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THE INHIBITION OF CITRATE SYNTHASE BY ADENOSINE TRIPHOSPHATE

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SUMMARY

1. Citrate synthase (citrate oxaloacetate-lyase (CoA-acetylating), EC 4.1.3.7) has been partially purified from beef liver, beef heart and *Escherichia coli*. The beef heart and beef liver enzymes were quite similar in their pH optima, substrate affinities and sensitivity to ATP inhibition. The *E. coli* enzyme had a lower substrate affinity and had a lower K_i for ATP than the mammalian enzymes. The *E. coli* citrate synthase differed markedly from the mammalian enzymes in its response to pH changes.

2. ATP was competitive with respect to CoASAc with the beef liver and beef heart enzymes. In *E. coli*, ATP was not competitive with CoASAc but changed both V and K_m for oxaloacetate. Mg^{2+} also inhibits the activity of the *E. coli* citrate synthase and tends to relieve the ATP inhibition.

3. The ATP inhibition of citrate synthase may act in concert with AMP and ADP stimulation of isocitrate dehydrogenase and with citrate stimulation of CoASAc carboxylase in partitioning CoASAc between oxidation by way of the citric acid cycle and storage as fat. It may also play a role in regulating liver ketone body formation.

INTRODUCTION

Citrate synthase (citrate oxaloacetate-lyase (CoA-acetylating), EC 4.1.3.7) from yeast has been reported to be subject to inhibition by ATP (ref. 1). This finding followed from a proposal that the AMP modulation of NAD-specific isocitrate dehydrogenase was part of a complex control system which determined the metabolic fate of CoASAc (ref. 2).

The adenine nucleotide modulation of these two enzymes, together with the citrate activation of CoASAc carboxylase[3], may contribute to the regulatory mechanism which determines whether CoASAc undergoes an energy-yielding oxidation through the citric acid cycle or is converted to fat, depending upon the energy needs of the cell.

Abbreviation: DTNB, 5,5-dithiobis-(2-nitrobenzoic acid).

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The citrate synthases from tobacco leaves (M. GLASER AND C. A. WEST, personal communication), rat liver[4], lemon mitochondria[5] and pig heart[1,6] have also been found to be inhibited by ATP. In this paper the kinetic properties of the enzymes from beef liver, beef heart and *Escherichia coli* have been examined. The properties of the enzymes from beef liver and beef heart are similar to those of the yeast enzyme. The *E. coli* enzyme is different from the other enzymes, particularly in its response to changes in pH.

EXPERIMENTAL PROCEDURE

Enzymes. The methods used for purification of the beef heart, beef liver and *E. coli* citrate synthases were modified from the procedure used by SRERE AND KOSICKI[7] in the purification of pig heart citrate synthase. Protein was determined by the Biuret method[8] or spectrophotometrically by measuring the absorption at 280 mμ (ref. 9). One unit of activity corresponds to the formation of 1 μmole of CoASH per min. Specific activity is defined as the units of enzyme per mg protein.

In the preparation of the beef liver and beef heart enzymes, diced frozen tissue was homogenized in a Waring blendor in 4 vol. of 0.4 M KCl in 20% ethanol. The starting temperature was —10° and this rose to 18° during the 10-min homogenization. The supernatant was allowed to cool and then was centrifuged for 15 min at 23 500 $\times g$. The pellet was discarded and the turbid red-brown supernatant solution was dialyzed overnight against 2 mM potassium phosphate buffer (pH 7.4). The dialysate was centrifuged as above to yield a clear red solution. $(NH_4)_2SO_4$ fractionations of this solution were made by carefully adding solid $(NH_4)_2SO_4$, stirring for 30 min, centrifuging for 15 min at 23 500 $\times$ g, and redissolving the pellet in a minimal amount of cold 0.1 M Tris buffer (pH 8.0). Fractionations were made at 0–35% saturation, 35–45% saturation, 45–70% saturation and 70–90% saturation based on a value of 70.6 g of $(NH_4)_2SO_4$ per 100 ml as 100% saturation. The fraction precipitating between 45% and 70% of $(NH_4)_2SO_4$ saturation contained the majority of the enzyme activity.

