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Carbamate Kinase from *Pseudomonas aeruginosa*: Purification, Characterization, Physiological Role, and Regulation

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Pseudomonas aeruginosa PAO1 possessed a carbamate kinase (CKase) distinct from carbamoylphosphate synthetase as well as from a constitutive acetate kinase which also catalyzes the phosphorylation of ADP by carbamoylphosphate. CKase was purified to homogeneity. Polyacrylamide gel electrophoresis of cross-linked CKase in the presence of sodium dodecyl sulfate showed that the enzyme consists of two subunits with identical molecular weights (37,000). The optimal pH of enzyme activity is 7.0. The double-reciprocal plot for carbamoylphosphate was linear at 2 mM ADP, yielding an apparent K_m of 5 mM. However, at 0.25 mM ADP, the plot was concave upward, and a Hill plot of the data yielded a coefficient of 1.4. This apparent cooperativity at low ADP concentrations might serve to reduce the extent of catabolism of carbamoylphosphate under growth conditions yielding high energy charge. Experiments on the regulation of synthesis under various growth conditions showed a response to three regulatory signals: CKase was induced to high levels by anaerobiosis, induced to moderate levels by arginine, and repressed by ammonia. Thus, CKase expression is regulated in a manner that allows the enzyme to function as a provider of ammonia under aerobic conditions and of ATP under anaerobic conditions. ATP was an effective inhibitor of CKase activity; this inhibition provides the cell with an effective mechanism for avoiding a futile cycle resulting from the simultaneous operation of CKase and carbamoylphosphate synthetase when cells are grown in the presence of exogenous arginine.

Pseudomonas aeruginosa and other fluorescent pseudomonads possess the arginine deiminase pathway (frequently referred to as the arginine dihydrolase pathway) for catabolism of arginine (1). Three enzymes, arginine deiminase (EC 3.5.3.6), catabolic ornithine carbamoyltransferase (cOTCase) (EC 2.1.3.3), and carbamate kinase (CKase) (ATP:carbamate phosphotransferase; EC 2.7.2.2), catalyze the three sequential reactions of the pathway:

$$\text{arginine} + H_2O \rightarrow \text{citrulline} + NH_3$$

$$\text{citrulline} + P_i \rightarrow \text{ornithine} + \text{carbamoylphosphate}$$

$$\text{carbamoylphosphate} + ADP \rightarrow ATP + CO_2 + NH_3$$

Ornithine is metabolized to glutamate via the reactions catalyzed by acetylornithine aminotransferase and pyrroline carboxylate reductase (Fig. 1).

The deiminase pathway has long been considered to be important under anaerobic conditions as a means of ATP generation by aerobic pseudomonads (27), and recent evidence (24, 31) suggests that it might be an important route for arginine utilization, also under aerobic conditions.

Arginine deiminase (26) and cOTCase (12, 29) from *Pseudomonas putida* have been characterized. However, CKase has only been assayed in crude extracts of pseudomonads, and adequate criteria were not employed to assure that this reaction was not attributable to other enzymes which catalyze the phosphorylation of ADP by carbamoylphosphate (1). Carbamoylphosphate synthetase (CPSase) (4, 13) and acetate kinase (7, 30) catalyze this reaction in enteric bacteria.

We have reported elsewhere (submitted for publication) on the purification and properties of CPSase from *P. aeruginosa*. The present paper establishes the presence in *P. aeruginosa* of a CKase that is distinct from both CPSase and a constitutive acetate kinase. CKase was purified to homogeneity and characterized with respect to kinetic and possible regulatory properties. The problem of regulating the flow of carbamoylphosphate in the opposing pathways of

