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SOLUBILIZATION OF ACTIVE ($H^+ + K^+$)-ATPase FROM GASTRIC MEMBRANE

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($H^+ + K^+$)-ATPase-enriched membranes were prepared from hog gastric mucosa by sucrose gradient centrifugation. These membranes contained Mg^{2+}-ATPase and *p*-nitrophenylphosphatase activities (68 ± 9 μmol P_i and 2.9 ± 0.6 μmol *p*-nitrophenol/mg protein per h) which were insensitive to ouabain and markedly stimulated by 20 mM KCl (respectively, 2.2- and 14.8-fold). Furthermore, the membranes autophosphorylated in the absence of K^+ (up to 0.69 ± 0.09 nmol P_i incorporated/mg protein) and dephosphorylated by 85% in the presence of this ion. Membrane proteins were extracted by 1–2% (w/v) *n*-octylglucoside into a soluble form, i.e., which did not sediment in a 100 000 × *g* × 1 h centrifugation. This soluble form precipitated upon further dilution in detergent-free buffer. Extracted ATPase represented 32% (soluble form) and 68% (precipitated) of native enzyme and it displayed the same characteristic properties in terms of K^+-stimulated ATPase and *p*-nitrophenylphosphatase activities and K^+-sensitive phosphorylation: Mg^{2+}-ATPase (μmol P_i/mg protein per h) 32 ± 9 (basal) and 86 ± 20 (K^+-stimulated); Mg^{2+}-*p*-nitrophenylphosphatase (μmol *p*-nitrophenol/mg protein per h) 2.6 ± 0.5 (basal) and 22.2 ± 3.2 (K^+-stimulated); Mg^{2+}-phosphorylation (nmol P_i/mg protein) 0.214 ± 0.041 (basal) and 0.057 ± 0.004 (in the presence of K^+). In glycerol gradient centrifugation, extracted enzyme equilibrated as a single peak corresponding to an apparent 390 000 molecular weight. These findings provide the first evidence for the solubilization of ($H^+ + K^+$)-ATPase in a still active structure.

Introduction

The ($H^+ + K^+$)-ATPase from the fundic part of the stomach is believed to sustain the process of gastric acid secretion. It is an H^+-translocating membrane-bound ATPase [1], the mechanism of which involves a phosphoryl enzyme intermediate sensitive to K^+. It has been suggested that this ATPase is an oligomeric protein of 100 kDa subunits as characterized by gel electrophoresis [2], sedimentation equilibrium analysis [3] and Sephadex chromatography [3] after solubilization by SDS. Whether its subunits are identical or different is still a matter of debate [3,4]. On the basis of trypsin fragmentation data, they were first proposed to account for three different peptides, one of them being a glycoprotein [4]. However, this proposal was recently questioned, because neither long-term centrifugation nor chemical modifications produced any difference in the SDS-solubilized 100 kDa protomers [3]. Data from radiation-induced enzyme inactivation suggested either a trimeric [5] or a tetrameric [6] structure, whereas titration of ATP- and vanadate-binding sites suggested a dimeric one [7]. None of these studies, essentially concerned with chemical structure and molecular size of the ATPase, provides direct information on the subunits' assembly or on the role of these subunits in catalysis and transport. Such

Abbreviations: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate.

information could probably be gained if one could dissociate the enzyme into (active) elementary units and study the recovery of functional capability while reconstituting the oligomer. As a first step in this approach, the present paper describes a method for solubilization of $(H^+ + K^+)$-ATPase by detergent with apparent preservation of the three characteristic properties of the enzyme, i.e., K^+-stimulated ATP phosphohydrolase activity, K^+-stimulated phosphatasic activity and formation of a K^+-sensitive phosphoenzyme. A preliminary account of this work has already appeared [8].

Material and Methods

Chemicals. ATP (Mg^{2+} or Na^+ salts), phospho*enol*pyruvate (monocyclohexylammonium salt), glycerol, ouabain, dithiothreitol, *p*-nitrophenyl phosphate (Tris salt), Hepes and glycine were obtained from Sigma Chemical Company. Pyruvate kinase and *n*-octylglucoside were purchased from Boehringer, [γ-^{32}P]ATP from New England Nuclear. Tris buffer was purchased from Merck; acrylamide and polyacrylamide were from BDH chemicals, sodium dodecyl sulfate (SDS) from Calbiochem.

