Proc. Nat. Acad. Sci. USA
Vol. 72, No. 12, pp. 4729–4733, December 1975
Biochemistry

Inhibition of citrate synthase by oleoyl-CoA: A regulatory phenomenon

(oleoyl-ethenoCoA/critical micelle concentration/ligand binding/fatty acid biosynthesis/biological membranes)

KUO-HOM LEE HSU* AND GARY L. POWELL†

Department of Chemistry and Department of Biochemistry, Clemson University, Clemson, South Carolina 29631

Communicated by P. Roy Vagelos, August 4, 1975

ABSTRACT **Fatty acyl-CoAs are good detergents (critical micelle concentrations = 3–4 μM) and can inhibit a number of enzymes, including some involved in fatty acid biosynthesis. The regulatory significance of fatty acyl-CoAs as negative effectors has been questioned largely because of the difficulties in distinguishing possible nonspecific detergent effects from more specific regulatory interactions with these enzymes. A new analogue of oleoyl-CoA, oleoyl-(1,N^6-etheno)-CoA, is a better detergent (critical micelle concentration = 3.2 μM) than oleoyl-CoA (critical micelle concentration = 4.7 μM). This new analogue is not as good (by an order of magnitude) an inhibitor of citrate synthase [citrate oxaloacetate-lyase (*pro*-3*S*-CH_2·COO^-→acetyl-CoA); EC 4.1.3.7] nor is it bound as well as oleoyl-CoA. Since the only difference between these two compounds is substitution of 1,N^6-ethenoadenine for the adenine of CoA, the difference in inhibition and binding implies a specific interaction between the adenine moiety of oleoyl-CoA and citrate synthase. Moreover, since oleoyl-(1,N^6-etheno)CoA is a better detergent than oleoyl-CoA, the detergency of oleoyl-CoA is not the sole cause of the fatty acyl-CoA inhibition of citrate synthase. These results support a physiological role for oleoyl-CoA as a negative effector for citrate synthase. An analogous physiological role for fatty acyl-CoA as negative effectors for other enzymes seems reasonable.**

A role has been suggested for fatty acyl-CoA as a negative effector in enzyme regulation (1–5; refs. 6 and 7 review this inhibition with respect to fatty acid biosynthesis). The physiological significance of this inhibition has been questioned because fatty acyl-CoA is an excellent detergent (8) and because a number of enzymes can be inhibited, some of which are seemingly unrelated to fatty acyl-CoA metabolism (9).

In this report we will test the regulatory significance of fatty acyl-CoA using pig heart citrate synthase [citrate oxaloacetate-lyase(*pro*-3*S*-CH_2·COO^-→acetyl CoA) EC 4.1.3.7]. This enzyme is localized within the matrix of mitochondria where it catalyzes the condensation of acetyl-CoA and oxaloacetate to form citrate. This product can activate acetyl-CoA carboxylase (6, 7), the first committed step of fatty acid biosynthesis, and has been found to serve as an efficient precursor for fatty acid biosynthesis (10, 11). The susceptibility of citrate synthase to fatty acyl-CoA inhibition has been well documented (12–14).

We have prepared a close analogue of fatty acyl-CoA, oleoyl-(1,N^6-etheno)CoA, which is a comparable detergent, but which is much less inhibitory (by an order of magnitude) to citrate synthase and which is not appreciably bound. Thus the detergency and the inhibition were experimentally separable, demonstrating that the inhibition was a specific characteristic of fatty acyl-CoA and was independent of its detergent properties.

