Regulatory Properties of Citrate Synthase from *Rickettsia prowazekii*

PAUL V. PHIBBS, JR.† AND HERBERT H. WINKLER*

*Department of Microbiology and Immunology, College of Medicine, University of South Alabama, Mobile, Alabama 36688*

Received 27 July 1981/Accepted 14 September 1981

Citrate synthase [citrate (sii)-synthase] (EC 4.1.3.7) was partially purified from extracts of highly purified typhus rickettsiae (*Rickettsia prowazekii*). Molecular exclusion and affinity column chromatography were used to prepare 200-fold-purified citrate synthase that contained no detectable malate dehydrogenase (EC 1.1.1.37) activity. Rickettsial malate dehydrogenase also was partially purified (200-fold) via this purification procedure. Catalytically active citrate synthase exhibited a relative molecular weight of approximately 62,000 after elution from a calibrated Sephacryl S-200 column. Acetyl coenzyme A saturation of partially purified enzyme was sensitive to strong competitive inhibition with adenylates (ATP >> ADP >> AMP). [β,γ-methylene]ATP, dATP, and dADP also caused strong inhibition, but guanosine and cytosine nucleotides were significantly less inhibitory. Adenylates had no effect on oxalacetate saturation kinetics when acetyl coenzyme A was present in high concentration (≥ 50 μM). Neither NADH nor α-ketoglutarate affected the saturation kinetics of rickettsial citrate synthase. Thus, citrate synthase from *R. prowazekii* exhibits greater similarity to the eucaryotic and gram-positive procaryotic enzymes than to citrate synthase from free-living gram-negative bacteria. These results represent the first characterization of a highly purified key regulatory enzyme from these obligate intracellular parasitic bacteria.

*Rickettsia* spp. are obligate intracellular bacteria that multiply inside the cytoplasm of a variety of eucaryotic host cells (6, 19, 20, 22). These organisms are morphologically typical, small gram-negative bacteria (1, 5, 14, 16) with a demonstrable outer cell wall membrane, periplasmic space, and inner cytoplasmic membrane. Outer membrane preparations obtained from purified cells of *Rickettsia prowazekii* are rich in 2-keto-3-deoxyoctulosonic acid, a compound that apparently is unique to gram-negative bacterial lipopolysaccharide (16).

Purified typhus and spotted fever rickettsiae have been shown to oxidize exogenously provided glutamate to CO₂ (4, 17, 21, 26, 27), and there is convincing enzyme evidence for the presence of an oxidative tricarboxylic acid cycle (7; and P. V. Phibbs, Jr., and H. H. Winkler. In W. Burgdorfer and R. L. Anacker, ed., *Rickettsia and rickettsial diseases*, in press). These bacteria generate ATP as a consequence of glutamate oxidation (2, 3, 24), but the mechanisms for energy coupling have not been described. *R. prowazekii* also contains an ADP-ATP transport system by which these intracellular parasites may accumulate ATP directly from their environment via obligatory exchange with intrarickettsial ADP (25). Thus, typhus rickettsiae possess dual mechanisms for obtaining and generating endogenous ATP.

Bovarnich (2) reported some evidence that respiration of glutamate was significantly inhibited by exogenously provided ADP or ATP. Williams and Weiss (24) more recently provided suggestive evidence that exogenous adenylates may modulate endogenous ATP synthesis supported by glutamate respiration in *Rickettsia typhi*. There is a dearth of additional information on the regulation of metabolism in *Rickettsia* spp. However, the possible significance of metabolic regulatory properties to the obligatory intracellular parasitic nature of *Rickettsia* spp. and the urgent need for more information on their mechanisms of metabolic control have been emphasized by Weiss (21).

Citrate synthase [citrate (sii)-synthase] (EC 4.1.3.7), the first enzyme unique to the oxidative tricarboxylic acid cycle, has been studied extensively in a diversity of representative procaryotic and eucaryotic cells (23). In this report, we describe the partial purification of this key regulatory enzyme from cell extracts of *R. prowaze-
kii and present evidence that its regulatory properties contrast with those of citrate synthase from most free-living gram-negative bacteria. The possible relationship of citrate synthase catalytic regulatory properties to control of the dual mechanisms for ATP generation and accumulation in these obligate intracellular bacteria is discussed.

