INHIBITION OF CITRATE-SYNTHASE BY PALMITYL-COENZYME A *

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The inhibition of the citric acid cycle,which is a decisive factor in the process of excess ketone body formation,has been accounted for on the basis of decreased liver oxalacetate with resultant impairment of the citrate-synthase (CS)** step (Wieland et al.,1961). In addition to this control by reduction in substrate concentration,the possibility that CS may be inhibited directly by some metabolic product(s) must also be considered. Since the concentration of palmityl-CoA is expected to increase in the liver under conditions leading to ketosis,we investigated the effect of this compound upon CS activity.

METHODS

CS was prepared according to Srere and Kosicki (1961) from pig heart.The crystallised enzyme was further purified by repeated washings with ammoniumsulfate solutions an recrystallisation (Löffler and Wieland,1963).For preparation of CS from rat

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** Abbreviations: CS = citrate-synthase(nomenclature according to the "Report of the Commission on Enzymes of the IUB".Pergamon Press,1961;formerly termed "citrate condensing enzyme"); CoA = coenzyme A; DPN = diphosphopyridine nucleotide; MDH = malate dehydrogenase; tris = tris(hydroxymethyl)-aminomethane.

liver,acetone powder was extracted with $KHCO_3$-solution. By adsorption on calcium phosphate gel and ammonium sulfate fractionation only a partial purification of the enzyme could be achieved the preperations containing still considerable palmityl-CoA deacylase activity. Palmityl-CoA and octanoyl-CoA were synthesised by reaction of the corresponding fatty acid chlorides with CoA (Seubert,1955).Acetyl-CoA was prepared from acetic anhydride and CoA (Simon and Shemin,1953). CoA,DPN and MDH were products of Boehringer u.Soehne,Mannheim,Germany. CS activity was measured optically by the method of Ochoa et al.(1951) in an Eppendorf photometer with attached recorder.

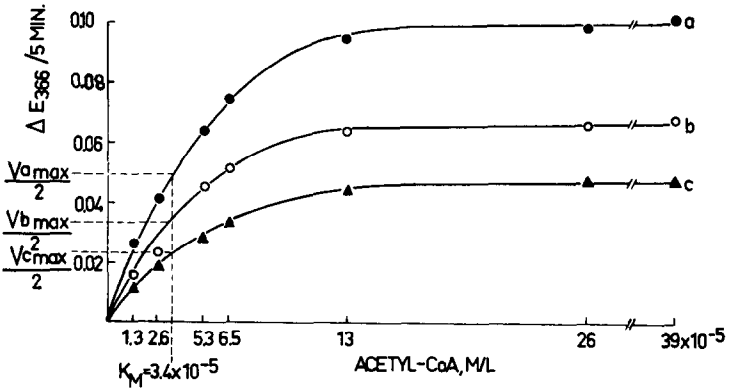

Fig.1 Inhibition of CS by palmityl-CoA. The assay system contained,in a final volume of 2 ml,tris-HCl buffer,pH 7.4:336 µM; potassium -L- malate: 20 µM; DPN: 2 µM; MDH: 50 µg; CS (heart muscle): 1 µg; acetyl-CoA as indicated.Curve a shows the saturation of CS with increasing concentrations of acetyl-CoA without added palmityl-CoA.In curve b palmityl-CoA in a final concentration 4.2 x 10^{-6} M/L,in curve c 4.6 x 10^{-6} M/L was added. The reaction was started by addition of acetyl-CoA after preliminary incubation of the assay mixture for exactly 5 min.at 25°C. Light path 1 cm; wave length 366 mµ; enzyme activity is expressed as Δ E366/5 min.at 25°C on the basis of the initial reaction velocity.

RESULTS

As shown in Fig.1, palmityl-CoA in low concentrations inhibits CS activity. Since the affinity of the enzyme to acetyl-

CoA is not altered by palmityl-CoA as indicated by the unchanged K_M in Fig.1, the inhibition is non-competitive with respect to acetyl-CoA. When the palmityl-CoA was pre-treated for 90 min. at pH 13 and 50°C to cleave the thioester bond, no inhibition of CS was observed. From Fig.2 where the per cent inhibition of CS activity has been plotted against palmityl-CoA concentration, an inhibition constant $K_i = 4.2 \times 10^{-6}$ (M/L) is obtained. Octanoyl-CoA has a much weaker effect with $K_i = 2.5 \times 10^{-4}$ (M/L). From the curve of Fig.2 it can be concluded that the action of palmityl-CoA on the enzyme differs from the usual inhibition kinetics, indicating that some additional factor(s) influence the dissociation equilibrium between enzyme, inhibitor and enzyme-inhibitor complex. It could be assumed for example that one enzyme molecule combines with several inhibitor molecules and that the occupation of one binding site enhances the affinity of the others for palmityl-CoA by altering the structure of the protein. Such an interaction has been shown for instance to occur when oxygen is bound by hemoglobin (Perutz, 1962).

