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SOLUBILIZATION AND MOLECULAR WEIGHT DETERMINATION OF THE (Na^+ + K^+)-ATPase FROM RECTAL GLANDS OF *SQUALUS ACANTHIAS*

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Summary

The membrane-bound (Na^+ + K^+)-activated ATPase (ATP phosphohydrolase, EC 3.6.1.3) system was treated with the nonionic detergent octaethyleneglycoldodecyl ether, yielding a transparent supernatant after centrifugation. The supernatant was highly active with both ATPase and *p*-nitrophenylphosphatase, with initial specific activities of 2300 μmol P_i released · mg^{-1} protein · h^{-1} and 350 μmol *p*-nitrophenol released · mg^{-1} protein · h^{-1}, respectively. The supernatant was purified to 95—100%, with respect to the 96 000 dalton and the 56 000 dalton peptides. The solubilized enzyme was gel filtered in Sepharose 4B-CL and displayed 2 peaks, both with catalytic activity. The low molecular weight particles eluted at K_{av} = 0.54, corresponding to a molecular weight of approximately 500 000 daltons and the particles had a specific activity of 2100 μmol P_i · mg^{-1} protein · h^{-1}. Both peaks contained phospholipid with 60 mol phospholipid bound per 300 000 g protein. The low molecular weight particles had a molecular weight of 276 000 as determined by sedimentation equilibrium analysis.

Introduction

One of the important problems in elucidating the properties of the (Na^+ + K^+)-activated ATP hydrolysing enzyme system ((Na^+ + K^+)-ATPase) (ATP phosphohydrolase, EC 3.6.3.1) is the lack of knowledge of the molecular weight of the integral system. [1]. Several attempts have been made to obtain this parameter, such as(Na^+ + Mg^{2+})-dependent phosphorylation, ouabain and ATP binding [1,2]. The working hypothesis has been that one enzyme molecule has one

binding site for each of these labels. Binding site concentrations as high as 3.7—4.0 nmol · mg^{-1} protein have been achieved [2], corresponding to a molecular weight of 250 000—280 000 daltons. Other means of obtaining the molecular weight has been radiation inactivation, yielding a molecular weight of 250 000 daltons [3].

As shown in the present paper, the enzyme has been solubilized in a purified active form in the nonionic detergent octaethyleneglycoldodecyl ether ($C_{12}E_8$), and an attempt has been made to measure the molecular weight by gel filtration and sedimentation equilibrium centrifugation. This detergent has been used for solubilization of the Ca^{2+}-ATPase [4,5], and it has an advantage over other commonly used detergents since its partial specific volume is 0.973 [6], which is very close to that of water. This makes the effect of the detergent bound to the enzyme negligible in the calculation of the molecular weight from sedimentation equilibrium data.

Methods

Preparation of membrane-bound enzyme. ($Na^+ + K^+$)-activated ATPase was purified as previously described [7] from rectal glands of the spiny dogfish (*Squalus Acanthias*) to a specific activity of 1500 μmol ATP hydrolysed · mg^{-1} protein · h^{-1}. The preparation is stable for several months when stored at 4°C.

Solubilization of membrane-bound enzyme. The membrane-bound enzyme was solubilized as follows: To 4 vol. 20 mM histidine, 25% glycerol (pH 7.0), containing 1—3 mg protein · ml^{-1}, was added 1 vol. of detergent ($C_{12}E_8$) * in 20 mM histidine (pH 7.0) to a final concentration of 2 mg detergent · mg^{-1} protein. The solubilization was instantaneous and was carried out at 10—12°C. The amount of enzyme solubilized is taken as the protein in the supernatant after 280 000 X g centrifugation, for 60 min at 10—12°C.

