Biochimica et Biophysica Acta, 382 (1975) 465–478

BBA 76909

MEMBRANE ATPase OF *PROTEUS* L-FORMS
SOLUBILIZATION AND MOLECULAR PROPERTIES

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(Received October 14th, 1974)

SUMMARY

The Mg^{2+}-dependent ATPase (EC 3.6.I.3) of *Proteus* L-form membrane has been solubilized according to various procedures (Tris · HCl shock-wash with or without Mg^{2+}, EDTA, Triton X-100). The best results were obtained by the same 33 mM Tris · HCl (pH 7.5) shock-wash without Mg^{2+} used for ATPase of protoplasts from *Streptococcus faecalis*. The solubilized enzyme after 105 000 × *g* centrifugation was purified on acrylamide/agarose. The molecular weight was estimated to be 360 000 by gel filtration and by sedimentation coefficient (12.5 S). Polyacrylamide disc-gel electrophoresis in sodium dodecylsulphate revealed two classes of subunit of mol. wt. 64 000 (α) and 58 000 (β), associated in the ratio 1 : 1. We propose a formula $\alpha_3\beta_3$ for the native ATPase of *Proteus* L-forms. Structural similarities to ATPase of various origins are discussed.

INTRODUCTION

Previous investigations on the growth inhibition of stable *Proteus* L-forms by antisera led us to postulate that these antibodies were most effective against the membrane-bound ATPase, Adenosinetriphosphatase (EC 3.6.1.3) [1]. Understanding and elucidation of the biological functions of ATPase require that the enzyme be available in purified form, and its physical and chemical properties be known. We had recently reported the biochemical properties of the soluble enzyme [2]. This Mg^{2+}-dependent ATPase was activated by two monovalent cations K^+ (100 mM) and Na^+ (100 mM), taken alone or together. Solubilized enzyme exhibits allotopic properties, an initial increase in activity followed by a fairly rapid inactivation at 0 °C. The enzyme was solubilized from membrane ghosts by repeated washings in Mg^{2+}-free 33 mM Tris · HCl buffer, pH 7.5.

We would now like to compare this procedure with methods of solubilization used for an other Gram-negative bacteria. This paper reports in addition the amino-acid composition, molecular weight and subunit structure of *Proteus* L-form ATPase.

MATERIAL AND METHODS

Stable L-forms of *Proteus* P 18 were grown in a liquid synthetic medium and harvested as previously described [2].

Release of soluble ATPase

Abrams method [3]. A modification was used. The different phases of enzyme extraction have been reported in our recent publication [2], and are referred to in Fig. 1. Membrane ghosts were washed twice with 2.0 M LiCl/0.25 M Tris · HCl, pH 7.5. After washing with 1 mM $MgCl_2$, the sedimented membranes from the preceding step were left overnight in 33 mM Tris · HCl, pH 7.5. After three washes in this buffer free of divalent cations, the ATPase was released into the solution. The crude soluble ATPase was centrifuged at 105 000 × *g* for 90 min and the supernatant was concentrated to 1 mg protein/ml by ultrafiltration with the Pellicon apparatus (PSJM 02510).

Simplified method of solubilization. The method of Abrams [3] was thus simplified. The membrane ghost pellet C_2 was washed with 10 ml of 1 mM $MgCl_2$, and sedimented by centrifugation at 40 000 × *g* for 30 min. The ATPase was released from the membrane by two successive 30 min washings at 37 °C with 10 ml of 33 mM Tris · HCl buffer, pH 7.5, both preceding a 30 min centrifugation at 40 000 × *g*. Both supernatants were pooled and the membrane residues were removed by centrifugation at 105 000 × *g* for 30 min.

Carreira et al. method [4]. The pellet C_2 was treated by seven times its own volume of 3 mM EDTA/50 mM NH_4HCO_3, pH 9.0. The enzyme was solubilized after a 30 min contact at 37 °C and then centrifuged at 27 000 × *g* for 20 min at room temperature.

Hanson and Kennedy method [5]. The pellet C_2 was resuspended in 8 ml of 50 mM Tris · HCl buffer, pH 7.8, containing 10 % Triton X-100. After overnight contact at room temperature, the suspension was submitted to centrifugation at 100 000 × *g* for 30 min. The Triton X-100 was eliminated from the supernatant by adsorption on polystyrene Bio-Beads SM-A 20-50 Mesh, according to the procedure of Holloway [6]; two 1-h contacts at 4 °C, under continuous agitation. The soluble enzyme was then obtained by centrifugation at 105 000 × *g* for 45 min.

Roisin and Kepes method [7]. The pellet C_2 was resuspended in 1 mM $MgCl_2$/1 mM Tris · HCl (pH 7.6) buffer and then diluted in 20 vols of 1 mM Tris · HCl buffer, pH 7.6. The solubilized ATPase was retrieved by centrifugation at 100 000 × *g* for 20 min.

