Molecular Cloning and Sequencing of cDNAs Encoding the Proteolipid Subunit of the Vacuolar H\(^{+}\)-ATPase from a Higher Plant*

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To understand the molecular structure of the vacuolar H\(^{+}\)-translocating ATPase from plants, cDNAs encoding the 16-kDa proteolipid from oat (Avena sativa L. var. Lang) have been obtained. A synthetic oligonucleotide corresponding to a region of the bovine proteolipid cDNA (Mandel, M., Moriyama, Y., Huymes, J. D., Pan, Y.-C. E., Nelson, H., and Nelson, N. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 5521-5524) was used to screen an oat cDNA library constructed in agt11. The nucleotide sequences of several positive clones (VATP-P1, clones 12, 54, 93) demonstrated the presence of a small multigene family. The four clones showed extensive divergence in their codon usage and their 3′-untranslated regions; however, the deduced amino acid sequences of the proteins were 97-99% identical. These clones encoded the proteolipid subunit as one of them (clone 12) expressed a fusion protein that reacted with an antibody to the 16-kDa proteolipid. The open reading frame of one cDNA clone (VATP-P1) predicted a polypeptide of 165 amino acids with a molecular mass of 16,641. Based on hydrophathy plots, a molecule with four membrane-spanning domains was predicted, in which domain IV was especially conserved among different species. This domain showed 80% identity in nucleotide or amino acid sequences between the oat and the bovine proteolipids and contained a glutamate residue that is the putative N,N′-dicyclohexylcarbodiimide-binding residue. The presence of a small multigene family of the 16-kDa proteolipid was confirmed by Southern blot analysis showing that several distinct restriction fragments of oat nuclear DNA hybridized with the VATP-P1 cDNA.

The tonoplast H\(^{+}\)-pumping ATPase in higher plants utilizes the energy of ATP hydrolysis to pump protons into the vacuolar lumen (1, 2). The proton motive force drives secondary active transport of various ions and metabolites such as Ca\(^{2+}\), anions, amino acids, and sugars into vacuoles (2). In plant cells vacuoles not only serve as storage compartments but are also important for regulating cytoplasmic pH and cytoplasmic ion levels (e.g., Ca\(^{2+}\)) and thus maintaining cytoplasmic homeostasis and cell turgor (3). The tonoplast ATPase belongs to a class of vacuolar-type ATPases (V-ATPases) found on membranes of acidic compartments in many eukaryotes such as fungal vacuoles, bovine chromaffin granules, and brain clathrin-coated vesicles (for review see Ref. 4) and on the plasma membrane of archaeabacteria (5).

The eukaryotic V-ATPase is a multimeric enzyme consisting of 3-10 different subunits with a mass of about 450-759 kDa (see 4 and references therein). Three subunits common to the eukaryotic V-ATPases are the major polypeptides of 67-73 (70) and 57-60 (60) kDa and a 16-17-kDa proteolipid (e.g., 6-11). The primary amino acid sequences of the 70 kDa and the 60-kDa polypeptides have been deduced recently from cDNA or genomic DNA sequences (e.g., 12-15). Identification of nucleotide binding domains supported previous experiments showing nucleotide binding sites on both the 70- and 60-kDa subunits (e.g., 16-18). The 70- and 60-kDa polypeptides are thought to be catalytic and regulatory subunits, respectively, which participate in catalysis analogous to the interaction between the α and β subunits of the F,Fo-ATPase.

These two large polypeptides form part of a peripheral sector that can be removed fromvacuolar membranes by chaotropic anions (e.g., 10, 19, 20), an observation confirmed by the hydrophilic nature of the deduced amino acid sequences of cloned 70- and 60-kDa subunit genes (12-15).