Gel chromatography. A sample of 2—6 ml containing 4—20 mg solubilized protein was gel filtered on Sepharose 4B-Cl. A column (2.5 X 90 cm), equilibrated at 4°C with 20 mM histidine, 0.2 M KCl, 20% v/v glycerol and 150 μg detergent · ml^{-1} (pH 7.0) was eluted at a rate of 9 ml · h^{-1} and 6.25-ml fractions were collected. Dextran Blue and 2-mercaptoethanol were used for measuring the void and total volumes, respectively, and Apoferritin (M_r = 466 000 [8]) was a protein marker. Sephadex G-25 was used for desalting and freeing the Sepharose 4B-Cl fractions from glycerol, which interfered with the subsequent phospholipid determination. A column (2.5 X 20 cm) was equilibrated with 10 mM histidine and 60 μg detergent · ml^{-1} (pH 7.5) and eluted at room temperature at a rate of 1.5—2 ml · min^{-1}. The sample was usually 3 ml of the Sepharose 4B-Cl eluate diluted with 3 ml H_2O in order to decrease the viscosity difference between the sample and the elution medium. The fractions from the G-25 column containing the void volume were used for phospholipid determination. A control experiment employing phosphatidylserine showed all the inorganic phosphate bound in the lipid to elute in the void volume.

* Octaethyleneglycoldodecylether ($C_{12}E_8$) was prepared by Nikko Chemicals, Tokyo, Japan, and was obtained through The Kuoyoh Trading Company, LTD., Tokyo, Japan. Albumin was obtained from Behring Werke AG.

ATPase assay. $Na^+ + K^+$ dependent hydrolysis of ATP was measured as the difference between the rates of production of inorganic phosphate with and without 1 mM ouabain. Other constituents of the assay were 20 mM histidine, 0.2 mM EGTA and Na^+, K^+, Mg^{2+}, albumin, ATP, detergent, and pH as indicated in the legends. Standard conditions were an incubation time of 2 min and the following concentrations: 130 mM Na^+, 20 mM K^+, 6 mM Mg^{2+}, 660 μg albumin · ml^{-1}, 3 mM ATP, 90 μg detergent · ml^{-1}, (pH 6.8) for the solubilized enzyme and 130 mM Na^+, 20 mM K^+, 4 mM Mg^{2+}, 330 μg albumin · ml^{-1}, 3 mM ATP, (pH 7.4) for the membrane-bound enzyme. The reaction was started by the addition of enzyme to the assay medium and was run at 23°C or at 37°C. The reaction was terminated by the addition of 1/10 vol. 50% trichloroacetic acid, and inorganic phosphate was determined by the method of Fiske and Subbarow [9].

p-Nitrophenylphosphatase assay. The K^+-dependent hydrolysis of *p*-nitrophenylphosphate was measured as the difference in the rate of production of *p*-nitrophenol with or without 150 mM KCl in the test medium. Other constituents of the assay were 20 mM histidine, 10 mM *p*-nitrophenylphosphate, 20 mM Mg^{2+}, 0.2 mM EGTA, 330 μg albumin · ml^{-1}, 80 μg detergent · ml^{-1} and pH as indicated in the legends. The assay was performed analogous to the ATPase assay, and the liberated *p*-nitrophenol was measured as the absorbance at 410 nm after the addition of 2 vol. 0.5 M Tris base. ϵ was taken to be 18 100 $M^{-1} \cdot cm^{-1}$.

Protein was measured according to Lowry et al. [10], using bovine serum albumin as a standard. The absorbance at 280 nm was also used as an estimate of enzyme protein. The optical density was 1.096 for protein solubilized in either sodium dodecyl sulphate (SDS) or in octaethyleneglycoldodecyl ether at 1 mg protein · ml^{-1}, based on a Lowry determination as above.

Phospholipid was measured by the wet ashing method of Bartlett [11], using the small volume modification.

SDS-gel electrophoresis was carried out according to the method of Weber and Osborn [12] using 7.5% gels. Staining was according to Fairbanks et al. [13], and the gels were scanned at 555 nm in a Beckman Acta III with scanning equipment, and the aperture set at 0.1 mm. Samples containing K^+ were used without dialysis prior to the addition of SDS as the potassium laurylsulphate precipitate did not interfere with the electrophoresis.

