Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 9818–9822, November 1993
Biochemistry

Subunit structure of a class A aspartate transcarbamoylase from *Pseudomonas fluorescens*

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Communicated by Mary Ellen Jones, June 7, 1993 (received for review March 11, 1993)

ABSTRACT **The class A aspartate transcarbamoylase (ATCase, EC 2.1.3.2) from *Pseudomonas fluorescens* was purified to homogeneity with retention of full catalytic and regulatory functions. Careful determinations under conditions that minimized proteolysis showed that the molecule is a 1:1 stoichiometric complex of two polypeptide chains of 34 and 45 kDa. Pyridoxal phosphate is a competitive inhibitor of the enzyme (K_i = 1 μM). Reduction of the pyridoxal phosphate enzyme adduct with sodium boro[^{3}H]hydride showed that the active site is located on the 34-kDa polypeptide. Affinity labeling with 5′-[*p*-(fluorosulfonyl)benzoyl]adenosine, an ATP analog, suggested that the regulatory site is also located on the 34-kDa species. While the function of the 45-kDa subunit is unknown, neither carbamoyl phosphate synthetase nor dihydroorotase activities are associated with the ATCase. The molecular mass of the enzyme was determined by gel filtration, sedimentation velocity, and electron microscopy to be 464 kDa. Thus the enzyme is composed of six copies of the 34-kDa polypeptide and six copies of the 45-kDa polypeptide. The molecule has a Stokes' ratio of 70.9 Å and a frictional ratio of 1.37, suggesting a compact globular shape. We propose that the *P. fluorescens* ATCase is composed of two trimers of 34-kDa catalytic chains and is likely to be a D_3 dodecamer with an arrangement of subunits analogous to that of the class B ATCase molecules.**

Aspartate transcarbamoylase (ATCase; aspartate carbamoyltransferase, carbamoyl-phosphate:L-aspartate carbamoyltransferase; EC 2.1.3.2) catalyzes the formation of carbamoyl aspartate in *de novo* pyrimidine biosynthesis (1). Although the enzyme occurs universally, there are several structural variants. Bethell and Jones (2) identified three classes of bacterial ATCase that differ in size and regulatory properties.

Class A ATCases are the largest (average Stokes' radius, 85 Å) and are sensitive to allosteric effectors but, unlike the class B enzymes described below, the catalytic and regulatory functions cannot be readily separated. *Pseudomonas fluorescens* ATCase, a class A protein originally characterized by Adair and Jones (3), was found to be a dimer composed of two 180-kDa subunits.

Class B ATCases have an average Stokes' radius of 64.8 Å. *Escherichia coli* ATCase (310 kDa), a typical class B protein, consists of six copies of two polypeptides, 33-kDa catalytic chains and 17-kDa regulatory chains (4). X-ray studies (5–7) showed that two catalytic trimers are stacked above each other in nearly eclipsed configuration and are held together by the three regulatory dimers, which are clustered around the periphery of the molecule.

Class C ATCases are the smallest, with a Stokes' radius of only 40 Å, and are insensitive to allosteric effectors. *Bacillus subtilis* ATCase has been shown (8) to be a trimer composed of 33.5-kDa subunits with a tertiary structure that is very similar to the *E. coli* catalytic subunit (9). The ATCase from higher plants has a similar size and subunit structure (10).

In mammals ATCase activity is associated with a multifunctional mammalian polypeptide with carbamoyl phosphate synthetase (CPSase), ATCase, and dihydroorotase (DHOase) activities, called CAD, which catalyzes the first three steps in pyrimidine biosynthesis (11–13). The ATCase domain was isolated from controlled proteolytic digests (14) and has also been cloned, sequenced (15), expressed in *E. coli*, and purified (J. Molina and D.R.E., unpublished data). The mammalian ATCase domain is an unregulated trimer composed of 34-kDa subunits.