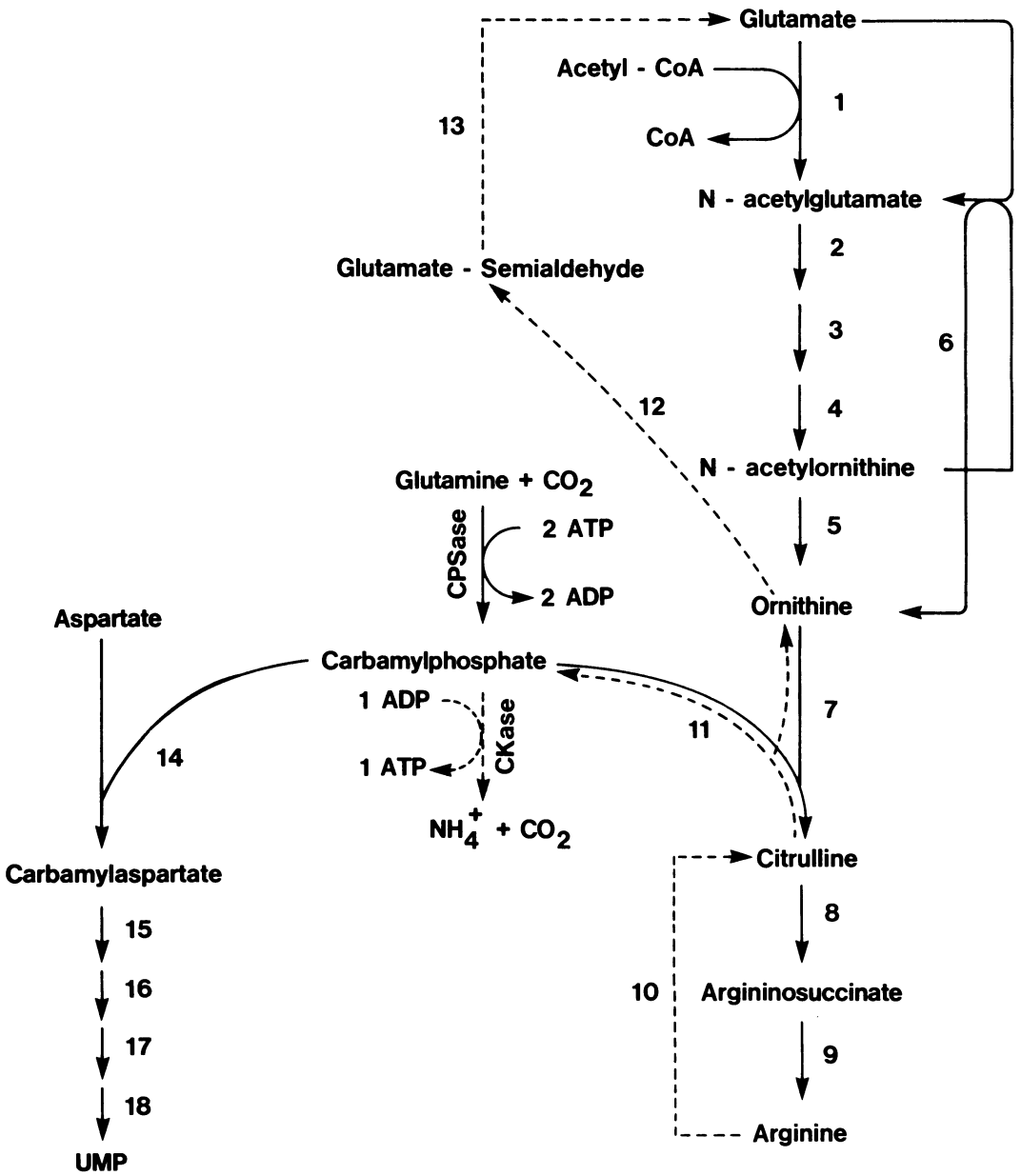

FIG. 1. Carbamoylphosphate metabolism in *P. aeruginosa*. Solid arrows, anabolic reactions; broken arrows, catabolic reactions. Enzymes: 1, acetylglutamate synthetase; 2, acetylglutamate phosphotransferase; 3, acetylglutamate semialdehyde dehydrogenase; 4 and 12, acetylornithine aminotransferase; 5, acetylornithinase; 6, ornithine acetyltransferase; 7, anabolic ornithine carbamoyltransferase; 8, argininosuccinate synthetase; 9, argininosuccinate lyase; 10, arginine deiminase; 11, cOTCase; 13, pyrroline carboxylate oxidase; 14–18, steps in pyrimidine biosynthesis.

arginine biosynthesis and catabolism is examined.

MATERIALS AND METHODS

Reagents. Amino acids, nucleotides, acetylphosphate, carbamoylphosphate, NAD^+, NADH, $NADP^+$, and NADPH were all obtained from Sigma Chemical Co. [^{3}H]ADP was purchased from New England Nuclear Corp. Ethylagarose and hexylagarose were obtained from Miles Laboratories.

Cultures and preparation of cell extracts. All strains were derivatives of *P. aeruginosa* PAO1 and were obtained from B. Holloway. Cells were grown in the basal salts medium described by Stalon et al. (28) supplemented with trace elements (28 mg of H_3BO_3,

7.5 mg of $Na_2MoO_4 \cdot 2H_2O$, 2.4 mg of $ZnSO_4$, and 2.5 mg of $CaSO_4 \cdot 5H_2O$ per liter of medium). Carbon and nitrogen sources were sterilized separately and used at a concentration of 20 mM. Cells were harvested, washed once with water, suspended at 0.4 g (wet weight) per ml in 0.1 M potassium phosphate (pH 7.6) containing 1 mM EDTA and 1 mM phenylmethylsulfonylfluoride. Cells were ruptured by passage through an Aminco French pressure cell or by sonication. The crude cell extract was centrifuged at 27,000 × *g* for 30 min at 4°C. Protein was determined by the method of Lowry et al. (15) with crystallized bovine serum albumin as the standard.

