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STUDIES ON ($K^+ + H^+$)-ATPase

V. CHEMICAL COMPOSITION AND MOLECULAR WEIGHT OF THE CATALYTIC SUBUNIT

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(1) A ($K^+ + H^+$)-ATPase preparation from porcine gastric mucosa is solubilized in sodium dodecyl sulfate, and is subjected to gel filtration. (2) A main subunit fraction is obtained, which is a protein carbohydrate lipid complex, containing 88% protein, 7% carbohydrate and 5% phospholipid. The detailed composition of the protein and carbohydrate moieties are reported. (3) Sedimentation analysis of the subunit preparation, after detergent removal, reveals no heterogeneity, but the subunits readily undergo aggregation. (4) Acylation of the subunit preparation with citraconic anhydride causes a clear shift of the band obtained after SDS gel electrophoresis, but the absence of broadening and splitting of the band pleads against subunit heterogeneity. (5) Treatment of the subunit preparation with dansyl chloride indicates that the NH_2 terminus is blocked, which favors the assumption of homogeneity of the protein. (6) Binding studies with concanavalin A indicate that at least 86% of the subunit preparation is composed of glycoprotein. (7) These findings, taken together, strongly suggest that there is a single subunit which is a glycoprotein and which represents the catalytic subunit of the enzyme. From sedimentation equilibrium analysis a molecular mass value of 119 kDa (S.E. 3, $n = 6$) is calculated for protein + carbohydrate and of 110 kDa (S.E. 3, $n = 6$) for protein only. (8) In combination with the molecular mass of 444 kDa (S.E. 10, $n = 4$) obtained for the intact enzyme by radiation inactivation we conclude that the enzyme appears to be composed of a homo-tetramer of catalytic subunits.

Introduction

The gastric ($K^+ + H^+$)-ATPase is thought to be involved in the acid secretion process in the stomach [1–3]. The enzyme is able to generate a proton gradient [4], most probably via an electroneutral exchange of H^+ and K^+ [5]. The reaction mechanism of this gastric H^+-pump resembles that of the ($Na^+ + K^+$)-ATPase in many respects [6], with H^+ replacing Na^+ [7].

The enzyme can be isolated from the gastric mucosa of various species as a vesicular membrane fraction by isopyknic gradient centrifugation and subsequent free flow electrophoresis [8] or zonal electrophoresis [9]. SDS-polyacrylamide gel electrophoresis of the purified membrane fraction reveals a protein band with an apparent molecular mass around 100 kDa, which comprises more than 70% of the total amount of protein and which can be phosphorylated by ATP [5,8,10].

Upon isoelectrofocussing of the ($K^+ + H^+$)-ATPase preparation, purified by means of free flow electrophoresis, Sachs et al. [11] obtained a pattern which suggested heterogeneity of the 100 kDa band. From the results obtained by tryptic digestion, Saccomani et al. [12] concluded that the 100 kDa band would consist of three separate proteins: a catalytic subunit, a glycoprotein and a

third unknown protein. A minimum molecular mass of about 300 kDa for the protein part of the enzyme would thus be required. This interpretation appeared to be supported by an estimation of the target size of $(K^+ + H^+)$-ATPase by means of radiation inactivation yielding a molecular mass value of 270 kDa at −50°C and 324 kDa at 20°C [13].

We have solubilized the purified enzyme preparation in SDS and isolated the 100 kDa subunit fraction by gel filtration. The isolated protein fraction thus obtained has been studied for heterogeneity by various approaches. No evidence for heterogeneity has been found. The chemical composition and the molecular weight of the subunit have been determined.

Materials and Methods

Enzyme preparation. The enzyme is isolated from porcine gastric mucosa according to Schrijen et al. [10] and is stored in 0.25 M sucrose at −20°C. The specific activity ranges from 80–110 μmol ATP hydrolyzed per mg protein per hour.

Solubilization and gel filtration. The enzyme is solubilized in SDS as described for $(Na^+ + K^+)$-ATPase [14]. Gel filtration is carried out on a Sephadex G-200 superfine (Pharmacia, Uppsala, Sweden) column, 2.6 × 95 cm, under the same conditions as previously described for $(Na^+ + K^+)$-ATPase [14]. After gel filtration the fractions are, if necessary, concentrated to the desired volume on a Minicon-B concentrator (Amicon, Lexington, MA, U.S.A.).