Sedimentation equilibrium analysis. A Beckman model E analytical centrifuge with a photoelectric scanner was used. According to Tanford et al. [6], the slopes of equilibrium plots (log concentration vs. r^2) yield and quantity that is proportional to $M(1 - \phi' \cdot \rho)$, where M is the molecular weight of the protein component of a mixed particle, ϕ' is the effective specific volume per gram of protein, and ρ is the density of the solvent. The contribution of the bound lipid, sugar and detergent can be allowed for by the relation [6]:

$$(1 - \phi' \cdot \rho) = (1 - \overline{v}_P \cdot \rho) + \frac{g_D}{g_P}(1 - \overline{v}_D \cdot \rho) + \frac{g_L}{g_P}(1 - \overline{v}_L \cdot \rho) + \frac{g_S}{g_P}(1 - \overline{v}_S \cdot \rho)$$

where $\overline{v}_P$, $\overline{v}_D$, $\overline{v}_S$ and $\overline{v}_L$ are the partial specific volumes of the protein, the detergent, the sugar and the lipid, respectively, and g_D/g_P, g_S/g_P and g_L/g_P are the weight ratios of detergent, sugar and lipid to protein, respectively.

$\overline{v}_P$ = 0.732 cm^3/g was calculated on the basis of the amino acid composition of the ATPase by the method of Cohn and Edsall [14], taking 65% of the protein to be the 96 000 dalton peptide (α-chain) and 35% to be the 56 000 dalton glycoprotein (β-chain), see Table II. The values were $\overline{v}_\alpha$ = 0.734 and $\overline{v}_\beta$ = 0.728, using the amino acid analyses given by Perrone et al. [15]. $\overline{v}_S$ = 0.628 is based on the carbohydrate composition given Perrone et al. [15] and calculated as given by Gibbons [16]. g_S/g_P = 38 000/300 000 is based on the values given in [15]. The value of $\overline{v}_D$ = 0.973 was taken from Tanford et al. [17]. g_L/g_P = 46 000/300 000 is measured as above [10], assuming all the bound lipid to be phospholipid, see Table III. The value of $\overline{v}_L$ cannot be calculated, as the composition of the bound lipid is not known. Assuming a mixture of lipids as given in [18] we have used a value of $\overline{v}_L$ = 0.975. We cannot measure the amount of detergent bound to the protein, but as the detergent-term together with the lipid-term is much smaller than the $(1 - \overline{v}_P \cdot \rho)$ term, the amount of bound detergent does not play a great role in the calculation of M. We have used a value of g_D/g_P = 50 000/300 000 (see Discussion). By using these parameters we arrive at a value for $(1 - \phi' \cdot \rho)$ of 0.251.

Results

Solubilization of membranes

The membranes were solubilized as described in Methods. Optimal solubilization was achieved with a detergent to protein ratio of 2 (w/w). Increasing this ratio further did not solubilize more protein, nor was the solubilization or the catalytic activities affected by the addition of 1 mM dithiothreitol, 0.2 M KCl or 0.2 M NaCl to the membranes before the addition of detergent.

The supernatant after a 280 000 × g centrifugation usually contained 60—65% of the protein starting material. The initial specific activity of the ATPase increased from about 1500 to about 2300 μmol P_i released · mg^{-1} protein · h^{-1} and the initial specific activity of the p-nitrophenylphosphatase increased from about 210 to about 350 μmol p-nitrophenylphosphate hydrolysed · mg^{-1} protein · h^{-1} (Table I), when the starting material and the supernatant enzyme were compared. This is comparable to the activities obtained with deoxycholate solubilized enzyme [7].

TABLE I

ACTIVITY AND PURITY OF MEMBRANE-BOUND AND SOLUBILIZED ENZYME

At 37°C the specific activity of the membrane-bound enzyme were measured at pH 7.4 and the specific activities of the supernatant enzyme at pH 6.8 at 23°C and 37°C. The specific activities of the supernatant enzyme were all initial specific activities. Test media were as given in the legend to Fig. 1. Purity was estimated as the sum of the areas in the 96 000 dalton and the 56 000 dalton peaks in SDS-gels, taken as percent of the total amount of protein in the gel.

	ATPase (μmol · mg^{-1} · h^{-1})	p-Nitrophenylphosphatase (μmol p-nitrophenol · mg^{-1} · h^{-1})	Purity (%)
Membrane-bound enzyme	1488 ± 20 (n = 5)	208 ± 6 (n = 5)	72
Supernatant enzyme 37°C	2317 ± 63 (n = 5)	352 ± 12 (n = 5)	95—100
Supernatant enzyme 23°C	949 ± 27 (n = 3)	160 ± 6 (n = 3)	
Pellet 37°C	120 ± 23 (n = 3)	60 ± 14 (n = 3)	10—20