MATERIALS AND METHODS

Rickettsial growth and purification. The Madrid E strain of R. prowazekii was propagated in antibiotic-free, embryonated hen eggs, harvested from the yolk sacs, and purified as described previously (25). The resultant rickettsial suspension was passed slowly through a 47-mm, type AP20 microfilter glass filter (Millipore Corp., Bedford, Mass.) as described by others (22). Filtered suspensions retained >70% of hemolytic activity, approximately 70% of turbidity at 545 nm, and 85 to 90% of total protein, as compared with unfiltered suspensions. However, contaminating cytochrome c oxidase activity from homogenized yolk sacs was reduced at least 13-fold to undetectable or insignificant trace levels (0 to <1.5 nmol min^{-1} mg of suspension protein) by AP20 filtration.

Filtered rickettsial suspensions were purified further by Renografin density gradient centrifugation by a variation (16) of the method of Weiss et al. (22). Rickettsiae collected from gradient tubes were sedimented at 20,000 × g for 15 min and washed once in ice-cold 0.25 M sucrose in 40 mM potassium phosphate (pH 7.2). Washed cell sediments were used immediately or stored at −70°C; storage under these conditions had no obvious effects on citrate synthase and malate dehydrogenase (MDH) (EC 1.1.1.37) activities in cell extracts.

Extract preparation and enzyme purification. Sediments of purified rickettsiae (fresh or frozen) were suspended in ice-cold sucrose-free buffer (8 to 10 ml per 0.5 to 1.0 g of packed cells) with a Teflon tissue homogenizer. Crude extracts were prepared at 0 to 4°C by passing the rickettsial suspension through a precooled miniature French pressure cell twice at 20,000 lb/in², centrifuging at 10,000 × g for 10 min, and collecting the supernatant fluid. The soluble cytoplasmic fraction was prepared by centrifuging the crude extract for 2 h at 105,000 × g and collecting the supernatant fluid. This fraction was concentrated three- to fivefold in an ice water bath-chilled 10-ml Amicon ultrafiltration cell equipped with a PM10 membrane filter and was pressurized with nitrogen gas. The concentrated cytoplasmic fraction was used immediately or stored overnight at −70°C, with little loss of enzyme activity. In a typical experiment, approximately 1 g (wet weight) of purified rickettsiae in 11.2 ml of suspension yielded 9.5 ml of crude extract (76 mg of protein) that yielded 2.3 ml of concentrated soluble cytoplasmic fraction containing 37 mg of protein. Approximately 150 yolk sacs were required to obtain this quantity of rickettsial extract.

The concentrated soluble cytoplasmic fraction was applied to a column (1.5 by 88 cm) containing Sephacryl S-200 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) (pH 7.2) and eluted at a constant flow rate maintained by a peristaltic pump. Fractions were collected at timed intervals and assayed immediately for absorbance at 280 nm (protein) and for citrate synthase and MDH activities. Fractions containing citrate synthase and MDH activities were pooled, concentrated to a final volume of 2 to 3 ml by Amicon PM10 ultrafiltration, and stored at −70°C or used immediately.

