Partial Purification and Some Properties of ATP: Citrate Lyase from the Oleaginous Yeast *Lipomyces starkeyi*

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ATP: citrate lyase has been purified some 20-fold from the oleaginous yeast, *Lipomyces starkeyi* CBS 1809. The enzyme, which is labile, was stabilized by the addition of ATP and citrate to extraction buffers. Michaelis–Menten kinetics were exhibited towards ATP and citrate. The molecular weight of the enzyme was determined to be approximately 510 000. There was a specific requirement for ATP for activity. No activity was observed in the absence of Mg^{2+} , although Co^{2+} and Mn^{2+} could partially substitute for Mg^{2+} . ADP inhibited activity (50% inhibition at 1 mM), as did long-chain fatty acyl-CoA esters (50% inhibition with 4 μ M oleoyl-CoA). The possibility of control of ATP: citrate lyase activity by fluctuations in the prevailing energy charge or by changes in the intracellular concentrations of long-chain fatty acyl-CoA esters is discussed.

INTRODUCTION

ATP: citrate lyase (EC 4.1.3.8; ATP: citrate oxaloacetate-lyase CoA-acetylating and ATP-dephosphorylating) catalyses the ATP-dependent cleavage of citrate to acetyl-CoA and oxaloacetate. The enzyme, which is widely distributed in both plant and animal tissues (Srere, 1975) catalyses the first reaction in the pathway of cytosolic lipogenesis by providing precursor acetyl-CoA units which are themselves derived from intramitochondrial citrate. Evidence for the involvement of ATP: citrate lyase in lipogenesis has principally been provided by studies with mammalian systems. In early studies (Kornacker & Lowenstein, 1963, 1965) it was reported that the observed activity of rat hepatic ATP: citrate lyase positively correlated with altered rates of lipogenesis produced by dietary manipulation. Subsequently, it has been shown that this is an induction-repression process (Yen & Mack, 1980). Rat hepatic ATP: citrate lyase has a molecular weight of 540 000, consists of four identical subunits and has been identified as a phosphoprotein (Linn & Srere, 1979). The structural phosphate is distinct from enzyme-bound phosphate derived from ATP during the reaction and may appear as a consequence of hormone-mediated phosphorylation reactions (Janski *et al.*, 1979).

Our interest in ATP: citrate lyase has arisen as a consequence of studies of the biochemistry of lipid accumulation in oleaginous micro-organisms. In a previous report (Boulton & Ratledge, 1981a) we have provided evidence that ATP: citrate lyase is found only in yeasts capable of accumulating substantial amounts of lipid under optimal cultural conditions ($\geq 20\%$, w/w). In the same report evidence was provided that ATP: citrate lyase may catalyse the rate-determining step in the sequence of reactions leading to triacylglycerol biosynthesis in a typical oleaginous yeast, *Lipomyces starkeyi*. In a preliminary report (Boulton & Ratledge, 1981b), it was shown that ATP: citrate lyase from *L. starkeyi* was strongly inhibited by long-chain fatty acyl-CoA esters and that this phenomenon may be of regulatory significance in controlling triacylglycerol biosynthesis in oleaginous yeasts. The present paper presents the results of a more detailed study of the properties of ATP: citrate lyase partially purified from the same yeast.

METHODS

Yeast and growth. *Lipomyces starkeyi* CBS 1809 was grown at 30 °C in a five litre chemostat using a glucose/salts medium under nitrogen-limitation, as previously described by Botham & Ratledge (1979), with a dilution rate of 0.05 h⁻¹ and the pH maintained at 5.5 by the automatic addition of NaOH.

Preparation of extracts. Washed yeast was suspended in 50 mM-Tris/HCl, pH 7.5, containing 10 mM-MgCl₂, 10 mM-dithiothreitol, 1 mM-ATP and 20 mM-tripotassium citrate and a cell-free extract prepared using a French pressure cell (Boulton & Ratledge, 1980).