MATERIALS AND METHODS

Citrate synthase was prepared from fresh frozen pig heart after the method of Srere (15), using gel filtration on Sephadex G-100 (Pharmacia Chemical Co.) after the calcium phosphate gel step and before crystallization. The enzyme was homogeneous by disc gel electrophoresis and gave a specific activity of 110 units/mg of protein. Oleoyl-εCoA and oleoyl-CoA were prepared (16, 17) from oleoyl chloride and (1,N^6-etheno)CoA (εCoA) or CoA, respectively. The desired product was purified by gel filtration on a 2.5 × 60 cm column (Teflon end plates, Glenco Co.) of Sephadex LH-20 (Pharmacia Chemical Co.) equilibrated with water/tetrahydrofuran (3:7 vol/vol). The εCoA was prepared by first reacting CoA (Li salt, P-L Biochemicals, Inc.) with a 2-fold molar excess of 5,5′-dithiobis 2-nitrobenzoic acid (Aldrich) at pH 8.1 followed by chromatography on Sephadex G-15 in 0.01 M sodium acetate (pH 4.5) to separate the mixed disulfide from the yellow 5-thio-2-nitrobenzoate and unreacted 5,5′-dithiobis 2-nitrobenzoic acid. The adenine of the CoA mixed disulfide was then converted to 1,N^6-ethenoadenine by reacting with chloroacetaldehyde (18) until the 260 nm absorbing peak was replaced by peaks at 265 and 275 nm (19) (24–36 hr). Gel filtration on Sephadex G-15 as above separated the product from reactants. A 2-fold molar excess of dithioerythritol (P-L Biochemicals, Inc.) at pH 8.1 reduced the mixed disulfide; gel filtration on Sephadex G-15 separated the desired reduced εCoA from 5-thio-2-nitrobenzoate and the other products. The fluorescence excitation and emission spectra of εCoA are identical to those reported for other 1,N^6-ethenoadenine-containing compounds (19). The purity of oleoyl-CoA and oleoyl-εCoA were estimated at 87% and 80%, respectively, based on the molar extinction coefficient for CoA (20) and ε-adenine (6.0×10^3) (19) and the expected moles of -SH released by pancreatic lipase treatment (21). The yields were 43% and 36%, respectively, for the acylation. Oleoyl-[*G*-^{3}H]CoA was prepared enzymically from [*G*-^{3}H]CoA (New England Nuclear Corp.) by an unpublished procedure (S. B. Tove, personal communication) using the long chain fatty acyl CoA synthase (22) from fresh chicken liver (the gift of Marshall Farms, Greenville, S.C.). The product of the enzymic synthesis was purified by gel filtration on Sephadex LH-20 in water/tetrahydrofuran (3:7 vol/vol) as above, followed (after lyophilization) by butanol extraction (22). A 40% yield based on recovery of ^{3}H as oleoyl-[^{3}H]CoA was attained. The product was chromato-

Abbreviations: The general term, "fatty acyl-CoA," means the coenzyme A thioesters of palmitic, stearic, or oleic acid, the common fatty acyl residues of mammalian tissue. CMC, critical micelle concentration; εCoA, the 1,^6N-ethenoadenosyl derivative of CoA.

* Current address: Department of Microbiology, University of Alabama at Birmingham, Birmingham, Ala. 35233.

† On leave as Visiting Professor of Chemistry in the Laboratory of O. H. Griffith, Molecular Biology Institute, Department of Chemistry, University of Oregon, Eugene, Or. 97403, where reprint requests should be sent.

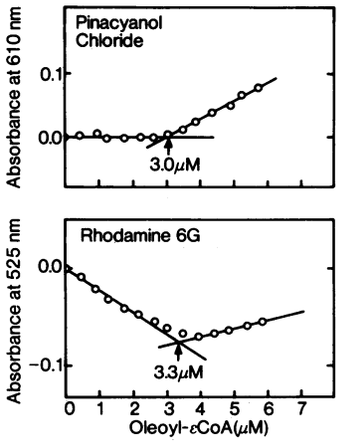

FIG. 1. CMC estimation for oleoyl-εCoA. Increasing concentrations of oleoyl-εCoA were added to 0.5 μM pinacyanol chloride (Eastman) (8) (upper graph) or to 0.5 μM rhodamine 6G (Allied Chemical Co.) (25) (lower graph) in 20 mM Tris·HCl (pH 8.1) in 10-cm glass cuvettes. The same ordinate is used for both graphs. The intersection of the two linear portions of the curves (indicated by an arrow) was taken as the value of the CMC. The average of these two values is referred to in the body of the paper.

graphed on a thin layer of silica gel G using *n*-butanol/acetic acid/water (5:2:3 vol/vol) (22). About 96% of the ^3H cochromatographed with authentic oleoyl-CoA. Acetyl-CoA was prepared by the method of Simon and Shemin (23). Radioactivity was determined using the scintillation solution of Patterson and Greene (24). Fluorescence was routinely assayed using a fluorocolorimeter (American Instrument Co.).