Enzyme assays. CKase frequently has been assayed by a spectrophotometric assay in which the production of ATP is coupled to the reduction of $NADP^+$ in the presence of glucose and hexokinase. However, the use of this method for assay of CKase in crude extracts or *P. aeruginosa* was not satisfactory because of the high rate of $NADP^+$ reduction in the absence of carbamoylphosphate. The inclusion in the reaction mixture of P^1,P^5-di(adenosine 5')pentaphosphate, an inhibitor of adenylate kinase (14), did not significantly reduce the control values. Accordingly, CKase was assayed by a procedure based on the conversion of [^{3}H]ADP to ATP. For assay of CKase activity in crude extracts or during purification, the reaction mixture (final volume, 0.15 ml) contained enzyme, 100 mM sodium citrate (pH 5.4), 5 mM ADP (3×10^5 to 5×10^5 cpm/μmol), 15 mM carbamoylphosphate, and 20 mM $MgCl_2$. Under these conditions of buffer and pH, the contribution of acetate kinase was minimal. The reaction was started by the addition of carbamoylphosphate, and at appropriate times samples were spotted onto polyethyleneimine-Avicel cellulose thin-layer plates and dried with hot air to stop the reaction. For kinetic experiments, CKase was assayed in 0.1 M HEPES buffer (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.0), and the reaction was stopped by the addition of 25 μl of 2 M HCl, chilled in ice for 10 min, and neutralized with 25 μl of 2 M Tris before being spotted onto polyethyleneimine-Avicel cellulose plates. The plates were developed in 0.5 M potassium phosphate (pH 3.4) to 15 cm above the origin. The dried plates were exposed overnight to X-ray films. The spots corresponding to ADP and ATP were cut out, extracted with 0.25 ml of 2 M NH_4OH for 20 min, and counted in a toluene-based scintillation mixture containing 10% BioSolv (Beckman Instruments, Inc.). For each assay a parallel reaction was run in the absence of carbamoylphosphate, and the amount of conversion obtained under these conditions was subtracted to obtain the reported values for CKase.

Acetate kinase was assayed by the procedure described for CKase except that acetylphosphate was substituted for carbamoylphosphate. CPSase was assayed as previously described (2). cOTCase was determined as described by Stalon et al. (29).

Purification of CKase. CKase was purified from *P. aeruginosa* PAO1 grown in the basal salt medium containing arginine as the sole source of carbon and nitrogen. Cells were grown aerobically in a 14-liter New Brunswick microfermentor. Cells (200 g) were harvested, washed once with water, and suspended at 4.0 g (wet weight) per ml in 0.1 M potassium phosphate (pH 7.6). This buffer, as well as all solutions coming in contact with the enzyme in subsequent steps, contained 1 mM EDTA, 1 mM phenylmethylsulfonylfluoride, 1 mM dithiothreitol, and 4 mM $MgCl_2$. The presence of dithiothreitol and $MgCl_2$ increased the stability of the enzyme.

Streptomycin sulfate (1 ml of a 10% solution per 10 ml of centrifuged extract) was added at 0°C with stirring and equilibrated for 10 min. After centrifugation at 18,000 × *g* for 30 min, the supernatant was adjusted to a final concentration of 0.02 M potassium phosphate (pH 7.6). The enzyme solution was pumped at a rate of 120 ml/h onto a column (70 by 2.6 cm) of DEAE-cellulose (Whatman DE-52) equilibrated with 0.02 M potassium phosphate (pH 7.6). After the column was washed with 240 ml of the equilibrating buffer, protein was eluted with a linear gradient of potassium phosphate (pH 7.6). CKase was eluted between 0.03 and 0.06 M with the peak of activity at 0.04 M. Fractions containing CKase activity were combined and dialyzed against 0.02 M potassium phosphate (pH 7.6).

The dialyzed solution was pumped at a rate of 30 ml/h onto a column (40 by 1.6 cm) of ethylagarose. This step separated CKase, which does not absorb to ethylagarose, from acetate kinase, which absorbs tightly to the column. The unadsorbed protein solution containing CKase was then applied to a column (40 by 1.6 cm) of hexylagarose equilibrated with 0.2 M potassium phosphate (pH 7.6). Development of the column with a linear gradient of potassium phosphate (pH 7.6) eluted CKase between 0.08 and 0.15 M with the peak of activity at 0.12 M. Fractions containing CKase activity were combined and dialyzed against 0.02 M potassium phosphate (pH 7.6).