Preparation of the membrane fraction. Microsomes were prepared from fresh hog stomachs as previously described [9]. They were diluted in 0.25 M sucrose (8.3% (w/w) sucrose)/2 mM dithiothreitol/50 mM Hepes-Tris (pH 7.2) (buffer 1). They were layered upon a discontinuous two-step 30 and 38% (w/w) sucrose gradient buffered with 50 mM Hepes-Tris (pH 7.2)/2 mM dithiothreitol. The gradient was centrifuged at 0–4°C for 2 h at 40000 rpm (70 Ti rotor, L 5-65 centrifuge, Beckman Instruments). Fraction I refers to the 8.3% sucrose layer, fraction II to the 8.3–30% interface, fraction III to the 30% layer and the 30–38% interface, fraction IV to the 38% layer and fraction V to the pellet. Fraction II was diluted 1 : 3 (v/v) with 50 mM Hepes-Tris (pH 7.2)/2 mM dithiothreitol and pelleted for 1 h at 40000 rpm. The pellet (F II) was stored in buffer 1 at 15–20 mg protein/ml and kept at −20°C for up to 6 months.

Solubilization by n-octylglucoside. Solubilization was carried out at 0–4°C in the presence of 1.3–2% (w/v) *n*-octylglucoside. Proteins and *n*-octylglucoside were combined in a protein/detergent ratio of 1. After 30 min incubation at 0–4°C, the mixture was either (a) centrifuged for 1 h at 100000 × *g* (40000 rpm, 50 Ti rotor) or (b) diluted 1 : 20 (v/v) in buffer 1 and centrifuged for 45 min at 40000 rpm (0–4°C). The pellets were suspended in buffer 1. This fraction was kept frozen at −40°C, in the presence of 20% (v/v) glycerol.

Glycerol gradient. Glycerol gradient was formed by eight successive 1.35 ml layers of 20, 23, 23, 26, 29, 32, 35 and 38% glycerol solutions in buffer 1 containing 0.5% (w/v) *n*-octylglucoside over a cushion of 41% glycerol. Detergent-treated material (1.7 ml fraction) was layered on top of the gradient which was run for 2 h at 40000 rpm (70 Ti rotor). Fractions of 1 ml were collected and glycerol concentrations were measured by refractometry. Gradients were calibrated with the soluble markers ferritin, thyroglobulin, catalase and aldolase (Pharmacia, Uppsala, Sweden) centrifuged under the same conditions.

ATPase activity. ATPase activity was determined by measuring the release of P_i from ATP. The standard incubation medium (final volume 450 µl) contained 2 mM MgATP, 4 mM phospho*enol*pyruvate and 25 µg/ml pyruvate kinase as ATP-regenerating system, and 50 mM Hepes-Tris (pH 7.2). Potassium-stimulated activity, referred to as K^+-ATPase, was estimated as the difference between activity in the presence of 2 mM $MgCl_2$ plus 20 mM KCl and basal activity in the presence of 2 mM $MgCl_2$ alone. When used, ouabain was 10^{-4} M. The reaction was initiated by the addition of 50 µl of F II (15–20 µg protein). It was run at 37°C for 10 min and stopped by the addition of 10% ice-cold trichloroacetic acid. Released P_i was determined by the Fiske and SubbaRow procedure [10]. Since high concentrations of *n*-octylglucoside were found to inhibit $(H^+ + K^+)$-ATPase (1% (w/v) resulted in full inhibition), samples were diluted 1 : 10 in detergent-free medium prior to assays for enzyme activity.

