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HYDRODYNAMIC PROPERTIES OF SOLUBILIZED ($Na^+ + K^+$)-ATPase FROM RECTAL GLANDS OF *SQUALUS ACANTHIAS*

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Summary

(1) ($Na^+ + K^+$)-ATPase from the rectal glands of *Squalus acanthias*, solubilized in octaethylene glycol dodecyl monoether (2 mg detergent/mg protein), retains its activity for days when stored at 0°C both with and without 20% glycerol. Glycerol protects partially against inactivation at higher temperatures.

(2) Solubilization leads to a decrease in the amount of lipids bound per mg protein. 50 mol phospholipids and 40 mol cholesterol are bound per 265 000 g protein. 90% of the phospholipid is phosphatidylcholine (72%) and phosphatidylethanolamine (18%) and there is about 1 mol acidic phospholipid per 265 000 g. In addition, the protein has about 27 000 g carbohydrate as hexose, hexosamine and sialic acid bound per 265 000 g.

(3) The calculation of the molecular weight from an ln C vs. r^2 plot obtained by sedimentation equilibrium centrifugation in the presence of 560 μM detergent gives a molecular weight of the protein part of the active solubilized enzyme of 265 000 using the measured values for bound detergent, lipid (phospholipid + cholesterol) and carbohydrate. The sedimentation coefficient ($s_{20,w}$) is 10.1 S, giving a Stokes' radius of 77 Å.

(4) An increase in detergent concentration to 56 mM dissociates the 10.1 S particle into particles with a sedimentation coefficient of 5.8 S and a molecular weight of 139 000 (Stokes' radius, 66 Å). In the presence of this detergent concentration the enzyme is inactive.

(5) The molecular weights of the sodium dodecyl sulphate-solubilized, isolated α- and β-chains are found to be 106 000 and 40 000, respectively.

Abbreviations: $C_{12}E_8$, octaethylene glycol dodecyl monoether; EGTA, ethylene glycol bis(β-aminoethyl) ether)-*N*,*N*'-tetracetic acid; α and β, the catalytic subunit and the glycoprotein, respectively.

(6) It is concluded that the active solubilized enzyme is an $(\alpha\beta)_2$ structure and that it dissociates into an enzymatically inactive $\alpha\beta$ structure when the detergent-to-protein ratio is increased.

Introduction

In a previous paper, it was shown that the $(Na^+ + K^+)$-ATPase (EC 3.6.3.1) isolated from the rectal glands of *Squalus acanthias* can be solubilized in a fully active form in octaethylene glycol dodecyl monoether ($C_{12}E_8$) and that gel filtration gives a homogenous population of particles [1]. The molecular weight of the protein of the purified particles as measured by sedimentation equilibrium analysis is 270 000. This differs from the value obtained by Hastings and Reynolds [2] (380 000) of a Lubrol-dissolved $(Na^+ + K^+)$-ATPase from the rectal glands of another shark species, *Carcharhinus obscurus* [2].

The calculation of the value of 270 000 daltons from the equilibrium plots was undertaken with some assumptions necessitated by (a) the presence of 20% glycerol in the buffer used and (b) the lack of precise values for the amount of detergent, lipid and carbohydrate bound in the particles.

In the present paper, the bound detergent, lipid and carbohydrate have been measured and it has been possible to transfer the solubilized enzyme in an active form from a glycerol to a glycerol-free buffer and thereby to perform the sedimentation studies without glycerol present. This has been carried out in order to obtained a more precise value for the molecular weight and also to see whether this can eliminate the discrepancies between the previously reported values for the molecular weight [1,2].

The molecular weights of the solubilized fully active enzyme complex, of an inactive complex observed at very high $C_{12}E_8$ concentrations and of the sodium dodecyl sulphate (SDS)-solubilized isolated α- and β-chains have been determined.

From sedimentation data combined with the molecular weight and data on detergent binding to the enzyme and the lipid and carbohydrate composition, the Stokes' radius (R_S) and the minimal radius (R_{min}) have been calculated in order to obtain information about the overall shape of the molecule.

