SHORT COMMUNICATION

ATP: Citrate Lyase – The Regulatory Enzyme for Lipid Biosynthesis in *Lipomyces starkeyi*?

By CHRISTOPHER A. BOULTON AND COLIN RATLEDGE*

Department of Biochemistry, University of Hull, Hull HU6 7RX, U.K.

(Received 27 July 1981)

ATP: citrate lyase from *Lipomyces starkeyi* was inhibited by oleoyl-CoA (50% inhibition at approx. 3 μ M) and other long-chain fatty acyl-CoA esters but not by other fatty acyl compounds. The inhibition was readily reversed by adding bovine serum albumin. The molecular size of the protein was not altered even in the presence of 200 μ M-oleoyl-CoA. It is concluded that this is a genuine feedback inhibition effect which may be significant in regulating the amount of triacylglycerol being produced by an oleaginous yeast.

INTRODUCTION

The possession of ATP: citrate lyase by eukaryotic oleaginous micro-organisms can account for their ability to accumulate lipid (up to 70% of the biomass in certain instances) (Botham & Ratledge, 1979; Boulton & Ratledge, 1981). The enzyme, which is absent in non-oleaginous micro-organisms, may well be the rate-limiting step for lipid biosynthesis as its specific activity varies in parallel with changes in the specific rate of lipid formation in oleaginous yeasts grown in continuous culture (Boulton & Ratledge, 1981; and unpublished work). We present here further evidence that the enzyme is of key importance in lipid biosynthesis as it is now shown to be under fine feedback inhibitory control.

METHODS

Yeast and growth. *Lipomyces starkeyi* CBS 1809 was grown in batch culture under nitrogen-starvation conditions on a glucose/salts medium as previously described (Boulton & Ratledge, 1981). The lipid content of the cells was about 35% (w/w). Cells were disrupted using a French press (see Boulton & Ratledge, 1980).

Enzyme purification. ATP: citrate lyase [EC 4.1.3.8; ATP: citrate oxaloacetate-lyase (CoA-acetylating and ATP-dephosphorylating)] was purified some 20-fold from crude extracts by precipitating with $(\text{NH}_4)_2\text{SO}_4$ (collecting the 30–50% saturation fraction) followed by chromatography, first through Sepharose 4B and finally through DEAE-Sephadex. The enzyme was stabilized by adding ATP (about 1 mM) and citrate (20 mM) to all buffers. It was assayed at pH 8.3 and 30 °C as previously described (Boulton & Ratledge, 1981).

Effect of oleoyl-CoA on molecular size of ATP: citrate lyase. Crude extract (2 ml) was incubated with 200 μ M-oleoyl-CoA for 30 min at 20 °C. It was then chromatographed through Sephacryl S300 (25 $\times$ 300 mm) equilibrated and eluted with 50 mM-Tris/HCl, pH 7.5, containing 1 mM-MgCl₂, 1 mM-ATP, 20 mM-tripotassium citrate, 10 mM-mercaptoethanol and 5 μ M-oleoyl-CoA. Fractions of 6 ml were collected: protein was determined by measuring A_{280} and the enzyme activity was determined both before and after adding bovine serum albumin (1 mg ml⁻¹). A control sample of crude extract was chromatographed through the same column but without oleoyl-CoA in the preincubation mixture and in the elution buffer.

CoA esters and other chemicals were from Sigma.

RESULTS AND DISCUSSION

When over 25 different compounds were tested against ATP: citrate lyase (an abridged list is given in Table 1), significant inhibition was only produced by long-chain fatty acyl-CoA

Table 1. Effect of lipid intermediates on the activity of ATP: citrate lyase from *Lipomyces starkeyi* CBS 1809

Partially purified enzyme [specific activity $2.1 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] was assayed at pH 8.3 and 30°C (Botham & Ratledge, 1979). The enzyme was preincubated for 5 min with the test compound at the concentration indicated prior to initiation of the reaction by addition of CoA.

Compound	Concentration (μM)	Inhibition (%)
Acetyl-CoA	10	0
Lauroyl-CoA	10	48.2
Myristoyl-CoA	10	95.2
Palmitoyl-CoA	10	84.6
Oleoyl-CoA	10	79.5
Stearoyl-CoA	10	55.1
Oleic acid methyl ester	100	2.8
Oleoyl alcohol	100	0
Oleoyl laurate	100	10.4
Trioleoylglycerol	100	1.4

Fig. 1

Fig. 2

Fig. 1. Inhibition of ATP: citrate lyase from *Lipomyces starkeyi* CBS 1809 by oleoyl-CoA. The enzyme was preincubated with oleoyl-CoA at the concentration indicated for 5 min at 30°C , pH 8.3, prior to initiation of the assay by addition of CoA. The specific activity of the enzyme was the same as in Table 1.