Enzyme assay. ATP : citrate lyase activity was measured by the coupled spectrophotometric procedure of Srere (1962).

Protein estimation. Protein was assayed by the dye-binding procedure of Bradford (1976) using γ -globulin as standard.

Enzyme purification. In a typical purification (see Table 1), 250 ml culture was removed from the chemostat which yielded approximately 20 g (wet wt) cells after harvesting and washing. The extract was prepared, and all procedures were carried out, at 4 °C. Solid (NH₄)₂SO₄ [2.26 g (ml extract)⁻¹] was added to the stirred extract over a period of 15 min to give 40% saturation. After a further 15 min stirring, the precipitate was sedimented by centrifuging at 30000 *g* for 10 min. The precipitate was dissolved in a minimum volume of 20 mM-Tris/HCl, pH 7.5, with the additives already described, and applied to a Sepharose-4B column (2.5 × 40 cm) equilibrated in similar buffer. After elution, active fractions were pooled and applied to a DEAE-Sephadex (A50) column (2 × 50 cm) equilibrated in 20 mM-Tris/HCl, pH 7.5, containing additives. ATP : citrate lyase was recovered in the wash-through peak and was stored at 4 °C.

Determination of enzyme purity. The purity of the enzyme preparation was gauged by polyacrylamide disc gel electrophoresis, as previously described (Boulton & Ratledge, 1980).

Calibration of Sephacryl-S300 column for determination of molecular size of ATP : citrate lyase. The column (2.5 × 90 cm) was equilibrated in 50 mM-Tris/HCl, pH 7.5, and the void volume determined using 5 ml 0.2% (w/v) Blue Dextran (Pharmacia) in the same buffer. The peak of the eluted dye was determined spectrophotometrically at 630 nm. The following proteins were used as standards, assumed molecular weights are given in parentheses: β -galactosidase (Sigma, grade VI), 3 mg (520000); urease (Sigma, type III), 30 mg (490000); phosphorylase-a (Sigma), 5 mg (370000); pyruvate kinase (Sigma, type II), 5 mg (235000) and thyroglobulin (Sigma, type I), 30 mg (669000). Proteins were dissolved in 5 ml 50 mM-Tris/HCl, pH 7.5 and, following elution in similar buffer, were detected spectrophotometrically at 280 nm and also by the specific assay procedures as described by Bergmeyer (1974).

RESULTS AND DISCUSSION

Stabilization of ATP : citrate lyase

Initial attempts to purify ATP : citrate lyase from *L. starkeyi* using only Tris/HCl buffer were hindered by the apparent instability of the enzyme such that even the simplest fractionation procedures resulted in unacceptable losses of activity. The addition of thiol-containing reagents and/or Mg²⁺ ions to extraction buffers, which have been reported to stabilize rat hepatic ATP : citrate lyase (Inoue *et al.*, 1966; Plowman & Cleland, 1967), did not alleviate the problem. It has been reported more recently that ATP : citrate lyase from germinating castor bean endosperm is also unstable but that the addition of citrate to extraction buffers exerted a stabilizing effect (Fritsch & Beevers, 1979).

Citrate was also observed to stabilize ATP : citrate lyase from *L. starkeyi*, as did ATP, but to a lesser extent. However the maximum effect was noted when the two substrates were used in combination. Thus, enzyme stored at 4 °C in 50 mM-Tris/HCl, pH 7.5, containing 1 mM-MgCl₂ and 10 mM-2-mercaptoethanol lost 83% of its activity in 48 h. However, the addition of 1 mM-ATP or 20 mM-tripotassium citrate to extracts reduced the loss of activity, over the same period, to 52% and 30%, respectively. Furthermore, when 1 mM-ATP and 20 mM-tripotassium citrate were added in combination, 91% of the activity remained after 48 h storage at 4 °C. Consequently, tripotassium citrate and ATP at final concentrations of 1 mM and 20 mM, respectively, were included in all buffers used for the extraction, purification and subsequent storage of the enzyme.