Materials and Methods

$C_{12}E_8$ is obtained from Nikko Chemicals, Tokyo, and the ^{14}C-labelled derivative (specific activity 46 mCi/mmol) from CEA, France. $(Na^+ + K^+)$-ATPase is prepared from the rectal glands of *S. acanthias* as previously described [3], omitting saponin in the deoxycholate treatment step and omitting the 38 000 $\times g$ centrifugation in the differential centrifugation. The resultant membrane-bound enzyme has a specific activity of 1514 ± 55 μmol $P_i \cdot mg^{-1} \cdot h^{-1}$ (S.E., $n = 4$).

Membrane-bound enzyme is solubilized in the presence of 2.74 M glycerol (20%, w/v) with 2 mg $C_{12}E_8$ per mg protein. The solubilized enzyme is purified by gel filtration in Sepharose 6B or CL-4B in the presence of 560 μM (0.3 mg/ml) $C_{12}E_8$ and 2.74 M glycerol as described in Ref. 1). Filtration in Sephadex

G-25 is used for removal of glycerol from the solubilized enzyme. The specific ATPase activity of the solubilized, purified enzyme is 2158 ± 49 μmol $P_i \cdot mg^{-1} \cdot h^{-1}$ at 37°C (S.E., $n = 3$) and 910 ± 18 μmol $P_i \cdot mg^{-1} \cdot h^{-1}$ at 23°C (see Ref. 1).

Protein determinations, ATPase assays and polyacrylamide gel electrophoresis in the presence of SDS are performed as described in Ref. 1).

The α- and β-subunits are isolated after removal of $C_{12}E_8$ with BioBeads (Bio-Rad); solubilization in SDS and gel filtration in Sepharose 6B are as described by Kyte [4].

For lipid analysis, the enzyme preparations are extracted for 30 min at 60°C is $CHCl_3/CH_3OH/H_2O$ (8:4:3, v,v). The extract is dialysed against tap water for 5 days and after evaporation the dry residue is extracted seven times and fractionated on DEAE-cellulose as described elsewhere [5]. The two fractions obtained (acidic and non-acidic lipids) are analysed by thin-layer chromatography on HPTLC plates (Merck) using actual reference samples and anisaldehyde or copper acetate for detection [5]. Quantitation is performed by densitometry of thin-layer chromatograms (copper acetate) with a Zeiss KM 3 scanner. Phosphatidylcholine and sulphatide are used as standards. Total P_i is determined by using the procedure of Bartlett [6]. Hexose is measured by the anthrone method [7], sialic acid by the thiobarbituric acid method [8] and hexosamine after 4—12 h of hydrolysis in 4 M HCl at 100°C by using the method described in Ref. 9.

The specific radioactivities of the detergents used in the binding experiments are about 2000 cpm/mg, and are identical both in the solubilization step and in the gel-filtration steps.

The viscosity of the solution used for sedimentation velocity experiments (histidine, 30 mM, pH 7.0; KCl 200 mM; $C_{12}E_8$, 560 μM) is measured in a Ubbelhode viscosimeter and is found to be 0.998 relative to the viscosity of water ($T = 293$ K). The relative viscosity of a 200 mM KCl solution is 0.998 (calculation from standard tables) and 560 μM $C_{12}E_8$ thus does not contribute to the viscosity.

A Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanner is used for sedimentation equilibrium and sedimentation velocity experiments. Sedimentation equilibrium measurements are made with a multichannel centerpiece and the centrifugations are at 9000—15 000 rev./min. The scanning is at 280 nm, and the temperature 287 K when measuring the $(\alpha\beta)_2$ and $\alpha\beta$ particles, and 295 K when the SDS-solubilized α- and β-subunits are measured. Sedimentation velocity experiments are performed in the Model E or in an MSE Centriscan ultracentrifuge, and rotor speeds are 44 000 or 48 000 rev./min at 287 K.

Results

Detergent binding

The method of gel filtration has been used as described in Ref. 10) for measuring the amount of detergent bound to the protein.

