RELIEF OF PALMITYL CoA INHIBITION OF CITRATE SYNTHASE BY LONG-CHAIN ACYLCARNITINE DERIVATIVES*

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We have recently observed that (+)-palmitylcarnitine stimulates fatty acid synthesis by liver supernatant fractions prepared from either fed or starved rats (Fritz and Hsu, 1966). One of the possible mechanisms by which (+)-palmitylcarnitine could restore lipogenesis by livers from starved animals to normal levels would include a release from an inhibition of fatty acid synthesis by a "physiological" inhibitor such as palmityl CoA (Bortz and Lynen, 1963), or a less well defined microsomal entity described by Masoro et al (1962). The concentrations in liver of both palmityl CoA and the microsomal inhibitor are reported by these authors to rise during starvation. Since palmityl CoA is also an inhibitor of citrate synthase activity (Wieland et al, 1964; Tubbs, 1963; Srere, 1965), and since citrate is involved in several aspects of fatty acid metabolism (Srere and Bhaduri, 1962; Martin and Vagelos, 1962), it appeared that this reaction would be an interesting one to investigate in relation to the above possibility. Accordingly, citrate synthase (E. C. 4. 1. 3.7) activity was assayed in the presence and absence of palmityl CoA, and various chain-length acylcarnitine derivatives were incubated with the enzyme during these procedures. Results to be presented demonstrate that long-chain O-acylcarnitine derivatives protect citrate synthase from inhibition by palmityl CoA if they are incubated with enzyme during the incubation period prior to oxaloacetate addition.

METHODS

Citrate synthase was purchased from Boehringer Mannheim Corporation, and activity was assayed by following the absorbance decrease at 232 mμ indicative of thioester cleavage after the addition of oxaloacetate to cuvettes containing acetyl CoA and enzyme together with various components

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listed in Results. Palmityl CoA was prepared by the method of Seubert (1960), and was also purchased from Sigma Biochemicals. (+)- and (-)-Palmitylcarnitine were generous gifts from Professor E. Strack, Leipzig. Various DL-acylcarnitine derivatives were prepared either as previously described (Fritz and Yue, 1963), or by methods of Bremer (1962). A sample of purified α-ketoglutarate dehydrogenase was generously provided by Dr. Vincent Massey, and enzyme activity was assayed by his procedures (Massey, 1960).

RESULTS AND DISCUSSION

Data presented in Fig. 1 confirm results of others (Wieland et al, 1964; Tubbs, 1963; Srere, 1965) that prior incubation of palmityl CoA with citrate synthase inhibits enzyme activity. Addition of acylcarnitine derivatives did not influence enzyme activity under conditions indicated when palmityl CoA was absent. In the presence of palmityl CoA, however, long-chain acylcarnitine derivatives offered varying degrees of relief of inhibition, while butyrylcarnitine was without effect at concentrations employed. DL-octanylcarnitine (10^{-4}M) was associated with a slight lessening of inhibition of citrate synthase by 2 x 10^{-5}M palmityl CoA (69% inhibition when