RESULTS

Critical micelle concentration of oleoyl-εCoA is slightly lower than that of oleoyl-CoA

Dye binding was the most appropriate technique to estimate the critical micelle concentration (CMC) of oleoyl-CoA and oleoyl-εCoA because of the low concentration ranges of these values. Pinacyanol chloride was used by Zahler, Barden, and Cleland (8) to estimate the CMC of palmitoyl-CoA (3–4 μM), and this value has been verified using rhodamine 6G (25). Both dyes absorb in the visible region well away from absorption or emission bands of oleoyl-CoA or of the fluorescent analogue. The average CMC for oleoyl-εCoA (3.2 μM, Fig. 1) was slightly lower than the average value for oleoyl-CoA (4.7 μM, Fig. 2).

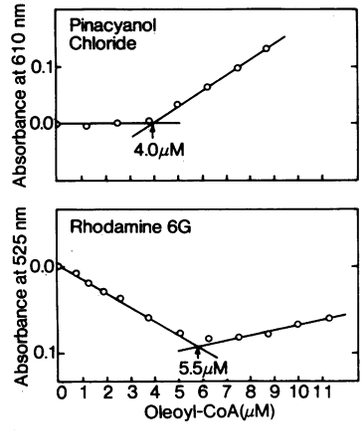

FIG. 2. CMC estimation for oleoyl-CoA. Increasing concentrations of oleoyl-CoA were used, as in Fig. 1.

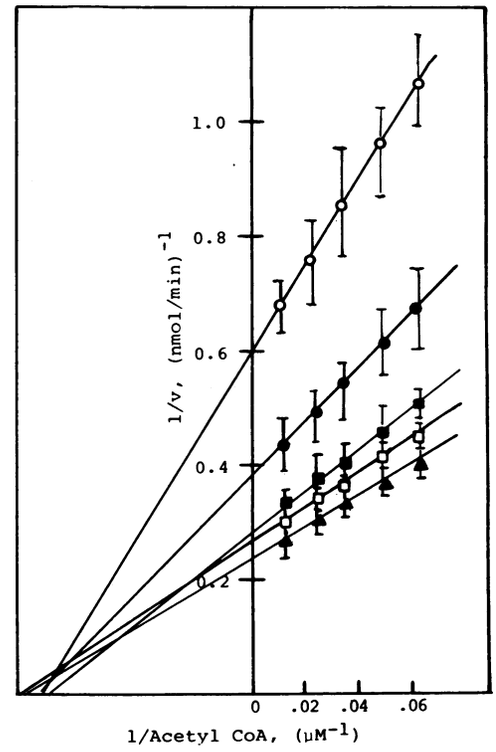

FIG. 3. Noncompetitive inhibition of citrate synthase by oleoyl-CoA. Assays (15) were carried out in 0.1 M Tris·HCl (pH 8.1) containing 0.1 mM 5,5′-dithiobis 2-nitrobenzoic acid and 40 ng of enzyme, and were started after 3 min of preincubation using a final concentration of 0.5 mM oxaloacetate. Each point on the graph is the mean of five assays; the bars represent the standard deviation, and the lines are the best least squares fit. Concentration of oleoyl-CoA: (▲) none, (□) 1.0 μM, (■) 2.0 μM, (●) 5.0 μM, (○) 10.0 μM.

Inhibition of citrate synthase by oleoyl-CoA is time dependent and is similar to inhibition by oleoyl-εCoA but occurs at lower concentrations

The inhibition of citrate synthase by fatty acyl-CoA compounds is complex since the degree of inhibition is time dependent (13, 14). We found that kinetically detectable concentrations of citrate synthase (20 ng) lost 15% of their maximal velocity immediately in the presence of 2 μM oleoyl-CoA. The inhibition increased to a maximum of 50% after 5 min unless oxaloacetate, one of the substrates, was present; then only 30% inhibition was achieved after 5 min under the latter condition. Bovine serum albumin could protect from oleoyl-CoA inhibition (ref. 14 and unpublished results of R. N. Ma) but could not restore full activity after complete inactivation (5 min incubation).

If the preincubation time was carefully reproduced, mixed, noncompetitive inhibition could be demonstrated using both oleoyl-CoA (Fig. 3) and oleoyl-εCoA (Fig. 4). Dixon plots (Figs. 5 and 6) curved upwards, suggesting cooperative inhibition. The concentrations of oleoyl-εCoA required to obtain comparable inhibition in each case were an order of magnitude higher than required for oleoyl-CoA.