Fig. 1 shows the elution profile of the $C_{12}E_8$-solubilized protein gel filtered in Sepharose 6B. As can be seen from the figure, the detergent binding is

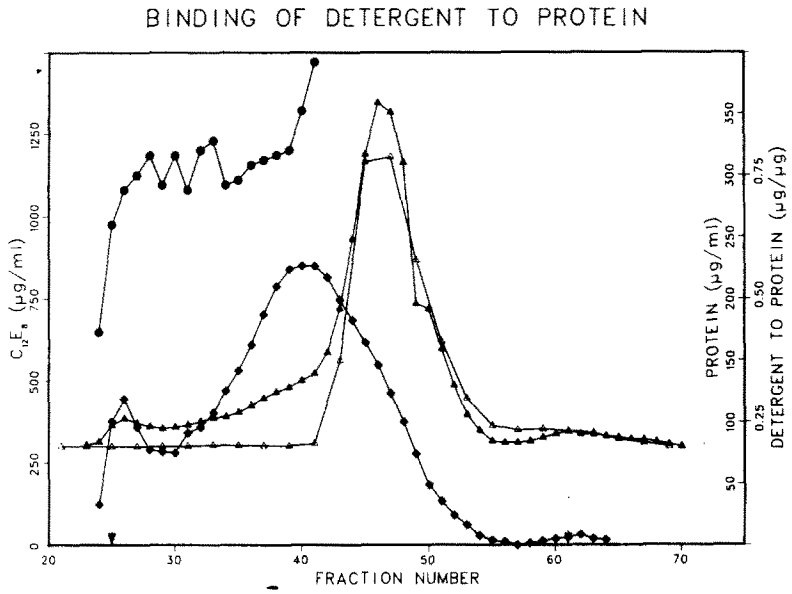

Fig. 1. Determination of the amount of detergent bound to the $(Na^+ + K^+)$-ATPase. A Sepharose 6B column is equilibrated with 30 mM histidine, pH 7.0 200 mM KCl and with 560 µM $[^{14}C]C_{12}E_8$ at 4°C. ATPase protein (9.5 mg) dissolved in 4 ml column buffer with 29 mg $[^{14}C]C_{12}E_8$ (spec. act. 2250 cpm/mg $C_{12}E_8$) is filtered through the column and 2.5 ml fractions are collected. The protein concentration is determined by the absorbance at 280 nm (♦———♦) and the concentration of $C_{12}E_8$ (▲———▲) is measured by radioactivity. In the following experiment, 29 mg $[^{14}C]C_{12}E_8$ in 4 ml column buffer are filtered through the column and the detergent concentration determined (△———△). The excess detergent in fractions 28—40 is only present in the experiments where the ATPase protein is included and represents the amount of detergent bound to the protein (●———●). The arrow to the left indicates the void volume and to the right the total volume.

constant over a fairly large range of protein concentrations. The amount of detergent bound to the protein (the detergent : protein ratio) has been determined with 560 µM $C_{12}E_8$ present with and without glycerol and is found to be 0.77 g/g (Table I). The detergent : protein ratio is not affected by glycerol.

The protein peak shown in Fig. 1 is asymmetric. The absorption at 277 nm relative to that at 295 nm is, in fractions from the ascending side, 3.26 (e.g., No. 35) while for fractions from the descending side it is 2.85 (e.g., No. 45). The weight ratio between the α- and β-subunits (estimated from SDS-polyacrylamide gel electrophoresis) is the same in all fractions across the protein peak, which would indicate that the absorption at 277 nm relative to that at 295 nm should be the same in all fractions. The difference in the absorption ratio is due to light scattering from lipid and detergent micelles eluting on the descending side of the protein peak, which means that the shoulder in the protein peak is due to an overestimation of the amount of protein.

$[^{35}S]$SDS binding to the α- and β-subunits is determined by the same method, and the results are given in Table I.

Lipid and carbohydrate composition

Table II shows the phospholipid composition of the rectal gland, of the purified membrane-bound enzyme and of the $C_{12}E_8$-solubilized purified

TABLE I

MOLECULAR WEIGHTS OF ACTIVE ($Na^+ + K^+$)-ATPase AND OF THE α, β AND $\alpha\beta$ SUBUNITS

$\bar{V}$, partial specific volume; δ, amount of substance bound to the protein (g/g); ρ, solvent density; $(1 - \phi\rho)$, buoyant density factor; $\bar{V}_{lipid}$, calculated from Table II, including cholesterol.

