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Lysosomal H^+-translocating ATPase has a similar subunit structure to chromaffin granule H^+-ATPase complex

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Subunit structure of the lysosomal H^+-ATPase was investigated using cold inactivation, immunological cross-reactivity with antibodies against individual subunits of the H^+-ATPase from chromaffin granules and chemical modification with *N,N'*-dicyclohexyl[^{14}C]carbodiimide. The lysosomal H^+-ATPase was irreversibly inhibited when incubated at 0°C in the presence of chloride or nitrate and MgATP. Inactivation in the cold resulted in the release of several polypeptides (72, 57, 41, 34 and 33 kDa) from the membrane, which had the same electrophoretic mobility as the corresponding subunits of chromaffin granule H^+-ATPase. Cross-reactivity of antibodies revealed that the 72, 57 and 34 kDa polypeptides were immunologically identical to the corresponding subunits of chromaffin granule H^+-ATPase. Dicyclohexylcarbodiimide, which inhibits proton translocation in the vacuolar ATPase, predominantly labeled two polypeptides of 18 and 15 kDa, which compose the membrane sector of the enzyme. These results suggest that the lysosomal H^+-ATPase is a multimeric enzyme, whose subunit structure is similar to the chromaffin granule H^+-ATPase. The subunit structure of other vacuolar H^+-ATPases, revealed by cold inactivation and immunological cross-reactivity, is also presented.

Introduction

The lysosomal H^+-translocating ATPase (H^+-ATPase) plays a crucial role in pH homeostasis of lysosomes, maintaining an acidic interior [1,2]. The H^+-ATPase is electrogenic, sensitive to nitrate (Moriyama, Y. and Nelson, N., unpublished data) and *N*-ethylmaleimide, and insensitive to oligomycin, azide and vanadate [3–8]. These observations indicate that the H^+-ATPase is of the vacuolar type, which has been recently classified as a third type of H^+-ATPase [9–15]. However, no structural information on lysosomal H^+-ATPase has been obtained mainly due to the extreme instability of the enzyme.

Recently a few vacuolar H^+-ATPases have been purified in a form which, upon reconstitution, are active as ATP-dependent proton pumps [16–19]. One of them, the H^+-ATPase from chromaffin granules, is composed of at most nine different polypeptides with apparent molecular mass of 115, 72, 57, 41, 39, 34, 20 and 17 kDa [16,23] *. H^+-ATPases from clathrin-coated vesicles and kidney microsome have similar subunits [17–19]; except that the latter lacks the 115 kDa polypeptide. Every vacuolar H^+-ATPase purified so far contains subunits equivalent to the 72, 57 and 17 kDa polypeptides. Gene sequencing analyses provided evidence that these three polypeptides contain sequences homologous to the β, α and DCCD binding subunits of F_0F_1-ATPase, respectively [20–22].

It was found that vacuolar H^+-ATPases exhibited sensitivity to cold under specific conditions such as presence of nitrate and MgATP. The inhibition is irreversible and results in the release of the water-soluble sector of H^+-ATPase from membranes [23]. Five polypeptides with apparent molecular weights of 72 kDa (subunit A), 57 kDa (B), 41 kDa (C), 34 kDa (D) and 33

Abbreviations: Mops, 4-morpholinepropanesulfonic acid; NEM, *N*-ethylmaleimide; DCCD, *N,N'*-dicyclohexylcarbodiimide; DTT, dithiothreitol; SDS, sodium dodecylsulfate.

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* These values are apparent molecular weights of each of the subunits, which are determined on SDS gels. The exact molecular weights of 39 kDa and 17 kDa polypeptide were calculated from the deduced amino acid sequence as 31495 and 15849, respectively [21,40].

kDa (E) were cold-released from chromaffin granules. The phenomena can be observed in all vacuolar membranes from plant or animal sources tested so far, therefore it was expected that the lysosomal H^+-ATPase may yield similar results. Using cold inactivation, immunological cross-reactivity and chemical modification, we investigated the subunit structure of lysosomal H^+-ATPase. In this communication we report that the lysosomal H^+-ATPase has subunit structure similar to the chromaffin granule H^+-ATPase.