The dialyzed solution was applied to a column (32 by 1.6 cm) of hydroxylapatite and eluted with a linear gradient of potassium phosphate (pH 7.6). CKase was eluted between 0.08 and 0.14 M. The enzyme was concentrated by application to a short column of DEAE-cellulose (2 by 2.6 cm) and elution with 0.3 M buffer (attempts to concentrate CKase by ultrafiltration or ammonium sulfate precipitation resulted in high losses of activity). The concentrated enzyme solution was finally applied to a Sephadex G-200 column (110 by 1.6 cm) and eluted with 0.1 M potassium phosphate (pH 7.6). The elution volume for CKase was 153 ml.

Partial purification of acetate kinase. Cells were grown and extracts were prepared as described above for CKase. Streptomycin sulfate precipitation and chromatography on DEAE-cellulose were carried out; acetate kinase was eluted from DEAE-cellulose between 0.05 and 0.14 M with the peak of activity at 0.08 M. Fractions containing acetate kinase activity were pooled, dialyzed against 0.02 M potassium phosphate buffer (pH 7.6), and applied to an ethylagarose column (40 by 1.6 cm). Development of the column with a linear gradient of potassium phosphate (pH 7.6) eluted acetate kinase between 0.12 and 0.3 M with the peak of activity at 0.24 M.

Sucrose gradient ultracentrifugation. Sucrose gradient ultracentrifugation was carried out as described by Martin and Ames (17) with an SW40 rotor in a Beckman L8-70 ultracentrifuge. Samples (0.3 ml) of enzyme preparation were layered on 13-ml linear sucrose gradients (5 to 20%, wt/vol) in 0.1 M triethanolamine buffer (pH 8.0) containing 5% glycerol, 1 mM EDTA, 1 mM dithiothreitol, 1 mM phenylmethylsulfonylfluoride, and 4 mM $MgCl_2$. Yeast alcohol dehydrogenase

(molecular weight, 148,000) was used as a standard. Gradients were centrifuged at 39,000 rpm for 24 h at 4°C.

RESULTS

Presence of CKase, distinct from CPSase and acetate kinase, in *P. aeruginosa*. Ultracentrifugation experiments established the presence in extracts of *P. aeruginosa* PAO1 of two ADP kinases that utilize carbamoylphosphate as a phosphoryl donor. Only one of them utilized acetylphosphate as a phosphoryl donor to a significant extent, and when it was partially purified as described above, the maximal velocity with acetylphosphate was eightfold higher than that with carbamoylphosphate at pH 7.0. The affinity of this kinase for acetylphosphate was also higher than that for carbamoylphosphate (K_m values with saturating ADP were 0.6 and 2.5 mM, respectively). Accordingly, this enzyme was considered to be an acetate kinase similar to that characterized from *Escherichia coli* (30).

When extracts of cells grown in citrate minimal medium were applied to sucrose gradients and fractions were assayed for glutamine-dependent CPSase, CKase, and acetate kinase (Fig. 2a), a peak of CKase activity that was distinct from those of CPSase and acetate kinase was revealed. The molecular weights of CKase and acetate kinase were estimated (17) to be 70,000 and 98,000, respectively.

Extracts prepared from cells grown in basal salts medium with arginine as the sole source of carbon, energy, and nitrogen had about eight times as much CKase activity as did extracts of cells grown in citrate minimal medium, but acetate kinase activity was essentially unaffected (Fig. 2b). These results are consistent with CKase playing an important role in arginine catabolism.

Purification of CKase. CKase was purified as described above. Table 1 summarizes the various steps in the purification of CKase with an overall purification of 90-fold. The final enzyme preparation showed a single band after electrophoresis on 7.5% polyacrylamide gels at pH 7 (9).

Properties of purified CKase. (i) Subunit composition. Electrophoresis of homogeneous CKase in the presence of sodium dodecyl sulfate (32) yielded a single band corresponding to a molecular weight of 37,000 (Fig. 3). When homogeneous CKase was cross-linked in 0.05 M triethanolamine (pH 8.5) by dimethyl suberimidate (8) before treatment with sodium dodecyl sulfate and mercaptoethanol, two bands corresponding to molecular weights of 37,000 and 74,000 were observed. The molecular weight of the dimer is in reasonable agreement with that obtained for the native enzyme by sucrose density gradient centrifugation. Thus, the enzyme is made of two subunits with identical molecular weights.