Partial purification of ATP : citrate lyase

The average values of five individual purifications, all of which gave broadly similar results, are shown in Table 1. The purified enzyme as judged by polyacrylamide disc gel electrophoresis,

Table 1. *Partial purification of ATP : citrate lyase from L. starkeyi*

Fraction	Volume (ml)	Total units ($\mu\text{mol min}^{-1}$)	Total protein (mg)	Specific activity [$\mu\text{mol min}^{-1}$ (mg protein $^{-1}$)]	Purification factor	Yield (%)
Crude	27.5	11.0	236.0	0.047	1.0	100
0–40% $(\text{NH}_4)_2\text{SO}_4$	4.8	9.0	68.2	0.132	3.0	90
Pooled Sepharose 4B eluate	60.0	8.4	29.4	0.286	6.0	76
Pooled DEAE-Sephadex eluate	48.2	6.3	6.8	0.922	20.0	57

was not homogeneous; at least seven protein bands were visible and no single major band was evident. However, it was free from contaminating activities of citrate synthase, adenylate kinase, acetyl-CoA deacylase and acetyl-CoA synthetase. The preparation did, however, contain malate dehydrogenase [specific activity $0.5 \mu\text{mol min}^{-1}$ (mg protein $^{-1}$)], but as this enzyme was a necessary constituent of the assay system it was not considered to be of serious consequence. It is appreciated that this purification was modest compared with those made with ATP : citrate lyase purified from mammalian sources, where affinity techniques such as the use of Cibacron blue Sepharose have been successfully applied (Hoffmann *et al.*, 1979; Redshaw & Loten, 1981). However, the necessity to stabilize the yeast enzyme by the addition of substrates, during the purification, had the consequence that no binding to this gel was observed, thus rendering this particular method inapplicable.

Properties of the enzyme

Variation of enzyme activity with pH. The response of ATP : citrate lyase activity to changes in pH was similar to that reported for the rat hepatic enzyme (Inoue *et al.*, 1966). Maximum activity was observed between pH 8.2 and pH 8.6 and activity was reduced to 50% at pH 7.3 and pH 9.5. Consequently, pH 8.4 was judged suitable for subsequent assays.

Initial velocity as a function of substrate concentrations. Lineweaver–Burk plots were linear with respect to both ATP and citrate concentrations. Apparent K_m values were calculated to be $135 \mu\text{M}$ and $70 \mu\text{M}$ for ATP and citrate, respectively. It was not possible to determine the apparent K_m for coenzyme A because non-saturating concentrations of this substrate were so low that rates rapidly became non-linear, thus making initial velocity measurements unreliable.

The apparent K_m values for ATP and citrate were of a similar order to those reported for the human, rat and avian enzymes (Hoffmann *et al.*, 1980*a, b*; Inoue *et al.*, 1966) as well as those reported for the enzyme from the mould *Penicillium spiculispurum* (Mahlén, 1973). However, the non-linear response, reported when citrate was the variable substrate, with the enzyme from the rat (Plowman & Cleland, 1967) and chicken enzymes (Hoffmann *et al.*, 1980*b*) was not observed. This was irrespective of the chloride concentration and, therefore, contrary to the observations of Plowman & Cleland (1967) who reported that the non-linear response was abolished in the presence of 0.25 M-chloride. Thus it is possible that the yeast ATP : citrate lyase may differ mechanistically from rat hepatic enzyme. However, this question can only be resolved by detailed kinetic studies which have not yet been performed.

Effect of divalent metal ions. The activity of the enzyme was absolutely dependent on the presence of Mg^{2+} . Mn^{2+} and Co^{2+} could partially substitute, giving rates 22.6% and 37.1%, respectively, of that observed in the presence of Mg^{2+} , when tested in the standard assay at a final concentration of 10 mM.