Following dialysis against 1 mM potassium phosphate buffer (pH 7.0), the 45–70% fraction was placed on a DEAE-cellulose column which had been previously equilibrated in the same buffer. The column was washed with 500 ml of 1 mM potassium phosphate buffer (pH 7.0). The enzyme was removed from the column by linear gradient elution. The mixing vessel contained 2 l of 1 mM phosphate buffer (pH 7.0), and the reservoir contained 2 l of 0.5 M KCl in 10 mM phosphate buffer (pH 8.0). The column eluant was collected in 20-ml fractions with the peak of enzyme activity appearing in tube 60.

The purification of the *E. coli* enzyme was similar to that used for the beef enzymes. The cells were disrupted by freezing and thawing in the presence of lysozyme in 0.1 M Tris buffer (pH 8.0), followed by two 60-sec periods of sonication with a Branson probe sonifier. The cell debris was removed by centrifugation for 40 min at 27 500 $\times$ g and the supernatant solution was fractionated with solid $(NH_4)_2SO_4$. The fraction precipitating between 40% and 80% of $(NH_4)_2SO_4$ saturation was chromatographed on DEAE-cellulose, as described above, with the peak of enzyme activity being eluted in fraction 144.

The procedures described above resulted in a 23-fold purification of the liver

enzyme, 96-fold purification of the heart enzyme and a 20-fold purification of the *E. coli* enzyme. The preparations were stable for several weeks when frozen.

E. coli growth conditions. A culture of *E. coli* B was obtained from the collection of the Bacteriology Department, University of California, Los Angeles. The cells were grown on a defined medium containing 1.5% $(NH_4)_2SO_4$, 2% glucose or lactate, and mineral ions as described by KEMP[10]. The medium was adjusted to a final pH of 7.1 with concentrated ammonium hydroxide. The glucose or lactate solutions and the $(NH_4)_2SO_4$–metals solutions were autoclaved separately. The *E. coli* cells were grown under two conditions, anaerobically on glucose in 13-l carboys, or aerobically on lactate in 2-l erlenmeyer flasks. The cells were harvested in the late phase of exponential growth with an air-driven Sharples Super Centrifuge.

Assay. The enzyme assay was that described by SRERE, BRAZIL AND GONEN[11] using ELLMAN'S reagent[12], 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB). The standard reaction mixture contained 50 μmoles of Tris–HCl at pH 8.1, 50 μmoles of CoASAc, 100 mμmoles of oxaloacetate, 125 mμmoles of DTNB and 0.05 ml of enzyme preparation in a final volume of 1.0 ml. Because of its low solubility in unbuffered water, DTNB was prepared in 0.01 M Tris–HCl (pH 8.1). The oxaloacetate and adenine nucleotides were also prepared in buffer and neutralized prior to use. All reagents other than the DTNB were prepared fresh daily. The rate of the reaction was followed by measuring the increase in absorbance at 412 mμ, using a Hitachi Perkin–Elmer Model 139 spectrophotometer or a Gilford Model 2000 spectrophotometer. Reagents and chemicals were obtained commercially.

RESULTS

Beef liver enzyme. The Michaelis constant of the enzyme for both substrates has been determined. The K_m for CoASAc was found to be 5.8 μM in the presence of 74 μM oxaloacetate and the K_m for oxaloacetate in the presence of 10 μM CoASAc was found to be 5.0 μM. The K_m of either substrate was found to vary depending on the concentration of the other substrate in a manner analogous to that reported for the pig heart enzyme by KOSICKI AND SRERE[13].