Materials and Methods

Materials

Most of the chemicals were purchased from Sigma. ^{125}I-labeled protein A, [γ-^{32}P]ATP, [^{14}C]DCCD and Amplify were from Amersham. Rats (Wistar, male, body weight 150–250 g) were supplied from Charles River Co. Bovine adrenal glands, kidney and brain were obtained from local slaughter house. Red beets (*Beta vulgaris* L), tomatoes and carrots were purchased from the local supermarket.

Analytical methods

Published procedures were used for determination of protein concentrations [24,25], assay of ATP-dependent proton uptake [16,26], SDS-gel electrophoresis and fluorography in the presence of Amplify [27], silver staining [28] and immunoblotting [29]. ATPase activities in lysosomal membranes were measured at pH 8.5 [7,26] using [γ-^{32}P]ATP as substrate.

Preparations

Chromaffin granule membranes were prepared from bovine adrenal glands as previously described [16,26]. The membranes were frozen in liquid nitrogen and stored at $-85°C$. The H^+-ATPase was purified from chromaffin granule membranes and reconstituted as previously described [16,30].

Lysosomal membrane vesicles were prepared from rat liver with minor modifications as described previously [7]. Proteinase inhibitors (pepstatin A, chymostatin, leupeptin and antipain) were used throughout the preparation at 5 μg/ml. Lysosomal membrane vesicles (3–4 mg/ml) were suspended in 20 mM Mops-Tris (pH 7.0), 0.25 M sucrose, 0.5 mM DTT, 0.5 mM EDTA and 5 μg/ml of proteinase inhibitors and used on the day of preparation. In some experiments, lysophosphatidylcholine (0.2 mg/ml) was added to the lysosomal membrane vesicles to remove proteins associated with membranes. The mixture was then centrifuged at 250000 $\times g$ for 20 min. The pellets were washed twice with 20 mM *N*,*N*-bis(2-hydroxyethyl)glycine-Tris (pH 8.5), 0.3 M NaCl, 0.5 mM DTT, 0.5 mM EDTA and 5 μg/ml of proteinase inhibitors. Most proteins associated with membranes were removed from the lysosomal membrane by these treatments without loss of Mg^{2+}-ATPase activity [31,32]. Lysosomes from rat kidney cortex were purified as described by Harikumar and Reeves [6], and their membranes were isolated by hypo-osmotic treatment as described in [7].

Microsomes from bovine kidney medulla were prepared as described according to Ref. 33. Crude clathrin-coated vesicles from bovine brain were prepared as described in [34] omitting the last sucrose ^2H_2O gradient centrifugation step. Synaptic vesicles from rat brain and pituitary granules from bovine brain were prepared according to Refs. 33 and 36, respectively. Microsomes, containing vacuolar membranes, from red beet, tomato and carrot were prepared as previously described [37,38]. All of those membrane preparations were frozen in liquid nitrogen and stored at $-85°C$. Antibodies against the 115 kDa subunit of chromaffin granule H^+-ATPase were prepared as described previously [39]. Antibodies against the 72, 57 and 39 kDa subunits of the chromaffin granule H^+-ATPase were prepared following electroelution of the subunits from SDS gels as described previously [40]. Antibody against the 34 kDa subunit of the chromaffin granule H^+-ATPase was raised after several injections of the polypeptides of the water-soluble sector of chromaffin granule H^+-ATPase [23]. The antibody also weakly recognized both 72 and 57 kDa subunits of the chromaffin granule H^+-ATPase. IgG fractions of these antisera were prepared by protein A-sepharose column chromatography according to the manufacturer's manual.

Results

ATP driven H^+-transport activity was found in lysosomal membranes, however, the H^+-ATPase has not been purified due to the extreme instability of the enzyme. The H^+-pumping activity decreases rapidly with time even in membranes vesicles and no activity was found after overnight incubation either at $4°C$ or at $-80°C$. The instability may be due in part to the presence of many kinds of hydrolase associated with membrane. Furthermore, although relatively high amounts of ATP hydrolytic activities are present in highly purified liver lysosomal membrane, only around 10% of the total ATPase activity was sensitive to NEM, which represents H^+-ATPase activity [2,4,7,8]. Therefore, to obtain the structure of the lysosomal H^+-ATPase, it is necessary to isolate the H^+-ATPase rapidly and specifically from lysosomal membrane even though it is inactive form. Cold inactivation may be useful for this purpose. A short incubation of vacuolar membranes with salt and MgATP at $0°C$ results in inhibition of the H^+-ATPase due to the specific release of water-soluble subunits from the membrane [23].