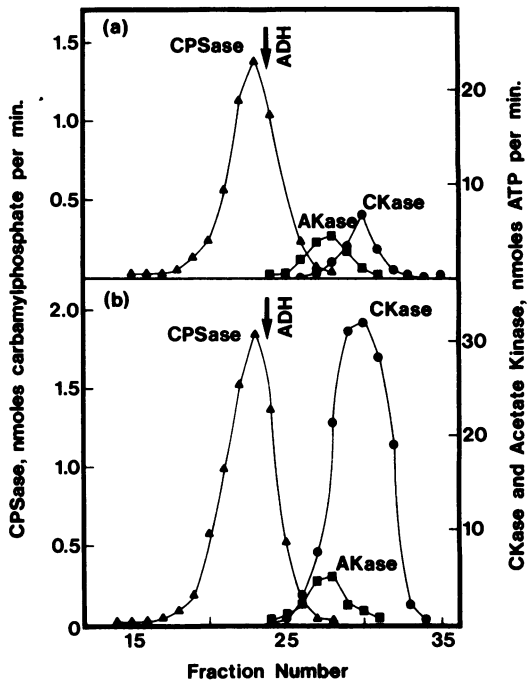

FIG. 2. Sucrose density gradient ultracentrifugation of extracts of *P. aeruginosa* PAO1. Cells were aerobically grown on basal salts medium with citrate and ammonia (a) or with arginine as the sole source of carbon and nitrogen (b). In either case a sample extract containing 5.0 mg of protein was applied. A total of 38 fractions were collected and assayed for CPSase, CKase (CKase was assayed in 0.1 M sodium citrate [pH 5.4], conditions under which acetate kinase activity is minimal), and acetate kinase. Yeast alcohol dehydrogenase (ADH) was used as a marker.

(ii) pH optimum. The optimal pH for activity of CKase in HEPES buffer was 7.0; activity decreased 60% at pH 8.0 and 50% at pH 5.0. The

TABLE 1. Purification of CKase from *P. aeruginosa*

Fraction	Vol (ml)	Protein (mg/ml)	Sp act (μmol/h per mg of protein)	Recovery (%)
Extract	410	33.0	52.8	100
DEAE-cellulose	290	5.1	527	109
Ethylagarose	340	2.4	610	70
Hexylagarose	170	2.0	1,350	64
Hydroxylapatite	197	0.9	2,550	54
Sephadex G-200	40	0.6	4,731	16

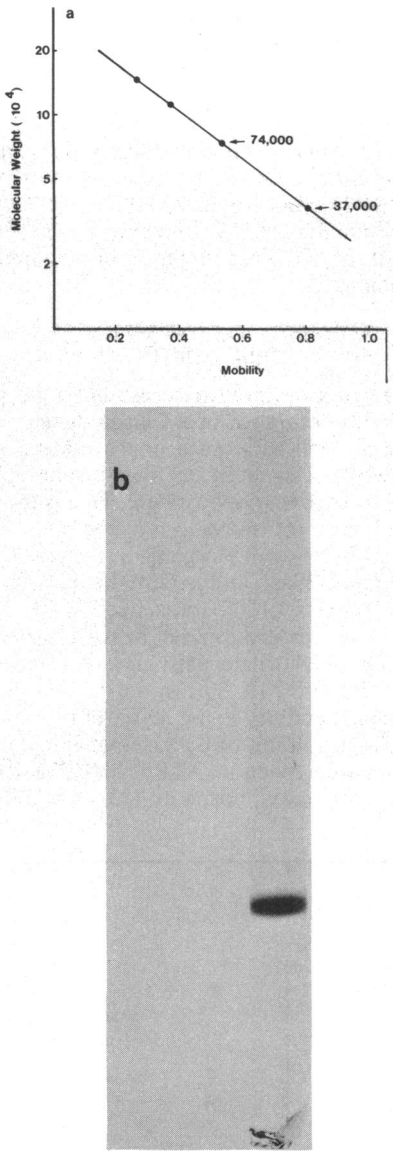

FIG. 3. (a) Estimation of the molecular weight of CKase subunit and cross-linked enzyme. Yeast alcohol dehydrogenase was cross-linked by dimethyl suberimidate (8). The cross-linked yeast alcohol dehydrogenase (bands at 37,000, 74,000, 111,000, and 148,000) was used as a standard. The electrophoretic mobilities were determined as described by Weber and Osborn (32). The arrows indicate the mobilities for the monomer and dimer of CKase after cross-linking and electrophoresis in the presence of sodium dodecyl sulfate. (b) Polyacrylamide gel after electrophoresis of CKase in the presence of sodium dodecyl sulfate (50 μg of protein was applied).

activity of CKase in HEPES buffer was twice that in potassium citrate buffer at pH 6.0.

(iii) Dependence of CKase activity on ADP and carbamoylphosphate concentrations. Carbamoylphosphate saturation curves were determined at 0.25 and 2 mM ADP (Fig. 4). At 2 mM ADP, the double-reciprocal plot was linear, yielding an apparent K_m of 5 mM for carbamoylphosphate. However, at the lower ADP concentration (0.25 mM), the double-reciprocal plot was concave upward, and a Hill plot of the data (Fig. 4, inset) yielded an interaction coefficient of 1.4, indicating positive cooperativity under these conditions.