SDS gel electrophoresis. SDS gel electrophoresis is performed on 16 × 16 × 0.075 cm polyacrylamide gradient gels (7–15% acrylamide, w/v) according to Laemmli [15].

Removal of SDS. SDS is removed by dialysing the concentrated protein solution (280 nm absorbance 0.3–1.2) at 4°C for 24–28 h against a suspension of 30–40 ml Dowex AG 1-X2 resin (200 × 400 mesh, acetate form; Biorad, Richmond, CA, U.S.A.) in 200 ml 50 mM Tris/acetate buffer of pH 7.4 [14].

Molecular weight determination. Molecular weights and sedimentation coefficients are determined in 0.05 M Tris/acetate buffer (pH 7.4, $\rho = 1.003$ g/cm^3) as previously described [14], except that the centrifugal speed during sedimentation equilibrium analysis is reduced to 6000–6800 rev./min in order to obtain proper equilibrium. Note that the equation for the calculation of the molecular weight in Ref. 14 should have read dA/dr^2 rather than A/r^2.

Determination of the NH_2 terminal amino acid. About 0.5 mg subunit protein is used for NH_2 terminal amino acid determination. Coupling with dansyl chloride, hydrolysis and chromatography are performed as described by Weiner et al. [16].

Coupling to concanavalin A. 0.5 ml subunit protein solution (280 nm absorbance: 0.2–0.6) is dialysed for 2–4 h against 100 ml buffer containing 10 mM Tris/acetate (pH 7.4), 0.5 mM magnesium acetate, 1.0 mM dithioerythritol (Sigma Chemicals, St. Louis, MO, U.S.A.) and 1.0 M NaCl. Coupling to Con A-Sepharose beads (Pharmacia, Uppsala, Sweden) is performed as described by Poliquin and Shore [17]. After the coupling reaction the beads are sedimented (1 min at 15000 × g). Before and after the coupling reaction the 280 nm absorbance is measured to determine the amount of binding. Blanks without subunit protein are used to correct for 280 nm absorbance due to concanavalin A released from the sepharose beads. To check the specificity of the concanavalin A binding, bovine albumin solutions have been incubated with the beads as described above, but no binding of albumin could be detected.

Acylation with citraconic anhydride. 0.5–1 mg protein is dissolved in 1 ml of 0.3% aqueous SDS solution at room temperature, and 10 μl citraconic anhydride (Sigma Chemicals, St. Louis, MO, U.S.A.) is added. The solution is kept at pH 8.5 with 0.1 M NaOH. After 3 h another 10 μl citraconic anhydride is added, maintaining pH 8.5. When the pH has become stable, the solution is dialysed overnight against 3 μM NaOH (pH 8.5), is freeze-dried and stored at −20°C to prevent de-citraconilation of the protein [18].

Analytical methods. Enzyme protein is determined by the method of Lowry et al. [19]. Bovine serum albumin, dissolved in 0.25 M sucrose, serves as a standard, since the enzyme preparation also contains 0.25 M sucrose. In this way the slight enhancement of the color by sucrose is corrected for. Absolute protein determinations are performed by amino acid analysis, after correction for

incomplete hydrolysis and destruction of certain amino acids [20], while tryptophan is determined spectrophotometrically by the method of Edelhoch [21]. Comparison of the two protein determinations shows that the method of Lowry et al. for $(K^+ + H^+)$-ATPase gives values that are 42% (S.E. 3, $n = 5$) too high [22].

Sugar analysis is carried out after hydrolysis in 2 M HCl at 100°C in sealed evacuated glass tubes in darkness. When sialic acid is analysed, hydrolysis is performed in 0.1 M H_2SO_4 at 80°C for 1 h. Times of hydrolysis are 2.5 h for neutral sugars and 1–3.5 h for amino sugars. Neutral sugars are assayed by gas-liquid chromatography according to Langeveld et al. [23], amino sugars are determined on the amino acid analyser [14] and sialic acid is measured by means of the fluorimetric assay of Hammond and Papermaster [24]. The phospholipid content of the fractions after gel filtration is measured by determining the organic phosphate content, after evaporation of the solute, by means of a modified Fiske-SubbaRow method [25].