CMC of oleoyl-εCoA bears little relationship to the concentrations required for binding to and inhibition of citrate synthase

The binding of oleoyl-CoA and oleoyl-εCoA was studied by equilibrium dialysis over a wide range of concentrations starting well below the CMC of these compounds. A Scatchard plot of this data (Fig. 7) showed little binding of ole-

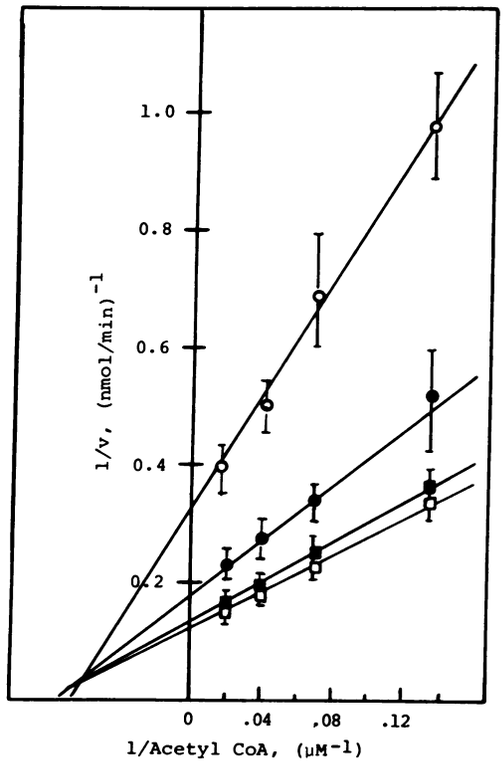

FIG. 4. Noncompetitive inhibition by oleoyl-ϵCoA. Each assay was carried out four times as in Fig. 3. Oleoyl-ϵCoA concentrations: (□) none, (■) 18 μM, (●) 45 μM, (○) 90 μM.

oyl-ϵCoA over a concentration range where oleoyl-CoA was appreciably bound. The graph for the oleoyl-CoA data was nonlinear at the higher concentrations, suggesting cooperative binding.

These binding data were also graphed as a function of concentration (Fig. 8). Oleoyl-CoA was appreciably bound

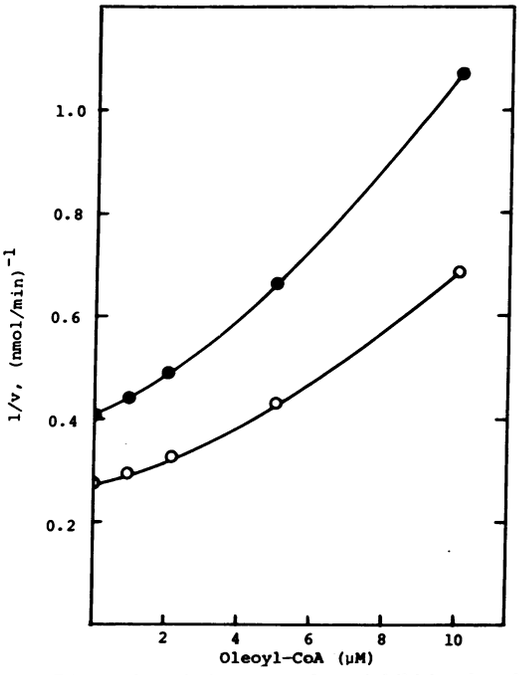

FIG. 5. Dixon plot of citrate synthase inhibition by oleoyl-CoA. See legend of Fig. 3. (●) 80 μM acetyl-CoA, (○) 16 μM acetyl-CoA.