Determination of molecular size. The apparent molecular weight of ATP : citrate lyase from *L. starkeyi* was determined by passing a sample of enzyme down a Sephacryl-S300 column and comparing the elution volume with the elution volumes of proteins of known molecular weight (see Methods). ATP : citrate lyase activity was recovered in a single peak with a molecular weight of 510000 (average of three determinations; highest 514000, lowest 505000). The molecular weight of the yeast enzyme was similar, therefore, to that reported for rat hepatic ATP : citrate lyase: 540000 (Linn & Srere, 1979).

Fig. 1.

Fig. 1. Activity of ATP: citrate lyase at various simulated energy charge values $[(\text{ATP} + \frac{1}{2}\text{ADP})/(\text{ATP} + \text{ADP} + \text{AMP})]$ using total adenine nucleotide concentrations of 0.1 mM, ○; 1 mM, ●, and 10 mM, ▲. Reactions were initiated by the addition of coenzyme A. The proportions of each nucleotide at each energy charge value were calculated according to Bomsel & Pradet (1968) assuming an equilibrium constant for adenylate kinase of 1.2.

Fig. 2.

Fig. 2. Inhibition of ATP: citrate lyase by: lauroyl-CoA, ■; oleoyl-CoA, ●; and palmitoyl-CoA, ▲. Enzyme [specific activity: $0.81 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$] was incubated for 5 min at 30°C with inhibitor, at the concentration shown, prior to initiating reactions by the addition of coenzyme A.

Effect of nucleotides on ATP: citrate lyase activity. With the exception of ADP and ATP, the mono-, di- and triphosphates of adenosine, guanosine, cytosine, uridine and inosine as well as cyclic AMP had no effect on enzyme activity when tested in the standard assay at 1 mM. ADP was a potent inhibitor of enzyme activity (50% inhibition with 1 mM-ADP when tested in the standard assay). None of the triphosphate nucleotides tested could replace ATP as substrate, an observation contrary to that made with the rat hepatic enzyme where the partial substitution of ATP by ITP, GTP or UTP as substrate has been reported (Srere, 1975).

Since ADP is a product of the ATP: citrate lyase reaction and is inhibitory towards it, it becomes evident that enzyme activity may be regulated by changes in the relative concentrations of adenine nucleotides, i.e. by changes in the energy charge of the cell (Atkinson, 1977). Thus ATP is utilized in the synthesis of lipid, via the action of ATP: citrate lyase and also acetyl-CoA carboxylase, thereby generating ADP which, if not phosphorylated back to ATP would ultimately reduce the rates of lipogenesis by product-inhibition of ATP: citrate lyase.

ATP: citrate lyase from *L. starkeyi* responded to mixtures of adenine nucleotides simulating various energy charge values such that activity increased with increase in energy charge (Fig. 1). Furthermore, the results indicated that the relative proportion of each nucleotide, and not their absolute amounts, was of importance as an increase of 100-fold from 0.1 mM to 10 mM total adenine nucleotide did not drastically (threefold, or so) affect enzyme activity at any particular energy charge value.

Whether this phenomenon is of regulatory importance in the control of lipogenesis in oleaginous yeasts is questionable. Previous studies with oleaginous yeasts have shown that when

Table 2. *Effect of acyl-CoA esters on ATP : citrate lyase activity*

Enzyme was pre-incubated for 5 min at 30 °C with the test compound prior to initiating reactions by the addition of coenzyme A. Activities are expressed relative to that observed in the absence of test compound after 5 min incubation at 30 °C [$0.81 \mu\text{mol min}^{-1} (\text{mg protein})^{-1}$]. In a control experiment no effect was observed when each compound was included in malate dehydrogenase assays at a concentration of 100 μM .