p-Nitrophenylphosphatase activity. The *p*-nitrophenylphosphatase activity was determined by measuring the *p*-nitrophenol formed from the hydrolysis of *p*-nitrophenylphosphate. The standard incubation medium of a final volume of 250 µl contained 5 mM *p*-nitrophenyl phosphate, 5 mM $MgSO_4$ and 50 mM Hepes-Tris (pH 6.9). Potas-

sium-stimulated activity, referred to as K^+-*p*-nitrophenylphosphatase, was estimated as the difference between activity in the additional presence of 20 mM KCl, i.e., ($Mg^{2+} + K^+$)-*p*-nitrophenylphosphatase, and the basal activity (Mg^{2+}-*p*-nitrophenylphosphatase). Reaction was initiated by the addition of 50 µl F II (15–20 µg protein), run at 37°C for 10 min and stopped by the addition of 1 ml 0.5 M NaOH. The *p*-nitrophenol was measured spectrophotometrically at 410 nm. Since the presence of *n*-octylglucoside in the assay was inhibitory (0.3% (w/v) decreased by 50% the *p*-nitrophenylphosphatase activity), samples were diluted 1 : 10 in detergent-free buffer.

^{32}P-phosphorylation. Phosphorylation experiments were carried out at 0–4°C according to Wallmark et al. [11]. The reaction mixture contained 2 mM dithiothreitol, 250 mM sucrose, 50 mM Hepes-Tris (pH 7.2) and 60–300 µg membrane proteins in a final volume of 110 µl. Phosphorylation was tested in the presence of either 1 mM EDTA or 2 mM $MgCl_2$ or 2 mM $MgCl_2$ plus 20 mM KCl. Mg^{2+}-activated phosphorylation was estimated as the difference between the activity in the presence of 2 mM Mg^{2+} and the activity in the presence of 1 mM EDTA; ($Mg^{2+} + K^+$) phosphorylation as the difference between the activity of 2 mM Mg^{2+} + 2 mM K^+ and the activity in the presence of 1 mM EDTA. Reaction was initiated by the addition of 5 µM [γ-^{32}P]ATP ($1.2 \cdot 10^5$ cpm), and after 10 s in ice terminated by expelling the reaction mixture into 4.0 ml of 7% (w/w) ice-cold $HClO_4$ containing 10 mM P_i. The precipitated protein was collected on a Millipore filter (pore size 0.45 µm) and the filter was washed with 5.0 ml of the ice-cold stop solution. The filter was then dissolved in 1.0 ml acetone and the ^{32}P radioactivity was counted using Aquassure (New England Nuclear) as scintillation fluid.

Electrophoresis. We used the technique described by Laemmli [12] with minor modifications. Plates and spacers were carefully washed in 1% Triton X-100. The gels were formed from a solution containing 6.9 g acrylamide, 0.027 g methylene bisacrylamide, 0.375 M Tris-HCl (pH 8.8), 2 mM EDTA, 0.1% SDS, 50 µl *N,N,N',N'*-tetramethylethylendiamine (TEMED), 0.5 g polyacrylamide, 0.16 mM NaF, 0.16 mM NaN_3, in 100 ml H_2O with 1 ml 10% ammonium persulfate. They were allowed to polymerize overnight at 0–4°C. Protein samples were suspended in 50 mM Tris HCl (pH 6.8)/2% SDS/2 mM EDTA/0.01% mercaptoethanol/10% glycerol. Solubilization was carried out for 1 h at 37°C. 0.25% bromophenol blue was added to localize the buffer front. The electrode buffer was 6 g/l Tris, 28.8 g/l glycine, 0.1% SDS and 2 mM EDTA. Samples (15 µl; 15–60 µg protein) were underlayed into wells and electrophoresis was run at 5–15°C. Gels were then incubated in 10% trichloroacetic acid, 20% methanol overnight to fix the proteins. These were afterwards stained by 30–60 min incubation at room temperature in the presence of 0.25% Coomassie blue in 10% acetic acid/50% methanol and clearing was carried out in 10% acetic acid/25% methanol.

Proteins were measured by the Coomassie blue staining according to the method of Bradford [13].

Cytochrome *c* oxidase activity was estimated as previously described [14].

Results

Purification of ($H^+ + K^+$)-ATPase-enriched membranes

($H^+ + K^+$)-ATPase enrichment was defined with reference to properties reported in the literature, i.e., K^+-stimulated, ouabain-insensitive ATPase and *p*-nitrophenylphosphatase activities, in addition to a glycoprotein of 100 kDa, which phosphorylates in the presence of Mg^{2+}-ATP and dephosphorylates in the presence of K^+.