Results

Subunit preparation

The solubilized $(K^+ + H^+)$-ATPase preparation is subjected to gel filtration on Sephadex G-200 superfine. The upper part of Fig. 1 shows the protein and phospholipid patterns thus obtained. The protein pattern shows one major sharp peak after 125–150 ml eluate followed by a low and diffuse protein band. Fig. 2 shows the SDS-polyacrylamide gel electrophoretic pattern of these bands, with the slot numbers corresponding to the fraction numbers at the top of Fig. 1. The sharp peak gives a single major band at about 100 kDa (slots 3 and 4), whereas the diffuse band is very heterogeneous (slot 5 and 6). Slot 7 represents the protein pattern of the original $(K^+ + H^+)$-ATPase preparation before gel filtration, indicating the true proportions of the different proteins seen in slots 2–6 in the native enzyme preparation. The low, diffuse protein band in the upper part of Fig. 1 is clearly due to several different proteins present in low concentrations in the enzyme pre-

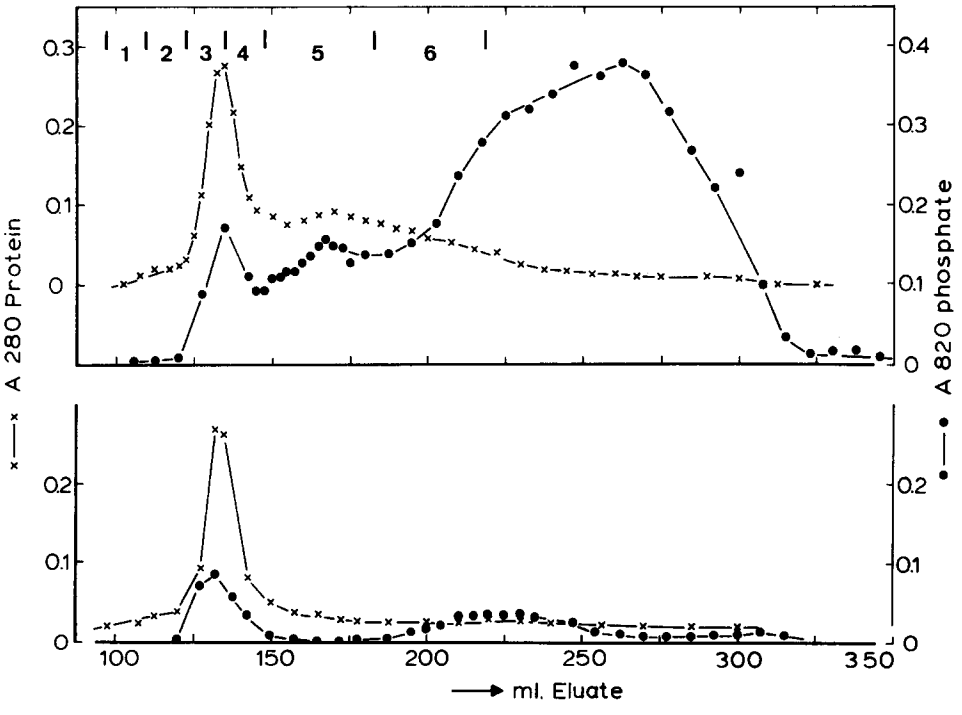

Fig. 1. Subunit separation by gel filtration. Upper part: Protein (×——×) and phospholipid (●——●) patterns of solubilized $(K^+ + H^+)$-ATPase after gel filtration on Sephadex G-200 superfine. Lower part: Protein (×——×) and phospholipid (●——●) patterns of the 100 kDa band of $(K^+ + H^+)$-ATPase after a second gel filtration on Sephadex G-200 superfine.