ATPase assay

Measurements of ATPase activity were carried out according to the procedure of Baginsky et al. [8] under conditions previously reported [2].

Proteins were determined by the Lowry procedure [9], using bovine serum albumine as a standard.

Purification of ATPase

The enzyme was purified by filtration on acrylamide/agarose (Indubiose) [10]. Different concentrations of acrylamide and agarose were used. Indubiose AcA 2-2

separated proteins of molecular weight between 90 000 and 1 200 000 and Indubiose AcA 3-4 separated proteins of molecular weight between 40 000 and 400 000. Columns (2×40 cm) were equilibrated and eluted at room temperature with 33 mM Tris · HCl (pH 7.5)/1.5 mM $MgCl_2$ buffer. 2.5 ml fractions were collected at a flow rate of 15 ml/h. The effluent was monitored at 280 nm for proteins and 730 nm for enzymatic reaction in aliquot fractions of 1.5 ml.

Analytical ultracentrifugation

Sedimentation coefficients were measured by ultracentrifugation analysis in a Spinco Model E analytical ultracentrifuge, equipped with an AN-E rotor, and a schlieren system. Centrifugations were run at 20 °C in the single-sector cells (16 mm), with rotation speed 47 660 rev./min. The position of the sedimenting boundary was recorded by taking photographs at intervals of 4–8 min.

Polyacrylamide disc gel electrophoresis

Different electrophoretic procedures were used for this study. Electrophoresis at alkaline pH in non-dissociating conditions according to the general procedure described by Davis [11], was carried out on separating gels with 7 % acrylamide and 0.18 % *N,N'*-methylene bisacrylamide (w/v). The polymerisation was performed at 20 °C for 1 h in 0.38 M Tris · HCl pH 8.8. No stacking gels were used. Electrophoresis were run at room temperature in 20 mM Tris/0.2 M glycine buffer (pH 8.5) for 12 h at 0.5 mA per gel. Bromophenol blue was used as tracking dye. Samples in 10 % glycerol were layered onto the gels with or without prior treatment by 1 % β-mercaptoethanol. These procedures were used to monitor the purity and native state of the ATPase preparations. Proteins were stained with Coomassie blue, and ATPase activity specifically located on the gel through an enzyme reaction: the gel was dipped into 33 mM Tris · HCl (pH 7.5)/1.5 mM ATP/0.75 mM $MgCl_2$ solution. After 30 min incubation at 37 °C, the gel was washed once in 33 mM Tris · HCl buffer, pH 7.5. A mixture of 4 % ascorbic acid/20 % trichloroacetic acid (8 ml) and 1 % ammonium molybdate (2 ml) revealed the released inorganic phosphate: a blue staining appeared about 15 min later, in the area of the protein band corresponding to ATPase activity.

Disc electrophoresis in the presence of protein denaturing agents according to the Weber and Osborn procedure [12] was carried out on gels of 5–10 % acrylamide concentrations, and containing 0.1 % sodium dodecylsulphate. Samples were treated beforehand by 1 % sodium dodecylsulphate for 2 min at 100 °C or by a combination 1 % sodium dodecylsulphate and 1 % β-mercaptoethanol. The location and intensity of the protein bands stained with Coomassie blue were revealed by spectrophotometric analysis at 600 nm in a Beckmann Acta III spectrophotometer.

Aminoacid analysis

The amino acid composition of ATPase was established following an automatic chromatography method. Samples containing 1 mg of enzyme protein were hydrolysed with 6 M HCl at 105 °C for 24, 36 and 48 h in sealed tubes, under vacuum. Hydrolysates were analysed in a Beckmann Multichrom Liquid Column Chromatograph 42-55. Cystein was determined as cysteic acid.

Chemicals

Indubiose AcA 2-2 and AcA 3-4 were purchased from Industrie Biologique

TABLE I

COMPARISON OF DIFFERENT METHODS OF SOLUBILIZATION OF ATPase

Identical pellets C_2 were obtained from centrifugation of aliquots of initial membrane suspension (see Fig. 1). One of them was taken apart and resuspended to a volume of 5 ml for initial determinations of proteins and ATPase activity. The others were treated according to each method referred. One unit of ATPase is defined as the amount of enzyme giving 1 μmol inorganic phosphate per min under the conditions previously reported [2].