(1) F II was the fraction most enriched in ($H^+ + K^+$)-ATPase (Fig. 1). It has 68 ± 9 µmol P_i per mg protein per h of Mg^{2+}-ATPase activity (Table I) which was 2.2-fold stimulated by 20 mM KCl. 10^{-4} M ouabain had no effect. Na^+ did not stimulate the activity but inhibited K^+ stimulation at high concentrations ([Na^+] ⩾ 50 mM), as previously reported [1]. F II was also the most enriched in *p*-nitrophenylphosphatase activity, which was 8- to 20-fold stimulated by K^+ (Table I) and not sensitive to 10^{-4} M ouabain. Potassium stimulation was maximal at pH 6.9; ATP and ADP (10^{-4} M to 10^{-3} M) were both inhibitory (not shown).

(2) In the presence of 5 µM ATP, F II incorporated up to 0.69 ± 0.09 nmol P_i per mg protein. P_i incorporation was 85% reduced in the presence of

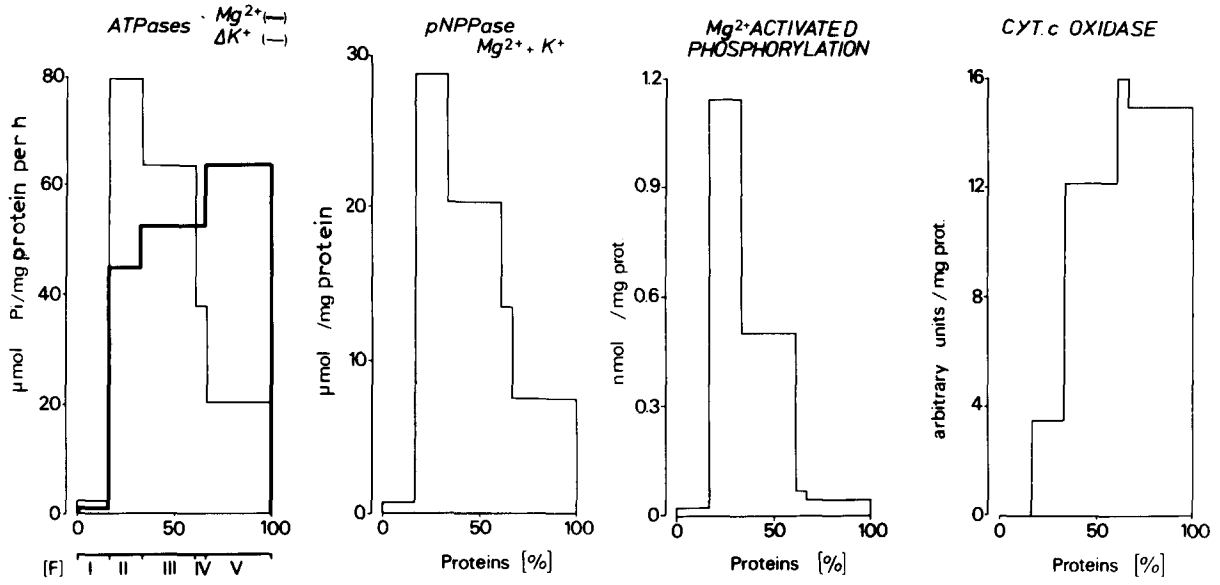

Fig. 1. Purification of hog gastric microsomes by discontinuous sucrose gradient. Microsomes (5–8 ml) were layered on top of a discontinuous sucrose gradient: 7 ml 30% (w/w) sucrose in 50 mM Hepes-Tris (pH 7.2)/2 mM dithiothreitol and 7 ml 38% (w/w) sucrose in the same buffer, Gradients were centrifuged 2 h at 40000 rpm (70 Ti rotor, Beckman L5 65 centrifuge). After centrifugation, the fractions (referred as F I, II, III, IV, V in the figure) were collected as written in Material and Methods. This scheme is representative of the equilibration of two microsomal preparations (13 and 15 mg protein/ml). The profiles are those found for Mg^{2+}-ATPase, K^+-ATPase, (($Mg^{2+} + K^+$)-ATPase minus Mg^{2+}-ATPase activities), ($Mg^{2+} + K^+$)-*p*-nitrophenylphosphatase (pNPPase), Mg^{2+}-phosphorylation and cytochrome *c* oxidase. Assays were performed as detailed in Material and Methods, but phosphorylation was tested with 6.5 µM ATP.

