Characterization of the Subunit Structure of the Maize Tonoplast ATPase

IMMUNOLOGICAL AND INHIBITOR BINDING STUDIES*

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Gradient purified preparations of the maize 400-kDa tonoplast ATPase are enriched in two major polypeptides, 72 and 62 kDa. Polyclonal antibodies were prepared against these two putative subunits after elution from sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel slices and against the solubilized native enzyme. Antibodies to both the 72- and 62-kDa polypeptides cross-reacted with similar bands on immunoblots of a tonoplast-enriched fraction from barley, while only the 72-kDa antibodies cross-reacted with tonoplast and tonoplast ATPase preparations from Neurospora. Antibodies to the 72-kDa polypeptide and the native enzyme both strongly inhibited enzyme activity, but the 62-kDa antibody was without effect.

The identity and function of the subunits was further probed using radiolabeled covalent inhibitors of the tonoplast ATPase, 7-chloro-4-nitro[14C]benzo-2-oxa-1,3-diazole ([14C]NBD-C1) and N,N'-[14C]dicyclohexylcarbodimide ([14C]DCCD). [14C]NBD-C1 preferentially labeled the 72-kDa polypeptide, and labeling was prevented by ATP. [14C]DCCD, an inhibitor of the proton channel portion of the mitochondrial ATPase, bound to a 16-kDa polypeptide. Venturicidin blocked binding to the mitochondrial 8-kDa polypeptide but did not affect binding to the tonoplast 16-kDa polypeptide. Taken together, the results implicate the 72-kDa polypeptide as the catalytic subunit of the tonoplast ATPase. The DCCD-binding 16-kDa polypeptide may comprise the proton channel. The presence of nucleotide-binding sites on the 62-kDa polypeptide suggests that it may function as a regulatory subunit.

Plant vacuoles are acidic organelles in which ions, sugars, organic acids, and hydrolytic enzymes are stored (1). Studies with isolated vacuoles and tonoplast (plant vacuolar membrane) vesicles have indicated that a proton-translocating ATPase present on the tonoplast generates an electrochemical gradient, which may be responsible for the observed accumulation of ions and solutes (reviewed in Ref. 2). A similar ATP-dependent proton pump is also present on the Golgi of maize coleoptiles (3). Several recent reports have described the partial purification of a novel ATPase from plant and fungal vacuolar membranes (4–12). The partially purified ATPases retain the same characteristics as the tonoplast proton pumps but appear to differ significantly from the other two types of H+-ATPases known to occur in these cells, the FoF₁ type found in mitochondria and chloroplasts and the ElE₂ type located on plasma membranes. The plant and fungal vacuolar membrane ATPases are not inhibited by the plasma membrane ATPase inhibitor, vanadate, or the mitochondrial ATPase inhibitor, azide, but are sensitive to KNO₃ and KSCN (4–12). The subunit composition of the vacuolar membrane ATPases also appears to be novel. Partial purifications have indicated enrichment in two major polypeptides, one of M₄ 66,000–89,000 and the second of M₄ 54,000–64,000 (4, 5, 7, 8, 10, 12, 13). In beet root, Neurospora, and yeast, a polypeptide of M₄ 15,000–19,500 has been shown to bind to [14C]-DCCD (5–7). The presence of multiple subunits resembles the FoF₁-type ATPases rather than ElE₂-type ATPases. However, sufficient differences are observed in the subunit composition and inhibitor effects to suggest that the tonoplast proton pumps may represent a third type of ATPase.

In animal cells, ATP-dependent proton-pumping activity has been measured in many endomembrane vesicles, including lysosomes (13), chromaffin granules (14), Golgi (15), coated vesicles (16, 17), synaptosomes (13), and multivesicular bodies (19). The characteristics of proton-pumping activity in the animal hydrolytic or storage organelles are similar to those of plant and fungal vacuoles, suggesting possible homology. An early report from Cidon and Nelson (14) postulated a novel subunit composition for the chromaffin granule ATPase. They reported enrichment of polypeptides of M₄ 125,000, 80,000, 40,000, and 20,000. More recently, Percy et al. (20) reported the presence of 5 polypeptides in preparations of chromaffin granule ATPase I of apparent M₄ 70,000, 57,000, 41,000, 33,000, and 16,000. The smallest polypeptide bound radioactive DCCD. Thus, there appears to be some similarity among plant, fungal, and animal endomembrane ATPases.

