme preparation was subjected to Dowex treatment to show the CoA effect, whereas the Witters-Vogt enzyme preparation was incubated in the double-reciprocal plots of velocity and acetyl-CoA of various forms of acetyl-CoA carboxylase was determined from the double-reciprocal plots of velocity and acetyl-CoA of the partially purified enzyme. The presence of 2 mM citrate did not affect CoA binding at all, indicating that the CoA binding site is different from that for citrate. This conclusion is in accordance with our findings concerning the mode of carboxylase activation by citrate and CoA using the partially purified enzyme (7). CoA binding to the carboxylase was inhibited by palmityl-CoA. The presence of 10 μM palmityl-CoA abolishes almost 85% of the CoA binding. Mg^{2+} also reduces the binding of CoA as shown in Table I. The same concentration of Mg^{2+} completely abolished the CoA activation, as shown in Fig. 4. However, the binding studies indicate only a 50% decrease in CoA binding (Table I).

The independence of CoA binding to the presence of citrate is supported by studies of the effects of various ligands on the binding of CoA to the purified enzyme (Table 1). In this experiment, the Dowex-treated enzyme (30 μg) was incubated with 1 mM CoA in the presence of different ligands. The addition of 2 mM citrate did not affect CoA binding at all, indicating that the CoA binding site is different from that for citrate. This conclusion is in accordance with our findings concerning the mode of carboxylase activation by citrate and CoA using the partially purified enzyme (7). CoA binding to the carboxylase was inhibited by palmityl-CoA. The presence of 10 μM palmityl-CoA abolishes almost 85% of the CoA binding. Mg^{2+} also reduces the binding of CoA as shown in Table I. The same concentration of Mg^{2+} completely abolished the CoA activation, as shown in Fig. 4. However, the binding studies indicate only a 50% decrease in CoA binding (Table I).

We cannot offer an explanation for the Mg^{2+} effect at this time.

**Effect of CoA Activation on the K_m for Acetyl-CoA**—The K_m for acetyl-CoA of the carboxylase varies extensively depending upon the purification procedure used and the degree of purification. As shown in column 1 of Table II, the K_m for acetyl-CoA is relatively high at 0.2 mM in the partially purified enzyme preparations. Such high K_m values are sustained until the enzyme solution is subjected to high citrate concentrations for 24 h during the purification procedure (9). Following this treatment, the K_m for acetyl-CoA is drastically lowered, i.e. about 10-fold (data are not shown).

The enzyme prepared by the procedure of Witters and Vogt (10) has a higher molecular weight than that prepared by the procedure of Nakanishi and Numa (9), but this procedure also involves a brief exposure of the enzyme to a high concentration of citrate. This enzyme has a K_m for acetyl-CoA of 80 μM, which is much lower than that of the partially purified enzyme (0.2 mM). When these highly purified enzyme preparations are activated by CoA, the K_m values for acetyl-CoA remain unchanged, as shown in Table II. In these experiments, the Nakanishi-Numa enzyme preparation was subjected to Dowex treatment to show the CoA effect, whereas the Witters-Vogt enzyme preparation was incubated in the double-reciprocal plots of velocity and acetyl-CoA of various forms of acetyl-CoA carboxylase was determined from the double-reciprocal plots of velocity and different concentrations of acetyl-CoA between 0.01 to 1 mM.

**Determination of low K_m** was approximated from Fig. 9.

**TABLE II**

| Enzyme preparation | K_m (μM) | K_m (μM) | K_m (μM) |
|--------------------|---------|---------|---------|
| Nonactivated       | 200     | 200     | 400     |
| Citrate-activated  | 20      | 20      | 40      |
| CoA-activated      | 200     | 200     | 400     |

*The K_m values for acetyl-CoA of various forms of acetyl-CoA carboxylase were determined from the double-reciprocal plots of velocities and different concentrations of acetyl-CoA between 0.01 to 1 mM.*

**FIG. 8. Scatchard plot for CoA binding of acetyl-CoA carboxylase.** Purified carboxylase (1 mg/ml) which has been dialyzed against buffer containing 50 mM Tris/C1 (pH 7.5), 0.25 mM sucrose, 1 mM EDTA, 5 mM β-mercaptoethanol at 4°C for overnight were incubated with different concentration of [3H]CoA (6400 cpm/nmol) at 37°C for 20 min. The samples were then placed on Millipore filters which have been washed with 10 ml of ice-cold buffer. The filters were then dried under IR lamp for 5 min and the radioactivity on the filters were counted in a scintillation counter.