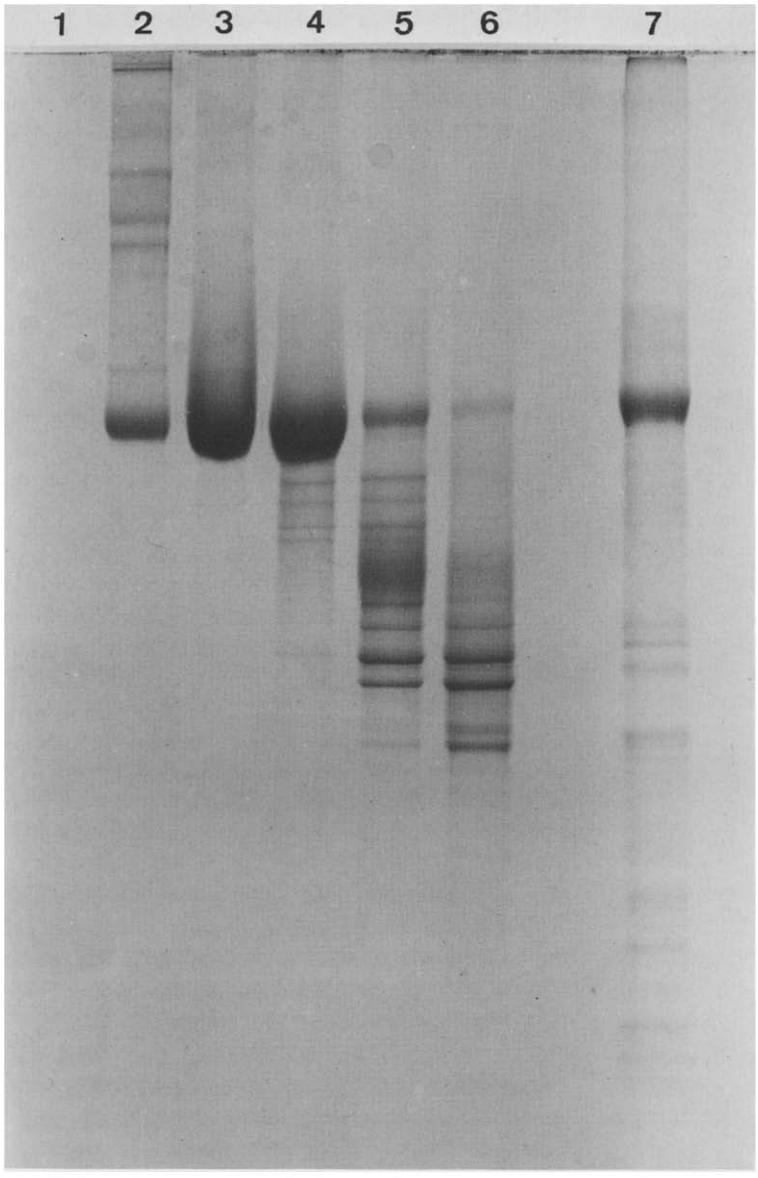

Fig. 2. SDS gel electrophoresis pattern of the different fractions of a purified ($K^+ + H^+$)-ATPase preparation after gel filtration. Slots 1–6 correspond to the fraction numbers indicated in the upper half of Fig. 1. Slot 7 represents purified ($K^+ + H^+$)-ATPase before gel filtration.

paration, hence presumably contaminating proteins.

The phospholipid pattern in the upper part of Fig. 1 indicates that the bulk of the phospholipids is separated from the protein. A small part of the phospholipids remains attached, even after a second gel filtration of the concentrated 100 kDa subunit fraction (Fig. 1, lower part), just as we have previously found for ($Na^+ + K^+$)-ATPase [14].

Chemical composition of the 100 kDa subunit preparation

The 100 kDa subunit fractions 3 and 4 (Fig. 1) are collected, concentrated and subjected to amino acid and sugar analysis. Table I presents the amino

TABLE I

AMINO ACID COMPOSITION OF THE 100 kDa SUBUNIT

Averages for analyses on two preparations after 10, 24, 48 and 72 h hydrolysis. Values for valine and isoleucine are extrapolated to infinite time of hydrolysis.

Amino acid	content in mol/100 mol amino acid
Aspartic acid	9.6
Threonine	6.2
Serine	6.7
Glutamic acid	10.9
Proline	5.0
Glycine	7.6
Alanine	8.4
Cysteine	0.8
Valine	6.0
Methionine	2.8
Isoleucine	5.2
Leucine	10.1
Tyrosine	2.7
Phenylalanine	4.5
Tryptophan	1.7
Histidine	1.6
Lysine	5.6
Arginine	5.0
Hydrophobic amino acid content [a]: 43.5%	

[a] Proline, alanine, valine, methionine, isoleucine, leucine, phenylalanine and tryptophan.