Adenine nucleotides have an inhibitory effect on the initial reaction rate of beef liver citrate synthase. As can be seen in Fig. 1, ATP is the most effective inhibitor, causing a 50% inhibition at 1 mM concentration in the presence of 10 μM CoASAc and

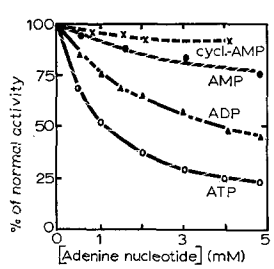
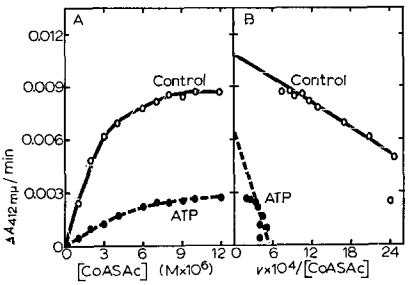


Fig. 1. Comparative effectiveness of the adenine nucleotides as inhibitors of beef liver citrate synthase. The reaction mixture contained 50 μmoles of Tris–HCl buffer (pH 8.0), 125 mμmoles of DTNB, 10 mμmoles of CoASAc, 19 mμmoles of oxaloacetate, nucleotides as indicated and 2.18 milliunits of enzyme (specific activity, 0.298) in a total volume of 1.0 ml.



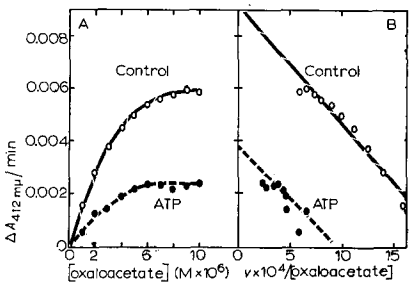


Fig. 2. Adenosine triphosphate effect on beef liver citrate synthase at varying concentrations of CoASAc. The reaction mixture contained 50 μmoles of Tris–HCl buffer (pH 8.1), 125 mμmoles of DTNB, 85 mμmoles of oxaloacetate, CoASAc as indicated, 6.7 milliunits of enzyme (specific activity, 0.1), 3 μmoles of ATP where indicated in a total volume of 1.0 ml.

Fig. 3. Effect of ATP on beef liver citrate synthase activity at varying concentrations of oxaloacetate. The reaction mixture contained 50 mμmoles of Tris–HCl buffer (pH 8.0), 125 mμmoles of of DTNB, 21 mμmoles of CoASAc, oxaloacetate as indicated, 4.4 milliunits of enzyme (specific activity, 0.1), 3 μmoles of ATP where indicated in a total volume of 1.0 ml.

19 μM oxaloacetate. ADP is less effective as an inhibitor, a 4.5 mM concentration being required to obtain a 50% inhibition under the same conditions. The degree of inhibition is dependent upon the CoASAc concentration, which is consistent with a competition between ATP and CoASAc. Other nucleoside triphosphates are from one-third to one-half as effective inhibitors as ATP. Considering the relative concentration of the nucleotides in the cell, it seems unlikely that inhibition of citrate synthase by the other nucleoside triphosphates has any physiological importance.

The effect of 3 mM ATP on the initial rate of reaction catalyzed by citrate synthase is shown in Fig. 2A. The oxaloacetate concentration is 85 μM. Changes in the oxaloacetate concentration had little effect on the ATP inhibition. These values are plotted in an Eadie plot in Fig. 2B with rate plotted as a function of rate/substrate concentration[14–16]. In this plot, V is indicated by the vertical intercept and the slope of the line gives K_m for ideal Michaelis behavior. As can be seen in Fig. 2B, ATP markedly changes the K_m of the enzyme for CoASAc. Thus ATP acts as a competitive inhibitor for CoASAc.

In Fig. 3A, the effect of 3 mM ATP on the reaction rate when the oxaloacetate concentration is varied and CoASAc is held constant at 21 μM is shown. An Eadie plot of the data, Fig. 3B, shows that the apparent K_m for oxaloacetate is not changed by ATP as the slopes of the lines are similar. The effect of ATP is less when higher concentrations of CoASAc are used. This is consistent with the competitive nature of the inhibition with respect to CoASAc.

A number of other metabolites were also tested as effectors. 3-Phosphoglyceric acid, β-hydroxybutyrate, α-ketoglutarate, glutamate, glucose 6-phosphate, fructose 6-phosphate, fructose diphosphate, phosphoenolpyruvate and pyruvate, tested at 1–5 mM, did not appreciably affect the rate of the reaction.