	Pellet C_2	Method of ATPase solubilization				
		Abrams [3]	Simplified Abrams	Roisin and Kepes [7]	Hanson and Kennedy [5]	Carreira et al. [4]
Protein (mg/ml)	3.28	0.16	0.55	0.55	1.43	1.52
Units per ml	0.70	0.20	0.20	0.31	0.46	0.70
Total vol. (ml)	5	3	3	3	3	3
Total proteins (mg)	16.40	0.48	1.65	1.65	4.29	4.56
Total Units	3.50	0.60	0.60	0.93	1.38	2.10
Spec. act. (units/mg proteins)	0.21	1.25	0.36	0.56	0.32	0.46
Purification degree		5.95	1.7	2.7	1.52	2.2
Yield (%)	100	17.1	17.1	26.6	39.4	60.0

Française. Acrylamide, bis-acrylamide, and non-enzymatic protein molecular weight markers were purchased from Serva-Feinbiochemica Heidelberg. Bio-Beads were purchased from Bio-Rad Laboratories, Richmond Calif.

RESULTS

ATPase solubilization

The results obtained for each procedure of solubilization are presented in Table I. The methods of solubilization by EDTA, Triton X-100 and Tris · HCl without Mg^{2+} displayed a high yield (60 %, 39.4 % and 26.6 % respectively). Both procedures of solubilization by shock-wash with Tris · HCl without Mg^{2+} have a far

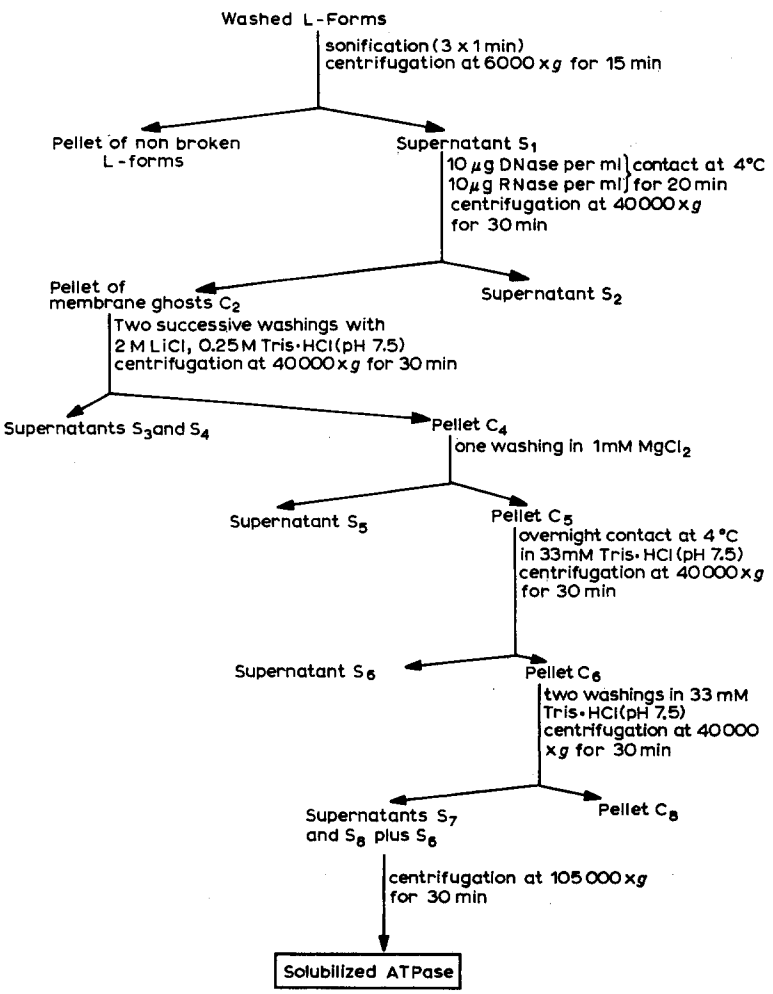

Fig. 1. Solubilization of ATPase according to the method of Abrams [3], slightly modified as described in the text.