acid composition and the percentage of hydrophobic amino acids. The amino acid composition resembles very closely that of the catalytic subunits of $(Na^+ + K^+)$-ATPase from rabbit kidney (Ref. 14, correlation coefficient 0.983) and $(Ca^{2+} + Mg^{2+})$-ATPase from rat sarcoplasmic reticulum (Ref. 26, correlation coefficient 0.961), while the similarity with the β-subunit of $(Na^+ + K^+)$-ATPase is much less (Ref. 14, correlation coefficient 0.81). There is also good agreement with the amino acid composition of the proteins in electrophoretically purified vesicles of Sachs et al. (Ref. 27, correlation coefficient 0.970). The higher serine value found by Sachs et al. (8.4 vs. our value of 6.7 mol%) is probably due to the presence of much more phosphatidylserine in their preparation compared to our almost delipidated 100 kDa subunit preparation (see Fig. 1). The slightly lower values for isoleucine, leucine and valine from Sachs et al. (4.5, 9.3 and 5.4 compared with our values of 5.2, 10.1 and 6.0, respectively) may be due to the use of a relatively short hydrolysis time (22 h) by Sachs et al. [27]. Hydrolysis of membrane proteins is not yet complete after 22 h [22], especially for amino acids like valine and isoleucine. The percentage of hydrophobic amino acids (see Table I) is 43.5%, which is virtually identical with that of the catalytic subunits of $(Na^+ + K^+)$-ATPase (43.2%, Ref. 14) and $(Ca^{2+} + Mg^{2+})$-ATPase (42.8% without tryptophan, Ref. 26) and only slightly higher than the value for total gastric vesicles (40.3% without tryptophan, Ref. 27).

Table II presents the carbohydrate composition of the 100 kDa subunit preparation, indicating the presence of the neutral sugars mannose, galactose, glucose and fucose, the amino sugars glucosamine and galactosamine and some sialic acid. Most of these carbohydrates are also reported for gastric vesicles by Sachs et al. [27], although there is much less carbohydrate present in our 100 kDa subunit preparation: 8.3 vs. 28 g/100 g protein. This difference may have two causes: (1) The minor proteins present in the purified $(K^+ + H^+)$-ATPase preparation of Sachs et al. may be predominantly composed of glycoprotein with a high carbohydrate content. (2) A considerable amount of glycolipid may be present in the pig gastric membrane, as reported by Sen and Ray [28].

The phospholipid content of the 100 kDa sub-

TABLE II

CARBOHYDRATE COMPOSITION OF THE 100 kDa SUBUNIT

Averages for three determinations on three preparations with standard error of the mean.

Carbohydrate	Content	
	mol/100 mol amino acid	g/100 g protein
Mannose	0.8 ±0.4	1.2 ±0.6
Galactose	1.2 ±0.25	2.0 ±0.4
Glucose	0.9 ±0.2	1.4 ±0.3
Fucose	0.3 ±0.1	0.5 ±0.2
Glucosamine	1.0 ±0.05	2.2 ±0.1
Galactosamine	0.4 ±0.05	0.8 ±0.1
Sialic acid	0.06±0.02	0.17±0.06
Total	4.7 ±0.5	8.3 ±0.8

TABLE III

COMPOSITION AND PARTIAL SPECIFIC VOLUME [a] OF THE 100 kDa SUBUNIT

	Content (% of total weight)	Partial specific volume [a] (cm^3/g)
Protein	88.3	0.7391
Carbohydrate	7.1	0.6366
Phospholipid	4.6	0.9759
Complete	100.0	0.7427

[a] Calculated as described earlier [14].

unit preparation after a second gel filtration procedure has been computed from the organic phosphate content. Assuming 90% of the phosphate originating from phospholipids, as was found for $(Na^+ + K^+)$-ATPase [14], and using a mean molecular weight of 753 for the phospholipids of $(K^+ + H^+)$-ATPase [29], then 5.2 g (S.E. 0.5, $n = 3$) of phospholipid is present per 100 g of protein. Table III summarizes the results of the chemical analyses and gives the protein, carbohydrate and phospholipid contents of the 100 kDa subunit preparation.

Homogeneity of the subunit preparation

Four different approaches have been used in order to test the homogeneity of the isolated subunit preparation: sedimentation analysis, NH_2-terminal amino acid analysis, citraconic anhydride acylation and concanavalin A-binding studies.

Sedimentation studies. Sedimentation analysis of the 100 kDa subunit preparations shows that immediately after detergent removal only the monomeric form is present, but after a few hours dimers appear. Sedimentation coefficients of 4.6 (S.E. 0.4, $n = 2$) for the monomeric form and 8.5 (S.E. 0.1, $n = 2$) for the dimer have been obtained. Other than aggregation, no evidence for heterogeneity like an asymmetric sedimentation profile, has been found in these sedimentation studies.