The 16-kDa polypeptide is a major subunit of the membranous sector that binds N,N′-dicyclohexylcarbodiimide (DCCD), an inhibitor of the V-ATPase activity and a potential H\(^{+}\) pore blocker (21-23). The DCCD-binding subunit can be extracted with chloroform/methanol, like the 8-kDa proteolipid of the F,Fo-ATPase (24). We have estimated that the 16-kDa proteolipid was present in about six copies/V-ATPase complex, and the binding of DCCD to one copy caused full inhibition of the ATPase activity (21). Sun et al. (25) presented evidence for H\(^{+}\) translocation by the purified and reconstituted 16-kDa proteolipid from coated vesicle V-ATPase; however, it is unclear whether an H\(^{+}\) pore is formed by 16-kDa alone or in combination with other integral subunits.

To understand the molecular structure of the vacuolar H\(^{+}\)-translocating ATPase(s), the amino acids in the 16-kDa proteolipid of oat, a higher plant. The nucleotide sequences and amino acid sequences from the oat proteolipid share obvious homology with those of yeast and bovine V-ATPase recently reported by Nelson and colleagues (26, 27). Our data also indicate that the 16-kDa proteolipid in oat is encoded by a small multigene family.

*The abbreviations used are: V-ATPase(s), vacuolar-type H\(^{+}\)-translocating ATPase(s); DCCD, N,N′-dicyclohexylcarbodiimide; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.
EXPERIMENTAL PROCEDURES

**Plant Material**—Oat seeds (Avena sativa L. var. Lang) were germinated in the dark (22°C) over an aerated solution of 0.5 mM CaSO4. Roots were harvested after 3 days of growth.

**Isolation of Total RNA and Poly(A)+ RNA**—Roots (2–10 g, fresh weight) were pulverized in liquid N2 and extracted with 4.0 M guanidine thiocyanate. The RNA was pelleted through a 5.66 M CsCl cushion (28). The yield was approximately 0.3 mg of RNA/g, fresh weight. Poly(A)+ RNA was purified from total RNA with oligo(dT)-cellulose (Sigma) (28). Total or poly(A)+ RNA was used subsequently for gel blot analysis, cDNA synthesis, or in vitro translation.

The presence of full-length mRNA of V-ATPase subunits was tested before constructing a cDNA library. The poly(A)+ RNA preparation from oat roots was transfected in a eukaryotic cell line (Panhcytes) with labeling of [32P]Sphingomyelin (Du Pont-New England Nuclear). The products were immunoprecipitated with polyclonal antibodies to oat V-ATPase (anti-ATPase) and Staphylococcus aureus Cowan I (antisera), separated by SDS-polyacrylamide gel, and fluorographed (29). Translation products at molecular masses of 70, 60 kDa, and occasionally at 18 kDa were detected as shown before (29). Such a poly(A)+ RNA preparation was then used for construction of a cDNA library.

**Constructs of cDNA Library in λgt11**—Double-stranded cDNA was synthesized from the poly(A)+ RNA preparation using the method of Young and Jackson (30) and cloned into the λgt11 vector (Stratagene) (31). The blunt ends of the double-stranded cDNA were created by filling in with T4 DNA polymerase (New England Biolabs). Potential internal EcoRI sites within the cDNA were protected by modifying the cDNA with EcoRI methylase (Promega). EcoRI linkers (Pharmacia) were added to the cDNA blunt ends with T4 DNA ligase (Promega) and cleaved with EcoRI (Promega) to generate cohesive EcoRI termini. The cDNAs were size-fractionated by disulfite acrylamide gel electrophoresis, and those longer than 300 base pairs were cloned into EcoRI-digested digested phosphorlylated λgt11 arms (Stratagene) with T4 DNA ligase (Promega) (32). The original cDNA library was estimated to contain 5 × 106 independent recombinants. The library was amplified (31) with Escherichia coli Y1088 (Stratagene) before screening.

