**N,N'-Dicyclohexylcarbodiimide-binding Proteolipid of the Vacuolar H+-ATPase from Oat Roots**

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In higher plant cells, vacuoles play a fundamental role in the maintenance and regulation of cell turgor and in the transport and storage of various ions and metabolites (1). An electrogenic H+-translocating ATPase is found in the vacuolar membrane (tonoplast) of several higher plant species as well as yeast and Neurospora (2,3). Our laboratory has recently shown that this proton pump can provide the proton motive force for active transport of solutes, such as Ca\(^{2+}\) (4), and various anions (5,6), across the tonoplast of oat root cells.

The tonoplast H+-ATPase from higher plants has been well characterized and represents a separate class of ATPase ("V-type" for vacuolar, see Ref. 7 and references therein) which differs from the "P-type" (plasma membrane and endoplasmic reticulum) and FIFo-type (mitochondria, chloroplast, bacteria) ATPases on immunoblots. From the purification studies, we estimated that the 16-kDa subunit was present in multiple (4–8) copies/holoenzyme. The purification of the proteolipid is a first step towards testing its proposed role in H\(^+\) translocation.

The inhibitor N,N'-dicyclohexylcarbodiimide (DCCD) was used to probe the structure and function of the vacuolar H\(^+\)-translocating ATPase from oat roots (*Avena sativa* var. Lang). The second-order rate constant for DCCD inhibition was inversely related to the concentration of membrane, indicating that DCCD reached the inhibitory site by concentrating in the hydrophobic environment. \(^{[14}C\)DCCD preferentially labeled a 16-kDa polypeptide of tonoplast vesicles, and the amount of \(^{[14}C\)DCCD bound to the 16-kDa peptide was directly proportional to inhibition of ATPase activity. A 16-kDa polypeptide had previously been shown to be part of the purified tonoplast ATPase. As predicted from the observed noncooperative inhibition, binding studies showed that 1 mol of DCCD was bound per mol of ATPase when the enzyme was completely inactivated. The DCCD-binding 16-kDa polypeptide was purified 12-fold by chloroform/methanol extraction. This protein was thus classified as a proteolipid, and its identity as part of the ATPase was confirmed by positive reaction with the antibody to the purified ATPase on immunoblots. From the purification studies, we estimated that the 16-kDa subunit was present in multiple (4–8) copies/holoenzyme. The purification of the proteolipid is a first step towards testing its proposed role in H\(^+\) translocation.

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\[^{[14}C\)Nbd-C1 specifically labeled the 72-kDa subunit of the oat tonoplast ATPase (18); this labeling could be protected by the substrate ATP or a potent competitive inhibitor, TNP-ATP, indicating that the 72-kDa subunit contains a substrate-binding site. The antibody to the 72-kDa polypeptide specifically inhibited the tonoplast ATPase and H\(^+\) pumping activities, consistent with the idea that the 72-kDa polypeptide contains a catalytic domain (18). Similar results had been reported for the vacuolar ATPase from corn coleoptiles (19) and Neurospora (17). In red beets, Bz-ATP bound to the 57-kDa polypeptide of the tonoplast ATPase, suggesting that this subunit also had a nucleotide-binding site (12). Since Bz-ATP was not a simple competitive inhibitor of the ATPase, Manoleon et al. (12) concluded that this binding site might be a regulatory site.

This paper describes our first study to understand the structure and function of the 16-kDa subunit of the plant tonoplast ATPase. This subunit of the purified vacuolar ATPase from several different tissues is preferentially labeled by \(^{[14}C\)DCCD (3,10,12,15,19) and is thought to be involved in proton translocation across the membrane (10). We present evidence that the inhibition of the tonoplast ATPase is caused by DCCD binding to the 16-kDa subunit and occurs after DCCD partitioning into the membrane. The 16-kDa subunit was purified 12-fold by chloroform/methanol extraction, indicating that this subunit is a proteolipid and is probably embedded within the membrane. Although there are several copies of the 16-kDa subunit per holoenzyme, 1 mol of DCCD/...
mol of ATPase is sufficient for inactivation. A preliminary report of this study has been presented (20).