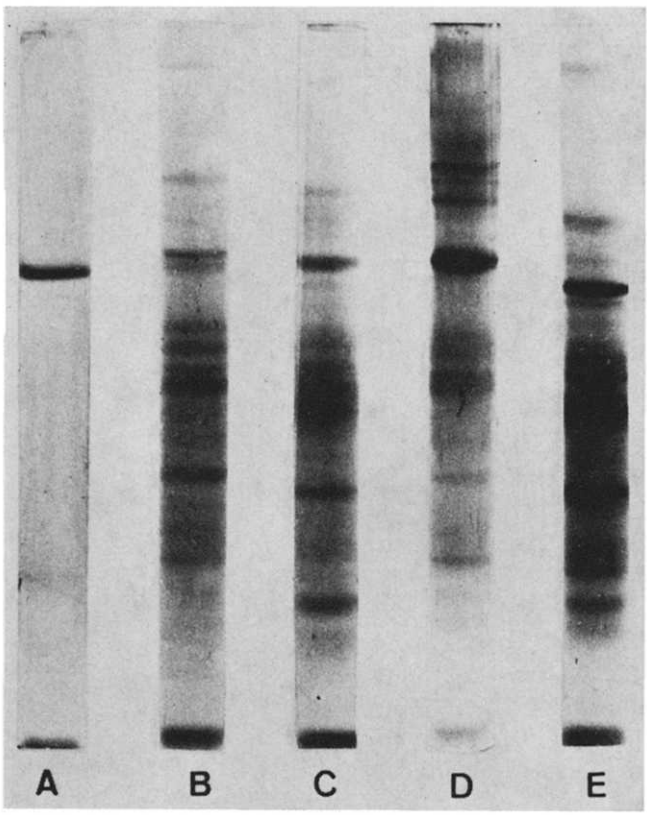

Fig. 2. Purity of ATPase Polyacrylamide gel electrophoresis on Tris/glycine gels, pH 8.8. Fractions of solubilized ATPase (200 μl) were layered on gels without pretreatment. The ATPase was solubilized according to the five methods referred in the text: (A) Abrams' modified method [3]; (B) simplified method; (C) Roisin and Kepes' method [7]; (D) Hanson and Kennedy's method [5]; (E) Carreira et al. method [4].

lower yield (17.1 %). We must also consider the degree of purification of the enzyme. It appears that purification of ATPase was less than three-fold when the enzyme was solubilized following high yield methods or our simplified method. In contrast, purification is about 6 fold when Abrams' method [3] is applied. These purification degrees were confirmed by the analysis of the electrophoretic behaviour of preparations conducted according to the five methods stated (see Fig. 2). The enzyme, when solubilized with the modified Abrams method [3] revealed one single protein band: at this stage, then, it was already substantially pure. When solubilized according to the four remaining procedures, the enzyme displayed 9–17 extra protein bands, with no ATPase activities. ATPase obtained in this way requires subsequent purifications of high complexity, with an increasing loss in proteins, in activity and thus in yield. Moreover, Triton X-100 used for solubilizing ATPase following Hanson and Kennedy's method [5] is hard to separate and when its concentration in the supernatant holding the solubilized enzyme is not short of 2 %, interferes with the determination of protein. Briefly, the Abrams [3] method of shock-wash by Tris · HCl

without Mg^{2+} has the impressive advantage of extracting a highly purified enzyme in a minimal number of steps (see Fig. 1).

Subsequently, the physical and chemical properties of the ATPase of *Proteus* L-forms were studied after solubilization following that method and purification.

Purification of ATPase

The behaviour of the crude ATPase in Indubiose AcA 2-2 before centrifugation

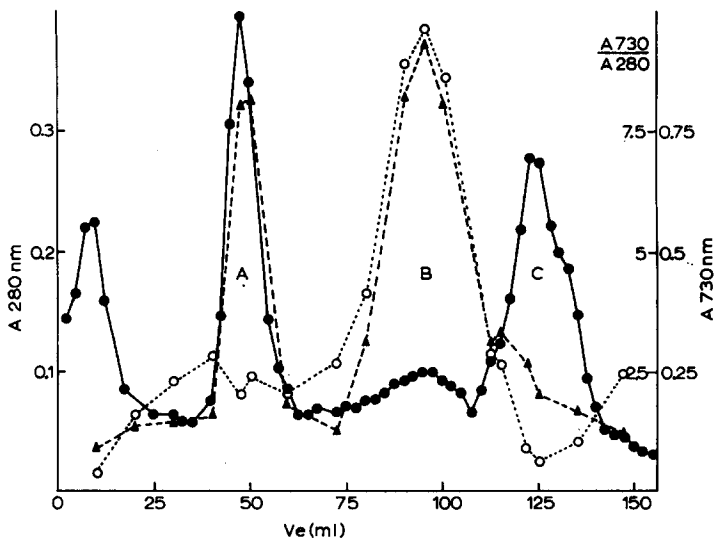

Fig. 3. Indubiose AcA 2-2 filtration of ATPase before centrifugation at 105 000 × *g*. Crude ATPase was charged on a column of the characteristics given under material and methods, and eluted as indicated there. Fractions of 2.5 ml were collected, assayed for ATPase activity at 730 nm (▲- - -▲) and monitored at 280 nm (●—●) for the proteins. The specific activity is given by the ratio absorbance at 730 nm/absorbance at 280 nm (○ · · · ○).