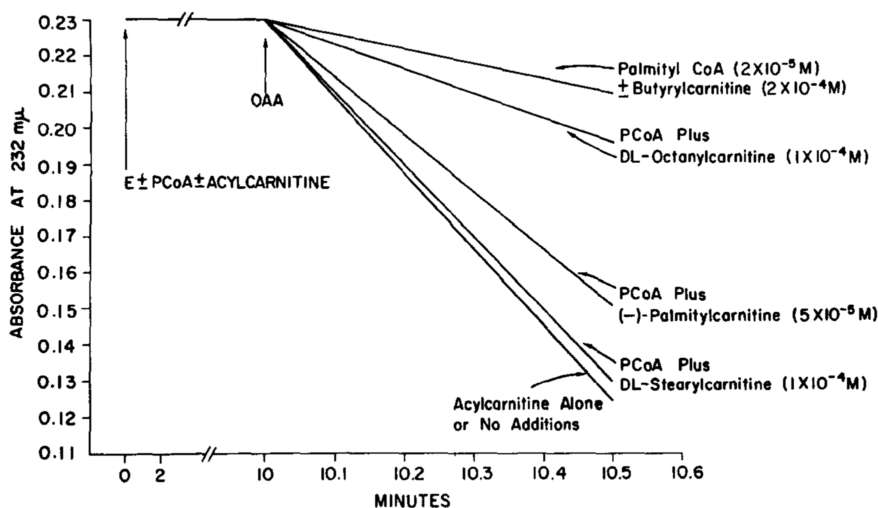
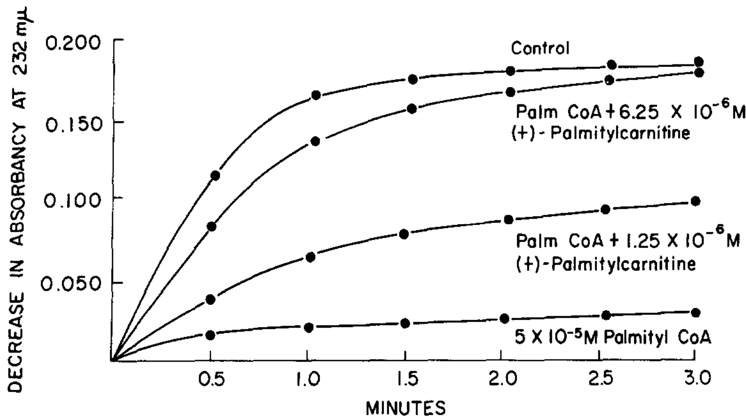
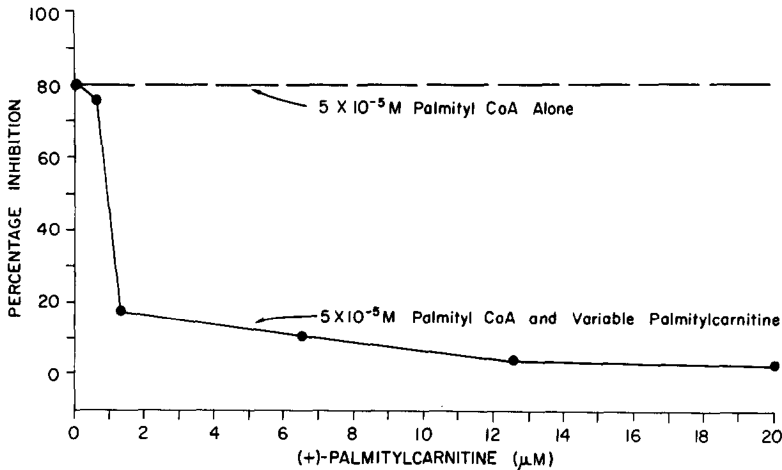


Fig. 1. Effects of various chain-length acylcarnitine derivatives on citrate synthase activity in the presence and absence of palmityl CoA. Crystalline enzyme (2 μg) was incubated for 10 minutes at 35° with 0.1M Tris-HCl buffer at pH 7.5, 5 μg crystalline bovine serum albumin (Armour), 5 x 10^{-5}M acetyl CoA plus or minus 2 x 10^{-5}M palmityl CoA and various acylcarnitine derivatives at concentrations indicated. The reaction was started by addition of potassium oxaloacetate to give a concentration of 5 x 10^{-5}M in a final volume of 1.0 ml. Absorbance changes were followed with a Beckman spectrophotometer having a Gilford automatic recorder attachment, with a full scale of 0.2 optical density units, and with the zero point adjusted to approximately 0.8 optical density units.





Figs. 2 and 3. Effects of various concentrations of (+)-palmitylcarnitine on citrate synthase activity in the presence of palmityl CoA. Conditions were the same as in Fig. 1 except that 50 μg albumin was present in cuvettes, and components were incubated at 35° for only six minutes before addition of oxaloacetate. **In Fig. 3, the percentage inhibition is calculated on the basis of initial velocity measurements.**

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palmityl CoA and octanyl carnitine were both present, and 81% when only palmityl CoA was incubated with the enzyme). Potassium palmitate with or without free carnitine did not influence palmityl CoA inhibition of citrate synthase (data not shown).

When higher concentrations of albumin were employed, palmityl CoA inhibition was relieved, confirming observations of others (Wieland et al, 1964; Srere, 1965). Under these conditions, relatively small amounts of

palmitylcarnitine were required to antagonize the palmityl CoA effect (Figs. 2 and 3). With 50 µg albumin per ml, it was necessary to increase the palmityl CoA concentration to 5 x 10^{-5}M to obtain a degree of inhibition comparable to that shown in Fig. 1. Either (+)- or (-)-palmitylcarnitine reversed palmityl CoA inhibition of citrate synthase activity, and no difference between the two isomers could be detected. Inhibition under conditions defined in Fig. 3 was approximately 80% with 50 µM palmityl CoA alone, and only 17% when 50 µM palmityl CoA and 1.25 µM (+)-palmitylcarnitine were present together.

Results depicted in Figs. 2 and 3 may be interpreted by assuming that palmityl CoA has a greater affinity for albumin than palmitylcarnitine does, thereby allowing palmitylcarnitine to be bound to citrate synthase in preference to palmityl CoA. It is also possible to imagine that palmitylcarnitine displaces palmityl CoA from specific portions of the enzyme molecule adjacent to the active center, and that this effect is more readily seen in the presence of high albumin because of competition between albumin and enzyme for palmityl CoA. In either case, the interpretation must remain highly speculative until additional information is obtained about the mechanism of palmityl CoA inhibition.

