INHIBITION OF GLUCOSE - 6 - PHOSPHATE DEHYDROGENASE BY LONG CHAIN ACYL-COENZYME A *

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Long chain acyl-thioesters of coenzyme A (LC acyl-CoA) have been shown to be effective inhibitors for several enzymes such as acetyl-CoA carboxylase (Bortz and Lynen, 1963) and citrate synthase (Wieland and Weiss, 1963; Tubbs, 1963; Wieland et al., 1964). Further studies in our laboratory have proved that glucose-6-phosphate dehydrogenase (G-6-PDH) is also highly sensitive to the inhibitory effects of LC acyl-CoA. In the present paper some results on the mechanism of this inhibition are presented.

METHODS

G-6-PDH (from yeast) was a commercial preparation of Boehringer u. Soehne, Mannheim Germany. For enzyme stability at higher dilutions, glycerol to 1% was added which did not affect the kinetic studies. No loss of activity over one week at 0° C was observed. G-6-PDH also was prepared from rat epididymal adipose tissue and liver and from human red cells according to the method of Kornberg and Horecker (1955). Detailed kinetic studies were done using the yeast enzyme. Enzyme activity was measured by following the reduction of TPN^+ optically at 366 mμ (Kornberg and Horecker, 1955). CoA-thioesters were synthesized by esterification of the corresponding fatty acid chloride with CoA (Seubert, 1960). Glucose-6-phosphate (G-6-P), TPN^+, and CoA were commercial products of Boehringer u. Soehne, Mannheim, Germany.

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RESULTS

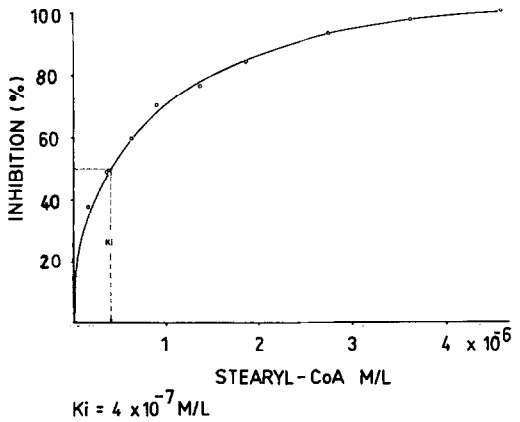

Fig. 1 Dependence of G-6-PDH inhibition upon increasing stearyl-CoA concentration. The assay system contained, in a final volume of 1.5 ml: Triethanolamine-HCl, pH 7.6: 60 μM; EDTA: 1 μM; G-6-P: 0.15 μM; TPN^+: 0.83 μM; G-6-PDH: 0.5 μg. Stearyl-CoA as indicated. The reaction was started by addition of TPN^+ after incubation of the assay mixture for 2 min at 25° C. Light path 1 cm; wave length 366 mμ. Mg^{++} was omitted in our system, due to the turbidity of the reaction mixture following the addition of stearyl-CoA.

As demonstrated in Fig. 1, G-6-PDH is inhibited at low concentrations of stearyl-CoA. From the curve, where the % inhibition of G-6-PDH activity is plotted against stearyl-CoA concentration, an inhibition constant $K_i = 4 \times 10^{-7}$ (M/L) is derived. G-6-PDH partially purified from rat epididymal adipose tissue, rat liver and human red cells was inhibited in a manner similar to the yeast enzyme. When the thioester bond of stearyl-CoA was hydrolyzed by alkaline pretreatment, no inhibition occurred. Other LC acyl-CoA derivatives showed a similiar behaviour when used in our assay system: with palmityl-CoA a $K_i = 6 \times 10^{-7}$ (M/L) was derived, with lauryl-CoA $K_i = 8.7 \times 10^{-6}$ (M/L). These specificity studies show that the extent of inhibition by LC acyl-CoA diminishes with shortening of the chain-lenghth of the fatty acid moiety. In this respect the inhibition of G-6-PDH resembles the inhibition of citrate synthase (CS) by LC acyl-CoA (Wieland et al., 1964).