EXPERIMENTAL PROCEDURES

Plant Material—Oat seeds (Avena sativa L. var. Lang) were germinated in the dark over an aerated solution of 0.5 mM CaSO4. After 3 or 4 days of growth, the roots were harvested.

Preparation of Tonoplast Vesicles, Tonoplast ATPase, and Mitochondria—Tonoplast vesicles were prepared from oat roots by the procedure of Churchill and Sze (8) with minor modifications. The tonoplast vesicle preparation was further separated by centrifugation on a 6% dextran cushion. Low-density vesicles collected from the turbid 0/6% (w/w) dextran interface were referred to as tonoplast vesicles (2, 9). The vesicles could be stored at -70°C for several weeks without loss of ATPase activity. The ATPase was purified by gel filtration (Sepharose CL-6B) after solubilization of tonoplast membrane proteins with 5% Triton X-100 (10).

Mitochondria obtained in the 8,000 X g pellet were further purified on sucrose step gradients consisting of 0.6, 1.2, 1.5, and 1.8 M sucrose (21). More than 90% of the ATPase activity of the 1.2/1.5 M interface was sensitive to 0.2 mM azide. Less than 1% of the activity originated from the tonoplast ATPase as judged by azide-resistant and nitratase-sensitive activity.

ATPase Assay—The release of ADP was monitored by measuring NADH oxidation spectrophotometrically at 340 nm in a coupled lactate dehydrogenase (LDH) and guinea pig brain lactate dehydrogenase (LDH) at 25°C in a 0.5 ml of reaction mixture. All assays contained 0.1 mM sodium molybdate to inhibit any contaminating acid phosphatase activity. The mitochondrial ATPase was assayed in the presence of 0.05% Triton X-100 and determined as azide-sensitive activity (±0.2 mM sodium azide) (9). The membrane-bound tonoplast ATPase was assayed in the presence of sodium valadate (200 mM) and sodium azide (200 mM) to inhibit activity from the plasma membrane and mitochondrial ATPases, respectively. Genticidin (2.5 mM) was added to prevent any inhibitory effect of the proton electrochemical gradient (2). The assay was initiated by addition of 10-18 μg tonoplast protein. After 4 min at 37°C, ATP, ADP, and AMP were determined. The tonoplast ATPase was routinely expressed as N2O-sensitive activity. Generally, more than 75% of the activity was N2O-sensitive.

To measure inhibition of the tonoplast ATPase by DCCD, tonoplast vesicles were diluted with resuspension buffer (2.5 mM Hepes-CH3CN containing 50 mM CH3CN) at 25°C in a 0.5 ml of reaction mixture. All assays contained 1 mM DCCD. ATPase activity was determined in the presence of sodium valadate (200 mM) and sodium azide (200 mM) to inhibit activity from the plasma membrane and mitochondrial ATPases, respectively. Genticidin (2.5 mM) was added to prevent any inhibitory effect of the proton electrochemical gradient (2). The assay was initiated by addition of 10-18 μg tonoplast protein. After 4 min at 37°C, ATP, ADP, and AMP were determined. The tonoplast ATPase was routinely expressed as N2O-sensitive activity. Generally, more than 75% of the activity was N2O-sensitive.

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RESULTS

Kinetics of DCCD Inhibition of the Tonoplast H+-ATPase—Incubation of the membrane-bound ATPase with DCCD resulted in rapid inactivation of the enzyme activity (Fig. 1A). The pseudo-first-order rate constants for DCCD inhibition determined from the semilog plot of Fig. 1A were proportional to the DCCD concentration (Fig. 1B), indicating noncompetitive inhibition by 1 mol of DCCD/mol of ATPase (30).