Beef heart enzyme. The kinetic properties of the beef heart citrate synthase are similar to those of the beef liver enzyme. The pattern of effectiveness of the various nucleotides was the same. The pH optima are similar as are the Michaelis constants.

The effects of 5 mM ATP, ADP and AMP on the initial reaction rate of beef

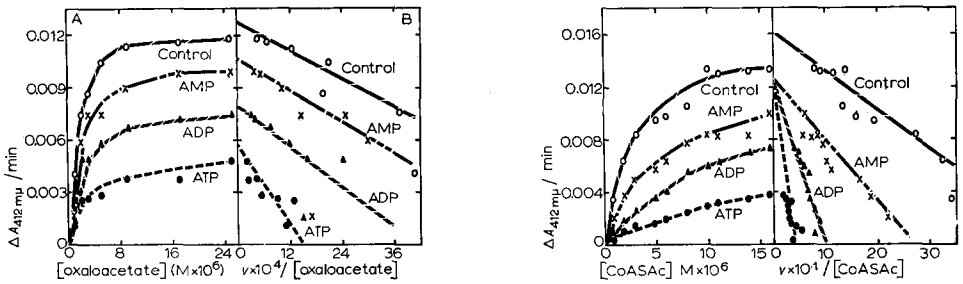


Fig. 4. Effect of adenylates on beef heart citrate synthase activity at varying oxaloacetate concentrations. The reaction mixture contained 50 μmoles of Tris–HCl buffer (pH 8.0), 125 mμmoles of DTNB, 21 mμmoles of CoASAc, oxaloacetate as indicated, 5 μmoles of adenine nucleotides as shown and 0.88 milliunit of enzyme (specific activity, 30) in a total volume of 1.0 ml.

Fig. 5. Effect of adenylates on beef heart citrate synthase activity as a function of CoASAc concentration. The reaction mixture contained 50 μmoles of Tris–HCl buffer (pH 8.0), 125 mμmoles of DTNB, 10 mμmoles of oxaloacetate, CoASAc as indicated, 5 μmoles of adenine nucleotides where shown, and 0.014 milliunit of enzyme (specific activity, 30) in a total volume of 1.0 ml.

heart citrate synthase at 21 μM CoASAc are shown in Fig. 4A. An Eadie plot of the data is shown in Fig. 4B. The ATP inhibition does not appear to be competitive with respect to oxaloacetate. The K_m of the beef heart enzyme for oxaloacetate is 2 μM at this CoASAc concentration. This value is very close to that reported by KOSICKI AND SRERE[13] for the pig heart enzyme and is slightly lower than that obtained with the beef liver enzyme.

The effect of the adenine nucleotides on the reaction rate at 10 μM oxaloacetate when CoASAc was varied is shown in Fig. 5A. Fig. 5B shows an Eadie plot of these data. The adenine nucleotides increase K_m for CoASAc, but do not affect V. In the

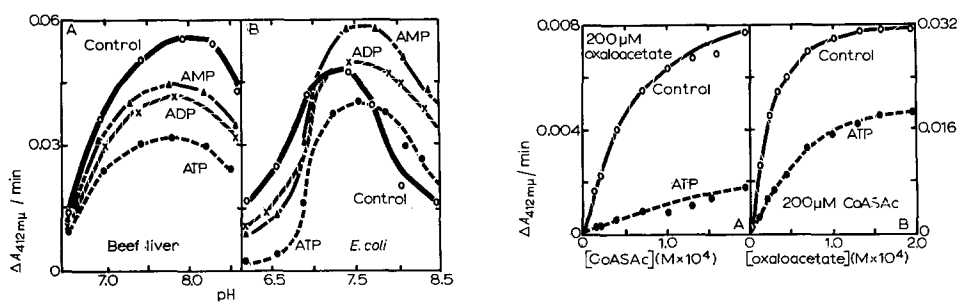


Fig. 6. Comparison of pH effect on beef liver and *E. coli* enzymes. A. The reaction mixture contained 60 μmoles of Tris–cacodylate buffer, 125 μmoles of DTNB, 50 mμmoles of CoASAc, 100 mμmoles of oxaloacetate, 3 μmoles of nucleotide as indicated and 4.8 milliunits of beef liver citrate synthase (specific activity, 0.1) in a total volume of 1.0 ml. B. Oxaloacetate, CoASAc and DTNB as above, 40 μmoles of Tris–cacodylate buffer, 2 μmoles of nucleotide as indicated and 5 milliunits of *E. coli* citrate synthase (specific activity, 0.0185) in 1.0 ml.