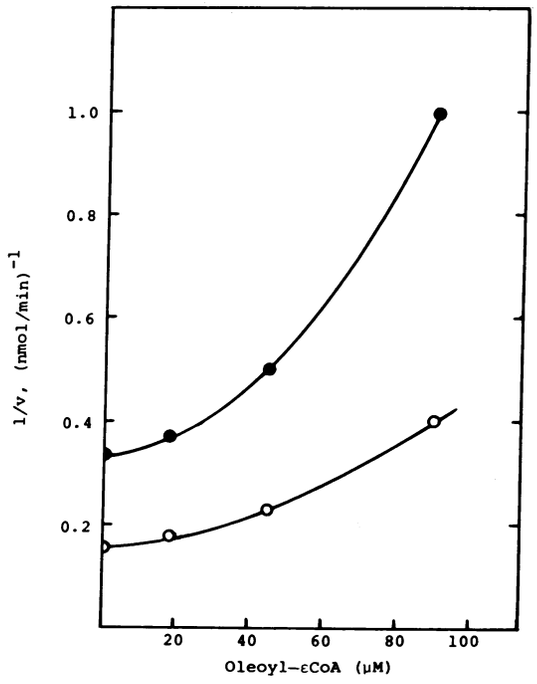

FIG. 6. Dixon plot of citrate synthase inhibition by oleoyl-ϵCoA. See legend of Fig. 3. (●) 50 μM acetyl-CoA, (○) 8 μM acetyl-CoA.

at concentrations well below the CMC. Cooperative binding was evident from the shape of the binding curve near and above the CMC. Citrate synthase bound much less oleoyl-ϵCoA within the concentration range used for oleoyl-CoA.

The percent inhibition of citrate synthase as a function of oleoyl-CoA concentration is superimposed upon the binding data for oleoyl-CoA in Fig. 8 by using a suitable (and arbitrary) choice of the scale for the binding data. By contrast citrate synthase was inhibited by oleoyl-ϵCoA only at concentrations an order of magnitude above the CMC. Thus, oleoyl-ϵCoA, a somewhat better detergent than oleoyl-CoA, was not bound and does not inhibit citrate synthase until concentrations well above the CMC were used. Oleoyl-CoA both binds and inhibits within that same concentration range near its CMC.

DISCUSSION

There is little question that citrate synthase can be strongly inhibited by low concentrations of fatty acyl-CoA. The controversy centers around whether the inhibition is a nonspecific detergent effect or a specific inhibition interaction. Specificity in such a context implies physiological significance, and a clear demonstration of a specific interaction for one enzyme would suggest the possibility of a role for fatty acyl-CoA as a negative effector for other enzymes known to be inhibited by fatty acyl-CoA.

To explore this specificity we have prepared an analogue, oleoyl-ϵCoA, which differs from the natural oleoyl-CoA only by the introduction of an 1,N^6-etheno group on the adenine moiety (Fig. 9). The 1,N^6-ethenoadenine is larger and possesses a quaternary, positively charged nitrogen. This analogue was also a good detergent, exhibiting a CMC slightly lower than the natural compound. Thus the new analogue is actually a slightly better detergent than oleoyl-CoA by one criterion, the CMC. We cannot rule out differences in aggregation number or micellar structure between the analogue and oleoyl-CoA without further measurements, but

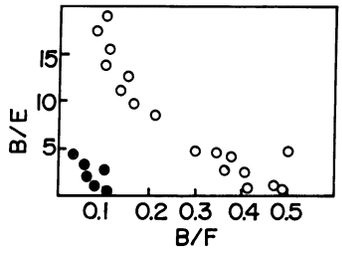

FIG. 7. Binding of oleoyl-CoA and oleoyl-ϵCoA to citrate synthase. Each point (except at the highest oleoyl-CoA concentration) represents the average of two equilibrium dialysis determinations, each with 25 nM citrate synthase. The ligand was placed in the same compartment as the enzyme in one determination and in the opposite compartment in the second determination. In every case the two determinations were within experimental error of each other. At oleoyl-CoA concentrations above the CMC, lengthy (8–12 days) equilibrations were required. Thus, oleoyl-ϵCoA binding was not measured above the CMC and the highest oleoyl-CoA datum shown was determined by gel filtration as follows: Citrate synthase (0.1 mg) was mixed with the indicated concentration of oleoyl-[G-^{3}H]CoA. The mixture was filtered through a 0.9 × 30 cm column of Sepharose 6B (Pharmacia Chemical Co.) equilibrated with unlabeled oleoyl-CoA of the same concentration. The binding was estimated from the molar extinction coefficient for citrate synthase and the ^{3}H that cochromatographed with the enzyme. Concentrations from 0.02 to 10.0 μM oleoyl-CoA (○) and from 0.13 to 3.45 μM oleoyl-ϵCoA (●) are shown.