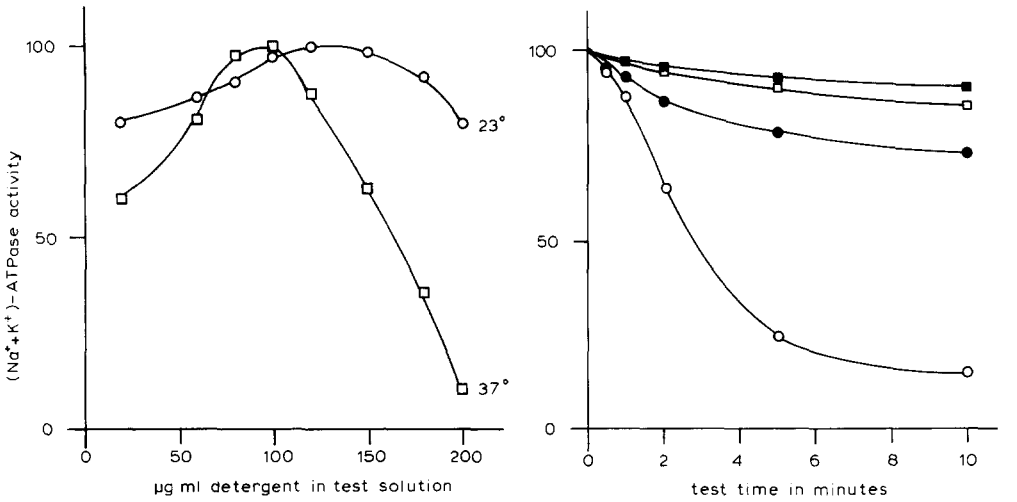

Fig. 1. The effect on the ATPase activity of varying the octaethyleneglycoldodecylether ($C_{12}E_8$) concentration in the test medium at 23° C (○———○) and 37° C (□———□). The specific ATPase activity is given as percent of activity at optimum detergent concentration. The test medium contained buffer and ligands as given in the legend to Fig. 2, and had an albumin concentration of 660 µg detergent · ml^{-1}. The reaction time was 2 min.

Fig. 2. The specific ATPase activity as a function of the test time at 37° C (●, ○) and 23° C (■, □). The activity is given as percent of the initial activity. Test medium was 30 mM histidine, 3 mM ATP, 130 mM Na^+, 20 mM K^+, 4 mM Mg^{2+}, 80 µg detergent · ml^{-1}, 0.2 mM EGTA and 660 µg albumin · ml^{-1} (■, ●) or 330 µg albumin · ml^{-1} (□, ○), pH 7.4. The amount of enzyme protein was 1—6 µg · ml^{-1}.

Activity measurements

The supernatant enzyme was stable for weeks upon storage at 4°C in the detergent solution. However, the catalytic activities were labile in the test-tube, and the lability depended on the detergent and albumin concentration in the test solution and on temperature. Detergent in the test solution is necessary for optimal activity, as is seen in Fig. 1. The optimum is broader at 23° C than at 37°C, and it is at a higher detergent concentration at 23°C. It is also necessary to include albumin in the test solution in order to stabilize the enzyme. Fig. 2 shows the effect of increasing the albumin concentration from 330 µg · ml^{-1} to 660 µg · ml^{-1} at both temperatures. Increasing the albumin concentration above 660 µg · ml^{-1} caused the specific activity to drop. The stabilizing effect of albumin is probably dual, since it is known both to increase the specific activity of the membrane-bound enzyme [7] and to bind detergents [19]. The marked effect of albumin on the supernatant enzyme probably stems from this last effect, it seems to act as a kind of buffer on the detergent. Optimal conditions were 660 µg albumin · ml^{-1} and 80—110 µg detergent · ml^{-1} in the incubation medium at 37°C. Incubation times as short as 15 s were used to extrapolate the curve showing the specific activity as a function of incubation time to zero, Fig. 2. This value represents the initial specific activity.