TABLE I

SOLUBILIZATION OF ($H^+ + K^+$)-ATPase BY *n*-OCTYLGLUCOSIDE

Gastric membranes (F II) (10–15 mg/ml) were treated with 1.3% *n*-octylglucoside for 30 min at 0–4°C. Then: (A) they were centrifuged (100000 × *g*, 1 h). The activity in the supernatant was tested. This supernatant was then diluted 20-times in buffer 1 and centrifuged (100000 × *g* for 45 min). The activity in the pellet was tested. (B) They were diluted 20-times in buffer 1 and pelleted at 100000 × *g* for 45 min (DF II). Conservation after freezing and effect of ouabain were tested on this DF II material. ATPase and *p*-nitrophenylphosphatase units are µmol/mg protein per h. Phosphorylation units are nmol P_i per mg protein. n.d. means non-determined. Mean ± S.E. of (a) eight, (b) four, (c) seven, (d) three experiments. Each experiment was done using a different preparation of F II.

	ATPase				*p*-Nitrophenylphosphatase				Phosphorylation		
	Mg^{2+}	$Mg^{2+} + K^+$	K^+		Mg^{2+}	$Mg^{2+} + K^+$	K^+		Mg^{2+}	$Mg^{2+} + K^+$	
F II	68 ± 9	151 ± 12	82 ± 4	(a)	2.9 ± 0.6	43.0 ± 3.0	40.0 ± 2.6	(c)	690 ± 90	106 ± 15	(b)
F II + *n*-octylglucoside (1.3%)	30 ± 3	54 ± 6	24 ± 3	(a)	3.7 ± 0.3	9.0 ± 1.1	5.3 ± 1.1	(c)	34 ± 7	33 ± 9	(b)
Supernatant (100000 × *g*, 1 h)	18 ± 1	31 ± 3	13 ± 2	(b)	5.0 ± 0.8	9.4 ± 2.0	4.4 ± 1.4	(d)	n.d.	n.d.	
Diluted and pelleted supernatant	26 ± 4	56 ± 7	30 ± 4	(b)	2.7 ± 1.0	11.1 ± 2.4	8.4 ± 1.8	(d)	n.d.	n.d.	
Diluted and pelleted detergent-treated F II (DF II)	32 ± 9	86 ± 20	54 ± 12	(b)	2.6 ± 0.5	22.2 ± 3.2	19.6 ± 3	(b)	214 ± 41	57 ± 4	(b)
DF II after freezing	32 ± 9	90 ± 20	58 ± 11	(b)	3.2 ± 0.6	23.2 ± 3.2	20 ± 3	(b)	n.d.	n.d.	
DF II + 10^{-4} M ouabain	29 ± 9	88 ± 19	57 ± 13	(b)	1.9 ± 0.5	20 ± 3.7	18.1 ± 3	(b)	n.d.	n.d.	