**TABLE I**

| Additions          | [H]CoA bound (μM) |
|--------------------|------------------|
| None               | 60.7             |
| Citrate (2 mM)     | 61.3             |
| Palmitoyl-CoA (10 μM) | 9.4           |
| Mg^{2+} (5 mM)     | 30.1             |

**FIG. 9. Double reciprocal plots of velocity and acetyl-CoA of CoA-activated enzyme.** The enzymes were partially purified in the absence of citrate as the first ammonium sulfate precipitation stage or CoA affinity column stage (□——□), or absence of 0.12 mM CoA (□——□) in a medium containing 50 mM Tris-HCl (pH 7.5) 1 mM diithiothreitol, 0.2 mM phenylmethylsulfonlf 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.
preparation was simply dialyzed free of citrate. With the latter preparation, the CoA effect was pronounced without requiring the Dowex treatment.

When the effect of CoA-affinity chromatography was examined with partially purified enzyme (35% ammonium sulfate stage) or more highly purified enzyme preparations, the K_m for acetyl-CoA was as high as 0.2 mM. When these enzymes were activated with 0.125 mM CoA, both enzyme preparations showed the presence of a species of enzyme with an extremely low K_m for acetyl-CoA. As shown in Fig. 9, the exact determination of low K_m values was difficult because of the gradual changes in the curve. However, one can estimate the lowest K_m for acetyl-CoA to be in the range of 2 to 5 μM.

Thus, it is clear that the purified enzymes which have a low K_m for acetyl-CoA are activated by CoA by a mechanism that only involves changes in the V_max. On the other hand, CoA activation of the enzyme in its more native state is accompanied by a decrease in the K_m for acetyl-CoA.

Effect of CoA on the Reactivation of Palmityl-CoA-inactivated Carboxylase—As indicated in our studies of CoA binding to carboxylase, the binding of CoA was effectively inhibited by palmityl-CoA but not by citrate. Since it has long been known that palmityl CoA inactivates carboxylase (16-18), it was of interest to determine whether CoA could reactivate palmityl-CoA inactivated enzyme. As shown in Table III, palmityl-CoA (0.24 μM) inactivates the carboxylase about 80%. Addition of CoA (108 μM) alone could not reverse the palmityl-CoA inhibition. The addition of BSA (0.1 mg/ml) restored enzyme activity. However, when both BSA and CoA were added the enzyme was activated to about 3 times the original activity. This experiment indicates that CoA alone cannot reverse the palmityl-CoA effect. Considering the extremely low K_m of palmityl-CoA compared to that of CoA, it is not surprising to see the lack of CoA effect in this regard.

However, BSA which is known to bind palmityl-CoA does restore the inactivated activity; thus, when BSA and CoA are both present, CoA can activate the palmityl-CoA-free enzyme.