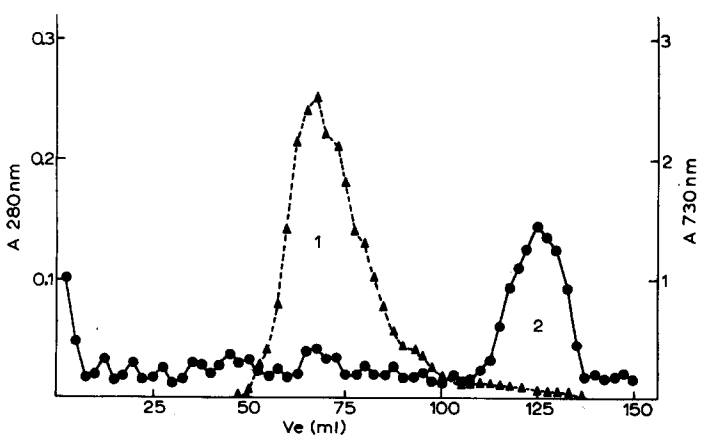

Fig. 4. Indubiose AcA 3-4 filtration of ATPase after centrifugation at 105 000 × *g*. Crude ATPase (1 mg protein/ml) was charged as previously. Fractions of 2.5 ml were also collected, assayed for ATPase activity at 730 nm (▲- - -▲) and monitored at 280 nm (●—●) for the proteins. The volume of elution for ATPase is V_e = 67 ml.

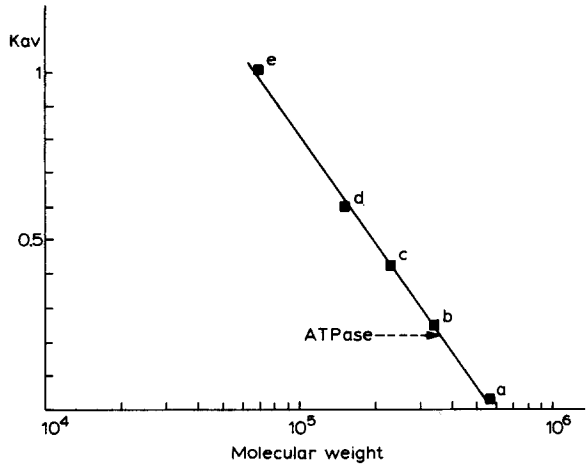

Fig. 5. Calibration of the Indubiose AcA 3-4 column equilibrated with 33 mM Tris · HCl (pH 7.5), 0.75 mM $MgCl_2$ buffer. Proteins (2 mg) of known molecular weight were applied to the Indubiose AcA 3-4 column (2×40 cm). The gel filtration properties of the compounds were expressed in terms of distribution coefficients $K_{av} = V_e - V_0 / V_t - V_0$ with V_e = elution volume of the standard, V_0 = void volume, V_t = total bed volume. The logarithm of the molecular weight of each standard is plotted as a function of its K_{av}. The standards are (a) ferritin, (b) fibrinogen, (c) catalase, (d) gamma-globulin, (e) bovine serum albumin. Arrow marks the distribution coefficient of the ATPase.

at 105 000×g is illustrated in Fig. 3. The heaviest component (Peak A) eluted with the void volume (V_0 = 46 ml) and contained a low specific activity for ATPase. Residual membrane particles were present as proven by negative staining in electron microscopy. The lightest component (Peak B) was eluted with an elution volume of V_e = 92 ml, and showed an enrichment in ATPase specific activity.

Ultracentrifugation at 105 000×g eliminated membrane particles and high molecular weight contaminants as can be seen in Fig. 4, showing the elution profile of crude ATPase on Indubiose AcA 3-4. A better resolution of the ATPase from low molecular weight contaminants was obtained by a single gel filtration step. From the elution volume value of Peak 1 (see Fig. 5), an approximate molecular weight of 360 000 was estimated for the native pure enzyme. Proteins with well-defined molecular weight were used as standards.

Analytical ultracentrifugation

The sedimentation coefficient $s_{20,w}$ of ATPase was 12.5 S for 1 mg protein/ml. By using the empirical relationship established by Paetkau and Lardy [13], between the molecular weight and the sedimentation coefficient: mol. wt. = $5.4 \cdot 10^3 (s_{20,w} \cdot 10^{13})^{1.66}$, an approximate value of 360 000 was calculated for the molecular weight of ATPase.

Polyacrylamide disc gel electrophoresis

Polyacrylamide gel electrophoresis of purified ATPase whether treated or not by β-mercaptoethanol, revealed only one electrophoretic band (see Fig. 6A). Enzymatic activity was tested by direct reaction on a second gel run in parallel. The results showed a single zone of liberated P_i for ATP. This zone coincided with the

position of the protein band. It was therefore ascertained that no protein contaminant remained associated with the enzyme molecule.