The appreciable similarity in sequence among the catalytic polypeptides of *E. coli* ATCase (16), *B. subtilus* ATCase (17), several other class B and C ATCases, and several eukaryotic ATCase domains (15, 18–20) suggests a highly conserved structure. Thus, although the overall structural organization of the ATCases from different organisms varies, a common theme appears to be that the catalytic activity is associated with 34-kDa domains that associate to form trimers. Moreover, a trimeric structure might be expected to be universal since the active site of the *E. coli* enzyme is composed of residues from adjacent subunits in the trimer (5–7). The unusually large dimeric class A enzymes are, therefore, especially interesting because they seem to be an exception to the rule.

Thus we have pursued the initial discoveries regarding the ATCase of *P. fluorescens* (3) to determine whether its activity is associated with a smaller domain and whether other catalytic or regulatory activities are present in the complex.

EXPERIMENTAL PROCEDURES

P. fluorescens, obtained from the American Type Culture Collection (ATCC strain 13525), was cultured in a Lab-Line Instruments Hi-Density Fermenter (model 29500). Four-liter stationary phase cultures yielded 40–100 g (wet weight) of cells. Alternatively, cultures were grown to midexponential phase in a 20-liter fermenter (New Brunswick Scientific) with a yield of 60–70 g of cells. The cells were suspended in two-thirds volume of the homogenizing buffer (3), frozen in a dry ice/ethanol bath, and stored at −70°C. Cell extracts were prepared by two passes through a French press.

The multifunctional protein CAD was purified from the overproducing hamster cell line 165-23 (13, 21). CAD (110 μg) was radiolabeled with 12 mM *N*-[^{14}C]ethylmaleimide (8.4 μCi/mmol; 1 Ci = 37 GBq) in 20 mM Hepes, pH 7.4/50 mM KCl/0.1 mM EDTA/30% (vol/vol) dimethyl sulfoxide/5% (vol/vol) glycerol at 30°C for 1 h, and the unreacted reagent was removed using a mini-Sephadex G-50 spin column (22). *E. coli* ATCase was purified (23) from an overproducing

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Abbreviations: ATCase, aspartate transcarbamoylase; CPSase, carbamoyl phosphate synthetase; DHOase, dihydroorotase; CAD, the mammalian protein having CPSase, ATCase, and DHOase activities; PALA, *N*-phosphonacetyl-1-aspartate.

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recombinant pEK2/EK1104 (Evan Kantrowitz, Boston College) with the additional final step of Sephacryl S-300 chromatography in 10 mM potassium phosphate, pH 7.5/100 mM KCl. Protein concentrations were determined by the method of Lowry *et al.* (24) or Bradford (25). Enzyme activities were assayed as described (13, 21, 26).

SDS/PAGE was carried out as described (27). For quantitative measurements, the Coomassie blue-stained gel was scanned with a Zeineh soft laser scanner. For autofluorography, the gels were preequilibrated in EN^{3}HANCE (NEN). For two-dimensional electrophoresis, protein samples were electrophoresed in the first dimension through 13 cm × 2.5 mm polyacrylamide tube gels under the nondenaturation conditions (28). Gels were electrophoresed in triplicate; one was stained for protein, a second was sliced and assayed for ATCase activity, and the third was carried through the second dimension—SDS/10% polyacrylamide gels (29). The proteins were electroblotted (30) onto an Immobilon membrane (Millipore). Edman degradation of membrane-bound protein samples was carried out in Wayne State University's Macromolecular Core Facility.

The Stokes' radius and molecular mass were determined by gel filtration (31) on a calibrated Sephacryl S-300 column. Centrifugation (120,000 × *g*, 12 h, 5°C) was carried out using 5.2 ml of 5–20% linear gradients of sucrose in 10 mM potassium phosphate, pH 7.5/100 mM KCl/0.5 mM 2-mercaptoethanol/20 μM EDTA. Sucrose concentration was determined by refractometry.