Fig. 7. Effect of ATP on the initial reaction rate of *E. coli* citrate synthase. A. The reaction mixture contained 60 μmoles of cacodylate (pH 6.4), 125 mμmoles of DTNB, 200 mμmoles of oxaloacetate, CoASAc as indicated, 5 μmoles of ATP were specified and 0.6 milliunit of enzyme (specific activity, 0.0185) in 1.0 ml. B. Buffer and DTNB as above, 200 mμmoles of CoASAc, varying amounts of oxaloacetate, 5 μmoles of ATP as indicated and 2.4 milliunits of enzyme in a final volume of 1.0 ml.

presence of 10 μM oxaloacetate, the K_m for CoASAc is 2.7 μM. This increases to 5.8 μM on the addition of 5 mM AMP, to 13.2 μM with 5 mM ADP, and to 26 μM with 5 mM ATP. Neither the K_m for CoASAc nor the effects of the adenine nucleotides are appreciably changed when the concentration of oxaloacetate is increased 6-fold.

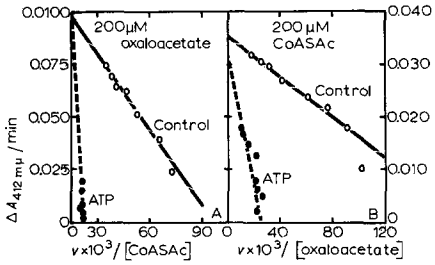
The other metabolites tested on the liver enzyme were also without effect on the heart enzyme. Concentrations of EDTA and $MgCl_2$ up to 5 mM had essentially no effect on the enzyme.

E. coli enzyme. The *E. coli* enzyme is also affected by adenine nucleotides, but its response differs from that of the beef liver and beef heart enzymes. Both ADP and ATP increase the rate of the reaction catalyzed by the *E. coli* enzyme at pH 8.0 in 0.5 M Tris–HCl buffer, but inhibit the same reaction at pH 6.0 in 0.05 M cacodylate buffer. The enzyme is approx. 50% as active in Tris buffer as in cacodylate buffer at pH 7.0. In Fig. 6 the pH profile of the *E. coli* enzyme in the presence of the adenine nucleotides is compared to the behavior of the beef liver enzyme under the same conditions. The oxaloacetate concentration was 100 μM and the CoASAc concentration was 50 μM. A Tris–cacodylate buffer was used and the pH was measured before and after each assay. As can be seen, the three adenine nucleotides inhibit the beef liver enzyme across the pH range measured, with ATP being the most effective inhibitor. The pH optimum for the *E. coli* enzyme in this buffer is 7.3. The effect of the adenine nucleotides on the reaction rate varies according to the pH. ATP strongly inhibits the enzyme from pH 6.0 to 7.3, then the inhibition decreases with increasing pH until ATP becomes slightly stimulatory above pH 7.6. AMP and ADP inhibit to a lesser degree than ATP at low pH (below 7.0) and stimulate to a greater extent above pH 7.2.

The inhibition of the *E. coli* enzyme by ATP in 0.06 M cacodylate buffer and its response to increasing levels of either substrate in the presence of a large excess of the other is shown in Fig. 7. The order in which the substrates were added had a bearing on the degree of inhibition seen. In Fig. 7A, the oxaloacetate was added last and, in the presence of a 200 μM concentration of both substrates, 5 mM ATP produces 74% inhibition. In Fig. 7B, the CoASAc was added last and the per cent inhibition at the same concentrations was 41%. Thus, it seems that the presence of oxaloacetate in the reaction mixture prior to initiating the reaction protects the enzyme from ATP inhibition in some fashion. This phenomenon has not been studied in detail.