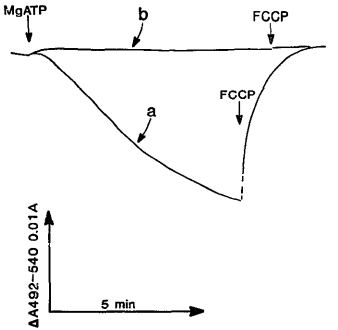

Fig. 1. Nitrate-induced cold inactivation of ATP-driven H^+-transport in lysosomal membrane vesicles. Lysosomal membrane vesicles (4 mg/ml) were suspended in 20 mM Mops-Tris (pH 7.0), 0.2 M sucrose, 0.1 M $NaNO_3$, 0.5 mM DTT, 5 μg/ml leupeptin, 5 μg/ml chymostatin, 5 μg/ml pepstatin A and 5 μg/ml antipain with (b) or without (a) 5 mM MgATP and kept at 0°C for 1 h. ATP-driven H^+-transport activities were measured by Acridine orange absorption change using an Aminco DW-2a spectrometer in 1 ml buffer containing 20 mM Mops-Tris (pH 7.0), 0.1 M KCl, 0.2 M sucrose, 1 μg valinomycin and 15 μM Acridine orange, and lysosomal membranes (100 μg protein) per assay. As indicated, MgATP (1 mM) was added to start H^+-transport and the reaction was terminated by the addition of FCCP (*p*-trifluoromethoxyphenylhydrazone) 1 μM.

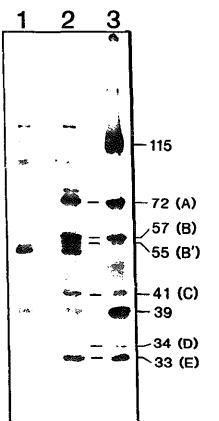

Fig. 2. Release of hydrophilic peptides from lysosomal membranes during cold inactivation of H^+-ATPase. Lysosomal membranes were treated with lysophosphatidylcholine and washed with alkaline buffer as described in Materials and Methods. Membranes (5 mg/ml) were suspended in 20 mM Mops-Tris (pH 7.0), 0.1 M $NaNO_3$, 0.5 mM DTT, 5 μg/ml leupeptin, 5 μg/ml chymostatin, 5 μg/ml pepstatin A and 5 μg/ml antipain with or without 5 mM MgATP. After incubation at 0°C for 1 h, the suspensions were centrifuged by Airfuge at 30 lbs/inch^2 for 30 min. Supernatant was carefully taken and a sample buffer containing SDS was added. Proteins in the supernatant were separated by SDS gel electrophoresis and visualized with Coomassie brilliant blue. Lane 1, supernatant from incubation without MgATP (20 μl). Lane 2, supernatant from incubation with MgATP (20 μl). Lane 3, reconstituted chromaffin granule H^+-ATPase (2 μg of protein).