**Synthesis of Oligodeoxynucleotides**—Oligodeoxynucleotides were synthesized by Dr. T. G. Kenne (Protein and Nucleic Acid Laboratory, University of Maryland) using Biosearch model 8750 DNA synthesizer and extracted with ether twice. Probes for screening a cDNA library or for blotting were labeled at the 5' end by T4 polynucleotide kinase (New England Biolabs) and synthesized with [γ-32P]ATP (Du Pont-New England Nuclear) or Amersham Corp.) (28) and separated from unincorporated labeled ATP by passing through a Bio-Gel P-60 column (Bio-Rad). Specific activity of labeled probes was about 1–5 × 108 cpm/nmol. Other synthetic oligonucleotides were used as sequencing primers.

**Slot Blotting**—Conditions for hybridization of a synthetic probe to oat root cDNA were determined by slot blotting using a Mini Slot blot device (Schleicher & Schuell) to transfer DNAs onto GeneScreen Plus membrane (Du Pont-New England Nuclear). Prehybridization and hybridization were carried out at 42°C in 5 × SSPE (1 × SSPE is 0.15 M NaCl, 10 mM Na2HPO4, pH 7.4, 1 mM EDTA-Na2) and 2 × Denhardt's solution (10 × Denhardt's solution is 1% polyvinylpyrrolidone, 1% bovine serum albumin, and 1% Ficoll 400), 0.1 mg/ml salmon sperm DNA, 0.1% SDS with varying concentrations of deionized formamide (0–50%). Oligodeoxynucleotide probes 5' end labeled with 32P were used at 1–5 × 106 cpm/ml. After hybridization the membranes were washed three times at room temperature successively with 1 × SSPE, 0.1% SDS; 0.5 × SSPE, 0.1% SDS; and 0.25 × SSPE, 0.1% SDS for 10 min each. Further washing was done at 42°C for 30 min in 5 × SSPE, 0.1% SDS with formamide at the same concentration as in the hybridization buffer.

**Screening cDNA Library with Oligodeoxynucleotide Probe**—The cDNA library was screened by plaque hybridization (29). Plaques at a density of 109 plaque-forming units (PFU) were transferred in a Biodyne II Colony/Plaque Screen (Du Pont-New England Nuclear). DNA on the membrane was denatured with 0.2 M NaOH, 1.5 M NaCl and neutralized with 2 × SSPE. Prehybridization, hybridization, and washing were performed as described above with 20% formamide in hybridization and wash buffer. Positive clones were plaque purified by two additional rounds of plaque hybridization with the same probe.

**Subcloning of cDNA Inserts**—DNA from the positive λgt11 recombinants were prepared in large scale from lysates of E. coli Y1090 grown in liquid culture (28). cDNA inserts were cleaved from recombinant phage DNA with EcoRI, separated by agarose gel electrophoresis, and recovered by electrophoresis onto DEAE-pitocellulose membrane (NA-45, Schleicher & Schuell). Plasmid DNA of pT7/T3 (Bethesda Research Laboratories) was dephosphorylated with alkaline phosphatase (Promega), and ligated to the cDNA inserts with T4 DNA ligase. Freshly made competent cells of E. coli DH5 α−p− (Bethesda Research Laboratories) were transformed with the subcloned cDNA pT7/T3-18 recombinants and selected on YT plates containing ampicillin, 5-bromo-4-chloro-3-indoly-β-D-galactoside and isopropyl β-D-thiogalactopyranoside (28). The lacZ+ colonies (white) were picked and plated on the same YT medium.

**Isolation of Plasmid DNA and DNA Sequencing**—Plasmid DNA (pT7/T3 α−18 recombinant) was isolated according to a method in Technical Manual of GeneSeq K/R system (Promega Biotec, 1986). Double-stranded plasmid DNA was denatured with 0.2 M NaOH, 0.2 mM EDTA and annealed with sequencing primers at 37°C for 20 min. Sequencing was done by the diideoxynucleotide chain termination method (32) using Sequense II (U. S. Biochemical Corp.) or T7 DNA polymerase (Pharmacia). Sequencing primers for both strands of pT7/T3 α−18 DNA (Bethesda Research Laboratories) and synthetic primers (20–30 bases) to internal sequences of the cDNA inserts (see Fig. 24 legend) were used to obtain the sequences of both strands.