**DISCUSSION**

As indicated in the introduction, the short term regulation of acetyl-CoA carboxylase appears to involve both allosteric control, mediated by cellular metabolites such as citrate and palmityl-CoA, and covalent modification by a phosphorylation-dephosphorylation sequence. Experimental evidence increasingly suggests that in those systems where covalent modification is involved in the control of enzyme activity such modifications result in changes in the enzyme's properties toward various allosteric cellular metabolites. High concentrations of citrate have long been known to activate acetyl-CoA carboxylase; thus, it has been proposed to be a physiological positive effector of this rate-limiting enzyme for long chain fatty acid synthesis (1). However, this theory has been criticized since the lack of a correlation between cellular concentrations of citrate and lipogenic activity made it difficult to understand how citrate alone could regulate carboxylase activity. When the CoA-affinity modification mechanism was discovered and the associated changes in the properties of the dephosphorylated carboxylase were examined (6) it was found that the active form of the enzyme required only 0.2 mM citrate for activation compared to 2.4 to 45 mM (6, 19). Thus, when lipogenesis is stimulated by a hormone such as insulin, the active species of the carboxylase can function with cellular concentrations of citrate. From these considerations, it becomes obvious that one cannot reject the role of citrate in the stimulation of lipogenesis. Gross changes in citrate concentration are not necessarily required for enzyme activity when the CoA-affinity modification mechanisms functions in conjunction with the allosteric molecules.

However, there was an additional problem in the short term regulation of acetyl-CoA carboxylase which could not be answered in terms of the allosteric and covalent modification mechanisms. Although one cannot easily assess the native state of acetyl-CoA carboxylase, earlier studies by Swanson et al. (11) showed that the majority of the rat liver enzyme occurs in an inactive state which can be activated by partial proteolysis. Indeed, in the crude state, the rat liver carboxylase is customarily activated by citrate before the enzyme is assayed (20). When the partially purified carboxylase was examined for the K_m of acetyl-CoA, the K_m was extremely high (0.2 to 0.6 mM) (6). A K_m of this magnitude, compared to the cytosolic concentration of acetyl-CoA of only a few micromolar (21, 22) poses serious questions about the functioning of the carboxylase under in vivo conditions. The highest cytosolic acetyl-CoA concentration reported is 87 μM (23).

Purification of the carboxylase progressively lowers the K_m for acetyl-CoA to about the 10 to 25 μM range. This suggests that proteolytic effects or the removal of some inhibitory molecule from the native enzyme occur during the purification. However, at present, the reason for the decrease in the K_m for acetyl-CoA during the purification of carboxylase is unknown. Given these considerations, the present studies showing that CoA activation of the relatively impure enzyme (which has probably undergone less required only a decrease in the K_m for acetyl-CoA suggests a possible physiological role for CoA in the control of acetyl-CoA carboxylase. In support of these in vitro studies, one can list a number of physiological experiments in which CoA concentrations are known to change in relation to the status of fatty acid synthesis. For example, conditions favoring gluconeogenesis result in a decrease in free CoA (24-26), whereas CoA concentration increases under conditions favoring lipogenesis (27). It is interesting to note that while 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.

A significant role for CoA in the control of lipogenesis becomes more apparent when one considers recent reports that fatty acid synthase requires CoA for activity (28) and that the phosphorylated form of citrate lyase shows decreased activity compared to the dephosphorylated form only in the presence of low CoA concentrations (29). Our studies on the effect of CoA on acetyl-CoA carboxylase (7), along with those of others on fatty acid synthase (28) and citrate lyase (29), indicate that cellular CoA may play a significant role in the control of lipogenesis at the three important steps catalyzed by the enzyme.
by these enzymes. The cytosolic concentration of CoA in normal cells has been reported to be 5 μM when liver cells were fractionated by lyophilization and homogenization in an organic solvent followed by density gradient centrifugation (21). However, when cells from fasted animals were fractionated by the modified digitonin method (30), the cytosolic concentration of CoA was about 23 μM. Since cells from fasted animals contain only about one-fifth of the normal concentration, about 115 μM in normal cells. Direct homogenization of liver tissue yielded about 2 ml used by others (24). The CoA concentration of CoA in the cytosol (31). In this case, the breakage of mitochondria was assessed by mitochondrial marker enzymes. Siess et al. (23) reported a value of 0.1 mM for CoA in normal fed cells. However, they used a cytosolic water content value of 1.05 ml/g of dry cells (23) in their calculation compared to 2 ml used by others (24). The CoA content in the cell fluctuates depending upon the medium in which the cells are prepared (23). However, the cytosolic concentrations are high enough to affect carboxylase activity.