By lowering the temperature to 23°C it is possible to work with the enzyme under conditions where small variations in the test time and the detergent concentration are not crucial. The specific ATPase activity is a factor 2.5 lower at 23°C than at 37°C, Table I, as is the specific activity of the membrane-bound

enzyme (not shown). The *p*-nitrophenylphosphatase activity showed the same dependence on detergent, albumin and temperature as the ATPase activity (not shown).

pH optimum

The pH optimum for ATPase activity changed from pH 7.4 for the membrane-bound enzyme to pH 6.8 for the solubilized enzyme. The solubilized enzyme was equally stable in the test tube at both pH. The shift in pH optimum is clearly of importance for obtaining the maximal specific activity, but also for the Na^+ concentration necessary for half-maximal activation of the enzyme [20]. The ATPase and *p*-nitrophenylphosphatase activities of the solubilized enzyme showed the same characteristic dependence on Na^+ and K^+ concentrations as the membrane-bound enzyme [1].

Protein composition

The increase in specific activity of the supernatant enzyme is parallelled by an increase in the percentage of two proteins seen on SDS-gels, namely from 47% to 64% for the 96 000 dalton peptide (α-chain) and from 25% to 32% for the 56 000 dalton peptide (β-chain), Fig. 3. Table II shows the results of scanning the Coomassie Blue stained gels; there is an increase in purity of the preparation from 72% to 95—100% taken as the amount of $\alpha + \beta$ chain in the preparation. The ratio α/β remained constant during purification.

Pellet

The pellet after clearing the solubilized membranes had very low specific activities of both the ATPase and the *p*-nitrophenylphosphatase, Table I. It also contained the impurities from the starting material, Fig. 3.

Gel filtration of solubilized ATPase

The elution profile of solubilized enzyme gel filtered on a column of Sepharose 4B-CL is shown in Fig. 4. A main peak with a shoulder (peak I), a smaller peak II, and a small peak at the void volume is seen, when the absorbance at 280 nm of the fractions is recorded. Peak I contains the majority of the gel filtered material and elutes at a position with $K_{av} = 0.54$. Peak II is a

TABLE II

PROTEIN COMPOSITION OF MEMBRANE-BOUND AND SOLUBILIZED ENZYME

The relative amount of the 96 000 dalton peptide and the 56 000 dalton peptide was determined by scanning the SDS-gels as described in Methods and shown in Fig. 3. The mean ± S.E. ($n = 8$) are given in percent of total protein in the gel.

	96 000 dalton peptide in percent of total	56 000 dalton peptide in percent of total	Other proteins in percent of total
Membrane-bound enzyme	47 ± 1	25 ± 2	28
Supernatant enzyme	64 ± 2	32 ± 2	0—5
Peak I protein	65 ± 1	35 ± 2	0
Peak II protein	65 ± 1	35 ± 2	0

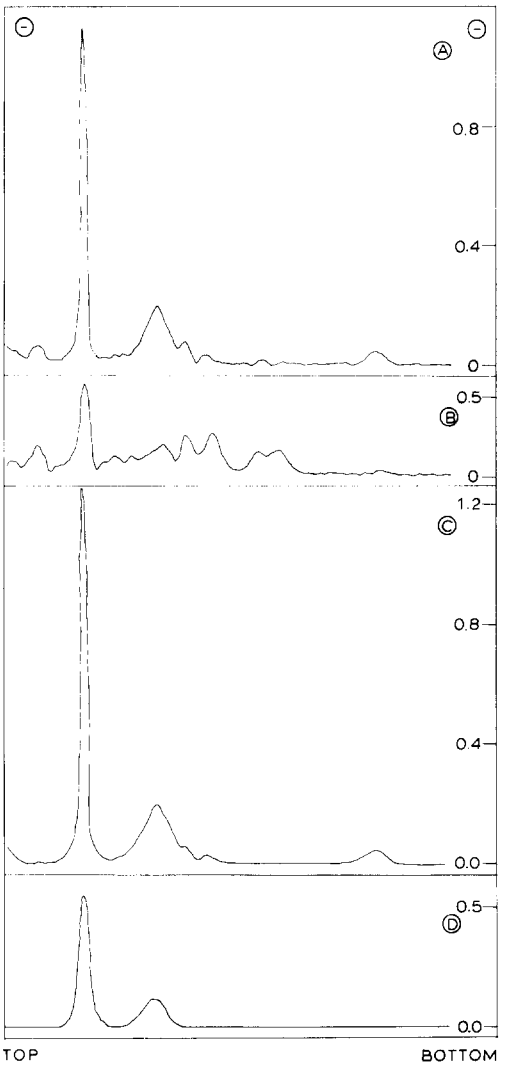

Fig. 3. Scans of SDS-gels with the enzyme at different stages of purification. The gels were stained with Coomassie Blue and scanned at 555 nm. The areas under the peaks were found to be proportional to the protein concentration from 0.5 to 4 μg protein per gel (not shown). The gels are A: membrane-bound enzyme, B: Pellet after solubilization in detergent and centrifugation, C: Supernatant enzyme, and D: Peak I protein after gel filtration in Sepharose 4B-CL.