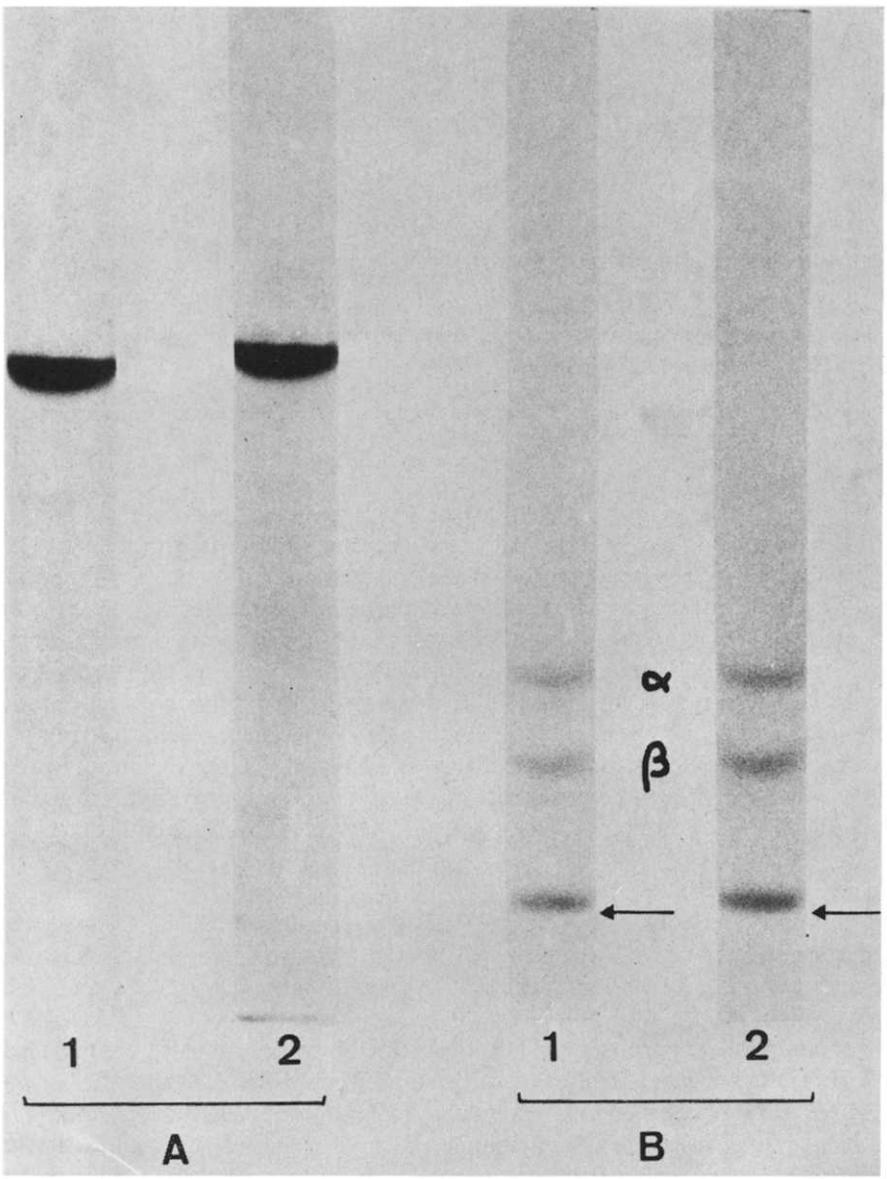

Fig. 6. (A) Disc electrophoresis of native ATPase. Electrophoresis was performed in the standard alkaline conditions stated in the text. Migration was toward the anode (bottom of the gels) in 7 % acrylamide. (1) Sample was not pretreated by β-mercaptoethanol. (2) Sample was pretreated by β-mercaptoethanol. (B) Subunit patterns of ATPase. Fractions of purified ATPase (40 μg) were heated for 2 min at 100 °C with 1 % sodium dodecylsulphate (gel 1) or with combination 1 % sodium dodecylsulphate and 1 % β-mercaptoethanol (gel 2). Migration was towards the anode (bottom of the gels) in 6 % acrylamide (←, front line).

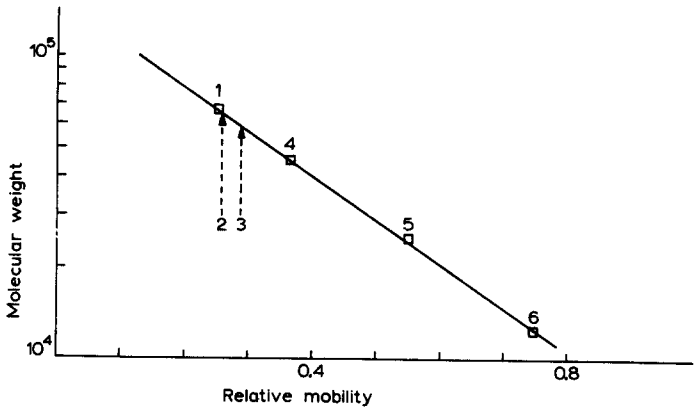

Fig. 7. Molecular weight determination using sodium dodecylsulphate/polyacrylamide (10 %) gel electrophoresis. (1) Bovine serum albumin. (2) Purified ATPase, subunit α. (3) Purified ATPase, subunit β. (4) Ovalbumine. (5) Cytochrome C dimer. (6) Cytochrome C. Mobility of standard proteins measured in the same electrophoresis run as that of the purified ATPase to minimize errors due to changes in the running conditions.