The second-order rate constants for DCCD inhibition (k) determined from Fig. 1B were inversely proportional to the concentration of membranes, being 1225 × 10-3 M-1·m for 500 μg protein/ml and 638 M-1·min-1 for 400 μg protein/ml. Identical second-order rate constants were obtained for both protein concentrations when the concentration of DCCD was expressed as nmols/milligrams of protein. Thus, the rate of enzyme inactivation was not dependent on the DCCD concentration in molar units, but rather in units of nanomoles of DCCD/milligrams of membrane protein, which are used in subsequent experiments. The dependence of the second-order rate constant on the concentration of vesicles suggests that DCCD reached the inhibitory site by partitioning into the
beled. We have found that labeling was more specific for the protein, Fig. 3).

plast proteins unselectively at high concentrations. Branes continued to increase (Fig. 2A).

labeling 30 min with low DCCD concentrations (80 nmol/mg radioactivity was associated with a 16-kDa polypeptide after gel electrophoresis and analyzed by fluorography. Most of the DCCD/mg of protein, the ATPase activity was completely inhibited (Fig. 2B); however, the binding of DCCD to membranes continued to increase (Fig. 2A). Since DCCD forms covalent bonds with hydrophobically located carboxyl groups (32), the results suggest that DCCD labeled numerous tonoplast vesicles and under the same experimental conditions. After 30 min incubation with low concentrations of DCCD (29–63 nmol/mg), there was a fairly good correlation between DCCD binding and ATPase inhibition. After incubation for 30 min with 486 nmol of DCCD/mg of protein, the ATPase activity was completely inhibited (Fig. 2B); however, the binding of DCCD to membranes increased as ATPase activity (Fig. 2). Both binding and activity assays were conducted using the same tonoplast vesicles and under the same experimental conditions. After 30 min incubation with low concentrations of DCCD (29–63 nmol/mg), there was a fairly good correlation between DCCD binding and ATPase inhibition. After incubation for 30 min with 486 nmol of DCCD/mg of protein, the ATPase activity was completely inhibited (Fig. 2B); however, the binding of DCCD to membranes continued to increase (Fig. 2A). Since DCCD forms covalent bonds with hydrophobically located carboxyl groups (32), the results suggest that DCCD labeled numerous tonoplast vesicles unselectively at high concentrations.

To determine which polypeptides were labeled by [14C]DCCD, the tonoplast membrane proteins were separated by gel electrophoresis and analyzed by fluorography. Most of the radioactivity was associated with a 16-kDa polypeptide after labeling 30 min with low DCCD concentrations (80 nmol/mg protein, Fig. 3). After 30-min incubation at 150 or 500 nmol of DCCD/mg of protein, many other polypeptides were labeled. We have found that labeling was more specific for the 16-kDa polypeptide at 0°C than at 20°C when tonoplast vesicles were incubated with [14C]DCCD to give the same degree of inhibition of ATPase activity (data not shown).

hydrophobic environment of the membrane.

Double-reciprocal plots of the half-time of inactivation (t½) versus 1/[DCCD] have been used to obtain evidence for a saturable noncovalent enzyme inhibitor complex preceding the covalent link of the inhibitor to the enzyme (31). An analogous plot (not shown) derived from the presented data gave a y intercept of zero, suggesting that there is no saturable noncovalent intermediate involved in the inactivation reaction.

DCCD Binding to the 16-kDa Subunit of the ATPase Is Directly Proportional to Inhibition of ATPase Activity—Although [14C]DCCD labeled the 16-kDa polypeptide of the purified tonoplast ATPase (10), the relationship between binding and inactivation has not been studied previously. To determine whether the inactivation of the ATPase was caused by covalent DCCD binding, the effect of incubation time on DCCD binding to membranes was compared to the inhibition of ATPase activity (Fig. 2). Both binding and activity assays were conducted using the same tonoplast vesicles and under the same experimental conditions. After 30 min incubation with low concentrations of DCCD (29–63 nmol/mg), there was a fairly good correlation between DCCD binding and ATPase inhibition. After incubation for 30 min with 486 nmol of DCCD/mg of protein, the ATPase activity was completely inhibited (Fig. 2B); however, the binding of DCCD to membranes increased as ATPase activity (Fig. 2). Both binding and activity assays were conducted using the same tonoplast vesicles and under the same experimental conditions. After 30 min incubation with low concentrations of DCCD (29–63 nmol/mg), there was a fairly good correlation between DCCD binding and ATPase inhibition. After incubation for 30 min with 486 nmol of DCCD/mg of protein, the ATPase activity was completely inhibited (Fig. 2B); however, the binding of DCCD to membranes continued to increase (Fig. 2A). Since DCCD forms covalent bonds with hydrophobically located carboxyl groups (32), the results suggest that DCCD labeled numerous tonoplast vesicles unselectively at high concentrations.

