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DIFFERENTIAL RESPONSE OF MITOCHONDRIAL AND GLYOXYSOMAL CITRATE SYNTHASE TO ATP

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SUMMARY

Citrate synthase (EC 4.1.3.7) in glyoxysomes and mitochondria from endosperms of 4-day-old castor bean seedlings was examined for inhibition by ATP. The mitochondrial enzyme responded in the way reported previously for enzymes of animal, plant and yeast origin; that is, inhibition occurred and was competitive with respect to acetyl-CoA. On the other hand, the glyoxysomal enzyme was not inhibited.

Since the function of mitochondria is to generate biochemical energy, as ATP, while that of the glyoxysome is to convert acetyl-CoA to succinate, the above results support the interpretation that inhibition of mitochondrial citrate synthase by ATP has regulatory significance.

INTRODUCTION

HATHAWAY AND ATKINSON[1] discovered that citrate synthases (EC 4.1.3.7) of beef and of pig heart are inhibited by ATP. They suggested that these might be additional examples of regulatory enzymes, which being in a pathway leading to energy generation, were responsive to energy charge[1,2]. Many other sources of ATP-responsive citrate synthase have since been described. These include, *inter alia*, beef and pig liver[3], yeast[4], trout liver[5], lemon fruit[6], and tobacco leaves[7].

The inhibition by ATP has been shown to be competitive with respect to acetyl-CoA. SRERE[8] as well as WU AND YANG[9] showed that citrate synthase from beef heart, typically inhibited by ATP, is also inhibited by various salts, competitively with respect to acetyl-CoA. While studies made with citrate synthases from various sources are consistent with ATKINSON'S[2] theory, an unequivocal demonstration that the inhibition by ATP is biologically significant has yet to be devised.

When castor beans are germinated, a rapid production of glyoxysomes and mitochondria occurs in the endosperm, which reaches a maximum in 4–5 days[10], when conversion of fat to sucrose is at its height. The glyoxysomes are the site of the glyoxylate pathway which converts the acetate arising from the reserve fatty acids (with which the seed is richly endowed) to succinate, preparatory to the formation of sucrose[11]. The acetate, as it arises from its precursor by β-oxidation in the glyoxysome[12] is present as the CoA thioester and is introduced into the pathway through the action of citrate synthase and malate synthase[11].

The mitochondria contain the conventional enzymes of the Krebs tricarboxylic acid cycle, including citrate synthase[11,13]. Thus there are conveniently present in a single tissue two kinds of organelles containing citrate synthase in pathways of differing function. The mitochondria serve to generate energy in the form of ATP. Hence mitochondrial citrate synthase may be a reasonable candidate for modulation by ATP. On the other hand, the end product of the glyoxylate pathway is not energy, but rather carbohydrate precursor. If the response to inhibition by ATP indeed corresponds to a genuine physiological control, citrate synthase in the glyoxysome might not then be subject to such regulation.