Double-reciprocal plots for ADP are shown in Fig. 5. At high carbamoylphosphate concentrations (15 mM), the double-reciprocal plot was linear, yielding a limiting K_m of 0.3 mM. In contrast, at lower carbamoylphosphate concentrations, the double-reciprocal plot for ADP was concave downward, indicating negative cooperativity.

(iv) Activity of CKase with acetylphosphate. CKase was assayed with acetylphosphate as described above. The activity with acetylphosphate was approximately 5% of that with carbamoylphosphate as phosphoryl donor.

(v) Inhibition by ATP. Several compounds indicative of arginine and pyrimidine availability or energy charge were tested for modulation of CKase activity at nonsaturating concentrations of ADP or carbamoylphosphate. The compounds tested (at 6 mM) were arginine, ornithine, glutamine, NAD^+, NADH, $NADP^+$, NADPH, uridine monophosphate, uridine triphosphate, cytidine triphosphate, phosphate, AMP, and ATP. Only ATP significantly affected

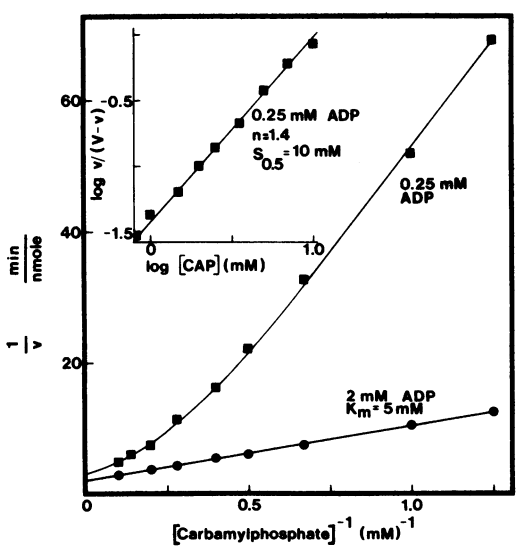

FIG. 4. Double-reciprocal plots for carbamoylphosphate (CAP). Inset, Hill plot for the data at 0.25 mM ADP. The enzyme was assayed as described in the text except that additional $MgCl_2$ equal to the ADP concentration was present in the reaction mixtures.

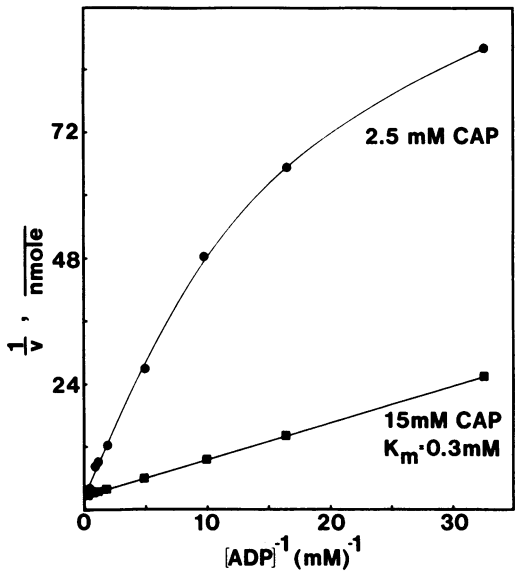

FIG. 5. Double-reciprocal plots for ADP. The enzyme was assayed as described in the text except that additional $MgCl_2$ equal to the ADP concentration was present in the reaction mixtures. CAP, Carbamoylphosphate.

CKase activity; CKase activity at 0.2 mM ADP and 15 mM carbamoylphosphate was inhibited 50% by 0.5 mM ATP (Fig. 6). Experiments on inhibition by ATP (data not shown) showed a competitive pattern when ADP was the variable substrate (K_i, 0.2 mM ATP).

(vi) Regulation of CKase synthesis. The levels of CKase were determined in extracts of cells grown in a basal salts medium supplemented by a number of carbon and nitrogen sources. Since other enzymes of the arginine deiminase pathway are derepressed under conditions of oxygen limitation (5, 19), in certain experiments cultures were grown aerobically to an optical density at 420 nm of 0.5 and then sparged with nitrogen for 2 h before being harvested. In all cases, chloroamphenicol (200 μg/ml) was added, and cells were chilled in ice before being harvested. CKase was assayed in 0.1 M sodium citrate buffer at pH 5.4, conditions which minimize the interference by acetate kinase. Under these conditions, the contribution of acetate kinase toward the CKase titer is approximately 10 to 20% under uninduced conditions and is negligible under induced conditions.