Fig. 2. Chromatography through Sephacryl S300 of crude extracts (2 ml) of *Lipomyces starkeyi* CBS 1809 containing ATP: citrate lyase activity: (a) column equilibrated with 50 mM-Tris/HCl, pH 7.5, containing 1 mM-MgCl₂, 1 mM-ATP, 20 mM-tripotassium citrate and 10 mM-mercaptoethanol; (b) the equilibration and elution buffer was supplemented with 5 μM -oleoyl-CoA and, prior to loading, the sample was preincubated with 200 μM -oleoyl-CoA for 30 min at room temperature. Fractions of 6 ml were collected and assayed for protein, A_{280} (●—●), and for ATP: citrate lyase activity without (○—○) or with (●—●) added bovine serum albumin (1 mg ml⁻¹) to eliminate the inhibitory effects of oleoyl-CoA (note, in b, the full activity of the enzyme was restored by this means).

esters (FACES). The effect of these compounds was much higher, by at least one order of magnitude, than has been reported for the inhibition of other enzymes in yeasts (Satyanarayana & Klein, 1973) including acetyl-CoA carboxylase (Lynen, 1967; White & Klein, 1965; Gill & Ratledge, 1973). Hitherto, the latter enzyme has been assumed to be the first committed, and therefore the controlling, enzyme of lipid biosynthesis (Volpe & Vagelos, 1976).

The effect of long-chain FACES on enzymes is always suspect as these compounds are powerful detergents and may exert their effect non-specifically by disrupting the quaternary or even the tertiary structure of the enzymes (Volpe & Vagelos, 1976; Bloch & Vance, 1977). However, there was no evidence for such an action in this case as (a) significant inhibition was produced at below the critical micellar concentration (CMC) of oleoyl-CoA (Fig. 1), the CMC being about 3 μM (Hsu & Powell, 1975); (b) using other detergents, such as sodium dodecyl sulphate and Triton X-100, no inhibition was achieved until very high concentrations (0.1%, w/v) were reached; and (c) inhibition was readily and instantaneously reversed by adding bovine serum albumin (at about 1 mg ml⁻¹) to the inhibited preparation.

Further, we were able to show that treatment of ATP: citrate lyase with 200 μM -oleoyl-CoA for 30 min at room temperature, which might have been expected to cause disruption of the quaternary structure of the enzyme, had no effect on the molecular size of the protein (Fig. 2). When oleoyl-CoA does act as a detergent to give pseudo-inhibition of enzyme activity this is often marked by disaggregation of the enzyme into sub-units (Kawaguchi & Bloch, 1974) and by the failure of bovine serum albumin to relieve inhibition; in addition, such pseudo-inhibition would be expected to occur only above the CMC of the FACE (Bloch & Vance, 1977). We should, though, point out that the inhibitory effect of oleoyl-CoA towards ATP: citrate lyase was not produced maximally until the mixture had been incubated for about 5 min. We interpret this as either indicating a slow rate of conformational change of the molecule or being due to the use of a non-homogeneous preparation of enzyme which then slowly acquired the oleoyl-CoA at the expense of other proteins to which the inhibitor may have become attached initially.

We thus conclude that ATP: citrate lyase shows all the properties necessary for it to be considered as the control point for lipid biosynthesis in oleaginous micro-organisms. The absence of this enzyme in non-oleaginous moulds and yeasts marks a significant biochemical difference between these two groups of micro-organisms (Boulton & Ratledge, 1981). The 'exquisite' sensitivity of the enzyme to feedback inhibition by long-chain FACES – and such sensitivity has not been found with acetyl-CoA carboxylase from oleaginous yeasts (Botham & Ratledge, 1979; Gill & Ratledge, 1973) – raises the question as to how FACES may be released to give our observations some physiological significance. It is possible that in answering this question an understanding may be gained as to how the total amount of lipid a cell can store may be controlled. The enzymes for triacylglycerol biosynthesis, which utilize FACES as substrates, are associated with the 'lipid particles' of baker's yeast (Christiansen, 1979) and are thus likely to be on the surface phospholipid which encompasses the droplet. If the same applies to oleaginous yeasts, then as these droplets expand with increasing lipid storage so might the enzymes become less securely attached to the droplet. This, in turn, would decrease triacylglycerol synthesis, lead to the appearance of free FACES in the cytoplasm and thus to the inhibition of fatty acid biosynthesis. Although the concentration of FACES in yeast cells remains to be determined, values of between 15 and 140 μM have been recorded in mammalian and avian liver (Volpe & Vagelos, 1976).

ATP: citrate lyase occupies a unique position in the schemes of fatty acid biosynthesis. Although this enzyme is also present in animals and plants, the microbial enzyme appears distinct with its high sensitivity to feedback inhibition as well as possibly being the rate-limiting step in lipid biosynthesis.

This work was supported by a grant from the Agricultural Research Council.

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