	SDS		$C_{12}E_8$		
	α (I)	β (II)	$\alpha\beta$ (III)	$(\alpha\beta)_2$ (IV)	$(\alpha\beta)_2$ [a] (V)
Detergent (mg/ml)	2	2	30	0.3	0.3
$\bar{V}_{protein}$	0.734	0.728	0.732	0.732	0.732
$\bar{V}_{lipid}$	—	—	0.968	0.968	0.968
$\bar{V}_{sugar}$	—	0.649 [c]	0.643	0.643	0.643
$\bar{V}_{detergent}$	0.864	0.864	0.973	0.973	0.973
δ_{lipid}	—	—	0.190 [b]	0.190	0.190
δ_{sugar}	—	0.259 [c]	0.105 [b]	0.105	0.105
$\delta_{detergent}$	0.539	0.794	0.767 [b]	0.767	0.737
$\rho_{solvent}$	1.004	1.004	1.011	1.008	1.064
$(1 - \phi\rho)$	0.3345	0.4661	0.3132	0.3184	0.2226
$M(1 - \phi\rho)$	35 579 ± 2100 [d]	18 489 ± 1560	43 195 ± 2600	84 416 ± 7400	57 136 ± 3600
M_r	106 363	39 665	138 000	265 100	257 000

[a] In the presence of 2.74 M glycerol.

[b] Assuming the same composition as $(\alpha\beta)_2$.

[c] Assuming all hexosamine is bound to β.

[d] Four independent determinations of α, β and $\alpha\beta$, and eight of $(\alpha\beta)_2$.

TABLE II

LIPID COMPOSITION OF THE RECTAL GLAND, OF THE PURIFIED MEMBRANE-BOUND ENZYME AND OF $C_{12}E_8$-SOLUBILIZED GEL-FILTERED ($Na^+ + K^+$)-ATPase

Values are given as per cent of total lipid omitting cholesterol and as μg lipid per mg protein. The number of moles lipid per mole of solubilized enzyme is calculated to the nearest integer using a molecular weight of 265 000.

	Gland * (%)	Membrane enzyme		Solubilized enzyme		
		(μg/mg)	%	μg/mg	%	mol/mol
Phosphatidylethanolamine	35	147	36	24	18	9
Phosphatidylcholine	43	225	55	94	72	36
Sphingomyelin	5	13	3	4	3	1
Phosphatidylinositol + phosphatidyl serine	9	7	2	2	2	1
Sulphatides	2	12	3	3	2	1
Ganglioside	1	5	1	3	2	1
Cholesterol		>200		60		42

* From Ref. 5.

enzyme. The phospholipid composition of the membrane-bound enzyme is identical to that reported by Perrone et al. [11] for a Lubrol-purified membrane-bound enzyme. The amount of phospholipid and cholesterol per mol enzyme drops upon solubilization. The phospholipid content decreases from about 160 to about 50 mol phospholipid/mol enzyme (M_r 265 000, see below). The relative amount of phosphatidylcholine increases (from 45 to 72%) with a concomitant drop in the amount of phosphatidylethanolamine(from 36 to 18%). The cholesterol content decreases to about 40 mol per mol enzyme.

It is noteworthy that about 1 mol or less of acidic phospholipid per mol enzyme is necessary for activity in the solubilized enzyme. No deoxycholate (used in the preparation of the mebrane-bound enzyme [3]) was detected in the solubilized purified enzyme.

Table III shows the carbohydrate composition of the membrane-bound enzyme and of the $C_{12}E_8$-solubilized enzyme. The amount of sialic acid in the membrane-bound enzyme is about 70 nmol per mg protein (28 mol per mol enzyme), in agreement with the data reported by Perrone et al. [11]. However, as the membrane-bound enzyme is solubilized and purified, the amount of sialic acid per mg drops to about 20 nmol per mg protein (5 mol per mol enzyme). This indicates the presence of glycolipids containing sialic acid in the

TABLE III

CARBOHYDRATE CONTENT OF ($Na^+ + K^+$)-ATPase PREPARATIONS (SEE METHODS)

Values are given as nmol per mg protein. S.E. values are between 4 and 10%, $n = 3$.