Citrate synthase was further purified at 0 to 4°C by column chromatography with immobilized Cibacron Blue F3G A (Pierce Chemical Co., Rockford, III.) by a variation of the procedure described by Kay and Down (11). The Cibacron Blue suspension was packed, in accordance with the directions of the manufacturer, in an Econocolumn 0.7 by 15 cm; Bio-Rad Laboratories, Richmond, Calif.) to a 5 ml bed volume and rinsed with at least 30 ml of 2.5 mM PEM buffer (2.5 mM sodium/potassium phosphate, 0.5 mM EDTA, 0.5 mM MgCl₂ final pH 7.4) (see reference 11). After Sephacryl S-200 chromatography, concentrated enzyme preparations (1 to 3 ml) were dialyzed twice against 100-volume changes of 2.5 mM PEM buffer and applied to the Cibacron Blue column. Failure to dialyze samples against the dilute PEM buffer resulted in poor binding of enzymes to Cibacron Blue. Proteins were eluted from the column at a constant flow rate, with either step or linear gradients of KCl in 2.5 mM PEM buffer, as specified below. Fractions were collected at timed intervals and assayed immediately for protein content (absorbance at 280 nm) and for citrate synthase and MDH activities. Appropriate fractions were pooled and concentrated to about 1 ml by Amicon PM10 ultrafiltration. Samples were then diluted to 10 ml in the filter chamber with 40 mM potassium phosphate (pH 7.2) and reconstituted three successive times to dilute out components of the gradient elution buffers. The final concentrated preparations of citrate synthase and MDH were stable for at least 2 months when stored at −70°C. Failure to remove the high concentration of KCl in concentrated samples upon elution from Cibacron Blue columns caused rapid loss of enzyme activities during storage at −70°C.

Molecular weight estimation. The molecular weight of catalytically active citrate synthase was estimated from the relative elution of partially purified enzyme from a calibrated column containing Sephacryl S-200. The column (1.5 by 87 cm) was calibrated with human immunoglobulin G (molecular weight, 153,000), bovine serum albumin (molecular weight, 67,000), and chymotrypsinogen A (molecular weight 25,000) standards. Reproducible molecular weight standard curves were obtained when the column was packed at a flow rate higher than that used for elution of the enzyme, in accordance with the directions of Pharmacia Fine Chemicals, Inc.

Enzyme assay and other assays. Citrate synthase was assayed spectrophotometrically at 412 nm essentially as described by Sreere (18). The standard reaction mixture (pH 8.1; final volume, 1.0 ml) contained the following: 0.1 mM 5,5′-dithiobis-(2-nitrobenzoate), 100 mM Tris-hydrochloride, 0.05 mM acetyl coenzyme A (acyetyl-CoA), 0.5 mM oxalacetate (OAA), and cell extract. Reactions were initiated by the final addition of OAA, and initial velocities were adjusted for any OAA-independent activity as described by Sreere (18). Citrate synthase activity was always totally dependent on the presence of acetyl-CoA and extract protein. MDH activity was determined spectrophoto-
metrically at 340 nm as described by Reeves et al. (15). Its activity was totally dependent on the presence of OAA, NADH, and extract protein. Cytochrome c oxidase activity in rickettsial suspensions was determined by the procedure of Minnaert (13). Enzyme specific activities were calculated from initial reaction velocities and expressed as nanomoles of substrate converted per minute per milligram of extract protein (mIU/mg).

Protein concentration in rickettsial suspensions and extract preparations was determined by the method of Lowry et al. (12) with crystalline bovine serum albumin as the standard. The direct spectrophotometric method of Kalb and Bernlohr (10) was employed to estimate protein concentration in the final enzyme preparations after Sephacryl S-200 and Cibacron Blue column chromatography. Hemolytic activity of rickettsial suspensions was assayed spectrophotometrically at 545 nm by a variation (25) of the method of Snyder et al. (17). Turbidity of rickettsial suspensions also was determined at 545 nm with an appropriate diluent.

RESULTS

Partial purification of citrate synthase. Exclusion chromatography of a 2.0-ml preparation of concentrated soluble cytoplasmic fraction derived from approximately 0.6 g (wet weight) of purified R. prowazekii is shown in Fig. 1. MDH and citrate synthase activities eluted unresolved from each other in markedly asymmetrical peaks. However, these activities were separated from approximately 90% of total soluble fraction protein, and significant purification was achieved. Fractions 30 to 42, containing most of the citrate synthase and MDH activities, were pooled, concentrated to 2.1 ml by Amicon PM10 ultrafiltration, and stored overnight at −70°C.