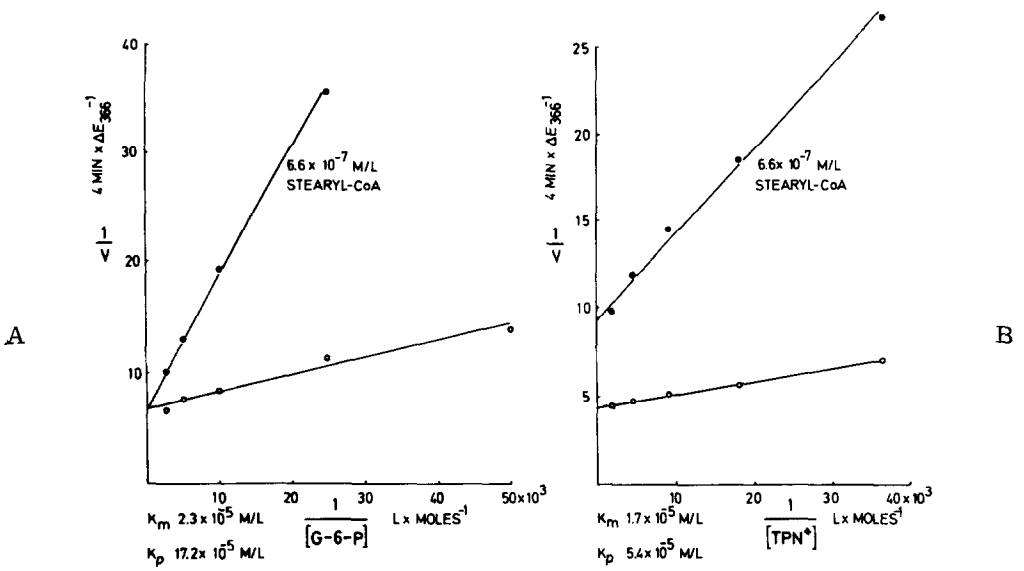

Fig. 2 Dependence of G-6-PDH inhibition by stearyl-CoA upon substrate concentrations: (A) G-6-P and (B) TPN^+. For experimental conditions in A, see Fig. 1; in B, identical except for simultaneous incubation of TPN^+, stearyl-CoA and enzyme; reaction was started by addition of G-6-P. Stearyl-CoA concentration as indicated. Enzyme activity is plotted against concentration of G-6-P and TPN^+ respectively, as described by Lineweaver and Burk (1934).

As shown in Fig. 2 A the affinity of the enzyme for G-6-P is reduced by stearyl-CoA. In this case the Michaelis constant increases from 2.3×10^{-5} (M/L G-6-P) under normal conditions to 17.2×10^{-5} (M/L G-6-P) when stearyl-CoA is present in the system. Maximal velocity of the enzyme reaction remains unchanged. Therefore, the stearyl-CoA-induced inhibition of G-6-PDH is competitive with respect to G-6-P. In contrast to the competition of stearyl-CoA and oxaloacetate in the case of CS-inhibition (Wieland et al., 1964), G-6-P also works by reactivation when added after stearyl-CoA. If TPN^+ concentration is varied (see Fig. 2 B) while G-6-P remains unchanged, the Michaelis constant increases from normal 1.7×10^{-5} (M/L TPN^+) to 5.4×10^{-5} (M/L TPN^+) in the presence of stearyl-CoA. Moreover, the maximal velocity of enzyme activity decreases to 46%. Thus the inhibition of G-6-PDH by stearyl-CoA is of the "mixed type" according to Dixon and Webb (1964) with respect to TPN^+.

Similar to reactivation studies with CS (Wieland et al., 1964), bovine serum albumin reverses the inhibitory effect of CoA thioesters upon G-6-PDH. In addition, sodium cholate and lysolecithin were found to reactivate G-6-PDH.

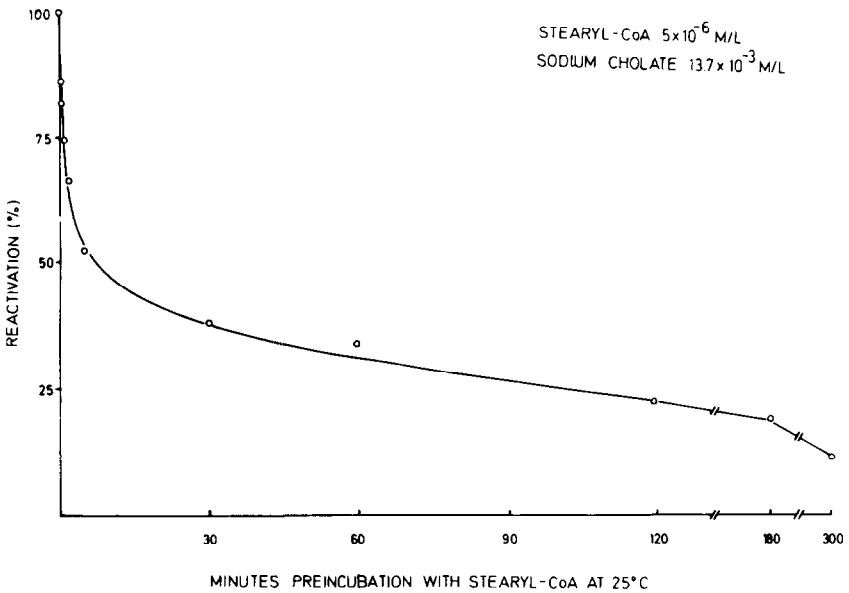

Fig. 3 Reactivation of G-6-PDH activity by sodium cholate dependent upon preincubation time of enzyme and stearyl-CoA. Incubation time of G-6-PDH with stearyl-CoA as indicated is plotted against per cent reactivation of initial reaction velocity. Otherwise, experimental conditions are as in Fig. 1. The amount of stearyl-CoA used gives total inhibition under conditions corresponding to those of Fig. 1. Before starting the reaction by addition of TPN^+, the mixture was incubated for 2 min with sodium cholate (13.7×10^{-3} M/L).