Electrophoresis in polyacrylamide gel (7 %) with sodium dodecylsulphate revealed dissociation of ATPase by sodium dodecylsulphate in two protein bands, the mobilities of which were greater than that of the native enzyme (see Fig. 6B). As no difference could be seen in the band patterns in the presence or absence of β-mercaptoethanol, there are no disulfide bridges in either of the two protein components. We have therefore regarded the L-Form ATPase as made up of two subunits. An evaluation of the molecular weight of these two subunits was calculated by comparing their electrophoretic mobilities with those of reference proteins (see Fig. 7). The two molecular weights were respectively 64 000 and 58 000. The ratio of the intensities of the two electrophoretic bands was 1 : 1. Thus the approximate molecular weight of 360 000, as determined, corresponds to the intrinsic ATPase complex $\alpha_3\beta_3$.

Aminoacid analysis

The aminoacid composition of ATPase is reported in Table II. Cystein was determined as cysteic acid. No product of degradation from methionine, such as sulfone and sulfoxide, were found. The results were the same after 24, 36 and 48 h hydrolysis.

For comparative purposes, the results obtained by other authors were included in Table II. These concern ATPases of beef heart mitochondria [14], *Bacillus megaterium* K.M. [15], *Bacillus stearothermophilus* [16], *Micrococcus lysodeikticus* [17] and *Streptococcus faecalis* [18]. The amino acid composition of ATPase of *Proteus* P 18 L-Forms is very different from that reported for Gram-positive microorganisms or beef heart mitochondria. We can particularly note a higher ratio of polar to non polar amino acids, though the ratio of basic to acid amino acids is about the same as that of other ATPases reviewed. It also contains the lowest amount of methionine.

TABLE II

MOLECULAR WEIGHT AND AMINO-ACID COMPOSITION OF ATPases OF VARIOUS ORIGINS

Amino-acid contents are expressed as amino-acid/100 mol total amino-acids. (–), not determined.

$$\frac{\text{Polar A.A.}}{\text{Non Polar A.A.}} = \frac{\text{Asp+Glu+Lys+Arg+Ser+Thr}}{\text{Val+Leu+Ile+Met+Phe}}$$

	Proteus L-Forms	Beef heart mitochondria [14]	*Bacillus megaterium* [15]	*Bacillus stearothermophilus* [16]	*Micrococcus lysodeikticus* [17]	*Streptococcus faecalis* [18]
Mol. wt.	360 000		379 000 to 410 000	280 000	340 000 to 350 000	385 000
Alanine	9.2	10.50	8.8	9.04	10.64	8.4
Valine	7.3	8.01	8.9	8.08	9.66	6.8
Leucine	8.6	9.25	9.3	8.12	8.44	9.3
Isoleucine	5.4	6.80	6.3	7.92	4.79	6.2
Proline	3.4	4.9	4.3	4.74	2.27	3.9
Phenylalanine	3.0	2.98	3.3	3.85	2.99	3.1
Méthionine	0.3	1.63	2.0	2.44	2.08	2.3
Glycine	8.6	9.39	9.0	7.78	10.13	8.7
Sérine	5.3	5.52	5.6	4.69	5.57	6.3
Threonine	5.3	5.49	5.9	5.34	6.57	6.7
Tyrosine	1.9	2.78	2.7	4.37	2.73	3.3
Aspartic acid	11.3	8.34	8.5	9.23	9.62	10.0
Glutamic acid	13.0	12.06	13.5	12.62	11.46	13.0
Arginine	6.9	5.41	5.6	4.88	7.02	4.5
Lysine	7.7	6.12	5.1	4.49	3.67	6.1
Histidine	2.0	1.56	1.6	2.43	1.89	1.7
Cysteine	—	—	0	—	0.16	—
Tryptophan	—	—	0.4	—	0.37	—
Cysteic acid	0.9	—	—	—	—	—
Polar A.A. / Non polar A.A.	2.01	1.49	1.48	1.35	1.57	1.68
Basic A.A. / Non basic A.A.	0.68	0.64	0.56	0.54	0.60	0.53