MgATP-driven H^+-transport in lysosomal membrane vesicles prepared from rat liver was inhibited completely after incubation of the membranes with nitrate and MgATP at 0°C (Fig. 1). Incubation of membranes with nitrate (0.1 M) and MgATP (1 mM) at 0°C gave maximum inhibition, while membranes incubated at room temperature retained about 50% of the H^+-transport activity. Chloride (0.3 M) was less effective than nitrate (0.1 M) for inhibition. About 60% of NEM sensitive ATPase activity in kidney lysosomes was also inhibited by this treatment *. These results show that lysosomal H^+-ATPase exhibits cold-sensitivity, as was observed with other vacuolar H^+-ATPase [23]. Fig. 2 shows that the cold inactivation resulted in the release of a set of polypeptides from membrane vesicles. Although several polypeptides were seen in the supernatant after centrifugation of the membranes, the appearance of the major polypeptides (72, 57, 41, 34 and 33 kDa) were dependent on treatment with MgATP in the cold. These polypeptides have almost identical molecular weights at the corresponding subunits of the chromaffin granule H^+-ATPase. One polypeptide (55 kDa, subunit B') migrating slightly faster than the 57 kDa peptide can always be seen on SDS gel. A polypeptide with the same molecular weight is also present in chromaffin granule H^+-ATPase, which might be proteolytic product of 57 kDa protein. The polypeptides released by cold treatment migrated together on glycerol density gradient following ultracentrifugation to a position corresponding to 400–500 kDa complex as was observed with the water-soluble sector of chromaffin granule H^+-ATPase [23] (not shown). This suggests that

* Lysosomal membrane vesicles from rat kidney cortex exhibited 0.08 units/mg protein of Mg^{2+}-ATPase activity. NEM at 0.1 mM inhibited more than 70% of the ATPase activity [32].

the released polypeptides maintain their multimeric structure.

The use of antibodies against subunits of chromaffin granule H^+-ATPase revealed immunological identity between the polypeptides released from lysosomal membranes and subunits of chromaffin granule H^+-ATPase. Fig. 3 depicts the cross-reactivity of these antibodies. The IgG fractions recognized the corresponding polypeptides specifically released from lysosomal membrane by cold inactivation (Fig. 3A, B, C). A polypeptide (39 kDa), which was recognized by IgG against the 39 kDa subunit of chromaffin granule H^+-ATPase, was present in the lysosomal membrane (Ref. 40 and Fig. 3D). However, this polypeptide was not released during cold inactivation, which was consistent with the results of chromaffin granule H^+-ATPase [23]. These results suggest that lysosomal H^+-ATPase has at least five different hydrophilic subunits whose composition is identical immunologically and electrophoretically to that of chromaffin granule H^+-ATPase.

It is well known that DCCD binds the hydrophobic membrane sector of F_0F_1-ATPase and inhibits the enzymatic activities. Recently, several lines of evidence have indicated the presence of a DCCD binding proteolipid in vacuolar H^+-ATPase [41–48]. The proteolipid (17 kDa polypeptide) from chromaffin granule H^+-ATPase and clathrin-coated vesicle H^+-ATPase may comprise at least part of proton channel of the enzymes [21,42]. Since lysosomal H^+-ATPase activity was also inhibited by this reagent [1,2,6–8,32], similar proteolipids may be components of the enzyme. As shown in Fig. 4, two low molecular mass DCCD binding proteins were identified in the membranes. Two polypeptides (18 and 15 kDa) were predominantly labeled with ^{14}C-DCCD under conditions which result in inhibition of H^+-ATPase. The hydrophobic nature of these polypeptides was demonstrated by the ability to extract them with chloroform/methanol (2:1, v/v) (Fig. 4B), and failure to extract them from membranes by chaotropic ion treatment or cold inactivation as stated above (not shown). DCCD binds to three kinds of hydrophobic polypeptides (115, 20 and 17 kDa) of chromaffin granule H^+-ATPase (Fig. 4). The major DCCD binding polypeptides (18 and 15 kDa) of lysosomal membranes thus might be equivalent of the hydrophobic polypeptides (20, 17 kDa) of chromaffin granule H^+-ATPase, suggesting the presence of a similar hydrophobic sector in lysosomal H^+-ATPase.

Finally, the subunit structures of various vacuolar H^+-ATPases were compared using cold inactivation, immunological cross-reactivity and binding of DCCD as above (Table I). Only the anti-115 kDa antibody did not cross react with any proteins from lysosomes or kidney microsomes. Although there are some minor

