ATP CONTROLLED ACETOACETATE AND CITRATE SYNTHESIS BY RAT LIVER MITOCHONDRIA OXIDISING PALMITOYL-CARNITINE, AND THE INHIBITION OF CITRATE SYNTHASE BY ATP*

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Acetyl-CoA in rat liver mitochondria may be converted either to acetoacetate or citrate, and the regulation of the relative flow rates through these divergent pathways has been explained either in terms of oxaloacetate deficiency (Lehninger, 1946; Wieland, Weiss & Eger-Neufeldt, 1964) at the site of citrate synthase (E.C.4.1.3.7) or of an inhibition of citrate synthase by long chain fatty acyl-CoA (Tubbs, 1963; Wieland & Weiss, 1963). Recently, it was shown that mitochondria from rat liver oxidised palmitoyl-carnitine in the presence of malate to citrate if oxidative phosphorylation was uncoupled and to acetoacetate if oxidative phosphorylation was coupled (Shepherd, Yates & Garland, 1965). This effect of uncoupling agents is largely reversed by ATP in an oligomycin-insensitive manner, and it would therefore appear that the activity of citrate synthase in intact mitochondria is inhibited by ATP. Although ATP could inhibit the activity of citrate synthase indirectly through a translocation of oxaloacetate (Tager, 1965), such a mechanism could not be operative in non-particulate systems. Accordingly we have studied the kinetics and sensitivity to inhibition by ATP of citrate synthase extracted from rat liver mitochondria. Inhibition by ATP of citrate synthase from yeast has been recently reported by Hathaway & Atkinson (1965).

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Methods. Reagents, preparation of mitochondria and modifications of the Eppendorf fluorimeter have been described (Garland, Shepherd & Yates, 1965). Citrate synthase was extracted from rat liver mitochondria by sonication followed by centrifugation at 70,000 x g for 20 min. Citrate synthase activity in the resultant supernatant was assayed fluorimetrically by following NADH production in the assay system of Ochoa, Stern & Schneider (1951). This procedure extracts 0.04 units of citrate synthase per mg. of mitochondrial protein when assayed under the conditions of Fig. 1, and the yield was not increased by the use of detergents. This crude preparation of citrate synthase does not exhibit ATPase activity unless Mg^{++} is added. ATP diphosphohydrolase (E.C.3.6.1.5) was prepared from potatoes as described by Krishnan (1955).

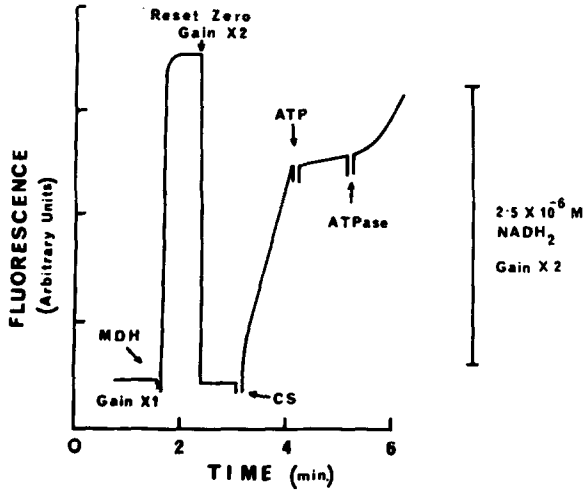

Fig. 1. Recorder tracing of fluorimetric assay of citrate synthase. Initially the 2.0 ml. reaction mixture at 25° contained 0.1M-tris-chloride pH 7.8; 5.0mM-L-malate; 0.1mM-NAD; 20 μM-acetyl-CoA. The addition of 25 μg. of malate dehydrogenase (MDH) was followed by citrate synthase (58 μg. of protein). Citrate synthase was inhibited by the addition of 2.0mM-ATP, and reactivated at the point marked "ATPase" by the addition of 0.5mM-$CaCl_2$ and 5 units of ATP diphosphohydrolase. During the experiment amplifier gain and recorder zero were altered as indicated

Results. The inhibitory action of ATP on rat liver citrate synthase is shown in Fig. 1. The inhibition was not released by Pi (10mM), ADP (2.5mM), AMP (2.5mM) or cyclic 3',5'-AMP (0.1mM). Concentrations of ATP, ADP and AMP required for 50% inhibition of citrate synthase were 0.42mM, 0.6mM, and greater than 2.0mM respectively. The addition of 5 units of ATP diphosphohydrolase to a citrate synthase assay system previously inhibited with ATP causes a ten-fold increase in activity (Fig. 1). The graph relating percentage inhibition of citrate synthase activity to concentration of ATP is sigmoid (Fig. 2). Use of the graphical method of Lineweaver & Burk (1934) demonstrated that the inhibition by ATP was non-competitive with regard to oxaloacetate or acetyl-CoA.

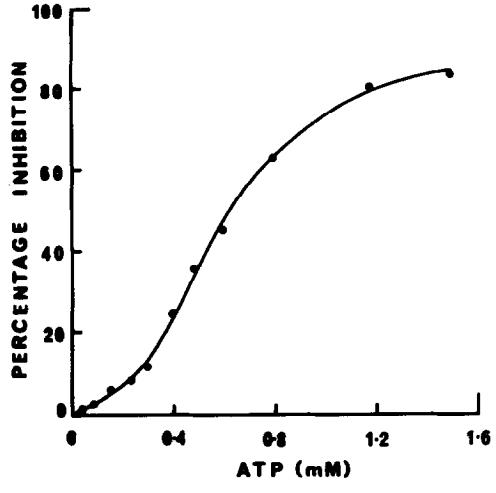

Fig. 2. Graph relating percentage inhibition of citrate synthase from rat liver mitochondria to ATP concentration. Assay conditions as in Fig. 1.

Discussion. These data show that citrate synthase isolated from rat liver mitochondria is inhibited by ATP, and a similar behaviour of the enzyme in its intramitochondrial site is consistent with the factors regulating citrate

synthesis in intact mitochondria (Shepherd, Yates & Garland, 1965). The significance of these findings regarding intact liver tissue may be considered in relationship to the modes of ATP synthesis which can be classified as "cycle dependent" or "cycle independent", according to whether or not the substrate oxidations concerned require the catalytic functioning of the tricarboxylic acid cycle. It is proposed that a sufficiently rapid mode of "cycle independent" ATP synthesis such as β-oxidation may inhibit citrate synthase through intramitochondrial ATP accumulation. Such a control mechanism has several important features:-

(i) it provides a basis for the switch from citrate synthesis to acetoacetate synthesis observed with increasing rates of fatty acid oxidation both in isolated mitochondria (Shepherd, Yates & Garland, 1965) and in the whole animal

(ii) it is sensitive to changes in the rate of ATP utilisation

(iii) it is applicable to other "cycle-independent"modes of ATP synthesis, such as the oxidation of certain amino acids.

The oxidation of glucose via glycolysis and pyruvate oxidation in normal rat liver does not result in significant acetoacetate synthesis. This may be accounted for by the relatively low rate of acetyl-CoA production from pyruvate by rat liver mitochondria (15-20% of the rate for palmitoyl-carnitine oxidation), inhibition of hepatic pyruvate dehydrogenase by acetyl-CoA (unpublished work), and the restraint on accumulation of ATP imposed through inhibition of phosphofructokinase by ATP (Passoneau & Lowry, 1963).

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