For studying the saturation kinetics and regulatory properties of citrate synthase, it is essential that the enzyme preparation be free of MDH because the latter enzyme will rapidly reduce one of the substrates (OAA) to malate when NADH is present in the reaction mixture. R. prowazekii citrate synthase and MDH were separated and further purified by Cibacron Blue column chromatography (Fig. 2). The concentrated preparation obtained after Sephacryl S-200 chromatography (Fig. 1) was dialyzed against 2.5 mM PEM buffer, and 2.0 ml was applied to the Cibacron Blue column. Most of the applied protein did not bind to the column and was eluted in the first eight fractions (12 ml) with 2.5-mL PEM equilibration buffer. Both citrate synthase and MDH bound tightly to Cibacron Blue but were eluted and completely resolved with KCl gradients as shown in Fig. 2. Fractions 41 to 46 and 55 to 62, containing citrate synthase and MDH, respectively, were pooled separately, desalted, and concentrated to small volumes by ultrafiltration.

A summary of results obtained in this purification run is shown in Table 1. The final citrate synthase preparation (200-fold purification) contained only 19 μg of total protein and was completely devoid of MDH activity. The specific activities varied in several batches of citrate synthase prepared at different times (from ca. 500 to 2,000 mU per mg of protein), but all of the batches were devoid of detectable MDH activity. Although not shown, these samples contained four to six distinct polypeptides when analyzed by sodium dodecyl sulfate-polyacrylamide disc gel electrophoresis by the method of Fairbanks et al. (9).

The relative molecular weight of partially purified, catalytically active citrate synthase was estimated by chromatography on a calibrated

![FIG. 1. Sephacryl S-200 column chromatography of the concentrated soluble fraction of R. prowazekii crude extract. The 2.0-ml sample applied to the column (1.5 by 88 cm) contained 10.3 mg of protein, 283 mU of citrate synthase activity, and 2,754 mU of MDH activity. Proteins were eluted with 40 mM potassium phosphate (pH 7.2) at a constant flow rate (27.8 ml/h). Fractions were monitored for absorbance at 280 nm (A280) (C) and assayed immediately for citrate synthase (CIT. SYN.) and MDH activities.](http://jb.asm.org/)
CITRATE SYNTHASE FROM RICKETTSIA

ILLUSTRATION

FIG. 2. Cibacron Blue affinity column chromatography of citrate synthase and MDH activities from R. prowazekii. A concentrated 2.0-ml sample containing 860 µg of protein, 54 mIU of citrate synthase, and 1,418 mIU of MDH was dialyzed against 2.5 mM PEM buffer (pH 7.4) and applied to the column. The column was eluted with 2.5 mM PEM containing various KCl concentrations. Fractions were collected at a constant flow rate of 14.8 ml/h and monitored for A₂₈₀ (○) and for citrate synthase (Cit. Syn.) and MDH activities.

A single symmetrical peak with a relative molecular weight of approximately 62,000. The results were identical in three separate experiments with different citrate synthase preparations.

Adenylate inhibition of citrate synthase. Citrate synthase activity in preparations at various stages of purification with a saturating OAA concentration exhibited hyperbolic acetyl-CoA saturation kinetics that conformed with the Michaelis-Menten equation. Apparent Kₘ values ranged from 2 to 4 µM acetyl-CoA in the most highly purified preparation to about 10 µM in preparations at earlier stages of purification.

TABLE 1. Citrate synthase and MDH purification from extracts of purified R. prowazekii

<table>
<thead>
<tr>
<th>Prepn</th>
<th>Sample vol (ml)</th>
<th>Total protein (mg)</th>
<th>Citrate synthase</th>
<th>MDH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract*</td>
<td>7.6</td>
<td>35.3</td>
<td>330 9 1 100 3,100</td>
<td>87 1 100</td>
</tr>
<tr>
<td>Concentrated soluble fraction*</td>
<td>2.0</td>
<td>10.3</td>
<td>280 27 3 84 2,800</td>
<td>190 2 89</td>
</tr>
<tr>
<td>Concentrated pool from Sephacyr*</td>
<td>2.0</td>
<td>0.86</td>
<td>91 100 11 27 2,900</td>
<td>2,200 37 96</td>
</tr>
<tr>
<td>Concentrated citrate synthase from Cibacron*</td>
<td>1.5</td>
<td>0.02</td>
<td>34 1,800 200 10d &lt;1*</td>
<td></td>
</tr>
<tr>
<td>Concentrated MDH from Cibacron*</td>
<td>1.6</td>
<td>0.02</td>
<td>&lt;1e</td>
<td>400 18,000 200 13d</td>
</tr>
</tbody>
</table>