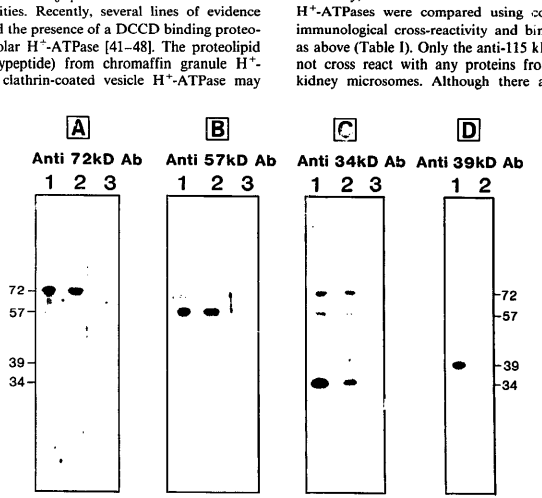

Fig. 3. Antibodies against subunits of chromaffin granule H^+-ATPase recognized the corresponding polypeptides released by cold inactivation. Polypeptide fraction released by cold inactivation was prepared as described in the legend of Fig. 2, and electrophoresed on 10% polyacrylamide gel, transferred onto nitrocellulose as described in Materials and Methods. Nitrocellulose sheets were decorated with 20 μl of anti 72 kDa IgG (A), anti 57 kDa IgG (B), anti 34 kDa IgG (C), and anti 39 kDa IgG (D), respectively. Treatment of ^{125}I-labeled protein A was carried out as described previously [28]. In A, B and C, lane 1, chromaffin granule H^+-ATPase; lane 2, supernatant from incubation with MgATP; lane 3, supernatant from incubation without MgATP. In D, lane 1, lysosomal membrane vesicle (40 μg of protein); lane 2, supernatant from incubation with MgATP.

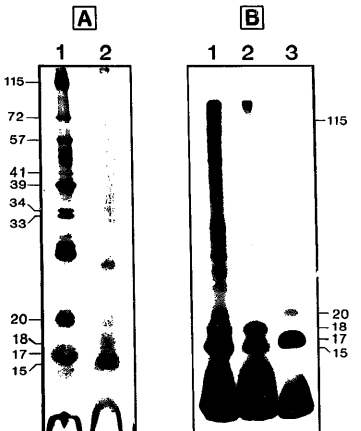

Fig. 4. ^{14}C-DCCD labeling of lysosomal membrane vesicles and extraction of DCCD binding proteins with $CHCl_3$/MeOH mixture. Lysosomal membranes (4 mg/ml) were treated with ^{14}C-DCCD (62 mCi/mmol, 0.4 mM) at 0°C for 1 h, then diluted 10 times with buffer containing 20 mM Mops-Tris (pH 7.0), 0.2 M sucrose, 0.5 mM DTT, 0.5 mM EDTA, 5 μg/ml leupeptin, 5 μg/ml pepstatin A, 5 μg/ml chymostatin and 5 μg/ml antipain. H^+-ATPase activity was completely inhibited by this treatment. The mixture was washed twice by centrifugation at 200000× g for 30 min. Pellets were suspended in 1 ml of the same solution. DCCD binding proteins were extracted with $CHCl_3$/MeOH. $CHCl_3$/MeOH (2:1, v/v) 5 ml was added to 1 ml lysosomal membranes and stirred at 4°C for 2 h. The mixture was centrifuged at 23000× g for 20 min. $CHCl_3$/MeOH layer was carefully taken. Samples were electrophoresed on 12.5% polyacrylamide gel and stained with silver (A) or visualized by fluorography in the presence of Amplify (B). (A) Lane 1, purified chromaffin granule H^+-ATPase (4 μg of protein); lane 2, $CHCl_3$/MeOH extract from lysosomal membrane (1 μg of protein). (B) Lane 1, lysosomal membrane (80 μg of protein); lane 2, $CHCl_3$/MeOH extract from lysosomal membrane (1 μg of protein); lane 3, purified H^+-ATPase from chromaffin granule membrane (4 μg of protein).

differences, the results clearly show that all vacuolar H^+-ATPases, especially of animal origin, have very similar subunit structure. Thus, it is concluded that lysosomal H^+-ATPase has a structure which is typical of vacuolar type H^+-ATPases.