Similar confusion exists as to the cytosolic concentration of acetyl-CoA (23). The cytosolic acetyl-CoA concentration of perfused rat liver has been determined as 0.005 mM (21, 22), whereas Siess et al. reported an acetyl-CoA concentration of 0.86 μM (23). It is difficult to assess which value is a more realistic representation of the in vivo situation. The Kₘ for acetyl-CoA of the partially purified enzyme is 0.2 mM, although that of the purified enzyme is about 10 to 25 μM in the presence of 10 mM citrate. Thus, during the purification of the carboxylase either a protein which affects affinity for acetyl-CoA is removed, or exposure to artificially high citrate concentrations or proteolysis affects the affinity. The purification procedure of Witters and Vogt (10) takes less time and yields a carboxylase with a higher molecular weight. This enzyme preparation, which appears to have experienced less proteolysis, has a much higher Kₘ for acetyl-CoA (80 μM), and is readily activated by CoA. However, the Kₘ for acetyl-CoA was not affected.

In our preliminary report, we tentatively concluded that the site of CoA action is different from that of citrate because under saturating concentrations of citrate, the effect of CoA activation was apparent with the partially purified enzyme (7). In the present studies, binding experiments with CoA using purified enzyme before or after Dowex treatment showed one binding site/subunit. Citrate did not interfere with CoA binding, supporting our previous conclusion that the CoA binding site is not the same as that for citrate. However, CoA binding was inhibited by palmityl-CoA, which brings up an interesting argument for the physiological role of palmityl-CoA. It has been suggested that palmityl-CoA is the negative feedback allosteric molecule for acetyl-CoA carboxylase (17). Using partially or highly purified chicken liver enzymes, it has been shown that palmityl-CoA inactivation is reversed by BSA (32). In the present studies, palmityl-CoA inactivation of the purified enzyme was reversed by BSA, which binds to palmityl-CoA. When CoA was added together with BSA, the palmityl-CoA-inhibited enzyme activity was restored by BSA, and the carboxylase is then activated by CoA. Since CoA alone cannot reverse the palmityl-CoA inhibition, even at a 50-fold higher concentration, it is very unlikely that CoA replaces palmityl-CoA which could then bind to BSA. It should be emphasized that rat liver acetyl-CoA carboxylase occurs in a more or less inactive state (11) and that it has been customary to activate the partially purified enzyme with citrate before enzyme assays are performed. It is not clear whether the inactive state of the acetyl-CoA carboxylase found normally occurring is due to palmityl-CoA or not. However, our attempt to show the presence of palmityl-CoA was negative. However, even in the event that some protein similar to BSA (33) could dissociate inhibitory molecules, such as palmityl-CoA, the de-inhibited enzyme still could not function in the presence of the very low concentration of acetyl-CoA in the cytosol. Therefore the present studies showing that CoA can lower the Kₘ for acetyl-CoA to the 2 to 4 μM range is very significant as an observation that might explain the functioning of acetyl-CoA carboxylase under in vivo conditions.

In the present studies, it was observed that the purified enzymes are activated by changes in the Vₘₐₓ, although the Kₘ for acetyl-CoA is not affected. Purification procedures for the carboxylase lower the Kₘ for acetyl-CoA, most likely by the effect of proteolysis. This hypothesis is based on the observation that BSA, alone or with citrate, does not lower the Kₘ for acetyl-CoA in the partially purified enzyme preparation. Finally, the relationship between the covalent modification mechanism and CoA activation should be mentioned.

Our preliminary experiments indicate that the phosphorylated species of carboxylase does not respond to CoA activation. It should be noted that the species of carboxylase does not respond to CoA activation. It should be noted that the Kₘ value for CoA is about 10-fold higher than the Kₘ for CoA (Figs. 3 and 8). Since the Kₘ value is affected by the substrates of an enzyme (34), it is difficult to assess the significance of this large difference in Kₘ values. It has been shown previously that the Kₘ value should not be directly compared to Kₘ (34).

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