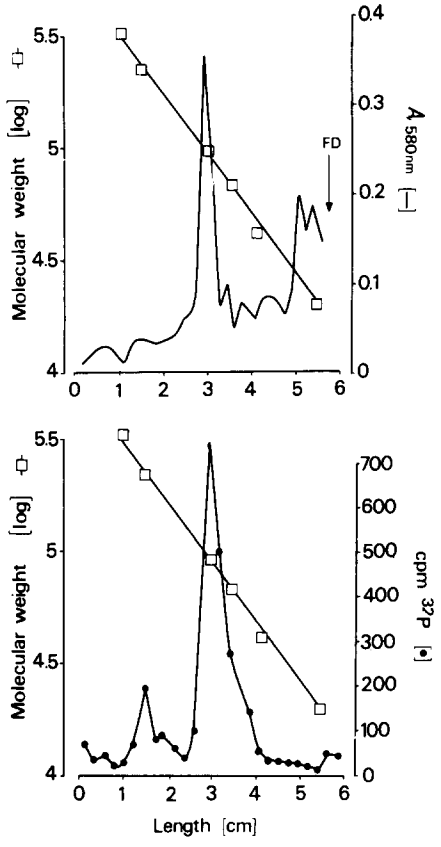

Fig. 2. Electrophoretic pattern of SDS-treated gastric membranes (F II). The higher plot shows the scanning of Coomassie blue staining absorbance at 580 nm, the lower one ^{32}P radioactivity in cpm; empty squares (□) stand for the proteic standards (thyroglobulin (330000), ferritin (220000), phosphorylase *b* (94000), albumin (67000), ovalbumin (43000) and trypsin inhibitor (20100)). F II was phosphorylated for 10 s in the presence of [γ-^{32}P]ATP as described in Material and Methods. Reaction was stopped by $HClO_4$ and the aggregated proteins were pelleted by centrifugation (Beckman Microfuge, 30 s). Pellets were resuspended in 50 mM Tris-HCl (pH 6.8)/2% SDS/2 mM EDTA/0.01% mercaptoethanol/10% glycerol. A sample was also made with non-radioactive ATP for Coomassie blue staining. Electrophoresis were run at 5–15°C, radioactive gels were then sliced every 2.5 mm. Each slice was incubated overnight in 0.5 ml H_2O_2 and the radioactivity was counted after addition of 3 ml scintillating fluid. FD stands for the dye front.

20 mM KCl (Table I) due to stimulation of the dephosphorylation rate. The protein pattern of electrophoresis or chromatography of the 1–2% SDS-treated F II showed a major protein band at 95 kDa (Fig. 2). This band represented at least 43% of the proteins of F II and contained most of the $^{32}P_i$ incorporated (Fig. 2).

Solubilization of ($H^+ + K^+$)-ATPase

n-Octylglucoside was efficacious in extracting ATPase from F II. Optimal yield was obtained with 1–2 mg detergent per mg protein in samples of 10–20 mg protein/ml. Extracted ATPase was still stimulated by K^+ (1.80-times in a series of eight experiments) although the detergent showed an inhibitory effect on both basal and K^+-stimulated activities (Table I). Extracted *p*-nitrophenylphosphatase was slightly stimulated by K^+ (2.43-fold as compared to 14.8-fold in untreated material), the detergent showing a stimulatory effect on basal *p*-nitrophenylphosphatase activity (1.27-fold) and an inhibitory effect on K^+-stimulated activity (0.21-fold) (Table I). At this stage, very low K^+-sensitive phosphorylation was found.

Extracted enzyme was in a soluble form: after detergent treatment, 82 ± 11% of total K^+-ATPase activity remained in the supernatant of 100000 × *g* (1 h) centrifugation (ten experiments). However, due to the inhibitory effect of *n*-octylglucoside on enzyme activities, this supernatant represented only 32 ± 4% of the K^+-ATPase and 11 ± 5% of the ($Mg^{2+} + K^+$)-*p*-nitrophenylphosphatase initially present in F II (ten experiments). Activity of the soluble ATPase (i.e., after detergent treatment and high-speed centrifugation) was stable at least 6 days when kept at 0–4°C in the presence of the

TABLE II

STABILITY OF THE SOLUBILIZED ATPase ACTIVITY AS A FUNCTION OF TIME

Gastric membranes (F II) (15.8 mg protein/ml) were treated with 3% *n*-octylglucoside 30 min at 0–4°C. They were then centrifuged (100000 × *g*, 1 h). The clear supernatant was collected and kept at 0–4°C. The activity was tested immediately (1st day) and on the 2nd, 5th and 6th day after preparation. ATPase units are μmol/mg protein per h.