rather broad peak with $K_{av} = 0.25$, which indicates a larger particle size than the peak I material. Both peaks contained protein with ATPase and *p*-nitrophenylphosphatase activity. The specific activity of the protein eluting at the positions indicated with arrows are given in Table III. Both the peak I and II proteins were stable in solution at 4°C, and could be stabilized under assay conditions, as could the starting material as described above. The gel filtration was conducted at 0.2 M KCl. The effect of substituting 0.2 M NaCl for 0.2 M KCl was investigated employing a Sepharose 6B column (2.5 X 35 cm), and no difference in the elution profiles was found, nor was the position of the peaks

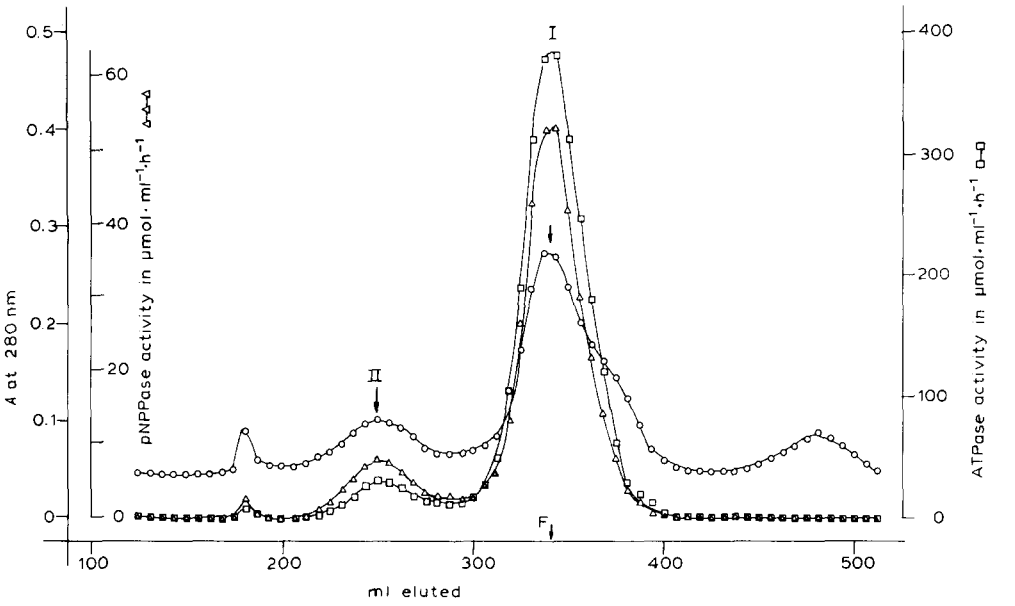

Fig. 4. Gel filtration of the supernatant enzyme in Sepharose 4B-CL. The column was equilibrated and run as described in Methods. The figure shows a typical elution profile when 14 mg protein in a volume of 10 ml is chromatographed. The absorbance at 280 nm (○———○): Activity of ATPase (□———□) and *p*-nitrophenylphosphatase (pNPPase) (△———△) are given in μmol · ml^{-1} · h^{-1}. Conditions for measuring the catalytic activities were as given in Methods. Peak I elutes at a K_{av} = 0.54, and peak II has a K_{av} = 0.25. The elution position of Apoferritin, M_r = 466 000, is indicated (F).

affected by the presence of 1 mM dithiothreitol. SDS-gels revealed that both peaks I and II are composed of α and β chains, and that these two peptides are present in the same relative amount in both peaks (65/35 (w/w), Table II). No other proteins were detectable in these peaks.

The elution profile was similar when membrane-bound enzyme treated with detergent was gel filtered in Sepharose 4B-CL without prior clarification by centrifugation. However, the amount of material in the void volume was larger and so was the amount of material in peak II.