CKase was induced when arginine was present as the sole nitrogen source under aerobic (cf. experiments 1 and 3) or anaerobic (cf. experiments 10 and 11) conditions (Table 2). The presence of ammonia prevented induction of the enzyme under aerobic conditions (experiments 1 and 2). CKase was induced to a much higher extent by anaerobiosis, regardless of whether arginine was present (cf. experiments 3 and 4; 8 and 9) or absent (cf. experiments 5 and 6).

cOTCase, which has been studied in detail previously (12, 19, 29), was also assayed in a number of experiments. In addition to the induction by arginine and anaerobiosis previously reported (19) and confirmed here (cf. experiments 1 and 3; 3 and 4), ammonia prevented the induction of cOTCase under aerobic conditions (experiment 2).

DISCUSSION

The results reported here establish the presence in *P. aeruginosa* of a CKase distinct from CPSase as well as from a constitutive acetate kinase which also catalyzes the phosphorylation of ADP by carbamoylphosphate. This is the only reported case of these three enzymes being produced by the same organism. Enteric bacteria contain CPSase and acetate kinase but lack CKase; reports of the presence of CKase in members of this group have been shown to be the result of alternate activities of CPSase or acetate kinase (7, 13, 30). In the case of yeasts, the reported activity is the result of presence of excess large subunit of CPSase which catalyzes the reversible reaction ATP + NH_3 + $CO_2 \rightleftarrows$ ADP + carbamoylphosphate (23). The physio-

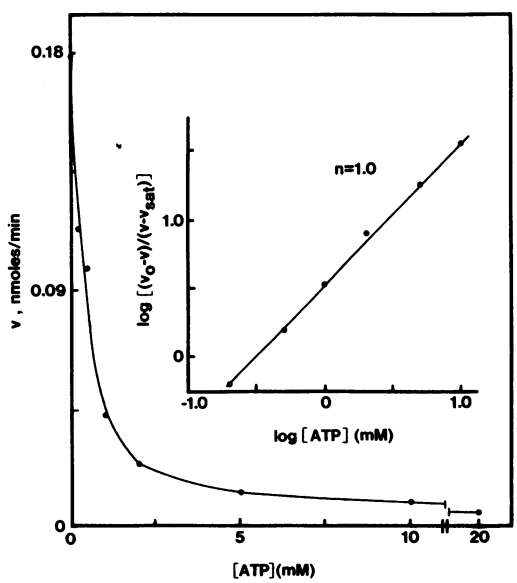

FIG. 6. Inhibition of CKase activity by ATP. The enzyme was assayed as described in the text except that the ADP concentration was 0.2 mM, and magnesium ATP was varied as indicated. Inset, Hill plot of the data where v_0 is the rate of the reaction in the absence of ATP and v_{sat} is the rate in the presence of a saturating level of ATP.

TABLE 2. Regulation of CKase synthesis

Expt no.	Growth conditions			Sp act[a] (μmol/h per mg of protein)	
	Carbon and energy source	Nitrogen source	Aeration[b]	CKase[c]	cOTCase
1	Pyruvate	Ammonia	+	4.9	19.2
2	Pyruvate	Ammonia and arginine	+	4.8	20.0
3	Pyruvate	Arginine	+	7.7	32.7
4	Pyruvate	Arginine	−	75.1	281.7
5	Glutamate	Glutamate	+	12.8	41.9
6	Glutamate	Glutamate	−	209.2	191.1
7	Glutamate	Arginine	−	479.6	
8	Arginine	Arginine	+	38.4	
9	Arginine	Arginine	−	208.3	
10	Citrate	Ammonia	−	112.4	
11	Citrate	Arginine	−	306.4	

[a] Before assay, extracts were diluted in 0.05 M HEPES buffer (pH 7.0) containing 0.5 mM EDTA and 0.05% bovine serum albumin.

[b] +, Aerated; −, cultures were sparged with nitrogen for 2 h before being harvested.

[c] The amounts of ATP produced in controls lacking carbamoylphosphate varied with growth conditions and were routinely subtracted to obtain the values reported here.

logical role of acetate kinase from *P. aeruginosa* is most likely in the utilization of exogenous acetate, as is the case for the enzyme from *E. coli* (6).

Detailed studies on CKase from microorganisms have been limited to streptococci and mycoplasmae (1). The enzyme from *P. aeruginosa* is similar in molecular weight to the enzymes from *Streptococcus faecalis* (16) and *Mycoplasma arthritidis* (25). Values for the sedimentation coefficient and the equivalent molecular weight of CKase from *S. faecalis* led to the suggestion that the enzyme from this organism is a dimer (16), as was shown here to be the case for *P. aeruginosa*. However, the enzyme from *P. aeruginosa* is characterized by a sharp pH optimum, in contrast to the constant activity between pH 5.0 and 8.0 exhibited by CKase from *S. faecalis* (16).