	Mg^{2+}	$Mg^{2+} + K^+$
Initial fraction F III	39	108
100000 × *g* (1 h) supernatant:		
1st day	17.9	32.2
2nd day	15.4	35.9
5th day	20.9	41.0
6th day	19.0	44.9

detergent (Table II). It was 1.6- to 3-fold stimulated by K^+. Other cations were also activators according to the sequence (referring to concentration for half-maximal effect): K^+ (5 mM) > NH_4, Rb^+ (7 mM) > Cs^+ (40 mM). Up to 20 mM, Na^+ did not affect ATPase activity. The soluble *p*-nitrophenylphosphatase was only slightly stimulated by K^+ (1.9-fold).

Inhibitory effect of *n*-octylglucoside on enzyme activities was mostly reversed by dissociating the protein-detergent complex. This was readily achieved by 1 : 20 dilution in detergent-free buffer 1. As a consequence of this dilution, proteins precipitated as shown by the formation of a pellet upon 100 000 × *g* centrifugation for 45 min. This pellet accounted for either 98% or 10% of the non-diluted fraction proteins, depending on whether F II + *n*-octylglucoside or the 100 000 × *g* (1 h) supernatant of F II + *n*-octylglucoside was treated. The precipitated pellet obtained from the diluted, solubilized F II (referred to as DF II in the following and Table I) was enriched 2.25-, 3.7- and 6.1-times, respectively, in K^+-ATPase, K^+-*p*-nitrophenylphosphatase and Mg^{2+}-phosphorylation (Table I), while that obtained from the diluted-precipitated 100 000 × *g* supernatant of solubilized F II was enriched 2.3- and 1.9-times in K^+-ATPase and K^+-*p*-nitrophenylphosphatase, respectively (phosphorylating activity was not tested). DF II contained 68% of the K^+-ATPase, 48%of the K^+-*p*-nitrophenylphosphatase, and 30% of the Mg^{2+} phosphorylating activities of F II. This fraction was stable and could be kept frozen for up to 4 months without significant loss of enzyme activities.

Purification of solubilized ($H^+ + K^+$)-ATPase

DF II was suspended in 0.5% *n*-octylglucoside, layered on top of a 20–41% glycerol gradient and centrifuged for 2 h at 100 000 × *g*. The protein distributed as a single peak centered around 31% glycerol (Fig. 3) with, in addition, a small pellet accounting for non-soluble material. This peak was situated between that of ferritin (440 kDa) and that of the thyroglobulin subunit (330 kDa), which equilibrated at 36 and 23% glycerol, respectively (Fig. 3). It accounted for almost all ATPase activity, this being more than 3-times stimulated by 20 mM K^+. It also accounted for the *p*-nitrophenylphosphatase activity, which was 8-times stimulated by 20 mM KCl, and for phosphorylation that was sensitive to K^+-dephosphorylation (Fig. 3).