Phospholipid determination

The amount of phospholipid present in peak I and II was determined after removal of glycerol and salts on a Sephadex G-25 column. Assuming all the phosphorus is bound in phospholipids and using an average molecular weight of 775, a total of about 60 mol phospholipid per 300 000 g of protein was measured both on peak I and II (Table III). It should be noted that the phospholipid of peak I was measured on fractions eluting immediately before the position indicated with an arrow. In the following fractions the phospholipid/protein ratio increased, probably indicating the elution of mixed phospholipid-detergent micelles.

Sedimentation equilibrium studies

Fractions from peak I eluting immediately before the position indicated with

TABLE III

SPECIFIC ACTIVITIES AND PHOSPHOLIPID CONTENT OF GEL FILTERED SOLUBILIZED ENZYME

Activity of gel filtered enzyme was measured at 37°C, pH 6.8 and shown as initial specific activities. Test media were as given in the legend to Fig. 1. Values are given as the mean ± S.E. Phospholipid was determined as in Methods.

	ATPase (μmol · mg^{-1} · h^{-1})	*p*-Nitrophenylphosphatase (μmol *p*-nitrophenol · mg^{-1} · h^{-1})	K_{av} (n = 5)	mol phospholipid per 300 000 g protein
Peak I	2095 ± 44 (n = 4)	290 ± 12 (n = 4)	0.54 ± 0.01	60 ± 6 (n = 4)
Peak II	603 ± 86 (n = 3)	157 ± 11 (n = 3)	0.25 ± 0.02	65 ± 8 (n = 4)

an arrow were centrifuged at 9000 and 10 000 rev./min at 14°C in a short-channel equilibrium centerpiece. The results were identical, yielding a straight-line relationship between log C and r^2, and the enzyme retained 80% of its catalytic activities after centrifugation. Using the relation and values given in Methods we arrived at a molecular weight of 276 000 ± 2500 S.E. (n = 4) for the protein part of the particle. The molecular weight of the whole particle including bound lipid and carbohydrate, but omitting detergent, can then be calculated to be approx. 360 000. This is in reasonable agreement with the K_{av} = 0.54 obtained from gel filtration, which corresponds to a particle molecular weight of about 500 000, including the detergent.

We have not accounted for the water bound to the particle. The effect is probably small; determinations of the amount of water bound to proteins have given values in the range of 0.1—0.5 g H_2O bound per g protein [21]. Including as an assumption 0.4 g water/g protein in calculations, the molecular weight would increase from 276 000 to 307 000. Experimentally, the effect of bound water might be overcome by omitting glycerol from the sample, but this has not been possible.

Discussion

The ($Na^+ + K^+$)-ATPase can be dissolved in an active form in octaethyleneglycoldodecylether with a specific activity of about 2300 μmol P_i · mg^{-1} · protein · h^{-1} at 37°C and with a protein purity of 65% for the α-chain and 95% for $\alpha + \beta$ chains, which is equal to the activity and purity of purified membrane preparations (see Ref. 2). The enzyme is stable in solution at 4°C, but unstable in the test medium. This can be overcome by appropriate adjustments of detergent and albumin concentrations, and by lowering the incubation temperature to 23°C.

Gel filtration of the solubilized enzyme in Sepharose 4B-CL shows two populations of particles with K_{av} of 0.54 and 0.25, respectively. The smallest particle with K_{av} = 0.54 has the highest specific activity, 2100 μmol P_i · mg^{-1} protein · h^{-1} at 37°C. By sedimentation equilibrium centrifugation of these particles a linear equilibrium plot is obtained. It seems therefore likely that they represent a complex of molecules, which in the membrane are closely associated; they contain the catalytic part of the ($Na^+ + K^+$)-ATPase.

A problem in the calculation of the molecular weight of the protein part of the particles from the equilibrium plot is that the partial specific volumes of the components of the particle are unknown. In the present experiments the partial specific volumes of the protein and carbohydrate parts have been calculated from the composition of the α and β chains given by Perrone et al. [15] and from the weight ratio of the two components as obtained from gel electrophoresis. For the lipid component, it is assumed that all the phosphate in the particles stems from the phospholipids, and it has not been taken into account that the particles besides the phospholipid may contain other lipids, such as cholesterol. The composition of the bound lipid is not known. Finally it is unknown how many detergent and lipid molecules there are bound in the particles. However, in the calculation of the molecular weight the lipid and detergent terms are of very small importance, since the partial specific volumes are such that these terms become negligible compared to the term containing the partial specific volume of the protein $(1 - \overline{v}_P \cdot \rho)$. The amount of water bound is unknown and is therefore an important uncertainty in the calculatons. It is unlikely, however, that our conclusions will be altered by more precise data on this point.