Discussion

The vacuolar class of H^+-ATPases contain a large number of H^+-ATPase species in various endomembrane systems [1,2,9–15]. Recent immunological and molecular biological studies on the ATPase show the three major subunits (≅ 70, ≅ 60 and ≅ 17 kDa polypeptides) from Archaebacteria to mammalian are conserved [20–22,43,44,52,53]. Biochemical studies also suggest that both 70 and 60 kDa subunits function as the active site and 17 kDa subunit as the proton channel of the enzyme [16,18,30,41–45,48–51]. Since limited data is available about this class of ATPase, further studies are required to generalize about the structure and function of the class of ATPases.

Lysosomal H^+-ATPase is one of the structurally undefined H^+-ATPases. Lysosomes are derived from Golgi apparatus and may fuse with endosomes, as part of the endocytic network within the cell. Although these organelles are independent of each other, they have similar H^+-ATPase [1,2,9–15]. Moreover, mutant cells which are conditionally defective in endosomal H^+-pump activity, but are normal in lysosomal H^+-pump activity, have been reported [56–58]. Thus, it is interesting to see whether H^+-ATPase in these organelles are identical, similar or different.

In this paper, the subunit structure of lysosomal H^+-ATPase was investigated using cold inactivation. Cold inactivation resulted in the release of water-soluble sector of H^+-ATPases in all vacuolar ATPases tested so far [23]. The inactivation occurred under conditions in which the membraneous F_1-ATPase is protected against cold inactivation [55,56]. Since it is difficult to prepare lysosomal membranes without mitochondrial contamination [1,2], the use of the cold inactivation coupled with immunological techniques excludes the possibility that the F_1-ATPase is mistaken for lysosomal H^+-ATPase. As expected, lysosomal H^+-ATPase exhibited sensitivity to cold in the presence of salts and MgATP (Fig. 1), and resulted in the release of a set of polypeptides (Fig. 2). The identity of some of the related proteins was verified by cross-reactivity with antibodies against subunits of chromaffin granule H^+-ATPase (Fig. 3). It is worthwhile to note that when lysosomal membranes were applied directly to SDS gel and Western blotting, partial digestion by lysosomal proteinases occurred decreasing the immunoreactivity of the corresponding subunits. Furthermore, the DCCD binding protein was identified which migrated to be similar position on SDS gel as the counterparts from the chromaffin granule H^+-ATPase, but not as subunit c of F_0F_1-ATPase (Fig. 4). Northern blot analysis probing mRNA from various sources, by cDNA encoding the 39 kDa and the proteolipid, also indicated that the lysosomal H^+-ATPase is similar if not identical to the chromaffin granule enzyme [40]. The presence of only a single gene encoding the 57 kDa or the proteolipid in yeast gives credence to this assumption [22]. These results indicate that subunits of lysosomal H^+-ATPase are similar to those of chromaffin granule H^+-ATPase.

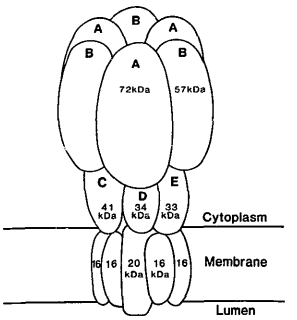

Fig. 5. A model for the subunit structure of vacuolar H^+-ATPases.

Thus, lysosomal H^+-ATPase may be composed of two distinct sectors; a hydrophilic (catalytic) sector and hydrophobic (membrane) sector, the former comprised of the 72, 57, 41, 34 and 33 kDa polypeptides and the latter containing at least 39, 18 and 15 kDa polypeptides.

By the same strategies, subunit structures of several unpurified H^+-ATPases from various vacuolar membranes can be partially elucidated (Table I). It was found that similar minor subunits, as well as three major subunits ($\cong$ 70, $\cong$ 60 and $\cong$ 17 kDa proteins), were present in all H^+-ATPases tested regardless of species, organs or organelles. These results support further the idea that vacuolar H^+-ATPase are highly conserved from Archaebacteria through mammals [22]. Fig. 5 depicts a model for the subunit structure of vacuolar H^+-ATPases. The 115 kDa protein and the 39 kDa protein are not included in the model because these polypeptides seemed to be accessory subunits of H^+-ATPase as discussed below and in Ref. 40. According to staining pattern and amino acids analysis of each subunit [59], stoichiometry of the subunits seems to be 72 kDa (3), 57 kDa (3), 41 kDa (1), 34 kDa (1), 33 kDa (1), 20 kDa (1) and 17 kDa (6). Water-soluble sector containing catalytic center (subunits A to E) is exposed to cytoplasm and membrane sector containing proton channel (subunit 20 kDa and 17 kDa protein) is present in membrane. This model is in line with the model presented by Arai et al. [59], except that subunits, C, D and E (41 kDa, 34 kDa, 33 kDa proteins) may be hydrophilic proteins by virtue of their release by cold inactivation (Fig. 2, Table I and Ref. 23). Thus, the basic subunit structure of vacuolar H^+-ATPase might resemble that of F_0F_1-ATPase.