RESULTS

Separation of Pyrimidine Biosynthetic Activities in *P. fluorescens* Extracts. The large size of the *P. fluorescens* ATCase suggested that the protein may be a complex of ATCase with CPSase, or DHOase, or both. Consequently, cell extracts were chromatographed on a Sephacryl S-300 gel-filtration column (Fig. 1). The ATCase, CPSase, and DHOase were found to be well separated from one another, clearly indicating that these activities are carried by separate nonassociated polypeptide chains.

Identification of ATCase Subunits. Partially purified *P. fluorescens* ATCase was fractionated by two-dimensional electrophoresis. The expected 180-kDa polypeptide was not detected. Instead the activity was solely associated with a region of the gel containing two polypeptides of 34 and 45 kDa.

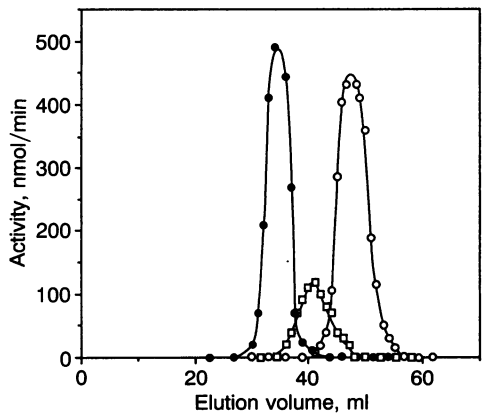

FIG. 1. Separation of the catalytic activities catalyzing the initial steps of pyrimidine biosynthesis in extracts of *P. fluorescens*. Frozen *P. fluorescens* cells were lysed in homogenizing buffer containing 1 mM phenylmethylsulfonyl fluoride and centrifuged at 15,000 × *g* for 1 h and then at 100,000 × *g* for 2.5 h. The supernatant was dialyzed overnight at 4°C against 20 mM Tris·HCl, pH 7.4/50 mM KCl/4 mM glutamine/4 mM aspartate/0.1 mM EDTA/1 mM dithiothreitol/5% glycerol/30% dimethyl sulfoxide. A 1-ml sample of the extract was applied to a 1.5 cm × 40 cm Sephacryl S-300 column equilibrated with dialysis buffer. Fractions (1 ml) were assayed for CPSase (□), ATCase (●), and DHOase (○) activities.

Because of the sensitivity of the large multifunctional proteins to proteolysis, we were concerned that endogenous proteases may have cleaved the parent polypeptide into active functional domains. Consequently, the proteolytic activity in *P. fluorescens* extracts was assayed by taking advantage of the extraordinary sensitivity of the mammalian pyrimidine biosynthetic polypeptide CAD to proteolysis (13, 14, 21, 32). Radiolabeled CAD was incubated in *P. fluorescens* cell extracts for various periods of time up to 6 h at 37°C and then analyzed by fluorography of SDS/polyacrylamide gels. The molecular mass of the CAD polypeptide remained the same and no traces of proteolytic fragments could be detected regardless of whether protease inhibitors had been added to the cell extracts. In contrast, no intact CAD remained after a 5-min incubation with low concentrations of trypsin [CAD/trypsin ratio, 100:1 (wt/wt)].

Protease activity was also assayed during a large-scale preparation (see below) by using a standard test kit (Boehringer Mannheim). No proteolytic activity was detected in any of the fractions containing *P. fluorescens* cells or extract (15-μl samples, 16-h assays at 31°C). These results strongly suggested that the 34-kDa and 45-kDa species were not fragments of a larger polypeptide.