Compound	Concentration (μM)	Activity (%)
Acetyl-CoA	10 or 100	100
Malonyl-CoA	10 or 100	100
Lauroyl-CoA ($\text{C}_{12:0}$)	10	52
Myristoyl-CoA ($\text{C}_{14:0}$)	10	5
Palmitoyl-CoA ($\text{C}_{16:0}$)	10	5
Steroyl-CoA ($\text{C}_{18:0}$)	10	45
Oleoyl-CoA ($\text{C}_{18:1}$)	10	21

cultural conditions favour lipogenesis (i.e. a deficit of nitrogen with a surfeit of carbon), the adenylate energy charge is considerably higher than that observed in cultures where lipogenic rates were low (Botham & Ratledge, 1979; Boulton, 1982), thus supporting the proposition that the control of ATP : citrate lyase activity by changes in the adenylate energy charge might be of importance in the regulation of rates of lipogenesis. However, the observed fluctuations in energy charge in the oleaginous yeasts were almost entirely due to variations in the intracellular AMP concentration rather than changes in ADP concentration. As AMP did not have any effect on ATP : citrate lyase activity, it may be that changes in the intracellular energy charge values would not influence the activity of the enzyme *in vivo*. Conversely, the determination of the energy charge values took no account of the undoubted intracellular compartmentalization of the adenine nucleotides. Therefore, the possibility that variations in the cytosolic pool-size of ATP and ADP might be sufficient to regulate the activity of ATP : citrate lyase *in vivo* cannot be entirely disregarded.

Effect of long-chain fatty acyl-CoA esters. Long-chain fatty acyl-CoA esters ($< \text{C}_{12}$) were potent inhibitors of enzyme activity (Table 2 and Fig. 2).

The following lipid intermediates and detergents had no effect on enzyme activity when tested at a final concentration of 100 μM : oleic acid, oleic acid methyl ester, oleoyl alcohol, oleoyl laurate, trioleoylglycerol, oleoylacetate, palmitic acid methyl ester, palmitic acid, myristyl ester, palmityl acetate, myristoyl chloride, sodium dodecyl sulphate and Triton X-100 (0.1%, w/v).

The inhibition produced by fatty acyl-CoA esters was time-dependent so that when reactions were initiated by the simultaneous addition of coenzyme A and 10 μM -oleoyl-CoA, the initial velocity was reduced by 55%, as compared with a control assay. However, when enzyme and 10 μM -oleoyl-CoA were pre-incubated for 15 min at 30 °C prior to initiating reactions by the addition of coenzyme A, the inhibition increased to 98%.

The inhibition by oleoyl-CoA was prevented by adding 1 mg bovine serum albumin to assay mixtures and, perhaps more importantly, the addition of 1 mg bovine serum albumin to mixtures of enzyme and oleoyl-CoA already pre-incubated readily reversed the inhibition.

The proposition that the inhibition of enzymes by fatty acyl-CoA esters is of regulatory significance is frequently criticized because these molecules are powerful detergents and may, therefore, exert their effects by non-specific binding or by disruption of the quaternary structure of the enzyme. However, in this case there was little evidence to support this premise. Hsu & Powell (1975) determined the critical micellar concentration of oleoyl-CoA, a typical fatty acyl-CoA ester, to be 3 μM in a solution whose pH and ionic strength were similar to those used in this present work. This value may then be taken as a measure of the detergency of these molecules. Yet oleoyl-CoA and other fatty acyl-CoA esters produced significant inhibition at concentrations below their critical micellar concentration values (Fig. 2). Furthermore, other detergents such as Triton X-100 and sodium dodecyl sulphate were not inhibitory even when tested at considerably higher concentrations. Other evidence for a regulatory role for fatty acyl-

CoA esters in the inhibition of ATP: citrate lyase is provided by the observation that inhibition by oleoyl-CoA was readily reversible by adding bovine serum albumin. This would not be expected if the inhibition was simply due to non-specific detergent effects. The time-dependency of the inhibition, in that approximately 5 min pre-incubation was needed to produce the maximum effect, is more difficult to explain, but we consider that this may be a consequence of using a non-homogeneous enzyme preparation such that the oleoyl-CoA may have bound non-specifically to other (inactive) proteins at the time of its addition and then been slowly and specifically acquired by the ATP: citrate lyase molecule during the pre-incubation period.