One of the major differences in the subunit structure is the 115 kDa protein, which is the highest molecular weight subunit of vacuolar H^+-ATPase. H^+-ATPase from lysosomes, kidney microsomes and plant vacuoles seem to be devoid of this subunit. The subunit does not seem to be involved in the catalytic processes since the purified enzyme from kidney microsome is active upon reconstitution [18]. We cannot conclude that the subunit is actually absent in the lysosomal H^+-ATPase because the antigenic activity of the subunit is extremely sensitive to proteinase treatment (not shown). Another major difference in the structure from various sources is the 39 kDa polypeptides, which is present in all animal sources

TABLE I

Subunit structures of vacuolar H^+-ATPases

Vacuolar membranes from plant or animal sources were prepared as described in Materials and Methods. Subunits of vacuolar H^+-ATPases were identified by cold inactivation followed by SDS gel electrophoresis of released polypeptides, immunoblotting using antibodies against 115, 72, 57, 39 and 34 kDa of subunits of chromaffin granule H^+-ATPase or ^{14}C-DCCD labeling followed by fluorography as described in the text. Apparent molecular weight of each polypeptide, which was determined on 10% or 12.5% SDS gels, was expressed. Polypeptides which are determined as equivalent subunits are circled. n.d., not detected; n.t., not tested; Ac1 and Ac2, accessory subunit 1 and 2, respectively.

Source of H^+-ATPase	Subunit								
	Ac1	A	B	C	Ac2	D	E		
Adrenal chromaffin granules (bovine)	[a] 115	[ab] 72	[ab] 57	[b] 41	[a] 39	[ab] 34	[a] 33	20	[c] 17
Liver lysosomes (rat)	n.d.	72	57	41	39	34	33	18	15
Kidney microsome (bovine)	n.d.	72	57	41	39	34	33	–	15
Brain clathrin-coated vesicles (bovine)	[a] 115	72	57	41	39	34	33	–	17
Brain synaptic vesicles (rat)	115	72	57	41	39	34	33	–	17
Brain pituitary granule (bovine)	115	72	57	41	39	34	33	n.t.	n.t.
Vacuolar membrane rich microsome (red beet)	n.d.	69	55	[b] 44	n.d.	n.d.	[b] 33	–	17
Vacuolar membrane rich microsome (tomato)	n.d.	69	55	44	n.d.	n.d.	33	–	17.5
Vacuolar membrane rich microsome (carrot)	n.d.	69	55	44	n.d.	n.d.	33	–	18

[a] Subunit identified with immunological cross-reactivity of anti 115 kDa, 72 kDa, 57 kDa, 39 kDa or 34 kDa antiserum.
[b] Polypeptides released by cold inactivation.
[c] ^{14}C-DCCD binding proteins.

but not from plant sources. This subunit is a membraneous protein with one hydrophobic region [40] and therefore is not released by cold inactivation (Fig. 3). Although we do not know the functional role of this subunit, it is possible that immunologically distinct counterpart is present in plant vacuole ATPases.

According to the highly conserved features of the subunit structure of vacuolar H^+-ATPases presented in this paper, it is possible to predict the subunit structure of unpurified H^+-ATPases. In eukaryotes, a single cell may contain Golgi apparatus, lysosomes, various endosomes and various secretory granules. It is highly probable that these organella have at least immunologically identical H^+-ATPases. It is of interest to determine if the subunits of different H^+-ATPases are the product of the same gene or not.

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