Purification of *P. fluorescens* ATCase. Although endogenous proteases could not be detected, the isolation of *P. fluorescens* ATCase was carried out under conditions designed to minimize proteolysis. Protease inhibitors [1 mM phenylmethylsulfonyl fluoride, soybean trypsin inhibitor II (250 μg/ml), and 2.5 mM benzamidine] were added to the cells and homogenizing buffer and the initial stages of the procedure were carried out at 5°C as rapidly as possible. The purification (Table 1) closely followed the method devised by Adair and Jones (3). However, their final step, preparative gel electrophoresis, which resulted in appreciable inactivation of the enzyme, was omitted. These preparations contained the 34- and 45-kDa ATCase subunits, a major 55-kDa protein, and several minor species. The 34- and 45-kDa subunits were isolated regardless of whether the cells were harvested from exponentially growing or stationary cultures. The results were similar to those as reported (3), although here the yield after the hydroxyapatite step was lower (18% vs. 44%) and the specific activity was slightly higher (19.6 vs. 14.4 μmol per min per mg). The contaminants could be removed by chromatography on a Superose 6 column (Fig. 2), yielding a homogeneous preparation of the enzyme with a specific activity of 54.5 nmol per min per mg.

Table 1. Purification of *P. fluorescens* ATCase

Purification step	Protein, mg/ml	ATCase, units/mg	Fold enrichment	Yield, %
Homogenate	64.8	0.04	1	100
Streptomycin sulfate	26.4	0.11	2.8	128
Ammonium sulfate fraction at 30–43%	64.5	0.20	5.0	93
Sephadex G-200	7.0	1.07	26.8	60
Hydroxylapatite	0.021	19.6	490	18

ATCase activity is expressed as units/mg, where 1 unit = 1 μmol/min. Hydroxyapatite columns used were 2.5 × 6–10 cm, depending on the amount of protein to be bound, and were used once. ATCase was bound at 5 mM phosphate (pH 7.5) and eluted with a gradient of 5–15 mM phosphate buffer.

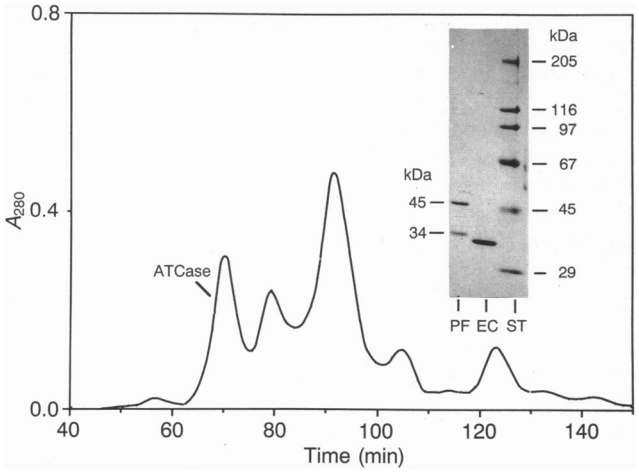

FIG. 2. Final purification of *P. fluorescens* ATCase. ATCase purified through the hydroxylapatite column step (Table 1) was applied to a Superose 6 HR 10/30 FPLC column (Pharmacia) and eluted with 50 mM sodium phosphate, pH 7.5/150 mM NaCl at 1 ml/min. The A_{280} was monitored. (*Inset*) The ATCase-containing fractions (70 min) were pooled (lane PF) and analyzed by SDS/PAGE along with *E. coli* ATCase catalytic subunit (lane EC) and standard proteins (lane ST). Molecular masses are indicated. The ordinate represents the observed absorbance ×10.

Stoichiometry of the *P. fluorescens* ATCase Subunits. Unlike the results obtained with the *E. coli* enzyme (33), *P. fluorescens* ATCase subunits could not be separated by reaction with mercurials. Sucrose gradient centrifugation (data not shown) indicated that reaction with 0.3 mM or 1 mM *p*-hydroxymercuribenzoate did not dissociate the enzyme. The subunits were separated only after SDS denaturation.

The relative amounts of the two subunits were determined by quantitative SDS/gel electrophoresis. A molar ratio of 1.00 ± 0.16 was obtained in 13 determinations, indicating that there are equivalent number of copies of the 34- and 45-kDa polypeptides in the complex.