Sodium cholate reactivation of stearyl-CoA-inhibited G-6-PDH is shown in Fig. 3. Within 5 min incubation of stearyl-CoA and enzyme, the activity is irreversibly reduced to about 50 % of the initial value, and progressively diminishes thereafter. When sodium cholate is added before stearyl-CoA, no inhibition occurs.

DISCUSSION

In vivo experiments suggest this described enzymic inhibition may have physiological significance in regulating the activities of G-6-PDH and, consequently, the functional status of the HMP-shunt. It is known

that glucose-1-C^{14} oxidation is impaired both in the liver and in adipose tissue in starvation and fat feeding * (Tepperman and Tepperman, 1963; Milstein, 1956). Increased fatty acid (FA) supply to the liver characterizes both conditions (as well as acutely decompensated diabetes) and there is a concomitant and marked rise in the tissue LC acyl-CoA content (Bortz and Lynen, 1963; Garland and Tubbs, 1963; Wieland, 1964; Felts et al., 1964). In addition, the concentration of liver G-6-P falls in the same conditions (Felts et al., 1964). Thus a reduction in available G-6-P plus an increase of LC acyl-CoA would provide an effective intracellular control for the initial enzymatic step of the pentose phosphate shunt.

The known stimulatory effect of insulin upon glucose-1-C^{14} oxidation in adipose tissue may well be due in part to the decrease in intracellular LC acyl-CoA accompanying heightened reesterification of FA. Similarly, the amounts of FA delivered to the liver abruptly decline with a reduction in the hepatic content of LC acyl-CoA following insulin administration to the diabetic animal (Wieland, 1964; Felts et al., 1964).

The activation of G-6-PDH from human erythrocytes by TPN^+ and warming has been shown to be accompanied by an increase in molecular weight, indicating conformational alteration of the protein (Kirkman and Hendrickson, 1961). Whether inactivation of G-6-PDH by stearyl-CoA also involves conformational transition is the subject of further investigation.

The observation of the time-dependent, irreversible enzyme inactivation by stearyl-CoA as shown in Fig. 3 possibly derserves attention with respect to the general phenomenon of intracellular enzyme degradation. Usually proteins are degraded only after prior denaturation. LC acyl-CoA may thus function as metabolic detergents which initiate the physiological (and pathological ?) proteolytic breakdown of certain enzyme proteins.

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* While similar results were obtained using liver slices from untreated, longstanding alloxan-diabetic rats (Glock et al., 1956; Ashmore et al., 1957) other mechanisms than the one proposed here may operate in such a special experimental condition where the total body fat is largely depleted and an acute influx of FA into the liver would not be expected.

REFERENCES

Ashmore, J., G. F. Cahill, jr., A. Baird Hastings and S. Zotta, J. Biol. Chem. 224, 225 (1957).

Bortz, W. M. and F. Lynen, Biochem. Z. 337, 505 (1963).

Dixon, M. and E. C. Webb, Enzymes p. 324, Longmans, Great Britain 1964.

Felts, P. W., S. S. Chernick, I. Eger-Neufeldt and O. Wieland, 1964 (in preparation).

Garland, P. B. and P. K. Tubbs, Biochem. J. 89, 25P (1963).

Glock, G. E., P. McLean and J. K. Whitehead, Biochem. J. 63, 520 (1956).

Kirkman, H. N. and E. M. Hendrickson, J. Biol. Chem. 237, 2371 (1961).

Kornberg, A. and B. L. Horecker, Methods in Enzymology Vol. 1, p. 323, Academic Press, New York 1955.

Lineweaver, H. and D. Burk, J. Amer. Chem. Soc. 56, 658 (1934).

Milstein, S. W., Proc. Soc. Exp. Biol. Med. 92, 632 (1956).

Seubert, W., Biochemical Preparations Vol. 7, p. 80, John Wiley and Sons, Inc., New York 1960.

Tepperman, H. M. and J. Tepperman, Advances in Enzyme Regulation Vol. 1, p. 121, Pergamon Press 1963.

Tubbs, P. K., Biochim. Biophys. Acta 70, 608 (1963).

Wieland, O., presented at the Vth Congr. of the Internat. Diabetes Feder., Toronto, July 20 - 24, 1964.

Wieland, O., L. Weiss and I. Eger-Neufeldt, Biochem. Z. 339, 501 (1964).

Wieland, O. and L. Weiss, Biochem. Biophys. Res. Comm. 13, 26 (1963).