Thus, there is considerable evidence for a physiological role for the inhibition of ATP: citrate lyase from *L. starkeyi* as a means of controlling lipogenesis. This hypothesis casts some doubt, therefore, on the generally accepted premise that acetyl-CoA carboxylase is the 'first' enzyme of lipid biosynthesis and the controlling enzyme for this pathway (Volpe & Vagelos, 1976). It is pertinent to note that the inhibitory effects of fatty acyl-CoA esters towards ATP: citrate lyase observed here are an order of magnitude higher than those reported for yeast acetyl-CoA carboxylase (Lynen, 1967; White & Klein, 1965) including that from another oleaginous yeast, *Candida* 107 (Gill & Ratledge, 1973). The inhibition of ATP: citrate lyase from *L. starkeyi* by fatty acyl-CoA esters represents one of the few features which so far distinguish the yeast enzyme from ATP: citrate lyase from mammalian sources, which are relatively insensitive to this form of inhibition (Bloch & Vance, 1977). This may be explicable in that long-chain fatty acyl-CoA esters are the ultimate end-products of fatty acid biosynthesis in yeasts, whereas in mammalian tissues it is the free fatty acids that are the end-products (Bloch & Vance, 1977). Alternatively, fatty acyl-CoA esters may arise as the first product of triacylglycerol hydrolysis. Mobilization of the lipid reserve would lead to fatty acyl-CoA esters being produced as the substrates for the β -oxidation process. Inhibition of ATP: citrate lyase by these metabolites would then be a means of preventing lipid biosynthesis whilst lipolysis was occurring.

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REFERENCES

- ATKINSON, D. E. (1977). *Cellular Energy Metabolism and its Regulation*. New York: Academic Press.
- BERGMEYER, H. U. (1974). *Methods of Enzymatic Analysis*. New York: Academic Press.
- BLOCH, K. & VANCE, D. (1977). Control mechanisms in the synthesis of saturated fatty acids. *Annual Review of Biochemistry* **46**, 263–298.
- BOMSEL, J.-L. & PRADET, A. (1968). Study of adenosine 5'-mono-, di- and triphosphates in plant tissues. Regulation of the level of nucleotides, *in vivo*, by adenylate kinase: theoretical and experimental study. *Biochimica et biophysica acta* **162**, 230–242.
- BOTHAM, P. A. & RATLEDGE, C. (1979). A biochemical explanation for lipid accumulation in *Candida* 107 and other oleaginous micro-organisms. *Journal of General Microbiology* **114**, 361–375.
- BOULTON, C. A. (1982). *The biochemistry of lipid accumulation in oleaginous yeasts*. Ph.D. thesis, University of Hull, U.K.
- BOULTON, C. A. & RATLEDGE, C. (1980). Regulatory studies on citrate synthase in *Candida* 107, an oleaginous yeast. *Journal of General Microbiology* **121**, 441–447.
- BOULTON, C. A. & RATLEDGE, C. (1981a). Correlation of lipid accumulation in yeasts with possession of ATP: citrate lyase. *Journal of General Microbiology* **127**, 169–176.
- BOULTON, C. A. & RATLEDGE, C. (1981b). ATP: citrate lyase – the regulatory enzyme for lipid biosynthesis in *Lipomyces starkeyi*? *Journal of General Microbiology* **127**, 423–426.
- BRADFORD, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248–254.
- FRITSCH, H. & BEEVERS, H. (1979). ATP: citrate lyase from germinating castor bean endosperm; localization and some properties. *Plant Physiology* **63**, 687–691.
- GILL, C. O. & RATLEDGE, C. (1973). Regulation of *de novo* fatty acid biosynthesis in the *n*-alkane utilizing yeast, *Candida* 107. *Journal of General Microbiology* **78**, 337–347.
- HOFFMANN, G. E., SCHIESSL, J. & WEISS, L. (1979). ATP: citrate (*pro*-3S)-lyase in the rat; two-step purification procedure, properties, organ distribution. *Hoppe-Seyler's Zeitschrift für physiologische Chemie* **360**, 1445–1451.
- HOFFMANN, G. E., ANDRES, H., WEISS, L., KREISEL, C. & SANDER, R. (1980a). Lipogenesis in man. Properties and organ distribution of ATP: citrate (*pro*-3S)-lyase. *Biochimica et biophysica acta* **620**, 151–158.
- HOFFMANN, G. E., KRAUPE, P., WEISS, L. & WITTMANN, J. (1980b). Avian ATP: citrate (*pro*-3S)-lyase. *Hoppe-Seyler's Zeitschrift für physiologische Chemie* **361**, 1117–1119.