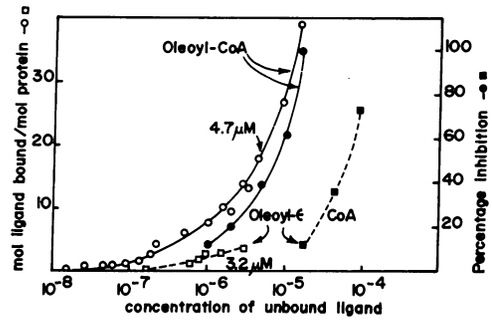

FIG. 8. Comparison of the binding with the inhibition of citrate synthase by oleoyl-CoA and by oleoyl-ϵCoA. The inhibition data were calculated from the intercept of the fitted least squares lines (infinite acetyl-CoA) in Figs. 3 and 4 at each inhibitor concentration. No inhibition could be measured for oleoyl-CoA (●) below 0.4 μM. No inhibition could be measured for oleoyl-ϵCoA (■) below 10 μM. The binding data were taken from Fig. 7 (□, oleoyl-ϵCoA) with the exception of the highest oleoyl-CoA concentration (○), which was determined by gel filtration. The CMC concentration from Fig. 1 for oleoyl-CoA (4.7 μM) is indicated by an arrow. The highest concentration tested for binding for oleoyl-ϵCoA (3.2 μM) was the average CMC concentration (Fig. 2).

marked differences would be unexpected because of the close structural similarities between these compounds.

The time dependence, inability to recover activity of fully inhibited enzyme, and the noncompetitive and cooperative nature of the inhibition observed with oleoyl-CoA and with the analogue all closely resemble the type of inhibition obtained in the presence of another anionic detergent, sodium dodecyl sulfate. The detergent effects of sodium dodecyl sulfate in perturbing protein structure (26) and inhibiting enzymes are well known. However, velocity ultracentrifugation and gel filtration experiments (refs. 14, 27, and our unpublished results) do not suggest unfolding or extensive dissociation of the dimeric citrate synthase. Most telling however was the order of magnitude difference in concentration required to observe inhibition of citrate synthase activity by the analogue. Thus the analogue is not as good an inhibitor as the natural compound even though it is a better detergent. We interpret this difference in effective inhibitory concentration as a specificity of the enzyme for an unmodified adenine for the inhibition.

Binding studies with oleoyl-CoA and oleoyl-ϵCoA further emphasized the necessity for an unmodified adenine for interaction with citrate synthase. Oleoyl-CoA was appreciably bound within the same concentration range near the CMC, as required for inhibition, while oleoyl-ϵCoA was not appreciably bound within that concentration range.

The shape of the oleoyl-CoA binding curve for citrate synthase also differs in some respects from that expected for nonspecific binding of detergents. Detergents such as sodium dodecyl sulfate have been shown recently to bind to the hydrophobic portion of cytochrome b_5 only over a narrow range near the CMC (28). Oleoyl-CoA binds to citrate synthase over several orders of magnitude but the binding becomes cooperative near the CMC. Moreover, cytochrome b_5 bound an amount of detergent corresponding to the aggregation number for the detergent (28). We observed binding of 20 molecules of oleoyl-CoA per mole of citrate synthase near the CMC. The only reported fatty acyl-CoA aggregation number is for palmitoyl-CoA and is >1000 (8). If the value for oleoyl-CoA is similar, citrate synthase must be binding aggregates smaller than the usual micelle at the CMC. The binding of oleoyl-CoA that occurred at concentrations well below the CMC implies available binding sites for oleoyl-CoA. The existence of a binding site specific for oleoyl-CoA has been suggested previously by Wieland (27). He reported that citrate synthase treated with p-chloromercuribenzoate [sic] desensitized the enzyme to stearyl-CoA