NH_2-terminal amino acid determination. Demonstration of more than one terminal amino acid would constitute direct proof for the existence of different protein species in the subunit preparation. The 100 kDa subunit preparation has been subjected to dansylation, hydrolysis and two-dimensional thin-layer chromatographic separation of the dansylated products. Only a serine spot is detectable, which is missing after prior removal of bound phospholipids by extraction with chloroform/methanol according to Folch et al. [30]. Thus, the serine spot must have originated from phosphatidylserine bound to the protein. The possibility that the dansylation reaction would not have proceeded normally can be ruled out, since the hydrolysate after thin layer chromatography reveals the normal reaction products resulting from dansylation of a protein (dansyl-OH, dansyl-NH_2 and dansylated non-terminal tyrosine and lysine). Therefore, we must conclude that the terminal NH_2 group is probably blocked, and that these experiments provide neither evidence for heterogeneity, nor proof of homogeneity, but statistically they favour homogeneity.

Acylation with citraconic anhydride. Fig. 3 shows the SDS-polyacrylamide gel electrophoresis pattern of the whole enzyme before and after treatment with citraconic anhydride. After citraconylation the mobility of the 100 kDa band is lowered, which is partly due to an increase of the molecular mass by maximally 7.0 kDa by acylation of the lysine ϵ-NH_2 groups. This effect must, however, be partly compensated by the change in surface charge of the protein due to citraconylation: positively charged amino groups are converted to negatively charged carboxyl groups, which should increase the mobility on the SDS gel.

In the case of protein heterogeneity of the 100 kDa subunit fraction one would expect the protein band to separate due to differences in lysine content or differences in reactivity towards citraconic anhydride of the components. The absence of splitting or even widening of the band renders heterogeneity of the 100 kDa subunit fraction unlikely.

Concanavalin A binding. Since we have found a considerable amount of mannose and glucose in the 100 kDa subunit fraction (Table II), we must assume the presence of one or more concanavalin A-binding glycoproteins. We have determined the percentage of the 100 kDa protein, which will bind to concanavalin A. We find that 86% (S.E. 3%, $n = 7$) of the subunit protein binds to concanavalin A, while a control protein (bovine serum albumin) does not bind at all. This means that at

least 86% of the 100 kDa band is composed of glycoprotein, quite a contrary to the value of 35% reported by Saccomani et al. [12], and pleading against their conclusion that a glycoprotein subunit and one or two non-glycoprotein subunits are present.

In conclusion, it can be stated that the combined evidence from these four approaches argues rather strongly against heterogeneity of the 100 kDa preparation, and thus that the enzyme most likely contains only a single subunit.

The presence of more bands after isoelectrofocussing of the $(K^+ + H^+)$-ATPase preparation, as performed by Sachs et al. [11], need not necessarily indicate heterogeneity of the 100 kDa subunit for the following reasons: (1) their vesicle preparation contains other minor proteins besides the 100 kDa band, (2) the carbohydrate part of a glycoprotein may be heterogeneous [31], (3) part of the 100 kDa (catalytic) subunit may be phosphorylated.

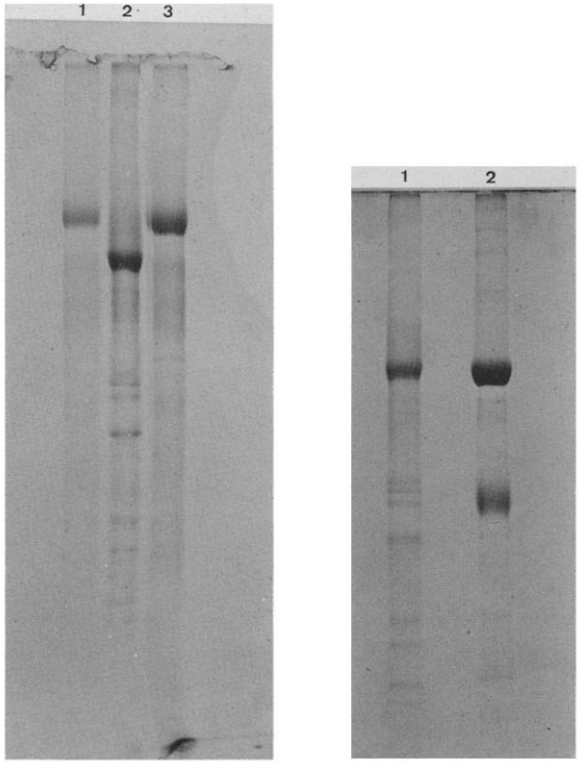

Fig. 3. SDS gel electrophoresis of purified $(K^+ + H^+)$-ATPase before (slot 2), and after citraconilation (slots 1 and 3).