DISCUSSION

We have previously reported the properties of the membrane ATPase of *Proteus* L-Forms [2]. The enzyme was solubilized by repeated washings with dilute buffers, according to the method of Abrams [3]. This author required lysozyme for the preparation of protoplasts of *Streptococcus faecalis* and removed it through LiCl. As stable L-forms possess an incomplete cell wall [19, 20], this prior preparation was not necessary. Consequently, we suppressed this 2 M LiCl step in the simplified method of solubilization. The yields obtained for solubilization in the presence or absence of LiCl are the same (17.1 %), but the degree of purification for the simplified method without LiCl is much lower. The analysis of the electrophoretic behaviour of ATPase confirms the presence of many contaminants. LiCl would be necessary to remove the ribosomes which remain present in the membrane ghosts. In these past few years, some workers have solubilized the ATPase of *Escherichia coli* by various procedures [4, 5, 7, 26]. We applied three of these methods to stable *Proteus* L-Forms. They were based on the use of a detergent such as Triton X-100 [5], EDTA [4], or of Tris · HCl plus Mg^{2+} shock-wash [7]. The yields obtained by use of these procedures were greater than those reported above (39.4 %, 60 % and 26.6 % respectively) yet all three methods showed a low degree of purification which was confirmed by electrophoretic analysis (see Fig. 2). It is also noteworthy to observe that the solubilization of ATPase by Tris · HCl shock-wash without Mg^{2+} gives better results than does Tris · HCl shock-wash with Mg^{2+}. The second procedure gives a higher yield (26.6 %) than the first (17.1 %), but the enzyme obtained is not pure. The final Mg^{2+} concentration used in releasing ATPase is $5 \cdot 10^{-5}$ M, lower than $4 \cdot 10^{-4}$ M which Abrams [3] found sufficient to inhibit a 50 % release of enzyme. Nevertheless, the concentration of Mg^{2+} is not the only factor to be considered, the concentration of Tris · HCl may play a prominent part in the specificity of release of ATPase. With the highest concentration of 33 mM, no other protein than ATPase is released whereas, with the lower concentration of 1 mM, some other proteins are solubilized. Thus we conclude that the procedure of solubilization of ATPase combining a low Mg^{2+} concentration with a high Tris · HCl concentration is the best to be applied to the *Proteus* L-Forms.

The molecular weight of membrane ATPase of *Proteus* L-Forms determined by sedimentation coefficient and by gel filtration on Indubiose was found to be 360 000. This high value is very close to that found for other ATPases, [3, 5, 15–18, 22, 23]. The enzyme could be dissociated by sodium dodecylsulphate into two subunits, which we called α and β.

The molecular weight of the subunits was estimated to be 64 000 and 58 000 respectively. Therefore, the ATPase of *Proteus* L-Forms seems to be very similar to that of *Streptococcus faecalis* [18] and to that of *Bacillus megaterium* KM [15]. These three ATPases consist of two types of subunits, which are associated following similar models: $\alpha_3\beta_3$ for the ATPase of *B. megaterium* KM [15] and that of *Proteus* L-Forms; $\alpha_6\beta_6$ for that of *S. faecalis* [18].

The ATPases of *E. Coli* [5] and *Micrococcus lysodeikticus* [17, 21] have also a molecular weight which is very close to that of the ATPase of *Proteus* L-Forms. However, more subunits have been found for these two ATPases.

It appears, therefore, that the ATPase of *Proteus* L-Forms is more similar to

that of Gram-positive microorganisms than to that of *E. coli*, a Gram-negative microorganism. This remark could explain why the procedure of solubilization of ATPase of Gram-positive bacteria was best suited to the solubilization of ATPase of *Proteus* L-Forms, when we were expecting the best results to be obtained by solubilizing the enzyme according to the method used for the ATPase of *E. coli* [4, 5, 7].

In other respects, the ATPase of *Proteus* L-Forms cannot be closely related to those of animal cells, which present a more complex pattern of subunits [22–25].

The ATPase of *Proteus* L-Forms presents an additional similarity to other ATPases: all are cold labile except that of the thermophilic bacterium *B. stearothermophilus* [16]. The ATPase of *E. coli* [5] dissociates in the cold into lower molecular subunits, as does the mitochondrial ATPase [22]. Yet the ATPase we studied does not dissociate when kept in the cold. The enzymatic activity disappears but the electrophoretic behaviour remains the same as the native active enzyme.

The results we have discussed concerning the molecular properties of *Proteus* L-Forms ATPase are in agreement with the point of view of Mirsky and Barlow [15]. These authors wrote that "*B. megaterium* KM ATPase seems to be part of a general class of ATPases with similar chemical and physical properties which are distributed through the bacterial, animal and plant kingdoms". The bacterial ATPases studied in the past few years belonged to Gram-positive bacteria, except for *E. coli*. We were the first to study the molecular properties of an other Gram-negative organism. Each of these enzymes was produced by facultatively anaerobic bacteria. We failed to show narrow similarities between ATPases of *E. coli* and *Proteus* L-Forms. It would be of a great interest to study the ATPase of other bacteria such as aerobes and anaerobes. These researches are under investigation in our laboratory. We expect by this way to help in the understanding of the role played in vivo by that kind of enzyme.

ACKNOWLEDGEMENTS

The authors wish to thank Dr A. Belcourt for performing the aminoacid analysis, and Miss R. Girardot for technical assistance.

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