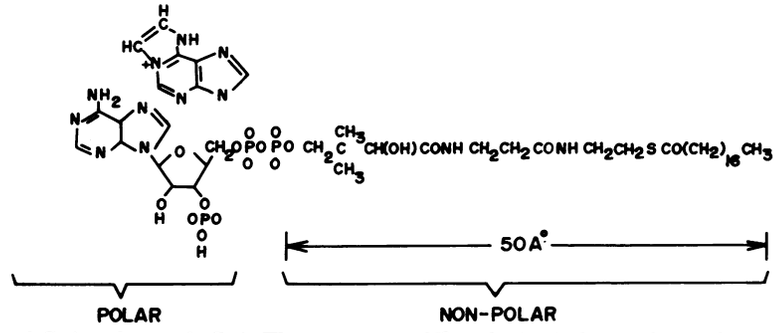

FIG. 9. Structures of stearyl-CoA and stearyl-ϵCoA. The structure 1, N^6-ϵ-adenine is drawn above adenosine diphosphate and would replace the adenine moiety in stearyl-ϵCoA. The nonpolar portion of the molecule with an approximate dimension is indicated suggesting what portion might be found inside a micelle or membrane. It should be noted that the -OH of pantoic acid, the amide linkages, and the thioester bond are all polar, making the indicated division between nonpolar and polar somewhat arbitrary.

inhibition while leaving the catalytic activity intact. Srere (10) has also postulated the existence of a general nucleotide binding site on citrate synthase. The noncompetitive inhibition observed with acetyl-CoA as the variable substrate is consistent with the existence of a binding site for fatty acyl-CoA separate from that of the CoA substrate. Further aggregation of oleoyl-CoA around the oleoyl-CoA more specifically bound at the lower concentration would probably account for the shape of the binding curve.

Good physiological arguments can be made for fatty acyl-CoA being a specific negative effector particularly for the enzymes of fatty acid biosynthesis. Thus citrate synthase provides citrate which can be converted to acetyl-CoA in the cytoplasm (10, 11). Acetyl-CoA carboxylase, which catalyzes the first committed step in fatty acid biosynthesis, is activated by citrate and inhibited by fatty acyl-CoA (6, 7, 29). Glucose 6-phosphate dehydrogenase, which is reversibly and competitively inhibited by palmitoyl-CoA (9, 30), may furnish reducing equivalents (NADPH) for fatty acid biosynthesis *in vivo* (31, 32). The isolation of polysaccharides from *Mycobacterium*, which apparently regulate the rate of fatty acid biosynthesis in the organism by sequestering the fatty acyl-CoA formed, also supports a physiological role for these compounds (5).

Major problems still remain in our understanding of the mechanism of the inhibition and the regulation of enzymes by fatty acyl-CoA. A consistent hypothesis must reconcile the apparent high sensitivity of some enzymes, such as glutamate dehydrogenase (9), to fatty acyl-CoA which are apparently unrelated to lipid metabolism, and the difficulty in demonstrating the reversal of inhibition of fatty acyl-CoA. We must recognize that mitochondrial enzymes such as citrate synthase and those of lipid metabolism *in vivo* are at least adjacent to membranes such as the inner mitochondrial membrane or to the endoplasmic reticulum. The nonpolar portion of fatty acyl-CoA (Fig. 9) apparently intercalates with the acyl moieties of a biological membrane (33–35). Thus the physiologically relevant interactions *in vivo* may be between the polar portion of the fatty acyl-CoA (including the adenine) which extends from the membrane and the extrinsic or intrinsic enzymes associated with that membrane. Some of the problems indicated above may be a consequence of our choice of pure enzymes in aqueous buffers in the presence of micellar fatty acyl-CoA for our demonstrations. Techniques that have been developed for investigating fatty acyl-CoA in membranes interacting with enzymes (35) may provide demonstrations more relevant to the situation *in vivo*.

We are indebted to S. B. Tove and C. B. Roberts, Department of Biochemistry, North Carolina State University, for instructing us in the preparation and use of the fatty acyl-CoA synthase to prepare radiotracer-labeled oleoyl-CoA. J. K. Zimmerman, Department of Biochemistry, Clemson University, and C. Tanford and J. A. Reynolds of the Department of Biochemistry, Duke University Medical Center, have helped us in the interpretation of our binding experiments. M. E. Haberland from the laboratory of J. A. Reynolds suggested the design of the dialysis cells. R. J. DeSa, Department of Biochemistry, University of Georgia, and D. G. Priest, Department of Biochemistry, Medical University of South Carolina, measured the fluorescence spectra of ϵCoA and oleoyl-ϵCoA for us. R. N. Ma of this laboratory has provided us with preliminary results on the inhibition of citrate synthase by oleoyl-CoA. A Clemson University Faculty Research Grant to G.L. allowed us to purchase the fluorocolorimeter. This work was supported largely by funds from the Department of Chemistry, Clemson University, and partially fulfills the M.S. degree requirements in Chemistry for K.L.H. at Clemson University.