- HSU, K. H. L. & POWELL, G. L. (1975). Inhibition of citrate synthase by oleoyl-CoA: a regulatory phenomenon. *Proceedings of the National Academy of Sciences of the United States of America* **72**, 4729–4733.
- INOUE, H., SUZUKI, F., FUKUMISHI, K., ADACHI, K. & TAKEDA, Y. (1966). Studies on ATP : citrate lyase of rat liver I. Purification and some properties. *Japanese Journal of Biochemistry* **60**, 543–553.
- JANSKI, A. M., SRERE, P. A., CORNELL, N. W. & VEECH, R. (1979). Phosphorylation of ATP : citrate lyase in response to glucagon. *Journal of Biological Chemistry* **254**, 9365–9368.
- KORNACKER, M. & LOWENSTEIN, J. M. (1963). The relation between the rates of citrate cleavage and fatty acid synthesis in rat liver preparations. *Biochemical Journal* **89**, 27P.
- KORNACKER, M. & LOWENSTEIN, J. M. (1965). Citrate and the conversion of carbohydrate into fat. The activities of citrate-cleavage enzyme and acetate thiokinase in livers of starved and re-fed rats. *Biochemical Journal* **94**, 209–215.
- LINN, T. C. & SRERE, P. A. (1979). Identification of ATP : citrate lyase as a phosphoprotein. *Journal of Biological Chemistry* **254**, 1691–1698.
- LYNEN, F. (1967). The role of biotin-dependent carboxylations in biosynthetic reactions. *Biochemical Journal* **102**, 381–400.
- MAHLÉN, A. (1973). Purification and some properties of ATP : citrate lyase from *Penicillium spiculisporum*. *European Journal of Biochemistry* **36**, 342–346.
- PLOWMAN, K. M. & CLELAND, W. W. (1967). Purification and kinetic studies of the citrate cleavage enzyme. *Journal of Biological Chemistry* **242**, 4239–4247.
- REDSHAW, J. C. & LOTEN, E. G. (1981). A rapid purification of hepatic ATP : citrate lyase using blue Sepharose. *FEBS Letters* **123**, 261–264.
- SRERE, P. A. (1962). Citrate cleavage enzyme. *Methods in Enzymology* **5**, 641–644.
- SRERE, P. A. (1975). Enzymology of formation and breakdown of citrate. *Advances in Enzymology* **43**, 57–101.
- VOLPE, J. J. & VAGELOS, P. R. (1976). Biosynthesis of fatty acids. *Physiological Reviews* **56**, 339–417.
- WHITE, D. & KLEIN, H. P. (1965). Factors affecting fatty acid synthesis in cell-free preparations from *Saccharomyces cerevisiae*. *Biochemical and Biophysical Research Communications* **20**, 78–84.
- YEN, C. S. & MACK, D. O. (1980). Immunoquantitation of hepatic ATP : citrate lyase in the starved, sucrose-refed, and fat-refed rat. *Nutrition Reports International* **22**, 245–252.