Fig. 4. SDS gel electrophoresis of purified $(K^+ + H^+)$-ATPase (slot 1), and purified $(Na^+ + K^+)$-ATPase (slot 2). Specific activity of $(K^+ + H^+)$-ATPase 98 μmol ATP hydrolyzed per mg protein per h and of $(Na^+ + K^+)$-ATPase 1150 μmol ATP hydrolyzed per mg protein per h.

Molecular weight of the main subunit

The molecular mass of the monomer of the main subunit of $(K^+ + H^+)$-ATPase must be about 130 kDa for the following two reasons: (1) the sedimentation constant (4.6 S) is equal to that of the α-subunit of $(Na^+ + K^+)$-ATPase, which has the same chemical composition and has a total molecular weight of 133 kDa [14], (2) the mobility of the main subunit of $(K^+ + H^+)$-ATPase during SDS-polyacrylamide gel electrophoresis (Fig. 4, slot 1) is about equal to that of the α-subunit of $(Na^+ + K^+)$-ATPase (Fig. 4, slot 2).

Aggregation in the subunit preparation is reflected by the increase of the sedimentation constant from 4.6 to 8.5 upon standing for 2 h after

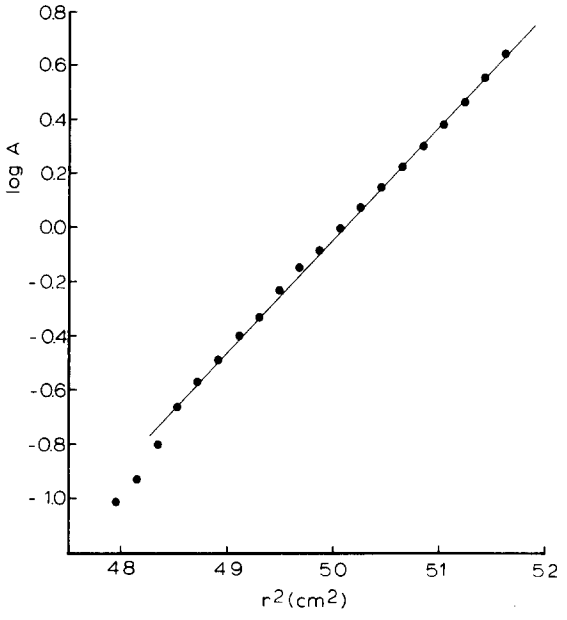

Fig. 5. Sedimentation equilibrium analysis of water-soluble, detergent-free 100 kDa band protein of $(K^+ + H^+)$-ATPase. The 280 nm absorbance of the starting solution was 0.45 in a 50 mM Tris/acetate buffer (pH 7.4, $\rho = 1.003$ g/cm^3). Rotor speed was 6000 rpm for 48 h at 20°C. The log A vs. r^2 plot represents the situation after 16 h centrifugation (A is 280 nm absorbance, r is distance to rotor axis). From this experiment which is representative for six experiments a molecular mass value of 476 kDa is calculated. Data from the same sample after 24 and 48 h yield broken lines, indicating further aggregation. So apparently after 16 h a metastable equilibrium exists.

detergent removal. Since sedimentation equilibrium analysis requires a much longer time, this method will always yield the molecular weight of an oligomer. Starting the collection of data after 7 h, we find only after 16 h a linear plot of $\log A$ vs. r^2 (Fig. 5) which indicates that at that time a metastable equilibrium exists. Using the partial specific volume, given in Table III, we calculate from the plots obtained in six experiments a mean molecular mass value of 498 kDa (S.E. 12, $n = 6$). In view of what has been said in the preceding paragraph, we must assume that the 498 kDa entity most probably represents a tetramer. The mean molecular mass value of the monomer including phospholipid must then be 125 kDa (S.E. 3, $n = 6$), for protein + carbohydrate 119 kDa (S.E. 3, $n = 6$) and for protein only 110 kDa (S.E. 3, $n = 6$). The latter two molecular mass values are not significantly different ($P < 0.05$) from the corresponding values for the α-subunit of $(Na^+ + K^+)$-ATPase (131 kDa, S.E. 4.5, $n = 7$ and 121 kDa, S.E. 4.6, $n = 7$, Ref. 14).