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purification of the mitochondrial proteolipid from yeast and proved to be more satisfactory. The chloroform/methanol that only 1 mol of DCCD bound per mol of ATPase is.

About 3-4 polypeptides were detected in the chloroform/methanol (6:l) extraction; 2.25%); lane 6, tonoplast membranes (15%; lane 7, P2 (6:1 extraction; 1%); lane 8, P2 (2:1 extraction; 3%); lane 9, P2 (6:1 extraction; 2%); lane 10, P2 (6:1 extraction, resuspended in sample buffer lacking urea).

To confirm that the 16-kDa DCCD-binding protein is a DCCD-binding protein.

We estimated the number of DCCD bound per mol of ATPase protein at 20 'C for 30 min and extracted with chloroform/methanol (61 extraction; 1%) extraction; 2%;)

To test whether the 16-kDa proteolipid bound DCCD, tonoplast vesicles were labeled with [14C]DCCD and then extracted with organic solvents. Although numerous polypeptides in the membrane fraction formed covalent bonds with DCCD, most of the radioactivity extracted by chloroform/methanol (6:1) and subsequently precipitated by ether was attributed to a polypeptide of 16-18 kDa (lane 9 in Fig. 5B). These results demonstrate that the 16-kDa proteolipid is a DCCD-binding protein.

Based on the total radioactivity recovered from each fraction, the DCCD-binding proteolipid after ether precipitation was only purified 4.5-fold relative to the tonoplast vesicles (Table I). But careful examination of the fluorogram (Fig. 5B, lane 6) clearly demonstrates that this would be an underestimate of the purification, since much of the radioactivity in the tonoplast vesicles was associated with other polypeptides.

By determining the relative specific activity of [14C]DCCD associated with the 16-kDa polypeptide alone in all fractions, the purity of the DCCD-binding proteolipid in the ether pellet would be about 12-fold. Since only about 36% of the radioactivity and 11.5% of the protein in the ether pellet was attributable to the 16-kDa band, the actual purification of the 16-kDa polypeptide in the gel may be as high as 105-fold (see legend to Table I). A similar maximal purification -fold was obtained when the 16-kDa proteolipid was extracted with chloroform/methanol at 2:1 (v/v) (three experiments, results not shown).

We had estimated before that there would be about 0.1 nmol of ATPase (50 µg/ml) when the molecular mass of the holoenzyme is about 500 kDa. If a 100-fold purification is required to purify the 16-kDa polypeptide, then there are about 10 µg or 0.6 nmol of the 16-kDa subunit/mg of membrane protein. Although these values are based on several estimates, we can conclude that there are several copies (perhaps as many as four to eight) of the 16-kDa subunit/ATPase holoenzyme.

To confirm that the 16-kDa DCCD-binding protein is part of the tonoplast ATPase, the proteolipid fraction was probed...
been determined, it is possible that the ATPase complex may
proteolipid fraction (Fig. 6, with antiserum made against the tonoplast ATPase.
diazophenylthioether paper and probed with antiserum made against
61 of chloroform/methanol.

Tonoplast vesicles or mitochondria were separated by SDS-PAGE (9-
15% acrylamide). Parallel gels were silver-stained
fraction; lanes 3 and 6, tonoplast proteolipid fraction extracted with 6:1 of chloroform/methanol.

with the antibody to the holoenzyme. The partially purified
ATPase (lane 4 of Fig. 6) showed intense reaction at 72, 60, 45 kDa and weaker reaction with 16 and 13 kDa. Although the functions of all these polypeptides have not been determined, it is possible that the ATPase complex may consist of all these subunits. The anti-ATPase antibody reacted with a single band of mass 16 kDa from the tonoplast proteolipid fraction (Fig. 6, lane 6), but not with the 8-kDa proteolipid from the mitochondria (Fig. 6, lane 5). We can therefore eliminate the possibility that the 16-kDa proteolipid was an aggregate of the 8-kDa proteolipid from the mitochondriald ATPase. These results support the idea that the 16-kDa DCCD-binding proteolipid is part of the tonoplast ATPase (10, 12) and also suggest that the DCCD-binding proteolipid of the tonoplast ATPase is immunologically different from the mitochondrial one.