* See text.
† See Fig. 1 and text.
‡ See Fig. 2 and text.
§ This loss in activity occurred primarily during freezing and thawing of the concentrated pool from Sephacyr S-200 chromatography.
¶ Below the limit of detection.
FIG. 3. Effects of adenylates on acetyl-CoA saturation kinetics of partially purified *R. prowazekii* citrate synthase. Standard reaction conditions were used with 500 μM OAA, 0.64 to 1.1 μg of enzyme protein per reaction mixture, and various concentrations of acetyl-CoA. Acetyl-CoA saturation kinetics were determined in the absence of adenylates and in the presence of AMP, ADP, and ATP. Lineweaver-Burk reciprocal plots of the data are shown.

Strong competitive inhibition by adenylates also was observed in less highly purified enzyme preparations and was shown to be saturable with respect to adenylate concentration (Fig. 4). Under these conditions, 1 mM ATP caused >50% inhibition of citrate synthase activity.

Nucleotide effects on citrate synthase activity were relatively specific for the adenylates (Table 2). GTP and GDP were 20 to 25% less inhibitory than ATP and ADP, respectively, whereas CDP and the various forms of NAD caused little or no significant inhibition. Adenylate potency was unaffected by modification of the ribosyl moiety (dATP and dADP) but was markedly reduced by modification of the adenine ring ([1-N^6-etheno]ADP). The length of the adenylate 5'-phosphate chain also was a major determinant in inhibitor specificity because the order of potency was ATP > ADP > AMP (see Fig. 4). The strong inhibition caused by [β,γ-methylene]-ATP is consistent with this interpretation and shows that adenylate inhibition does not involve an enzyme phosphorylation mechanism.

**Effects of other metabolites on activity.** Both NADH and α-ketoglutarate are known to be strong inhibitors of citrate synthase from diverse gram-negative bacteria (23). NADH (250 μM) had no effect on the OAA saturation kinetics of partially purified citrate synthase from *R.*

<table>
<thead>
<tr>
<th>EFFECTOR (5mM)</th>
<th>APPARENT Km (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NONE</td>
<td>4</td>
</tr>
<tr>
<td>AMP</td>
<td>10</td>
</tr>
<tr>
<td>ADP</td>
<td>36</td>
</tr>
<tr>
<td>ATP</td>
<td>50</td>
</tr>
</tbody>
</table>

**FIG. 4.** Effect of ATP concentration on the inhibition of citrate synthase activity. Standard reaction conditions were used with 5μM acetyl-CoA, 500 μM OAA, 7.2 μg of extract protein per ml, and various concentrations of ATP. The enzyme source was a concentrated, 30-fold-purified preparation obtained from Sephacryl S-200 column chromatography.
prowazekii (Fig. 5). Essentially identical results were obtained when acetyl-CoA was the variable substrate (see Table 2) and when NADH was further tested at concentrations of 1 and 2 mM. α-Ketoglutarate (2 mM) also caused no inhibitory effects on the OAA saturation kinetics of citrate synthase (Fig. 5), and nearly identical results were obtained when acetyl-CoA was the variable substrate. ATP (1 mM) had no discernible inhibitory effect on citrate synthase activity when OAA was the variable substrate and acetyl-CoA concentration was held constant at 50 μM (Fig. 5). In the preceding experiment with the same enzyme preparation (Fig. 4), 1 mM ATP caused potent inhibition when acetyl-CoA was present at a subsaturating concentration (5 μM). Thus, the activity of citrate synthase from R. prowazekii was unaffected by either NADH or α-ketoglutarate under all tested assay conditions, and the inhibition caused by adenylates was competitive with respect to acetyl-CoA.