An Eadie plot of the data is shown in Fig. 8. The *E. coli* citrate synthase has a lower substrate affinity than the mammalian enzymes. The K_m for CoASAc is 50 μM and the K_m for oxaloacetate is 22 μM. These values are approx. 10 times higher than those obtained with the enzymes from beef heart and beef liver. ATP decreases the apparent affinity of the enzyme for both CoASAc and oxaloacetate, in contrast to the mammalian enzymes where only the K_m for CoASAc is altered.

The kinetics of the *E. coli* enzyme are quite complex and have not been fully characterized. In addition to showing a different response to the adenine nucleotides as the pH is varied, the enzyme activity is also influenced by EDTA, Mg^{2+}, K^+ and changes in the molarity of the buffer. At pH 6.5 Mg^{2+} itself is inhibitory while the Mg–ATP complex appears to be much less inhibitory than free ATP. This is illustrated in Fig. 9A. At higher CoASAc concentrations the Mg^{2+} effects are less pronounced. In Fig. 9B it can be seen that Mg^{2+} also inhibits the enzyme at pH 7.8. The inhibition of the AMP–enzyme parallels that of enzyme alone, while in the presence of ATP or



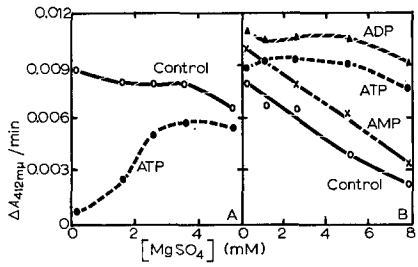


Fig. 8. Eadie plot of ATP effect on *E. coli* citrate synthase. Reaction conditions as in Fig. 7.

Fig. 9. Interaction of adenylates and magnesium with *E. coli* citrate synthase. A. The reaction mixture contained 31 μmoles of phosphate buffer (pH 6.7), 200 mμmoles of DTNB, 200 mμmoles of CoASAc, 100 mμmoles of oxaloacetate, 2.5 μmoles of ATP where shown, varying amounts of $MgSO_4$ and 0.6 milliunit of enzyme in 1.0 ml. B. Oxaloacetate, CoASAc, DTNB and enzyme as above, 31 μmoles of phosphate buffer (pH 7.8), 2.5 μmoles of adenine nucleotides as indicated and varying amounts of $MgSO_4$ in a total volume of 1.0 ml.

ADP, Mg^{2+} is much less effective as an inhibitor, presumably due to the formation of nucleotide–Mg complexes.

Varying the concentration of oxaloacetate and ATP sometimes resulted in sigmoid velocity curves. This sigmoidicity seemed to vary with the state of the enzyme and the conditions of the assay. In Table I, the effect of the adenine nucleotides and Mg^{2+} on the $S_{0.5}$ (ref. 17) and on the slope of the line obtained in a HILL plot[18] are shown. These values were obtained in the presence of 2.5 mM nucleotide, 5 mM Mg^{2+}, 50 μM CoASAc and 2.5 mM ATP–Mg in 31 mM phosphate buffer, pH 6.7. ATP inhibits

TABLE I

ADENYLATE AND MAGNESIUM EFFECTS ON KINETIC PARAMETERS OF *E. coli* CITRATE SYNTHASE

The reaction mixture contained 31 μmoles of potassium phosphate buffer (pH 6.7), 125 mμmoles of DTNB, 50 mμmoles of CoASAc, varying amounts of oxaloacetate, 2.5 μmoles of adenine nucleotide or magnesium sulfate as indicated and 0.8 milliunit of enzyme (specific activity, 0.0185) in 1.0 ml.

	Control	*AMP*	*ADP*	*ATP*	*ATP–Mg*	*Mg^{2+}*
$S_{0.5}$ (oxaloacetate)	0.07 mM	0.16 mM	0.09 mM	0.31 mM	0.12 mM	0.10 mM
HILL plot slope (oxaloacetate)	1.8	2.0	2.3	2.7	2.2	1.3

the enzyme, increasing the $S_{0.5}$ for oxaloacetate from 70 μM to 310 μM and the order of the reaction, as determined from the HILL plot slope, from 1.8 to 2.7. AMP and ADP also increase these values but to a lesser extent. The chelate formed by ATP and Mg^{2+} does not show the striking inhibition of the enzyme that free ATP does. The HILL plot slope is less, 2.2 rather than 2.7, and $S_{0.5}$ for oxaloacetate is 120 μM rather than 310 μM.