Location of the Catalytic and Regulatory Sites. Since the two subunits could not be readily separated, the functional sites were identified by affinity modification.

Reduction of the imine formed between pyridoxal phosphate and Lys-84 on the *E. coli* ATCase catalytic subunit, an active site residue (5–7) involved in carbamoyl phosphate binding, is accompanied by a complete loss of catalytic activity (34). *P. fluorescens* ATCase was also competitively inhibited by pyridoxal phosphate (apparent K_i of 1 μM) and was irreversibly inactivated by reduction of the adduct with sodium borohydride (data not shown).

The putative active site lysine was covalently modified by 25 mM pyridoxal phosphate followed by reduction with sodium boro[^{3}H]hydride. SDS/PAGE followed by autofluorography showed (Fig. 3) that the 34-kDa but not the 45-kDa polypeptide was modified. No incorporation of ^{3}H into the 34-kDa subunit occurred when pyridoxal phosphate was omitted or in the presence of carbamoyl phosphate, the bisubstrate analog *N*-phosphonacetyl-1-aspartate (PALA), or the weak competitive inhibitor ATP (3), indicating that the active site was specifically modified. A 55-kDa contaminant also incorporated ^{3}H but this occurred whether or not pyridoxal phosphate was present and carbamoyl phosphate and PALA had no significant effect on the labeling of this species. Thus the ATCase catalytic site must be located on the 34-kDa subunit.

The same approach was employed to locate the regulatory site using 5′-[*p*-(fluorosulfonyl)benzoyl]adenosine (FSBA), an analog of the allosteric inhibitor ATP (35). The autofluorograph showed that, after the reaction of purified *P. fluorescens* ATCase with [^{14}C]FSBA, there was no incorporation of the radiolabel into the 45-kDa subunit but the 34-kDa subunit was strongly labeled (data not shown). The labeling of the 34-kDa species was not affected when the reaction was carried out in 1 mM carbamoyl phosphate, indicating that FSBA was not reacting at the active site. On the other hand, the presence of 15 mM ATP significantly reduced the labeling of the 34-kDa species.

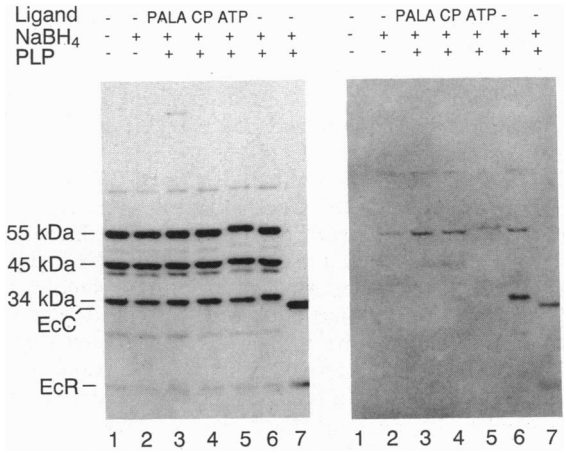

FIG. 3. Identification of the catalytic subunit by affinity labeling. *P. fluorescens* ATCase (9 μg) was incubated in the dark for 20 min at room temperature with 250 μM pyridoxal phosphate (PLP) in 0.01 M triethanolamine acetate, pH 8.0/0.5 mM 2-mercaptoethanol/20 μM EDTA or in the presence of 100 μM PALA, 2.5 mM carbamoyl phosphate (CP), or 10 mM ATP. Sodium boro[^{3}H]hydride ($NaBH_4$) at a final concentration of 5 mM was then added where indicated for 6 min. Controls lacking pyridoxal phosphate or sodium borohydride were also included as was *E. coli* ATCase (4 μg). The reaction mixtures were fractionated by SDS/PAGE (*Left*). The major bands in lanes 1–6 are the 55-kDa contaminant, the 45-kDa polypeptide, and the 34-kDa species migrating slightly more slowly than the *E. coli* ATCase catalytic chain (EcC) in lane 7, which also shows the regulatory chain (EcR). The autofluorograph (*Right*) was exposed for 17 days. +, Component added; −, component not added.