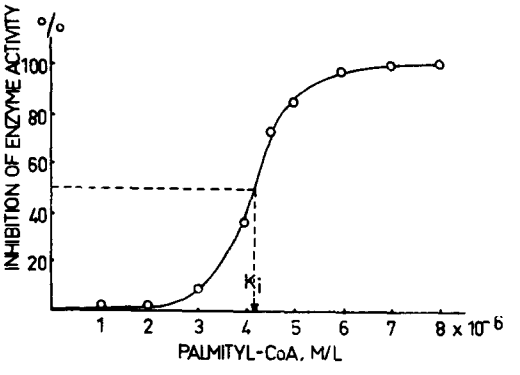

Fig.2 Dependence of CS inhibition upon palmityl-CoA concentration. Experimental conditions were the same as in Fig.1 except that the time of pre-incubation was 10 min. instead of 5 min.. From the curve where the per cent inhibition of CS is plotted against palmityl-CoA concentration, an inhibition constant $K_i = 4.2 \times 10^{-6}$ (M/L palmityl-CoA) is derived.

As illustrated in Fig.3 maximal inhibition occured only when CS was pre-incubated with palmityl-CoA before adding acetyl-CoA the incubation time necessary to give half maximal inhibition being dependent on the palmityl-CoA concentration.CS from rat liver was inhibited by palmityl-CoA in a manner similar to the heart muscle enzyme.The liver preparation however, contained considerable palmityl-CoA deacylase activity and exact kinetic data have therefore not yet been obtained.

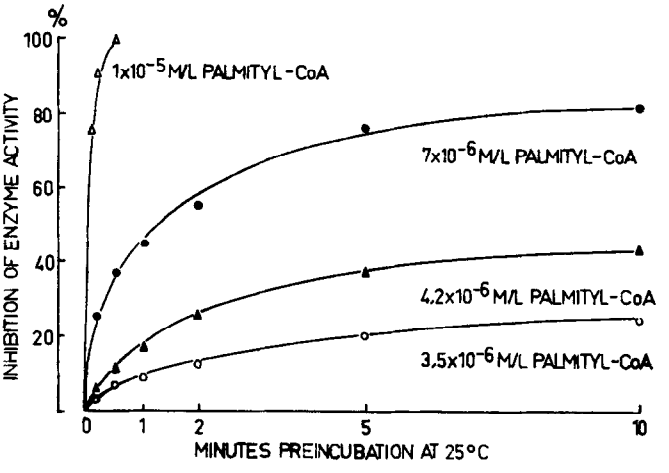

Fig.3 CS activity as a function of pre-incubation time with different palmityl-CoA concentrations. Experimental conditions see Fig.1.Acetyl-CoA concentration 3.9 x 10^{-4}(M/L) througout.

DISCUSSION

In contrast to the competitive inhibition of acetyl-carboxylase by long chain acyl-CoA (Bortz and Lynen,1963) the inhibition of CS by palmityl-CoA shown here is a non-competitive one in respect to acetyl-CoA.These experiments do not,however,exclude the possibility of a competitive mechanism involving oxalacetate.The dependency of enzyme inhibition upon pre-incubation with palmityl-CoA as well as the relationship of inhibitor concentration *vs.* enzyme activity suggest that changes in the con-

formation of the protein structure may play an essential role similar to observations on other enzymes such as phosphorylase (Madsen and Cori,1956),glutamic dehydrogenase (Frieden,1958) and acetyl-carboxylase (Martin and Vagelos,1962;Matsuhashi et al.,in preparation).

It may be assumed that the inhibition of CS by palmityl-CoA is of physiological importance in the regulation of aceto-acetate formation in liver by controlling the rate of acetyl-CoA oxidation via the citric acid cycle.In former investigations we have found an average long chain acyl-CoA content in the normal rat liver of 15-50 mμM per gm.wet weight (Wieland and Weiss,1958; Wieland et al. 1960).This would suffice to completely inhibit the citric acid cycle even under normal conditions.There is,however,no information available concerning the true intracellular concentration of acyl-CoA at the site of CS.In contrast to the liver,a reduction of CS activity in heart muscle by palmityl-CoA would be actually detrimental.Because the acyl-CoA content of the normal rat heart amounts to only about 1/10 of that of liver (Wieland and Eger-Neufeldt,unpublished),inhibitory concentrations of palmityl-CoA may not be reached at the site of CS in heart muscle.

SUMMARY

Palmityl-CoA inhibits CS from pig heart and from rat liver non-competitively with respect to acetyl-CoA.With the heart muscle enzyme an inhibition constant $K_i = 4.2 \times 10^{-6}$ (M/L) was found.The mechanism of the inhibition and its physiological significance as a possible regulatory factor in hepatic ketogenesis is discussed.

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