**Expression of β-Galactosidase Fusion Protein and Immunoblotting**—β-Galactosidase fusion proteins were expressed in E. coli Y1090 (Promega) by induction with isopropyl β-D-thiogalactopyranoside, and crude lysates were prepared (31). Specific antibody to the 16-kDa proteolipid (anti-16) was affinity purified from anti-V-ATPase (16, 19). Proteins in the E. coli lysates were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrottransferred onto Immobilon-P membrane (Millipore), and immunostained with anti-ATPase or anti-16 (19).

**Nuclear DNA Isolation and Southern Blotting**—The nuclear DNA from leaves of 7-day-old oat seedlings was isolated as described (33), digested with EcoRI, BamHI, or HindIII, separated by 10% agarose gel electrophoresis, and transferred onto Nytran membrane (Schleicher & Schuell). The cDNA insert of clone VATP-P1 was excised from plasmid pT7/T3 α−18 with EcoRI, purified by 1.0% low melting point agarose gel electrophoresis, and labeled with [α-32P]dATP (Amersham Corp.) and the random primer labeling kit (Boehringer Mannheim). Hybridization was done at 42°C in 6 × SSPE, 10 × Denhardt's solution, 100 μg/ml denatured salmon sperm DNA, 0.5% SDS, and 50% deionized formamide. Final washing was done at 65°C in 0.1 × SSPE and 0.5% SDS for 45 min.

**RESULTS**

**Isolation of cDNA Clones**—Our initial strategy for cloning the cDNA encoding the proteolipid of oat V-ATPase was to construct an oat cDNA library in λgt11. To ensure the presence of V-ATPase cDNAs we constructed the cDNA library using a poly(A)+ RNA preparation of oat roots from which V-ATPase subunits could be translated in vitro and identified by immunoprecipitation (not shown) (29). We screened the oat root cDNA library with a synthetic oligodeoxynucleotide probe corresponding to a presumptive conserved region of the proteolipid from bovine V-ATPase.2 The synthetic 90-mer (d(CAGCAGCCGCGGCTCTTCGTGGGCATGATCCTCATGCACGAGGGCTCGGGCAGTGGTCTTATTGAGCCCTATTACG) corresponded to nucleotides 499–588 of the cDNA sequence of bovine proteolipid (26). This region shares identical amino acid sequences corresponding to the nucleotide sequence (positions 583–672) of the yeast proteolipid cDNA (27) and includes the putative DCDC binding site, a glutamate residue at amino acid 139 of bovine proteolipid.

2 N. Nelson, personal communication.
...gencies (0–50% formamide). With 20% formamide in the hybridization and final wash buffers, the 90-mer hybridized with oat DNA and calf DNA but not XDNA and E. coli DNA (data not shown). We subsequently screened the oat cDNA library with the 90-mer using 20% formamide in 5 × SSPE at 42 °C for hybridization and washing. From screening about 500,000 plaques 16 positive clones were obtained at the intermediate stringency.

To confirm whether these positive clones encoded the proteolipid subunit, β-galactosidase fusion proteins were produced with isopropyl β-D-thiogalactopyranoside in E. coli Y1090 or Y1089 and examined by SDS-PAGE and immunoblot. Among the 16 clones only one (clone 12) produced a fusion protein with apparent molecular mass of 128 kDa which was detected with anti-ATPase (not shown) or anti-16 (Fig. 1), an immunoselected antibody from the polyclonal antiserum to the oat V-ATPase holoenzyme. Because the other positive clones cross-hybridized with clone 12 (data not shown) it was likely that they also encoded the proteolipid subunit. The DNA sequences of clone 12 (386 base pairs) and three other clones (designated clones 54, 93, and 16) exhibited extensive similarity to the bovine proteolipid cDNA (see below); however, none of these four clones appeared to contain a complete open reading frame.