1. Bortz, W. M. & Lynen, F. (1963) *Biochem. Z.* **337**, 505–509.
2. Guynn, R. W., Veloso, D. & Veech, R. L. (1972) *J. Biol. Chem.* **247**, 7325–7331.
3. Goodridge, A. G. (1972) *J. Biol. Chem.* **247**, 6946–6952.
4. Goodridge, A. G. (1973) *J. Biol. Chem.* **248**, 4318–4326.
5. Bergeron, R., Machida, Y. & Bloch, K. (1975) *J. Biol. Chem.* **250**, 1223–1230.
6. Lane, M. D., Moss, J., Ryder, E. & Stoll, E. (1971) *Adv. Enzyme Regul.* **9**, 237–251.
7. Volpe, J. J. & Vagelos, P. R. (1973) *Annu. Rev. Biochem.* **42**, 21–60.
8. Zahler, W. L., Barden, R. E. & Cleland, W. W. (1968) *Biochim. Biophys. Acta* **164**, 1–11.
9. Taketa, K. & Pogell, B. M. (1966) *J. Biol. Chem.* **241**, 720–726.
10. Srere, P. A. (1972) *Curr. Top. Cell. Regul.* **5**, 229–284.
11. Kornacker, M. S. & Lowenstein, J. M. (1965) *Biochem. J.* **94**, 209–215.
12. Tubbs, P. K. (1963) *Biochim. Biophys. Acta* **70**, 608–609.
13. Wieland, O. & Weiss, L. (1963) *Biochem. Biophys. Res. Commun.* **13**, 26–31.
14. Srere, P. A. (1965) *Biochim. Biophys. Acta* **106**, 445–455.
15. Srere, P. A. (1969) in *Methods in Enzymology*, ed J. M. Lowenstein (Academic Press, New York), Vol. 13, pp. 5–11.
16. Seubert, W. (1960) *Biochem. Prep.* **7**, 80–83.
17. Reitz, R. C., Lands, W. E. M., Christie, W. W. & Holeman, R. T. (1968) *J. Biol. Chem.* **243**, 2241–2246.
18. Secrist, J. A., Barrio, J. R., Leonard, N. J., Villar-Palasi, C. & Gilman, A. G. (1972) *Science* **177**, 279–280.
19. Secrist, J. A., Barrio, J. R., Leonard, N. J. & Weber, G. (1972) *Biochemistry* **11**, 3499–3506.
20. *Specifications and Criteria for Biochemical Compounds*, Supp. 1 (1963) National Academy of Sciences–National Research Council, Publication 719, Washington, D.C.
21. Barber, E. D. & Lands, W. E. M. (1971) *Biochim. Biophys. Acta* **250**, 361–366.
22. Banis, R. J. & Tove, S. B. (1974) *Biochim. Biophys. Acta* **348**, 210–220.
23. Simon, E. J. & Shemin, D. (1953) *J. Am. Chem. Soc.* **75**, 2520.
24. Patterson, M. A. & Greene, R. C. (1965) *Anal. Chem.* **37**, 854–861.
25. Wood, J. M. (1973) *Biochemistry* **12**, 5268–5273.
26. Reynolds, J. A. & Tanford, C. (1970) *J. Biol. Chem.* **245**, 5161–5165.
27. Wieland, O. (1966) *Adv. Enzyme Regul.* **4**, 281–284.
28. Robinson, N. C. & Tanford, C. (1975) *Biochemistry* **14**, 369–378.
29. Numa, S., Ringelmann, E. & Lynen, F. (1965) *Biochem. Z.* **343**, 243–257.
30. Kawaguchi, A. & Bloch, K. (1974) *J. Biol. Chem.* **249**, 5793–5800.
31. Rous, S. (1971) *Adv. Lipid Res.* **9**, 73–118.
32. Ziboh, V. A., Dreize, M. A. & Hsia, S. L. (1970) *J. Lipid Res.* **11**, 346–354.
33. Sumper, M & Trauble, H. (1973) *FEBS Lett.* **30**, 29–34.
34. Sumper, M. (1974) *Eur. J. Biochem.* **49**, 469–475.
35. Devaux, P. F., Bienvenue, A., Lauquin, G., Brisson, A. D., Vignais, P. M. & Vignais, P. V. (1975) *Biochemistry* **14**, 1272–1280.