The sequence of 19 and 17 aa at the amino end of the 34- and 45-kDa polypeptides, respectively (Table 2), showed no identifiable homology to any known ATCase.

The Molecular Mass of the Oligomer. The Stokes' radius of *P. fluorescens* ATCase was determined to be 70.9 Å by chromatography on a calibrated Sephacryl 300 column (Fig. 4). Replots of logarithm of the molecular mass vs. V_e/V_o gave a molecular mass of 460 kDa for the *Pseudomonas* enzyme. The $S_{20,w}$ of *P. fluorescens* ATCase, determined by sucrose gradient centrifugation, was 15.1×10^{-13} s. A molecular mass of 467 kDa was calculated from the observed $s_{20,w}$ and the Stokes' radius. This value was similar to the value of 477 kDa obtained by comparison with proteins of known molecular mass and sedimentation coefficient.

The mean molecular mass of the enzyme was also determined by scanning transmission electron microscopy at Brookhaven National Laboratory by Joseph Wall to be 451 kDa.

DISCUSSION

P. fluorescens ATCase was identified (2, 3) as a class A enzyme based on its large size and regulatory properties. We isolated this protein and found that it is a 1:1 stoichiometric complex of 34- and 45-kDa polypeptides. A 180-kDa polypeptide was never observed in cell extracts or during the isolation procedure carried out under conditions that minimized proteolysis. Moreover, no proteolytic activity was detected in cell extracts or partially purified preparations. We therefore conclude that the molecule consists of two distinct polypeptides.

Table 2. Properties of purified *P. fluorescens* ATCase

Parameter	Value or sequence
Kinetic property	
K_m (carbamoyl phosphate), μM	75.9 ± 9.2
K_m (aspartate), mM	1.08 ± 0.16
V_{max}, μmol per min per mg	
Aspartate saturation curve	42.3 ± 1.4
Carbamoyl phosphate saturation curve	45.1 ± 1.2
K_i (mixed CTP inhibition), mM	14.2
Structural property	
Molecular mass of catalytic chain, kDa*	34
Molecular mass of large chain, kDa*	45
N-terminal sequence of the catalytic chain	MTPLXAXRPLQ-LNAQGQLQ
N-terminal sequence of the 45-kDa chain	MKLSILGARVID-PASGLXXV
Ratio of 34-kDa/45-kDa chains, mol/mol*	1.0 ± 0.16
Stokes' radius (R_a), Å	70.9
Diffusion coefficient ($D_{20,w} \times 10^7$), $cm^2 \cdot s^{-1}$†	2.57
$s_{20,w} \times 10^{13}$, s	15.1
Molecular mass of the oligomer, kDa	
Gel filtration‡	460
Sucrose gradient centrifugation§	477
Calculated¶	467
Electron microscopy‖	451
Frictional ratio (f/f_o)†	1.37
Subunit structure	C_6P_6
Calculated from molecular mass of subunits, kDa	474

*Molecular mass obtained by SDS/PAGE.

†The diffusion coefficient (D) was calculated from the relationship $D = kT/(6\pi\nu a)$ and corrected to water at 20°C, where k is Boltzman's constant, T is the absolute temperature, ν is the viscosity, and a is the Stokes' radius (36). The frictional ratio (f/f_o) was calculated using the expression (37): $f/f_o = a[3VM/4\pi N]^{-1/3}$, where V is the partial specific volume assumed to be 0.735 (3), M is the molecular mass, and N is Avogadro's number.

‡Molecular mass obtained by gel filtration on a Sephacryl S-300 column calibrated with 10 proteins of known mass from linear plots of log molecular mass against V_e/V_o (31).

§Molecular mass (M) obtained by sucrose gradient centrifugation from the relationship $s_1/s_2 = (M_1/M_2)^{2/3}$ (38) using *E. coli* ATCase and catalase as reference proteins.