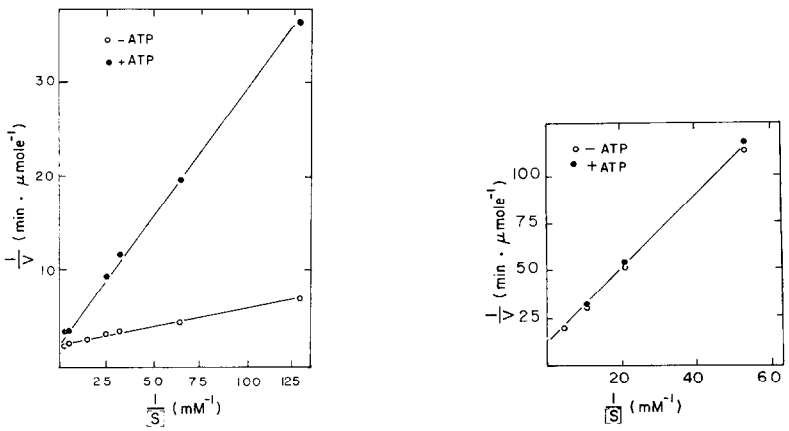

Fig. 1. Double reciprocal plot of velocity of mitochondrial citrate synthase reaction as function of concentration of acetyl-CoA, in the presence (●) and absence (○) of ATP. The reaction mixture contained in total volume of 1.0 ml: 0.10 ml of 1 mM 5,5′-dithiobis-(2-nitrobenzoate) in 1 M Tris–HCl buffer (pH 8.1); varying amounts of 9.4 mM acetyl-CoA; 0.1 ml 10 mM oxaloacetate in 0.1 M Tris–HCl buffer (pH 8.1); 0.1 ml of 0.047 M ATP, where indicated; and sufficient 0.1 M Tris–HCl buffer (pH 8.1) to make to volume. Reactions were run at 24°.

Fig. 2. Double reciprocal plot of velocity of glyoxysomal citrate synthase reaction as function of concentration of acetyl-CoA, in the presence (●) and absence (○) of ATP. Conditions as under Fig. 1.

Accordingly, mitochondria and glyoxysomes were separated from the endosperms of 4-day-old seedling and the responses of the citrate synthase activities of these organelles to ATP were compared.

EXPERIMENTAL

Mitochondria and glyoxysomes were obtained from endosperms of castor beans which had been germinated for 4 days at 30°. The organelles were isolated by isopycnic centrifugation on a sucrose–buffer gradient according to the procedure of GERHARDT AND BEEVERS[10]. Dithiothreitol was omitted from the buffer to avoid interference in the citrate synthase assay.

Citrate synthase was assayed by the method of SRERE[14]. Acetyl-CoA was prepared by the procedure of STADTMAN[15]. The concentration of acetyl-CoA in the preparation was determined from the increase in CoASH occurring when the citrate synthase reaction was taken to completion. Calculations were based on ε_M (412 nm) = 13600

for the anion of 5-thio-(2-nitrobenzoate). Corrections for mercaptide development in the absence of oxaloacetate were made when necessary.

RESULTS AND DISCUSSION

Citrate synthase activity in the mitochondrial fraction as a function of acetyl-CoA concentration is shown as a double reciprocal Lineweaver–Burke plot in Fig. 1. Activity when oxaloacetate was omitted was negligible indicating the absence of appreciable acetyl-CoA hydrolase activity. The K_m for acetyl-CoA was approx. $1.8 \cdot 10^{-5}$ M. Inhibition by ATP was competitive with respect to acetyl-CoA (Fig. 1). Thus the mitochondrial citrate synthase resembles its counterpart enzymes from the sources cited above. It was estimated that a 50 % inhibition was caused by ATP at $4.7 \cdot 10^{-3}$ M in the presence of $8.3 \cdot 10^{-5}$ M acetyl-CoA.

The glyoxysomal citrate synthase was not inhibited by ATP when assayed under identical conditions (Fig. 2). The K_m calculated for acetyl-CoA was $1.5 \cdot 10^{-4}$ M.

The observation that the mitochondrial enzyme is inhibited by ATP while the glyoxysomal enzyme is not, cannot be construed as a proof that the mitochondrial enzyme is regulated by ATP *in vivo*. It is, however, highly suggestive that sensitivity to ATP was shown only by the enzyme which was a component of an ATP-generating pathway.

In the endosperm of the germinating castor bean there is a massive production of sucrose from fat. During this time acetyl-CoA is generated and consumed in the glyoxysomes[11,12]. The lack of this substrate in the mitochondria probably accounts for the fact that the role of the mitochondria in this tissue is largely restricted to the electron transport and ATP production associated with the conversion of succinate to oxaloacetate and with NADH oxidation[16,17]. Inhibition of the mitochondrial citrate synthase by ATP would augment the restriction imposed by low acetyl-CoA supply on the operation of the tricarboxylic acid cycle.

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