To obtain a cDNA clone containing a complete open reading frame, a synthetic oligonucleotide (d(CGACGAGACCGCCCCCTCTTCGGCTTCCT)) corresponding to 30 bases at the 5’ end of the longest clone (clone 93, 754 base pairs in length) was synthesized and used as a hybridization probe to rescreen the oat cDNA library. From about 10⁴ plaques one cDNA clone of about 0.9 kilobases was obtained. This cDNA insert was cloned into ptT₇/T₈ α-18 to yield the plasmid pVATP-P1. DNA sequencing revealed that the cDNA insert contained a complete open reading frame encoding the proteolipid (Fig. 2B).

**Nucleotide Sequence and Deduced Amino Acid Sequence**—The nucleotide sequence of the pVATP-P1 was determined.
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by double-stranded DNA sequencing (Fig. 2, A and B). VATP-P1 consisted of 873 base pairs and was near full-length judging from a primer extension product of the oat mRNA. This primer extension product of about 300 bases (not shown) was obtained using a synthetic primer with reverse-complementary sequence starting at residue 280 in Fig. 2B. An mRNA of about 0.9 kilobases in length was also found in Northern blot analysis with the cDNA probe (not shown). If the first in frame AUG (Met-1 in Fig. 2B) served as the start codon, a polypeptide consisting of 165 residues with a calculated molecular mass of 16,641 was obtained. This molecular mass agreed with the 16-kDa apparent mass of a DCCD-binding proteolipid purified by chloroform/methanol extraction (21) and the DCCD-binding subunit of the oat V-ATPase (6).

Evidence for a Small Multigene Family of 16-kDa Proteolipid in Oat—We have obtained one complete cDNA clone VATP-P1 and three partial cDNA clones (clones 54, 12, and 93) encoding the 16-kDa proteolipid (Fig. 3). Of these four, only in clone 12 was the cDNA insert cloned in frame with the correct orientation relative to the lacZ sequence of the vector (Fig. 3). This explained our finding that only clone 12 encoded a β-galactosidase fusion protein recognized by the antibody to the 16-kDa proteolipid (Fig. 1). From the deduced amino acid sequences (Fig. 3) we concluded that clones VATP-P1, 12, 54, and 93 coded for the same polypeptide. The amino acid sequences deduced from the partial clones and VATP-P1 were 97-99% identical. In general, only one to two amino acids differed between their deduced sequences.

More extensive divergence among the four clones was found in their codon usages and the 3' untranslated regions (Fig. 3). Codon usage varied the most between clones 93 and 12, in which 34 out of 89 codons (38%) were different. In contrast, clone pVATP-P1 and 54 were so similar that only 3 out of 136 codons (2%) differed. These results provide evidence for a small multigene family encoding the V-ATPase proteolipid.
vealed four (I-IV) very hydrophobic domains. The four domains, corresponding to amino acid residues 11-33, 56-76, 96-117, and 130-155 (see Fig. 5), were remarkably similar to those in bovine (26) and yeast (27) proteolipids. These four stretches of 21–25 amino acids were highly conserved and were thought to span the membrane (26). Domain IV was especially conserved in which the oat amino acid and nucleotide sequences were 80% identical to the bovine sequences. It was therefore not surprising that a 90-mer corresponding to the bovine nucleotide sequence of the fourth membrane-spanning domain was a useful probe for identifying the proteolipid cDNA from diverse organisms such as oat.