**DISCUSSION**

Studies of rickettsial metabolism have been limited primarily to the determination of physio-

| Table 2. Effects of nucleotides on the activity of partially purified citrate synthase from R. prowazekii 
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effector</strong></td>
</tr>
<tr>
<td>None (control)</td>
</tr>
<tr>
<td>ATP</td>
</tr>
<tr>
<td>ATP</td>
</tr>
<tr>
<td>dATP</td>
</tr>
<tr>
<td>[βγ-methylene]ATP</td>
</tr>
<tr>
<td>GTP</td>
</tr>
<tr>
<td>ADP</td>
</tr>
<tr>
<td>ADP</td>
</tr>
<tr>
<td>dADP</td>
</tr>
<tr>
<td>[I-Nε-etheno]ADP</td>
</tr>
<tr>
<td>GDP</td>
</tr>
<tr>
<td>CDP</td>
</tr>
<tr>
<td>AMP</td>
</tr>
<tr>
<td>AMP</td>
</tr>
<tr>
<td>NAD</td>
</tr>
<tr>
<td>NADH</td>
</tr>
<tr>
<td>NADP</td>
</tr>
<tr>
<td>NADPH</td>
</tr>
</tbody>
</table>

A The reaction mixture contained 4.0 μg of extract protein, 2.0 μmol of OAA, 20 nmol of acetyl-CoA, and 100 nmol of 5,5'-dithiobis-(2-nitrobenzoate) in a 1.0-ml final volume. The MDH-free enzyme preparation was obtained from the Cibacron Blue column via step gradient elution with 0.35 M KCl instead of via linear gradient elution with 0 to 0.35 M KCl.

FIG. 5. Effects of putative regulatory metabolites on OAA saturation kinetics of rickettsial citrate synthase. (A) Standard reaction conditions were used with 100 μM acetyl-CoA, various OAA concentrations, and 1.1 μg of enzyme preparation per reaction mixture. The partially purified enzyme was totally free of MDH activity. OAA saturation kinetics were determined in the absence of putative effectors (●) and in the presence of 250 μM NADH (○) or 2 mM α-ketoglutarate (△). (B) Standard reaction conditions were used with 50 μM acetyl-CoA, various OAA concentrations, and 7.2 μg of extract protein per reaction mixture. This less-purified citrate synthase preparation is described in the legend to Fig. 4. OAA saturation kinetics were determined in the absence (▲) and presence (△) of 1 mM ATP.

The reaction mixture contained 4.0 μg of extract protein, 2.0 μmol of OAA, 20 nmol of acetyl-CoA, and 100 nmol of 5,5'-dithiobis-(2-nitrobenzoate) in a 1.0-ml final volume. The MDH-free enzyme preparation was obtained from the Cibacron Blue column via step gradient elution with 0.35 M KCl instead of via linear gradient elution with 0 to 0.35 M KCl.

The present investigations of rickettsial metabolism and its regulation was enhanced markedly by the recent development of methods for obtaining relatively large preparations of these bacteria free of contaminating host cell material (16, 22). The present results demonstrate that conventional methods for protein purification can be employed to obtain highly purified preparations of citrate synthase from Renografin-purified R. prowazekii that are
suitable for use in studies of the catalytic and regulatory properties of this key enzyme of intermediary metabolism.

Citrate synthase has been shown to occur in most (but not all) living cells (23), including the typhus rickettsiae (7; P. V. Phibbs, Jr., and H. H. Winkler, in press). Two distinct molecular forms of the enzyme have been recognized: an NADH-insensitive "small" type (molecular weight, ~100,000) in gram-positive bacteria and eucaryotic cells and an NADH-sensitive "large" type (molecular weight, ~230,000) in most gram-negative bacteria (23). Partially purified citrate synthase from R. prowazekii had a relative molecular weight of about 62,000, slightly larger than the 50,000- to 60,000-subunit molecular weight of the dimeric small type of enzyme. It is conceivable that larger, multimeric forms of active enzyme might have been observed had it been feasible to obtain much larger concentrated preparations. However, the addition of 3 mg of bovine serum albumin per ml of final enzyme preparation did not cause an increase in the apparent molecular weight of rickettsial citrate synthase.