**DISCUSSION**

DCCD is a very useful probe for understanding the structure and function of the tonoplast H\(^{+}\)-translocating ATPase. Here we have shown the following: (i) DCCD inhibits the tonoplast ATPase by partitioning into the hydrophobic environment of the membrane. (ii) Kinetic analyses of DCCD inhibition of the membrane-bound tonoplast ATPase suggests that 1 mol of DCCD bound per ATPase is sufficient to inactivate the enzyme (Fig. 1). This prediction was confirmed by binding experiments that showed 1 mol of DCCD was bound to the 16-kDa polypeptide when the tonoplast ATPase was completely inhibited (Fig. 4). (iii) The 16-kDa subunit of the tonoplast ATPase is a proteolipid, supporting the idea that it is an integral membrane protein. (iv) Purification studies indicate that the 16-kDa proteolipid is present in multiple copies per holoenzyme (perhaps four to eight copies). How DCCD interacts with the 16-kDa subunit(s) to inhibit ATP hydrolysis and \( H^{+}\) translocation is still unclear.

The similarities between the 16-kDa proteolipid of the tonoplast ATPase and the 8-kDa proteolipid of the mitochondrial, chloroplast, and bacterial F_{i}F_{o}-ATPase are striking. DCCD has been a useful probe for studying the structure and function of transport proteins (32), especially the F_{i}F_{o}-ATPase (36). Although DCCD can interact with carboxyls, sulphydryls, and tyrosines, it has been shown mostly to form covalent bonds with hydrophobically located carboxyl groups, such as glutamate and aspartate residues (32). When DCCD reacts with a carboxyl residue, an unstable O-acetylated adduct is first formed which can then rearrange to a stable N-acetylated. One of the results suggesting the participation of the 8-kDa proteolipid of the F_{i}F_{o}-ATPases in proton translocation came from bacterial mutants which had lost their ability to catalyze proton transport via the ATPase after the DCCD-sensitive carboxyl group had been eliminated by conversion to a glycine or asparagine (reviewed in Ref. 37). Based on our preliminary findings, it is tempting to infer that the 16-kDa proteolipid of the tonoplast ATPase is analogous in structure and function to the 8-kDa proteolipid of the F_{i}F_{o}-ATPases and that DCCD inhibits both enzymes by
a similar mechanism. However, there are indications that the two proteolipids vary. The tonoplast ATPase is less sensitive to DCCD than the mitochondrial enzyme from oat roots (9). The second-order inactivation rate constant of the tonoplast ATPase reported here is about a hundred times smaller than that of bovine heart mitochondria (30). The 16-kDa proteolipid of the tonoplast ATPase is two times larger than the mitochondrial proteolipid from oats, and they appear to be distinct immunologically (Fig. 6). Thus, determination of the complete molecular structure of the 16-kDa proteolipid is needed to establish its structural organization and its proposed role in proton translocation.

Note Added in Proof—After submission of this paper, two publications reporting similar characteristics of the DCCD-binding proteolipids of the vacuolar H+-ATPase from red beet tonoplast (38) and bovine brain clathrin-coated vesicles (39) have appeared. Another study has shown by reconstitution into proteoliposomes that the DCCD-binding polypeptide from bovine clathrin-coated vesicles has a role in H+-translocation (40).