	Hexose	Sialic acid	Hexosamine
Membrane-bound	237	69	
$C_{12}E_8$-purified	189	20	364
α-subunit	<20	<10	
β-subunit	333	45	1040 *

* Assuming all hexosamine to be bound in β-subunit.

membranes which are not present in the $C_{12}E_8$-solubilized purified enzyme. In accordance with this, most of the sialic acid present in the membrane-bound enzyme elutes with the phospholipids after gel filtration in the presence of SDS (not shown). This is in agreement with the data reported by Marshall [12] where it is shown that about 70% of the sialic acid in the membranes can be removed by lipid extraction. The values for the neutral carbohydrate content of the membrane enzyme as well as that of the solubilized enzyme are lower than the data reported by Perrone et al. [11] whereas the value for hexosamine is higher.

The amount of sialic acid as well as neutral carbohydrate in the α-subunit is less than 10 nmol/mg protein, which is less than 1 mol/mol subunit (M_r 106 000). The values are of the same order of magnitude as the detection limit. These low values suggest that the α-subunit of the rectal gland enzyme is not a glycoprotein, in contrast to the α-subunit of the eel electroplax enzyme [13], which contains about 16 mol/mol α-subunit.

Enzyme stability

In the previous experiments on the molecular weight determination, the enzyme was kept in 2.74 M glycerol [1]. When the glycerol is removed by

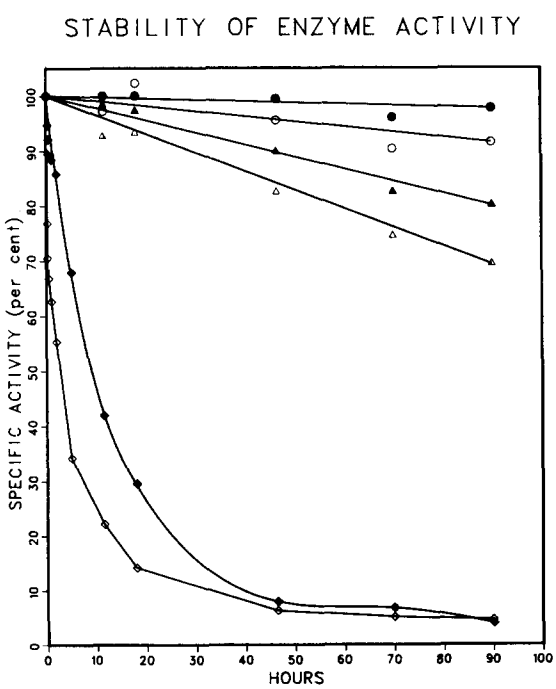

Fig. 2. The effect of temperature and of glycerol on stability of solubilized gel-filtered ATPase. Samples of enzyme (0.3 mg/ml) are incubated at 0°C (●,○), 23°C (▲,△) and 37°C (■,□) in a buffer containing 30 mM histidine, pH 7.0, 100 mM KCl, 560 μM $C_{12}E_8$ without glycerol (open symbols) and with 2.74 M glycerol (filled symbols). After the indicated times of incubation, the activity is tested in a solution containing 30 mM histidine, pH 7.1, 30 mM Tris-HCl, 130 mM NaCl, 20 mM KCl, 4 mM $MgCl_2$, 3 mM ATP, 0.2 mM EGTA, 0.66 mg/ml albumin and 0.1 mg/ml $C_{12}E_8$ (185 μM). Test time is 3 min at 23°C. Activity of control enzyme (100%) is 920 μmol/mg per h for the ATPase and 140 μmol/mg per h for the K^+-stimulated *p*-nitrophenylphosphatase at 23°C.