Hyperbolic saturation curves for both substrates were observed with rickettsial citrate synthase. These results are highly consistent with the kinetic characteristics of the small type of enzyme from other sources. In contrast, the large type of citrate synthase from diverse free-living gram-negative bacteria has a sigmoidal saturation curve and markedly higher \( K_m \) values for both substrates (23).

Rickettsial citrate synthase was shown to be sensitive to competitive inhibition by adenylates with respect to acetyl-CoA but not to OAA, as is typical of the small type of enzyme. Inhibitor specificity was dependent upon the nucleotide 5'-phosphate chain length (ATP > ADP >AMP), and chemical modification of the purine base or substitution with a pyrimidine base caused marked decreases in inhibitor potency. The inhibitory effects of ATP (at a subsaturating concentration of acetyl-CoA) increased most steeply at ATP concentrations of 1.0 to 1.5 mM (Fig. 4). The significance of this observation is consistent physiologically with reports of endogenous ATP pools of 1.5 to 2.9 mM in suspensions of actively metabolizing purified typhus rickettsiae (24, 25).

Rickettsial citrate synthase was insensitive to modulation by either NADH or \( \alpha \)-ketoglutarate, which is also consistent with the known properties of the small type of citrate synthase. This is in contrast to the large type of enzyme that is exquisitely sensitive to allosteric inhibition by both NADH (\( K_i \approx 20 \mu M \)) and \( \alpha \)-ketoglutarate (\( K_i < 100 \mu M \)) but is insensitive to inhibition by adenylates (23).

The present results demonstrate that the molecular size, substrate saturation kinetics, and sensitivity to metabolite effectors of citrate synthase from this obligate intracellular, morphologically typical gram-negative bacterium are remarkably similar to those of the enzymes from gram-positive bacteria and eucaryotic cells. These characteristics are in complete contrast to those of the large type of enzyme from Escherichia coli, a typical free-living gram-negative bacterium. The apparent relationship between the molecular form of citrate synthase and its kinetic and regulatory characteristics was demonstrated clearly in a recent report by Danson et al. (8). A mutant isolate of E. coli was shown to contain genetically altered citrate synthase that had converted from the wild-type large form to the small form, with respect to quaternary structure, substrate saturation kinetics, and catalytic and regulatory properties (8). Therefore, the observed divergence in the forms of citrate synthase between free-living and obligate intracellular gram-negative bacteria clearly is plausible.

Typhus rickettsiae can accumulate ATP directly from their external environment (i.e., host cell cytoplasm) via an obligatory ATP-ADP exchange transport system (25) and can generate endogenous ATP as a consequence of respiratory catabolism of glutamate, presumably via enzymes of the tricarboxylic acid cycle (21, 24). The adenylate-dependent regulation of citrate synthase activity demonstrated in this study may serve a critical role in modulating the relative contributions of the dual mechanisms in R. prowazekii for maintaining an adequate endogenous ATP pool under different environmental conditions. This rickettsiae grow and multiply only within the cytoplasm of living eucaryotic cells. Under these conditions, ATP accumulated directly from the host cell cytoplasm may cause inhibition of rickettsial citrate synthase activity, with a concomitant reduction in oxidative tricarboxylic acid cycle function. Rickettsiae also must survive transient periods outside of living host cells, between rounds of cellular parasitism, and they require metabolic energy for the successful penetration of new host cells (6, 20). Under these conditions, when abundant host cell-supplied (exogenous) ATP may not be available, adenylate inhibition of citrate synthase should decrease, thus allowing increased rates of oxidative metabolism for the generation of reducing power and ATP, which are essential for the maintenance of cell viability.

ACKNOWLEDGMENTS

P. V. P. is extremely grateful for the support and opportunity to conduct this investigation in the Department of Microbiology and Immunology, College of Medicine, University of South...
Alabama, while on educational leave from Virginia Commonwealth University. This research was supported by Public Health Service grant AI 15035 from the National Institutes of Health.

LITERATURE CITED