Domain IV might be a functionally conserved region since it included the putative DCCD-binding site. By analogy with the Fo proteolipid, DCCD is thought to inhibit H+-pumping vacuolar ATPase by reacting with carboxyl groups of glutamate or aspartate (36) found in the hydrophobic environment of the membrane (21). Glu-142 in oat proteolipid (Fig. 2), like Glu-139 in bovine (26) or Glu-137 in yeast (27) proteolipid, was an excellent candidate for DCCD modification for the following reasons. (i) The glutamate residue was in a hydrophobic domain. (ii) By computer analysis the DCCD-binding glutamate of an 8-kDa Fo proteolipid (Glu-61 in rice chloroplast ATPase proteolipid) was aligned to Glu-142 in oat V-ATPase proteolipid (Fig. 7). The 8-kDa proteolipid (81 amino acids) from rice chloroplast ATPase (37) shared 31% identity and 25% conserved substitution in an 81-amino acid overlap with the V-ATPase proteolipid (Fig. 7). The similarity among monocots (rice and oat) was also observed in comparisons between oat V-ATPase and the membrane sector of the chloroplast ATP synthetase proteolipids from dicots such as tobacco and soybean (not shown). Our results are remarkably similar to those obtained by Nelson and colleagues in their comparisons of the 16-kDa proteolipid from bovine chromaffin granule with the Fo proteolipids from yeast mitochondria and spinach chloroplasts (26).

Interestingly, two hydrophilic stretches, amino acid residues 34-55 and 118-129 (Fig. 5), were also very conserved among eukaryotic proteolipids, suggesting potential functional relevance. However, there was relatively less similarity between the oat and the archaeabacterium proteolipids (Fig. 5). The H+-ATPase from the archaeabacterium Sulfolobus is simpler than the eukaryotic enzyme, consisting of five peripheral subunits (66, 55, 25, 13, and 7 kDa) and a 10-kDa proteolipid (5, 34, 38). It has been suggested that gene duplication occurred when eukaryotic V-ATPase proteolipids evolved from an ancestral proteolipid gene (26). The align-
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We have isolated one cDNA (designated VATP-P1) and several partial cDNAs from oat that encoded the proteolipid subunit of V-ATPase. Several lines of evidence support this conclusion. (i) Clone 12 encoded a $\beta$-galactosidase fusion protein that reacted with the antibody against the 16-kDa proteolipid of oat V-ATPase (Fig. 1). (ii) Both cDNA and deduced amino acid sequences of VATP-P1 were nearly identical to clone 12 and two other cDNA clones (see Fig. 3). (iii) The deduced amino acid sequence of VATP-P1 showed extensive similarity with that of proteolipids of bovine (26) and yeast V-ATPase (27) (Fig. 5), particularly in domain IV with the putative DCCD binding site. (iv) Two internal polypeptide sequences from the proteolipid of another higher plant (red beet) V-ATPase showed 82 and 93% identity with amino acids 23–39 and 134–149 of oat proteolipid, respectively (Fig. 2B).

Nevertheless, we cannot eliminate the possibility that some of the cDNA clones encode a 16-kDa proteolipid-like polypeptide from a membrane protein complex other than the V-ATPase. To date there is no evidence for any other polypeptide with structure and function similar to the proteolipid of V-ATPase in higher plants. In contrast, a 16-kDa protein coisolating with gap junctions from bovine brain (39) and a 15-kDa proteolipid found in mediatophore preparations from Torpedo electric organ (40) show extensive similarity to the proteolipid of V-ATPase. Whether the proteolipid of V-ATPase is indeed a part of the gap junction complex or part of the mediatophore complex remains to be answered. In animals it is suggested that the proteolipid of V-ATPase plays a more diversified role in intercellular communication (39, 40).