Mg^{2+} decreases V and reduces the apparent interaction between sites on the enzyme, decreasing the slope of the HILL plot from 1.8 for control to 1.3.

DISCUSSION

The kinetic properties of the citrate synthases from beef liver and beef heart are generally similar to those of the enzymes from yeast[1], tobacco leaf (M. GLASER AND C. A. WEST, personal communication), lemon[5] and pig heart[1,6]. The *E. coli* citrate synthase differs from these enzymes. ATP at 1 mM does not inhibit above a pH of about 7.5, the apparent K_m's for oxaloacetate and CoASAc are higher, the pH profile is quite different, and the kinetic order of the reaction with respect to oxaloacetate, at least in phosphate buffer, is higher than 1.0.

KOSICKI AND LEE[6] have reported that Mg^{2+} inhibits the citrate synthase from pig heart and overcomes the ATP inhibition to some extent. It is highly probable that the same effects would be seen with the beef liver and beef heart enzymes. It would not have been observed in this study due to the low (0–5 mM) concentrations of Mg^{2+} tested. The effects of Mg^{2+} on the *E. coli* enzyme are qualitatively similar to those seen with the pig heart enzyme.

The *E. coli* citrate synthase has been shown by WEITZMAN[19] to be strongly inhibited by NADH. The NADH seemed competitive with CoASAc, a 50 μM NADH concentration causing a 70% inhibition at 0.16 mM CoASAc but only a 10% inhibition at 0.8 mM CoASAc. WEITZMAN indicates that ATP has little effect on citrate synthase, a 20 mM concentration being required to get a 78% inhibition. In view of the variable response to ATP reported in the present work, it seems likely that the lack of effect observed by WEITZMAN is due to differences in assay conditions. WEITZMAN found that NADH did not inhibit pig heart citrate synthase, yeast citrate synthase or *E. coli* malate synthase. The lack of a NADH effect on the pig heart enzyme has also been reported by KOSICKI AND LEE[6]. WEITZMAN proposes that in *E. coli* in contrast to mammals and yeast, NADH regulates the activity of citrate synthase. In view of WEITZMAN's work and the finding that NADH strongly inhibits pyruvate dehydrogenase activity in *E. coli*[20], it seems reasonable to assume that NADH concentration, as well as the adenylate system, may play an important role in regulating the entry of pyruvate and CoASAc into the citric acid cycle in *E. coli*.

Citrate synthase from rat liver mitochondria has been shown by SHEPHERD AND GARLAND[4] to be inhibited by ATP. These authors report two differences between this enzyme and the beef liver and other mammalian enzymes. The ATP inhibition of the rat liver enzyme is reported not to be competitive with respect to CoASAc and a plot of per cent inhibition against ATP concentrations gives a sigmoid curve. The differences between the observations on the rat liver enzyme and on the other mammalian enzymes may result from species differences, differences in enzyme preparation, contaminating enzymes, or the use of different assays. In their assay SHEPHERD AND GARLAND used malate dehydrogenase, which has been found to be subject to adenine nucleotide effects[21]. However, our comparison of the malate dehydrogenase coupled assay and the DTNB assay gave identical results with the beef liver enzyme. In both cases ATP was competitive with CoASAc, and plots of per cent inhibition against ATP concentration were not sigmoid.

Although it is difficult to determine the concentration of metabolites at the enzyme site, it seems that the ATP concentrations found to inhibit citrate synthase are within the expected physiological concentration range. The intracellular ATP concentrations reported for various species range from 2 to 5 mM (refs. 22–25). The

ratio of ATP:ADP observed is usually about 3:1 and the adenine nucleotides are present at much higher concentrations than the other nucleoside phosphates.