Sephadex gel filtration in the presence of 280 μM $C_{12}E_8$, the activity is retained but the sedimentation equilibrium plots become non-linear, indicating some sort of aggregation. An increase in the $C_{12}E_8$ concentration to 560 μM prevents, however, the aggregation in the absence of glycerol and is therefore used in the present experiments.

The stability of the solubilized enzyme at different temperatures without and with 2.74 M glycerol in the medium is shown in Fig. 3. At 0°C, more than 90% of the initial activity is retained for at least 4 days both with and without glycerol in the medium and at a detergent concentration of 560 μM (0.3 mg protein/ml). There is a slight decrease when the enzyme is stored at room temperature, whereas the activity decreases rapidly at 37°C: $t_{1/2}$ is 2—4 h. Glycerol gives a certain protection against inactivation at the higher temperatures (cf. Fig. 2).

The sedimentation equilibrium experiments are carried out at 14°C and last for 48—72 h. The ATPase activity after the centrifugation, measured at 37°C with albumin and 185 μM $C_{12}E_8$ in the test solution, is about 1950 μmol/mg protein per h (2150 before the centrifugation), i.e. the enzyme retains more than 85% of its activity during the centrifugation.

Removal of albumin from the test solution and an increase in $C_{12}E_8$ to 560 μM (the conditions of the centrifugation) have no effect on activity when

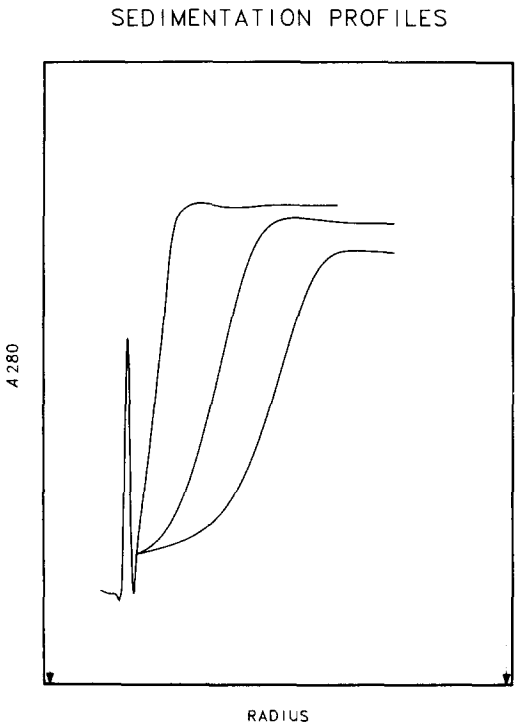

Fig. 3. Sedimentation profiles. Profiles of the sedimenting boundary of (Na^+ + K^+)-ATPase obtained at 9, 29 and 49 min after reaching a rotor speed of 48 000 rev./min. The temperature is 14°C. Arrows indicate the top and the bottom of the centrifuge cell and sedimentation is from left to right. The solvent for the experiment is 30 mM histidine, pH 7.0, 200 mM KCl, 560 μM $C_{12}E_8$. Glycerol is not included in the solvent.

TABLE IV

SEDIMENTATION COEFFICIENTS AND STOKES' RADII OF THE SOLUBILIZED ACTIVE ($Na^+ + K^+$)-ATPase AND OF THE $\alpha\beta$ SUBUNIT

	$\alpha\beta$	$(\alpha\beta)_2$
$s_{20,w}$	5.75 ± 0.27 (4)	10.1 ± 0.42 (7)
R_S (Å)	66.4	77.4
R_{min} (Å)	45.6	56.6
R_S/R_{min}	1.46	1.37

measured at 14°C, i.e., the enzyme is in its active form during the centrifugation. The enzyme loses activity as a function of time in the test solution at 37°C if tested with 560 μM $C_{12}E_8$ and no albumin (see Ref. 1).

Sedimentation velocity

Sedimentation velocity experiments are performed on the solubilized enzyme, gel-filtered in Sepharose CL-4B in the presence of 2.74 M glycerol and 560 μM $C_{12}E_8$ and the samples are freed from glycerol as described in Methods (see also Ref. 1 for details). It is concluded from the sedimentation profiles shown in Fig. 3 that the particles in the presence of 560 μM $C_{12}E_8$ sediment as a homogenous population with a sedimentation coefficient ($s_{20,w}$) of 10.1 S (Table IV). The S value is found to be independent of protein concentration in the range of 0.05—0.6 mg/ml.