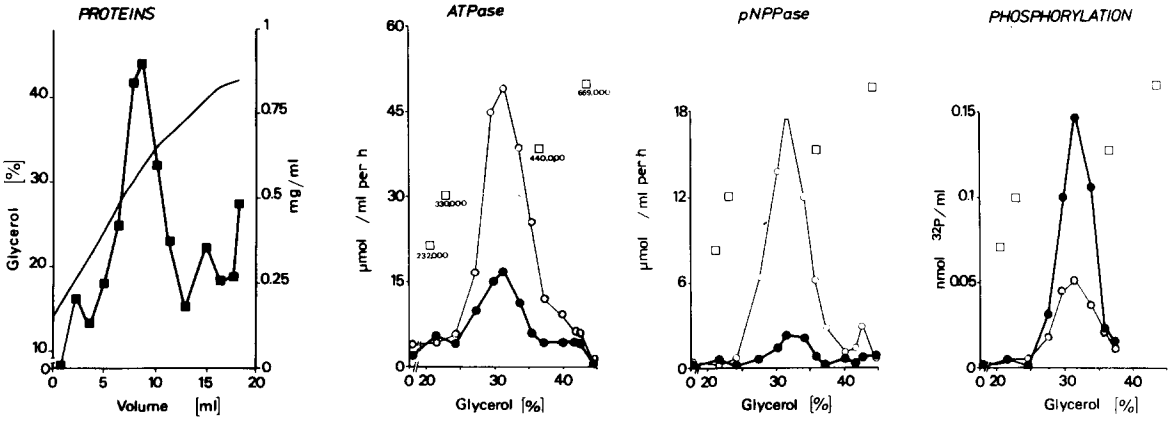

Fig. 3. Distribution of solubilized ($H^+ + K^+$)-ATPase on glycerol gradient. F II (14.8 mg/ml) was treated by 1.3% *n*-octylglucoside for 30 min at 0–4°C. It was then diluted 1 : 20 (v/v) in buffer 1 at 0°C and centrifuged 45 min at 40 000 rpm. The pellet was suspended (20–25 mg/ml) in buffer 1 + 0.5% *n*-octylglucoside, layered on top of a 20–41% glycerol gradient (see Material and Methods) and centrifuged 2 h at 40 000 rpm. The fractions were collected and tested for their protein content (■), glycerol concentration and for their activity in ATPase, *p*-nitrophenylphosphatase and phosphorylation as detailed in Material and Methods: Mg^{2+}- (●———●), ($Mg^{2+} + K^+$)- (○———○) ATPase; Mg^{2+}- (●———●), ($Mg^{2+} + K^+$)- (○———○) *p*-nitrophenylphosphatase; Mg^{2+}- (●———●), ($Mg^{2+} + K^+$)- (○———○) phosphorylation. Empty squares (□) represent the peak of equilibration of soluble markers plotting as ordinate the logarithm of their respective molecular mass.

Discussion

Previous attempts to extract ($H^+ + K^+$)-ATPase from gastric membrane led to full inactivation of the enzyme. Peters et al. [3] recently reported chemical characterization of ATPase subunits after SDS solubilization, but did not provide evidence for preservation of catalytic activity. The present study suggests that non-degradative extraction of ($H^+ + K^+$)-ATPase can be achieved by *n*-octylglucoside. This neutral detergent has already been proven useful in solubilization-reconstitution studies on a variety of membraneous enzymes, including bacteriorhodopsin [15], chloroplast [15] and bacterial [16] ATPases, bacterial NADH-dehydrogenase [16]. Its remarkable suitability for solubilizing fragile membrane-bound complexes is probably due to its mild action. As compared to the harsher detergents currently used (i.e., SDS and Triton X-100), protein extraction by *n*-octylglucoside proceeds more slowly and requires a higher detergent : protein ratio. These conditions facilitate monitoring of solubilization to obtain optimal and reproducible experimental conditions. Moreover, with respect to enzyme inactivation, which we think to be due to depolymerization of solubilized enzyme, *n*-octylglucoside offers the advantage of having a high critical micellar concentration (25 mM as compared to 0.015 mM for Triton X-100) and an apparently low affinity for membrane proteins. It is therefore easy to dissociate the protein-detergent complex (by simple dilution) which stops the reaction. Apparently, *n*-octylglucoside solubilization does preserve the ($H^+ + K^+$)-ATPase enzyme properties, since extracted enzyme displayed K^+-sensitive ATPase and *p*-nitrophenylphosphatase and K^+-sensitive autophosphorylation.

Glycerol gradient and sucrose gradient centrifugations have been used previously to purify solubilized ATPases from the neurospora [17] and yeast [18,19] plasma membranes. As we found that sucrose (30%, w/w) interfered with K^+-stimulation of the membraneous ($H^+ + K^+$)-ATPase (unpublished results), we chose glycerol gradient as a preliminary purification step. This method proved very convenient for our purposes because of its good reproducibility, enzyme recovery (70–120%) and conservation, and limited dilution of the material as compared to more conventional gel permeation methods [8]. In glycerol gradient centrifugation, *n*-octylglucoside-solubilized enzyme consistently distributed as a single peak containing K^+-sensitive ATPase, *p*-nitrophenylphosphatase and phosphorylation activities. The apparent molecular mass (390 kDa) is consistent with our preliminary report showing that *n*-octylglucoside solubilized ($H^+ + K^+$)-ATPase eluted as a 350 kDa complex on a cholate equilibrated-S400 Sephacryl column [8]. Studies dealing with further purification of the solubilized enzyme are in progress.

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