Discussion

Homogeneity of the catalytic subunit preparation

From four rather different approaches we must conclude that the 100 kDa subunit preparation is most probably homogeneous. Sedimentation studies do not reveal heterogeneity, no different NH_2-terminal amino acids are detected, acylation with citraconic anhydride gives a clear shift of the band upon SDS-polyacrylamide gel without band splitting, and concanavalin A binding shows that 86% is glycoprotein. This conclusion is further supported by the following observations: (1) the resemblance of the amino acid composition of the isolated subunit with that of the catalytic subunits of $(Na^+ + K^+)$-ATPase and $(Ca^{2+} + Mg^{2+})$-ATPase, which are both homogeneous, (2) the resemblance between the carbohydrate compositions of the 100 kDa subunit of $(K^+ + H^+)$-ATPase and the α-subunit of $(Na^+ + K^+)$-ATPase. Both the amino acid and carbohydrate compositions of the isolated subunit are rather different from those of the β-subunit of $(Na^+ + K^+)$-ATPase [14].

Hence, our findings contradict the conclusion of Saccomani et al. [12] that the 100 kDa subunit would be composed of three proteins, present in about equal amounts and one of them being a glycoprotein. Their conclusion is based in part on the observation that during digestion of the 100 kDa subunit by trypsin the periodic acid-Schiff staining intensity remains unchanged in the remaining one third part of it. However, this staining method is rather insensitive and irreproducible, so that no quantitative conclusions may be drawn from it. It is always dangerous to draw conclusions about the heterogeneity of a protein after first subjecting it to enzymatic digestion. Finally, their results are complicated by the use of a vesicular preparation of the enzyme. As they point out themselves, if there would be equal quantities of right-side-out and inside-out vesicles, which would be leaky to ATP, but not to trypsin, then their results could also be interpreted in terms of two subunits, a catalytic subunit and a glycoprotein. In view of our data for concanavalin A binding and our further evidence for homogeneity of the 100 kDa subunit fraction we conclude that only one catalytic subunit is present, which must be a glycoprotein, just like the α-subunit of $(Na^+ + K^+)$-ATPase from rabbit kidney outer medulla is a glycoprotein [14].

Molecular mass of the catalytic subunit

The molecular mass for the catalytic subunit of $(K^+ + H^+)$-ATPase calculated from sedimentation equilibrium analysis is 119 kDa (protein + carbohydrate). This value is higher than the value of 105 kDa obtained by SDS-polyacrylamide gel electrophoresis [8]. The high electrophoretic mobility may be due to the relatively high hydrophobic amino acid content, which tends to increase the mobility [32]. The value of 119 kDa is not significantly different from the corresponding value of 131 kDa for the α-subunit of $(Na^+ + K^+)$-ATPase. Recently, Niggli et al. [33] have determined by SDS gel electrophoresis a value of 125–140 kDa for the molecular mass of the catalytic subunit of $(Ca^{2+} + Mg^{2+})$-ATPase from erythrocyte plasma membrane. Together with the similar amino acid compositions of these subunits, this indicates a strong similarity of the catalytic subunits of these three transport ATPases.

Combining the data presented in this paper, we come to the following picture of the isolated puri-

fied $(K^+ + H^+)$-ATPase. The enzyme appears to contain only one subunit, the catalytic subunit. This is a glycoprotein with a protein molecular mass of 110 kDa (S.E. = 3), which in many ways resembles the α-subunit of $(Na^+ + K^+)$-ATPase from rabbit kidney. Radiation inactivation studies of the intact enzyme preparation in the absence of Mg^{2+} have yielded molecular mass values of 413–444 kDa for $(K^+ + H^+)$-ATPase [34], which suggests that the enzyme is a homo-tetramer, at least in the isolated, purified membrane preparation.

This assumption presupposes that only the 110 kDa subunit is required for the $(K^+ + H^+)$-ATPase activity. This presupposition is supported by the observation that SDS gel electrophoretic patterns of different stages in the purification of $(K^+ + H^+)$-ATPase demonstrate enrichment of the 110 kDa band only. Nevertheless, at this moment, with a preparation of about 80% purity, we cannot fully exclude the possible importance of proteins other than the 110 kDa protein.

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