However, an increase in the $C_{12}E_8$ concentration from 560 μM to 56 mM (and a constant protein concentration of 0.3 mg/ml) changes the sedimentation pattern drastically. At intermediate $C_{12}E_8$ concentrations the sedimentation profiles display a mixture of two components with sedimentation coefficients of about 10 and 6 S. The 10.1 S component is virtually absent with 56 mM $C_{12}E_8$ and the sedimentation pattern show a homogeneous population of particles with an S value of 5.8 S (Table IV). This dissociation of the 10 S component leads to loss of activity.

The sedimentation profiles of the isolated α- and β-subunits in the presence of SDS showed the particle population to be homogenous with sedimentation coefficients ($s_{20,w}$) of 4.2 and 3.0 S, respectively.

Sedimentation equilibrium

α- and β-subunits. From the linear ln C vs. r^2 plot, the molecular weight of the α-subunit is calculated to be 106 000 and the β-subunit 40 000 (see Table I). These values agree closely with the values of 106 000 and 36 000, respectively, obtained by Hastings and Reynolds [2].

High $C_{12}E_8$ concentration (solubilized inactive enzyme). The molecular weight of the subunit seen in the presence of 56 mM $C_{12}E_8$ and 0.3 mg protein/ml (5.8 S) is calculated to be 138 000 (Table I). It suggests an αβ structure.

Low $C_{12}E_8$ concentration (solubilized active enzyme). The previous sedimentation equilibrium experiments [1] were performed in the presence of 20% glycerol and with 280 μM $C_{12}E_8$. However, when the $C_{12}E_8$ concentration is increased to 560 μM the sedimentation equilibrium plots are linear in the absence of glycerol.

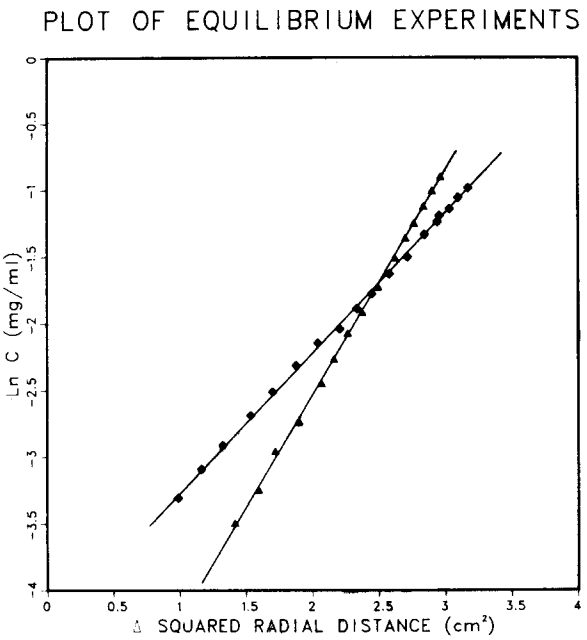

Fig. 4. Equilibrium plots. Equilibrium plots (ln C vs. r^2) of ($Na^+ + K^+$)-ATPase after centrifugation for 48 h at 9000 rev./min and at 14°C in a multichannel centerpiece. Two experiments are shown in the figure: one (▲) is an experiment using ATPase protein in 30 mM histidine, pH 7.0, 200 mM KCl, 560 μM $C_{12}E_8$ but without glycerol in the solvent (Table I, column IV); the other (■) includes 2.74 M glycerol in the solvent (Table I, column V).

The equilibrium distribution of the particles has been estimated in the presence of 2.74 M glycerol with 280 μM $C_{12}E_8$ [1] and in the presence of 560 μM $C_{12}E_8$ with and without glycerol. The linearity and the slope of the ln C vs. r^2 plot are independent of the protein concentration in the range of 0.05—0.3 mg/ml. The molecular weights and the parameters used in the calculations are listed in Table I. The molecular weight measured in the absence of glycerol (265 000) is essentially the same as that in the presence of glycerol (257 000; see Ref. 1).