The double-reciprocal plots for CKase from *P. aeruginosa* were not linear at low substrate concentrations. Apparent cooperativity in the binding of one substrate which is exhibited only at low levels of the second substrate can result from kinetic cooperativity through a random mechanism (20) or hysteresis (21). Reports on CKase from *S. faecalis* have suggested both an ordered (16) and a random (22) reaction mechanism. Additional kinetic and binding data are needed for the determination of the reaction mechanism for this enzyme. Regardless of the reaction mechanism, the apparent positive cooperativity in carbamoylphosphate binding at low ADP concentrations observed for CKase from *P. aeruginosa* is of possible physiological significance: it can serve to reduce the extent of catabolism of carbamoylphosphate under growth conditions yielding high energy charge. In contrast, the physiological significance of negative cooperativity in ADP binding at low carbamoylphosphate concentrations is not clear: it results in CKase being responsive over a wide range of ADP concentrations. However, CKase function is expected to be minimal in the lower range of ADP concentrations (growth conditions yielding high energy charge).

Experiments on the regulation of the synthesis of CKase under various growth conditions (Table 2) showed that the synthesis responded to three regulatory signals: it was induced to high levels by anaerobiosis, induced to moderate levels by arginine, and repressed by ammonia. These results suggest two roles for CKase in *P. aeruginosa*: it functions to provide ammonia under aerobic conditions and, more importantly, to generate ATP under anaerobic conditions. Mercenier et al. (19) have documented by detailed experiments the induction by low oxygen tension of certain enzymes of the deiminase pathway (arginine deiminase and cOTCase). Nutrient depletion has also been shown to result in partial induction of cOTCase (19). Accordingly, Mercenier et al. suggested that the deiminase pathway is used by *P. aeruginosa* to obtain energy under conditions of nutrient depletion. The mechanism by which anaerobiosis induces the deiminase pathway has not been elucidated. However, it is of interest that nitrate, which can replace oxygen as the terminal electron acceptor in *P. aeruginosa*, partially prevents enzyme induction by low oxygen tension in the wild-type strain, but not in a mutant lacking nitrate reduc-

tase (19). These results have led to the conclusion that the terminal electron acceptors (oxygen or nitrate) do not directly regulate the deiminase pathway (19).

P. aeruginosa possesses a second catabolic pathway for arginine involving its decarboxylation to agmatine, which is converted to *N*-carbamoylputrescine and putrescine (18). The relative contributions of the decarboxylase and deiminase pathways to the catabolism of arginine under aerobic conditions have not been determined. However, Voellmy and Leisinger (31) have shown that a mutant defective in *N*-acetylornithine transaminase (of the deiminase pathway; Fig. 1, step 12) grows very poorly on arginine and not at all on ornithine as a source of carbon and nitrogen under aerobic conditions. These results suggest that the deiminase pathway plays a major role in aerobic utilization of arginine. The finding that a mutant defective in cOTCase is still able to grow aerobically on arginine (10) does not preclude a major role for the deiminase pathway in arginine utilization under aerobic conditions if a critical concentration of putrescine is required for the induction of the subsequent enzymes of the decarboxylase pathway. Labeling experiments under various growth conditions would further clarify the functions of the deiminase and decarboxylase pathways in *P. aeruginosa*.

We have reported elsewhere (submitted for publication) the presence in *P. aeruginosa* of a single CPSase which is subject to activation by ornithine and *N*-acetylornithine and to feedback inhibition by uridine monophosphate. CPSase activity was repressed by pyrimidines but not by arginine. Thus, in the presence of exogenous arginine, both CPSase and CKase are present; their simultaneous operation would create a futile cycle resulting in loss of 1 mol of ATP per mol of carbamoylphosphate processed (see Fig. 1). The inhibition of CKase activity by ATP provides an effective mechanism for avoiding the operation of the cycle: at high energy charge, CKase activity is minimal, and the prevalent direction is the biosynthesis of carbamoylphosphate. Also, as shown by Stalon et al. (29), in *P. putida* ATP also inhibits cOTCase. Accordingly, at high energy charge, the operation of the arginine deiminase pathway is suppressed.

Haas and Holloway (11) have reported that arginine alone does not satisfy the requirement of carbamoylphosphate in mutants lacking CPSase; rather, these mutants require both arginine and uracil for growth. The inability of these mutants to utilize arginine as a source of carbamoylphosphate (confirmed by us) probably reflects the suppression of the enzymes of the deiminase pathway by high energy charge and the inhibition of cOTCase activity by ATP. Moreover, the small amount of carbamoylphosphate that is generated under these conditions must be preferentially utilized by CKase rather than by aspartic carbamoyltransferase. The basis for such a preferential utilization is not clear, particularly in view of the much lower K_m value reported for aspartic carbamoyltransferase (3).

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