¶Molecular mass (M) calculated from the measured Stokes' radius and $s_{20,w}$ using the relationship (36), $M = 6\pi\nu Nas/(1 - Vp)$, where p is the density of the solvent, s is the sedimentation coefficient, and the other parameters are defined above.

‖Molecular mass determined by scanning transmission electron microscopy mass measurements.

This conclusion agrees with recent sequencing studies that show that the *Pseudomonas aeruginosa* and *Pseudomonas putida* ATCase genes consist of two distinct open reading frames encoding polypeptides of about these sizes (John Vickrey, Michael Schurr, and Gerald O'Donovan, personal communication).

The molecular mass of the complex determined by several approaches was ≈464 kDa. Based on the mass of the constituent subunits and the stoichiometry of the complex, the protein must be a dodecamer, composed of six copies of the 34-kDa catalytic subunit and six copies of the 45-kDa polypeptide with a calculated molecular weight of 474,000.

We cannot account for our lack of agreement with the previous results (3); however, we were unable to obtain the strain of *P. fluorescens* used in the original studies so that it is possible that the subunit structure of the molecule is different in the current strain. In support of this explanation, the molecular mass (360 kDa) and Stokes' radius (85 Å) previously found for the protein from the lost strain are significantly different from the values reported here. Moreover, the K_m values were somewhat different and CTP was a noncompetitive inhibitor at saturating carbamoyl phosphate. The molecular mass had been determined quite accurately and an alternative possibility, suggested by M. E. Jones (personal communication), was that they had isolated a species lacking a 45-kDa dimer. However, it now seems clear that the ATCase molecules from one well-characterized standard *P. fluorescens* strain as well as the two other sequenced *Pseudomonas* enzymes are composed of two polypeptides.

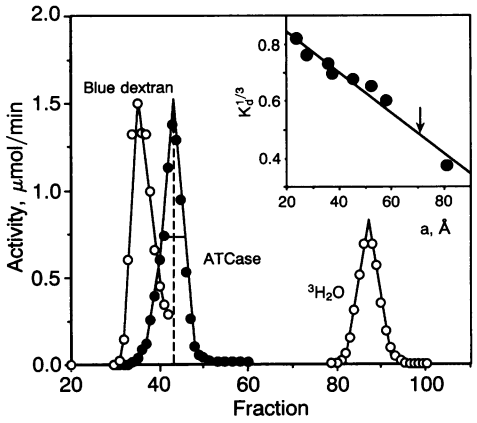

FIG. 4. Determination of the molecular mass by chromatography on a Sephacryl 300 gel-filtration column. *P. fluorescens* ATCase (●) was applied to a 1.4 cm × 56 cm Sephacryl S-300 column and eluted at 4 ml/hr at 5°C with 10 mM potassium phosphate, pH 7.5/100 mM KCl. The void volume (V_o) was determined by measuring the A_{315} of blue dextran and the internal volume (V_i) was determined with $[^3H]H_2O$ by measuring radioactivity. The ATCase activity is given as μmol/min; other ordinate units are arbitrary. (*Inset*) A plot (39) of $K_d^{1/3}$ vs. Stokes' radius (a) for eight standard proteins. The arrow represents the position of *P. fluorescens* ATCase.

The active site was found by affinity modification to be located on the 34-kDa polypeptide. This polypeptide is nearly the same size as the catalytic chain or domain of bacterial (16, 17), fungi (18, 20), insect (19), plant (10), and mammalian (15) ATCases. Thus a 34-kDa catalytic domain appears to be a universal occurrence in ATCase molecules.