Stokes' radii. The Stokes' radii (R_S) and the minimal radii (R_{min}) of the 10.1 and 5.8 S components are calculated as given in Refs. (14 and 15) and are listed in Table IV.

Discussion

The present experiments do not give the number of phospholipid and cholesterol molecules necessary for the enzyme activity. The 50 phospholipid and 40 cholesterol molecules may be more than that necessary, or if $C_{12}E_8$ can replace some of the required lipids, may be less than that necessary. It has been discussed whether acidic phospholipids are essential for activity [16]. If this is the case, the present experiments suggest that only 1 mol per mol of enzyme is required.

The M_r value of 106 000 for the SDS-solubilized purified α-chain is identical to the value obtained by Hastings and Reynolds [2]. The value of 40 000

daltons for the β-chain is a little higher than their value (36 000 daltons). In their calculations they used the carbohydrate content determined by Perrone et al. [11], which is higher than the value measured in our experiments. If the higher value given by Perrone et al. [11] is used then the molecular weight will decrease from 40 000 to 35 500.

From the measured values for the lipid, carbohydrate and detergent bound to the particle without glycerol in the medium, the molecular weight for the active solubilized particle is calculated to 265 000. This value is in agreement with molecular weights inferred from ligand binding to membrane-bound enzymes (see Ref. 17 for a review). Thus, the present experiments do not offer an explanation for the discrepancy between the previously determined of 276 000 and 380 000 daltons found by Hastings and Reynolds [2].

M_r (α) + M_r (β) is equal to 146 000, which is so close to the value measured for the complex in the high $C_{12}E_8$ concentration (138 000) that it seems likely that this is an αβ complex.

Accepting this will give 276 000 for $(\alpha\beta)_2$, which is identical to the observed 265 000 for the active molecule. However, there is a discrepancy between the values of 265 000 and 292 000 ($2\alpha + 2\beta$). Part of this difference can undoubtedly be explained by experimental error. There may also be a certain dissociation of $(\alpha\beta)_2$ into αβ even with the low (560 μM) $C_{12}E_8$ concentration; this would agree with the slight decrease in activity during the centrifugation. The effect of such a dissociation would be to decrease the calculated molecular weight of the active enzyme, and it might be difficult to see this as an inhomogeneity in the ultracentrifugation experiments.

Mg^{2+} (1 mM) has no effect on the sedimentation velocity of the 10 S particle (nor shown). No time-lag can be observed in activity measurements when the solubilized enzyme is transferred to the test solution, and the enzyme is active with 560 μM $C_{12}E_8$ in the test solution at the temperature of the ultracentrifugation. There is thus no indication that the transfer of the solubilized enzyme from the centrifugation medium to the test solution (with Mg^{2+} and ATP) leads to an oligomerization.

A tentative conclusion from the present experiments is that the solubilized enzyme at 560 μM $C_{12}E_8$ in the active from has an $(\alpha\beta)_2$ structure and is dissociated into an inactive αβ form by an increase in the $C_{12}E_8$ concentration.

The molecular weight of the active $(\alpha\beta)_2$ particle including the bound lipid, carbohydrate and detergent is calculated to be 546 000 (of which 265 000 is protein).

The amount of detergent bound in the particle (about 200 000 g per mol) indicates that the binding of detergent to the protein cannot be explained as a simple insertion of the protein into a detergent micelle as the micellar size of $C_{12}E_8$ is too small (about 65 000 g/mol [18]). The domains of protein which have to be covered by detergent are thus larger than the hydrophobic core of are detergent micelle (which is 22—56 Å, see Ref. 18).

Despite the large amount of detergent bound, the ratio R_S/R_{min} is 1.37 for $(\alpha\beta)_2$, indicating that the particle has a non-globular shape. The αβ particle has an even more non-globular shape (R_S/R_{min} 1.46), but this value is more uncertain because the detergent bound cannot be measured at the high $C_{12}E_8$ concentration; it is assumed to be the same as that for the $(\alpha\beta)_2$ particle.

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