With these assumptions the molecular weight of the protein part of the particles can be calculated to be 276 000. This is of the same size as the 250 000 daltons obtained from radiation inactivation experiments [3], and also as the values of 250 000—280 000 daltons calculated from studies on ^{32}P, ouabain and ATP binding (see Refs. 1, 2); in the binding studies it is assumed that there is one binding site per ATPase molecule.

The α and β chains occur in equimolar concentrations as judged from SDS gels using the molecular weights estimated by SDS gel electrophoresis, suggesting that the particles contain $2\alpha + 2\beta$ chains. The molecular weight of the β chain estimated this way is probably too high due to abnormal SDS binding for glycoproteins [22]; a value of 35 000 [23] more likely to be correct. This would lead to a molar ratio of 2α chains per 3β chains, and the particles in peak I would thus be composed of 2α chains + 3β chains. This is being investigated further.

Are the particles, with a molecular weight of 276 000 plus the bound lipid, the transport system for Na^+ and K^+?

The high specific activity of the particles show that they contains all the components necessary for catalytic activity, and it is therefore tempting to assume that they are the transport system. However, catalytic activity does not necessarily mean that they contain all the components necessary for the transport of cations. On the other hand, there are, at present, no means of specifically labelling more than one of the 2α-chains and none of the β-chains in the particle, and it is therefore impossible to exclude the possibility that some of the polypeptides may not be part of the system, but, rather, are located in the membrane and have such solubility properties that they follow the ATPase molecule in purification and solubilization. More information about the relationship between the protein and lipid component of this particle and the transport properties of the system is therefore needed before a final answer can be given.

References

1 Skou, J.C. (1975) Q. Rev. Biophys. 7, 401—434
2 Jørgensen, P.L. (1975) Q. Rev. Biophys. 7, 239—274
3 Kepner, G.R. and Macey, R.I. (1968) Biochim. Biophys. Acta 163, 188
4 Le Maire, M., Møller, J.V. and Tanford, C. (1976) Biochemistry 15, 2336—2342
5 Dean, W.L. and Tanford, C. (1977) J. Biol. Chem. 252, 3551—3553
6 Tanford, C., Nozaki, Y., Reynolds, J.A. and Makino, S. (1974) Biochemistry 13, 2369—2375
7 Skou, J.C. and Esmann, M. (1979) Biochim. Biochim. Acta 567, 436—444
8 Rothen, J. (1944) J. Biol. Chem. 152, 679
9 Fiske, C.H. and Subbarow, Y. (1925) J. Biol. Chem. 66, 375—400
10 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
11 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466—468
12 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406—4412
13 Fairbanks, G., Steck, T.L. and Wallach, D.F.H. (1971) Biochemistry 10, 2606—2617
14 Cohn, E.J. and Edsall, J.T., (1943) in Proteins, Amino Acids and Peptides, pp. 371—381, Rheinhold New York, NY
15 Perrone, J.R., Hackney, J.F., Dixon, J.F. and Hokin, L.E. (1975) J. Biol. Chem. 250, 4178--4184
16 Gibbons, R. (1966) Glycoproteins (Gottschalk, A., ed.), pp. 29—90, Elsevier Amsterdam
17 Tanford, C., Nozaki, Y. and Rohde, M.F. (1977) J. Phys. Chem. 81, 1555—1560
18 MacLennan, D.H., Seeman, P., Iles, G.H. and Yip, C.C. (1971) J. Biol. Chem. 246, 2702—2710
19 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29—79
20 Skou, J.C. (1979) Biochim. Biophys. Acta 567, 421—435
21 Timasheff, S.N., Lee, J.C., Pittz, E.P. and Tweedy, N. (1976) J. Colloid Interface Sci. 55, 658—663
22 Bretscher, M. (1971) Nat. New Biol. 231, 229—232
23 Kyte, J. (1972) J. Biol. Chem. 247, 7642—7649