Other reported inhibitors of citrate synthase are vasopressin and palmityl-CoA. Pig heart citrate synthase has been found to be inhibited by vasopressin[26] and this has been proposed as the mechanism by which vasopressin inhibits lipogenesis. Palmityl coenzyme A and other long chain acyl-CoA compounds have been reported to be inhibitors of citrate synthase[1,27–30]. This inhibition was suggested to be involved in determining whether CoASAc is oxidized or transformed into acetoacetate[31] although the physiological significance of the palmityl-CoA inhibition has been questioned by several authors[32–34].

The kinetic competititon between ATP and CoASAc seen with the mammalian enzymes does not necessarily result from a competititon for the same site on the enzyme. A kinetic competition could be seen if the two metabolites bind to the enzyme at different sites that interact with one another. The slope of a HILL plot of velocity as a function of oxaloacetate concentration for the reaction catalyzed by the *E. coli* enzyme is higher in the presence of ATP than in its absence. This behavior is consistent, for example, with an enzyme model which assumes that empty catalytic sites are inhibitory, and filled sites do not interact.

Regulation of citrate synthase by ATP may act in concert with the AMP and ADP stimulation of NAD-linked isocitrate dehydrogenase[36,37], and the activation of CoASAc carboxylase by citrate[3,38–41] to affect the tendency of CoASAc to be oxidized through the citric acid cycle or be stored as fat for future use, as discussed by HATHAWAY AND ATKINSON[1].

The effects of NADH in *E. coli* would complement these actions. The finding of a crossover point between pyruvate and citrate in *E. coli* grown aerobically on glucose[42] supports the existence of a control point between these two metabolites. Mg^{2+} might play a regulatory role as suggested by WYATT[43]. Citrate competes with ATP for Mg^{2+} and citrate levels rise as the energy needs of the cell are met. It is conceivable that citrate, whose concentration would increase due to diminished isocitrate dehydrogenase activity[1], would compete with ATP for Mg^{2+}, with the net production of free ATP. As free ATP seems to be more inhibitory than the ATP–Mg complex, this would lead to an inhibition of citrate synthase.

In the liver, CoASAc has a third fate, conversion to acetoacetate. SHEPHERD AND GARLAND[4] have suggested that the ATP inhibition of citrate synthase influences the rate of acetoacetate formation and consequently the development of ketosis. This would be consistent with the observation by KREBS[44], that the higher the ratio of ATP produced through citric acid cycle oxidations to ATP produced by other means, the more ketogenic the compound being metabolized.

The ATP inhibition of citrate synthase may also tend to conserve glucose and 3-carbon glucose precursors. In mammalian systems there is no appreciable conversion of acetate to 3-carbon units and, consequently, no net conversion of acetate to glucose. If the energy requirements of the cell were being met, it would be advantageous to the cell to prevent the physiologically irreversible conversion of pyruvate to CoASAc. The ATP–Mg–citrate regulation of citrate synthase may function in this regard. The ATP inhibition of citrate synthase would lead to an increased intracellular concentration of CoASAc due to the competitive nature of the inhibition. This would diminish pyruvate oxidation as GARLAND AND RANDLE[45] have shown that CoASAc

is an inhibitor of mammalian pyruviate dehydrogenase. In addition, the increased concentration of CoASAc would also stimulate the conversion of pyruvate to oxaloacetate as CoASAc is a positive effector for this enzyme[46,47]. The oxaloacetate thus produced can serve to replenish intermediates of the citric acid cycle used for biosynthetic purposes or be converted to phosphoenolpyruvate and, ultimately, to glucose. The observed metabolic effects on citrate synthase lend support to the proposal made by KREBS[48] in 1960, that the condensation of oxaloacetate and CoASAc is the rate-limiting step in the citric acid cycle.

It has not been established that the ATP inhibition of citrate synthase observed in extracts also occurs in the intact cell. The work of SHEPHERD, YATES AND GARLAND[33] indicates that the ATP inhibition of citrate synthase does occur within intact mitochondria, and plays a role in regulating ketone body synthesis. WILLIAMSON *et al.*[49] have recently come to the opposite conclusion. Thus, the physiological role of these effects remains unresolved.

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