REFERENCES

1. MacRobbie, E. A. C. (1979) in Plant Organelles (Reid, E. ed) pp. 61–67, Ellis Horwood Ltd., Chichester, Sussex, Great Britain.
2. Sze, H. (1985) Annu. Rev. Plant Physiol. 36, 175–208.
3. Bowman, B. J., and Bowman, E. J. (1986) J. Membr. Biol. 94, 83–97.
4. Schumaker, K. S., and Sze, H. (1986) J. Biol. Chem. 261, 12172–12178.
5. Kaehtner, K. H., and Sze, H. (1987) Plant Physiol. 83, 483–489.
6. Schumaker, K. S., and Sze, H. (1987) Plant Physiol. 83, 490–496.
7. Pedersen, P. L., and Carafoli, E. (1987) Trends Biochem. Sci. 12, 146–150.
8. Churchill, K. A., and Sze, H. (1983) Plant Physiol. 71, 610–617.
9. Wang, Y., and Sze, H. (1985) J. Biol. Chem. 260, 10434–10443.
10. Randall, S. K., and Sze, H. (1986) J. Biol. Chem. 261, 1364–1371.
11. Xie, X. S., and Stone, D. K. (1986) J. Biol. Chem. 261, 2492–2495.
12. Manolson, M. F., Rea, P. A., and Poole, R. J. (1985) J. Biol. Chem. 260, 12273–12279.
13. Mandalia, S., and Taiz, L. (1985) Plant Physiol. 78, 327–333.
14. Tognoli, L. (1985) Eur. J. Biochem. 146, 581–588.
15. Marin, B., Preissler, J., and Komor, E. (1985) Eur. J. Biochem. 151, 131–140.
16. Uchida, E., Ohsumi, Y., and Anraku, Y. (1985) J. Biol. Chem. 260, 1090–1095.
17. Bowman, E. J., Mandalia, S., Taiz, L., and Bowman, B. J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 48–52.
18. Randall, S. K., and Sze, H. (1987) J. Biol. Chem. 262, 7135–7141.
19. Mandalia, S., and Taiz, L. (1986) J. Biol. Chem. 261, 12850–12855.
20. Sze, H., Randall, S. K., Kaestner, K. H., and Lai, S. (1987) UCLA Symp. Mol. Cell. Biol. New Ser. 63, 195–207.
21. Douce, R., Christensen, E. L., and Bonner, W. D., Jr. (1972) Biochim. Biophys. Acta 275, 146–160.
22. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
23. Bensadoun, A., and Weinstein, D. (1976) Anal. Biochem. 70, 241–245.
24. Houšek, J., Svozbova, P., Kopecký, J., Kuzela, S., and Dráhota, Z. (1981) Biochim. Biophys. Acta 634, 331–339.
25. Laemmli, U. K. (1970) Nature 227, 680–685.
26. Kaplan, R. S., and Pederson, P. L. (1985) Anal. Biochem. 150, 97–104.
27. Marshall, T. (1984) Anal. Biochem. 139, 506–509.
28. Tsang, V. C., Peralta, J. M., and Ray Simons, A. (1983) Methods Enzymol. 92, 377–391.
29. Renart, R. T., and Sandoval, I. V. (1984) Methods Enzymol. 104, 455–460.
30. Kopecký, J., Dédina, J., Votruba, J., Svoboda, P., Houšek, J., Babitch, S., and Dráhota, Z. (1982) Biochim. Biophys. Acta 680, 80–87.
31. Meloche, H. P. (1967) Biochemistry 6, 2273–2280.
32. Nalecz, M. J., Casey, R. P., and Azzi, A. (1986) Methods Enzymol. 125, 86–108.
33. Sigrist, H., Sigrist-Nelson, K., and Gitler, C. (1977) Biochem. Biophys. Res. Commun. 74, 178–184.
34. Nelson, N., Eytan, E., Notsani, B. E., Sigrist, H., Sigrist-Nelson, K., and Gitler, C. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 2375–2378.
35. Sebald, W., Graf, T., and Lukins, H. B. (1979) Eur. J. Biochem. 93, 587–599.
36. Sebald, W., and Hoppe, J. (1981) Curr. Top. Bioenerg. 12, 1–64.
37. Schneider, E., and Altendorf, K. (1984) Trends Biochem. Sci. 9, 53–55.
38. Rea, P. A., Griffith, C. J., and Sanders, D. (1987) J. Biol. Chem. 262, 14745–14752.
39. Araï, H., Berne, M., and Forjac, M. (1987) J. Biol. Chem. 262, 11006–11011.
40. Sun, S.-Z., Xie, X.-S., and Stone, D. K. (1987) J. Biol. Chem. 262, 14790–14794.