Divergences in codon usage and in the 3' untranslated regions of the cDNA clones of oat proteolipid (Fig. 3) taken together with the Southern blot analysis of oat nuclear DNA (Fig. 4) provide strong evidence for the existence of a small multigene family in the oat genome. Sequence comparisons between the two most similar cDNA clones, VATP-P1 and 54, revealed three A to C substitutions, two A to G substitutions, and a G deletion (Fig. 3). These differences could not result from transcription of a single copy gene with different polyadenylation sites. More extensive differences among clones VATP-P1, 12, 54, and 93 further suggest that they perhaps represent different gene copies coding for isoforms of the 16-kDa proteolipid in oat. Since the oat genome is hexaploid, some of the cDNAs encoding the proteolipid could be allelic variants. However, clone 93 and VATP-P1 were most probably derived from two different loci because their 3' untranslated regions were completely different (Fig. 3). Genetic mapping and other studies are required to establish the extent and significance of this small multigene family in oat genome. In contrast, there is only a single copy of the DCCD-binding proteolipid gene in yeast genome (27). Recently, two reports showed that disruption of the cloned yeast proteolipid gene was conditionally lethal (41) and resulted in complete loss of V-ATPase activity and the ability to acidify the vacuole in vivo (42).

The significance of a small multigene family encoding the 16-kDa proteolipid in oat is not yet clear. One simple interpretation is the increase in gene dosage to meet the demands for the 16-kDa proteolipid in plant cells. Another one is that there perhaps are distinct types of V-ATPases associated with different tissues, cell types, or subcellular organelles. Multiple genes can provide isoforms with unique properties and levels of expression to satisfy the specialized requirement of H\textsuperscript{+} pumping activity for different tissues, cell types, or subcellular organelles. Biochemical evidence supports this notion. For example, some V-ATPases contain a 100-kDa subunit (7, 8, 10) whereas others do not (6, 43). Furthermore, a different type of V-ATPase may be associated with the Golgi (43, 44) than with the coated vesicles or vacuole/lysosome.

There is no evidence for the presence of a cleavable signal peptide in the deduced amino acid sequence of the oat 16-kDa proteolipid. The molecular mass deduced from the open read-

**Fig. 6.** Four membrane-spanning domains are suggested by hydropathy profiles of the proteolipid subunits from the oat, bovine, and yeast V-ATPase. Hydropathy data were analyzed by GenePro according to Kyte and Doolittle (38) with a window of 7. All sequences are numbered according to that of oat. Four hydropobic membrane-spanning domains are shown as I, II, III, and IV. E refers to the putative DCCD-binding glutamate residue in domain IV.

**Fig. 7.** Similarity between the amino acid sequences of the V-ATPase 16-kDa proteolipid (OAT) and a chloroplast ATPase 8-kDa proteolipid (RICE) (37). Vertical slashes indicate amino acid identity, and double or single periods indicate highly conserved or conserved substitutes, respectively. Amino acid residue numbers of the two peptides are shown. Gaps (shown as -) are introduced to align the sequences maximally.

| RESIDUE No. | OAT | BOVINE | YEAST |
|-------------|-----|--------|-------|
| 86          | 5FLFGYAVLRSLGLAGLIALAGLAAAGMAIGIVGAAGVRAANQPP...128 |
| 1           | NVPLAAAVTAAAGGLAAAGLSDGGQVQVQGAAGVAGLIARPEAE 46 |
| 129         | KLPVHLIRAAAALALALVIGVIFILSSLRAQQRAD 165 OAT |
| 47          | GIKGTLISLAPAELTIYVULAVALALLFPFP* 81 RICE |

| DISCUSSION |

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There is no evidence for the presence of a cleavable signal peptide in the deduced amino acid sequence of the oat 16-kDa proteolipid. The molecular mass deduced from the open read-
ing frame (16.6-kDa) agrees remarkably well with that estimated from SDS-PAGE analysis of the purified proteolipid (21). This finding would suggest that the proteolipid is synthesized and spontaneously inserted into the membrane bilayer either during or after translation. If so, then one interesting question is which of the internal sequences target the subunit to its final destination. The orientation of the proposed proteolipid structure (e.g. sidedness of the N and C termini), the organization of the integral subunits to form a H⁺- pore, and its functional residues all remain to be established. Having the primary structure of the V-ATPase proteolipid is a first step toward resolving these questions.

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