We do not know where the regulatory sites are located, but preliminary affinity-labeling studies suggest that the nucleotide binding sites are on the 34-kDa catalytic chain, not on the 45-kDa subunit. The extent of labeling of the 34-kDa subunit was not diminished by substrates, indicating that the reagent was not reacting at the active site but was significantly reduced when the reaction was carried out in the presence of the allosteric effector ATP. We tentatively conclude that both catalytic and regulatory sites are located on the 34-kDa species. This finding would not be surprising since the existence of a separate regulatory chain in *E. coli* ATCase is the exception. The *P. fluorescens* molecule does not bind substrates cooperatively and the heterotropic interactions are qualitatively different than those exhibited by *E. coli* ATCase, suggesting that the 45-kDa polypeptide does not function in a fashion analogous to the 17-kDa *E. coli* regulatory chain. Moreover, the *P. fluorescens* subunits were not dissociated by mercurials so the subunit interfaces probably lack zinc ions liganded to four cysteines (5–7), which are essential to maintain subunit association and regulation of the *E. coli* enzyme.

The function of the 45-kDa polypeptide remains unknown. Our initial hypothesis that the ATCase activity is associated with CPSase or DHOase, as in higher eukaryotic organisms, is incorrect since neither of these activities was cochromatographed with *P. fluorescens* ATCase. Although the allosteric sites may not be located on the 45-kDa species, this poly-

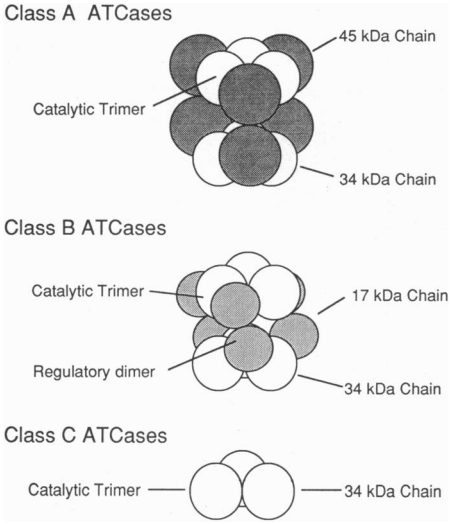

FIG. 5. Model of *P. fluorescens* ATCase. A schematic representation of the proposed subunit structure *P. fluorescens* ATCase, showing 34-kDa (unshaded) and the 45-kDa (shaded) polypeptides. An approximately globular shape was assumed based on the Stokes' radius and the frictional ratio. The subunit structures of the class B and the class C enzymes are shown for comparison.

peptide may be important for the maintenance of an oligomeric structure required for regulation.

The x-ray studies (5–7) of *E. coli* ATCase showed that the active site residues involved in binding the substrate analog PALA are found on adjacent subunits within the trimer. The importance of a shared active site was subsequently confirmed by biochemical studies (40). The strong sequence similarity of all known ATCase catalytic domains and subunits suggests that this structural organization is likely to be universal. A recent x-ray study of *B. subtilis* ATCase (9) and a model building study of the CAD ATCase domain (41) have shown that the active site of these trimeric molecules is also shared and that its topology is remarkably conserved. While the oligomeric structure of the catalytic subunit of *P. fluorescens* ATCase was not determined because the complex could not be dissociated without denaturation, we propose that the *P. fluorescens* ATCase, shown here to consist of a multiple of three catalytic chains, also has trimeric catalytic subunits (Fig. 5). Given the stoichiometry of the complex, C_6P_6, the class A ATCases are probably D_3 dodecamers, a structural organization analogous to that found in the class B enzymes.

We thank Dr. Mary Ellen Jones for helpful discussions and suggestions, Drs. Serge Vinogradov and Joseph Wall for making the electron microscopy measurements, Drs. George Stark and Evan Kantrowitz for mammalian and bacterial strains, and Emily Zaragoza for enthusiastic technical assistance. We also thank June Snow (Wayne State University Macromolecular Core Facility) for help and advice on protein sequencing and Dr. Allen Nicholson for the use of the high density Fermenter. This work was supported by the Office of Naval Research Contract N00014-87-K-0081 and Grants CA-27674 and GM-47399 from the U.S. Public Health Service.

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