Palmitylcarnitine also relieved palmityl CoA inhibition of α-ketoglutarate dehydrogenase (Fig. 4).

In experiments reported in an accompanying article, we have observed that palmitylcarnitine activated acetyl CoA carboxylase, and that it partially protected the enzyme from palmityl CoA inhibition under certain conditions. (Fritz and Hsu, 1966). Palmitylcarnitine did <u>not</u> prevent palmityl CoA inhibition of two other enzymes investigated, namely carnitine acetyltransferase and monoacyl glycerophosphate transferase* (unpublished observations). These findings suggest that palmitylcarnitine-palmityl CoA interactions with various enzymes inhibited by the CoA derivative are not necessarily general.

Palmityl CoA inhibits a wide variety of enzymes, and it is quite possible that the effect is non-specific (Taketa and Pogell, 1966). Similar conclusions have been made by Srere (1965) with respect to citrate synthase. Yet palmityl CoA inhibition of this enzyme is overcome, apparently in a competitive fashion, by oxaloacetate (Wieland et al, 1964), and these workers have also reported that palmityl dephospho-CoA did not inhibit citrate syn-

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*I wish to thank Dr. W. E. M. Lands for performing experiments with this enzyme.

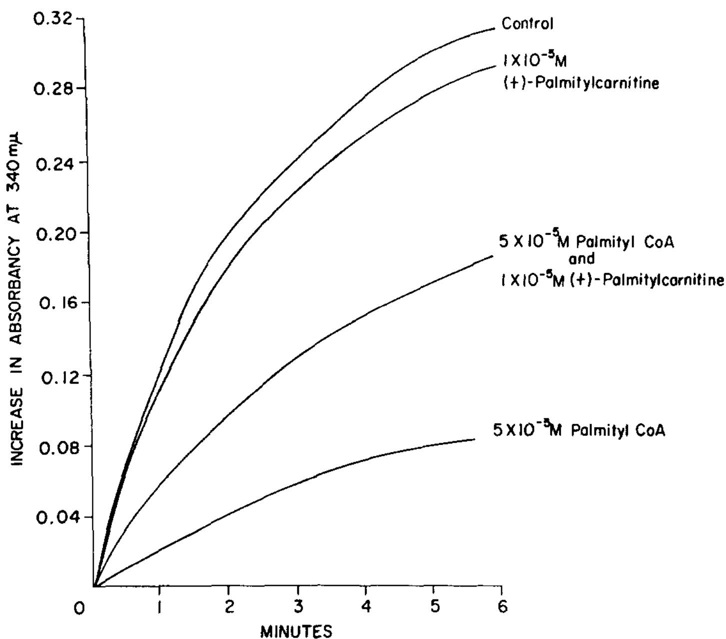

Fig. 4. Enzyme (25 μg) was incubated for 6 minutes at 35° with 100 mM phosphate buffer at pH 7.5, 1 mM α-ketoglutarate, 0.3 mM DPN, 30 mM glutathione plus or minus 5 x 10^{-5}M palmityl CoA and 1 x 10^{-5}M (+)-palmitylcarnitine. The reaction was initiated by addition of CoA to give a concentration of 0.054 mM in a final volume of 1.0 ml, and absorbance change at 340 mμ was followed with a Beckman spectrophotometer having a Gilford automatic recorder attachment.

thase. Similarly, palmityl CoA inhibition of acetyl CoA carboxylase is competitively overcome by citrate (Numa et al, 1965). It is therefore possible that palmityl CoA inhibition of these two enzymes may prove to be of physiological importance.

(-)-Palmitylcarnitine is known to be a substrate in the carnitine palmityltransferase reaction (Fritz and Yue, 1963; Bremer, 1963; Norum, 1965). Until now, attention has been concentrated on the importance of this reaction in fatty acid oxidation (Fritz, 1963; Fritz and Marquis, 1965). While (-)-palmitylcarnitine serves as a reservoir for the generation of palmityl CoA within mitochondria, it might also play a role in antagonizing palmityl CoA inhibition of such enzymes as citrate synthase or acetyl CoA carboxylase. This latter possibility appears attractive in accounting for the stimulation of fatty acid synthesis by (+)-palmitylcarnitine which is examined in an accompanying article (Fritz and Hsu, 1966).

SUMMARY

Inhibition of citrate synthase by incubation with palmityl CoA could be in large part prevented by concomitant incubation of enzyme with (+)-palmitylcarnitine, (-)-palmitylcarnitine or DL-stearylcarnitine. DL-Octanylcarnitine was less effective while butyrylcarnitine was without any protective action. The molar ratio of palmitylcarnitine to palmityl CoA required for relief of palmityl CoA inhibition was lowered at elevated concentrations of albumin.

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