Previously we have shown that an antibody produced to the M₄ 72,000 protein from maize cross-reacts to a similar polypeptide in Neurospora vacuolar membranes (5). In the present study, we have examined the effects of antibodies made to the M₄ 72,000 and 62,000 proteins on ATPase activity and have analyzed their cross-reactivity to barley tonoplast membranes. In addition, radioactively labeled inhibitors of the tonoplast ATPase were used as probes to characterize putative subunits on SDS gels.

MATERIALS AND METHODS

Enzyme Purification—Solubilization and purification of the tonoplast ATPase was conducted as previously described (4). Briefly, 1

1 The abbreviations used are: DCCD, N,N'-dicyclohexylcarbodiimide; NBD-Cl, 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole; SDS, sodium dodecyl sulfate; Mes, 4-morpholineethanesulfonic acid.
Protein and increased the specific activity of the tonoplast ATPase for 1 h in a Beckman SW 60 rotor. The pellet was resuspended in treated with 0.5 pCi of [14C]NBD-C1 (109 mCi/mmol) to give a final concentration of 75 mCi/ml ammonium per sulfate and resuspended in 50 mM Tris/HCl, pH 7.4. Following a 1-h incubation on ice in the presence of 50 pg/ml venturicidin for 10 min on ice. A 90-min incubation with 0.475 M, [14C]NBD-C1 at a concentration of 12 μM was terminated by dilution into 4 ml of resuspension buffer and centrifugation at -70 °C. We have previously shown that the ATPase fractions (C). Lanes: 1, gradient-purified maize tonoplast ATPase (-62K); 2, deoxycholate-washed maize tonoplasts (-72K); 3, Neurospora gradient-purified vacuolar ATPase (-72K); 4, barley tonoplast-enriched membranes (-72K); 5, Neurospora vacuolar membranes (-72K); 6, Neurospora vacuolar membranes (-72K). A, B, C. Anti-ATPase

RESULTS

Immunological Characterization—Antibodies to the M, 62,000 and 72,000 polypeptides were produced by immunizing rabbits against bands cut out from SDS gels. Specific cross-reaction to the appropriate proteins was detected by immunoblotting as shown in Fig. 1. Antibodies to the M, 62,000 polypeptide cross-reacted strongly with the M, 62,000 protein from deoxycholate-washed maize tonoplasts and the partially 3% acrylic, 0.08% bisacyclamide, 0.12 m Tri-HCl, pH 6.8. The running buffer was 0.2 m glycine, 0.25 m Tris, and 0.1% SDS. Proteins were mixed with sample preparation buffer to final concentrations of 0.1 m phenylmethylsulfonyl fluoride, 1.7% SDS, 1% β-mercaptoethanol, 8% glycerol, 42 mM Tris-HCl, pH 6.8, and incubated for 20 min at 70 °C. The gels were stained and destained by the method of Fairbanks et al. (25).

Fluorography—Gels of 14C-labeled material were incubated in 20 ml of ENHANCE (New England Nuclear) for 1 h and then rinsed with cold water for 1 h before drying. Intensifying screens (Du Pont) were used with 12P and 12I-treated material. Radioactive bands were detected with Kodak X-ray (XAR-5) film after 1-24-day storage in the dark at -70 °C.

Electrotransfer—Proteins from SDS gels were electrotransferred to nitrocellulose, and the blots were washed, incubated with antibody, and treated with 12I-Protein A, as described by Rott and Nelson (26). For the overnight incubation with antisera, a concentration of 20 μl of buffer was used for the antisera to the M, 62,000 and 72,000 proteins and 40 μl of antisera to gradient-purified ATPase.

Protein Assay—Protein was determined by the method of Lowry et al. (27) after trichloroacetic acid precipitation.

Neurospora and Barley Membranes—The Neurospora vacuolar membranes and ATPase fractions, prepared as described in Ref. 5, were provided by Dr. E. J. Bowman, University of California, Santa Cruz. The barley membranes were a gift from Dr. F. Du Pont (United States Department of Agriculture, Albany, CA) and were obtained from a 22/33% interface of a sucrose step gradient using a modification of the procedure described in Du Pont and Hurkman (28). The barley membranes were obtained from Difco. Venturicidin came from BDH Chemicals, Ltd., Great Britain. All other chemicals were obtained as previously described (4).

RESULTS

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\[
\text{A. Anti-62K} \quad \begin{array}{cccc}
\text{1} & \text{2} & \text{3} & \text{4} \\
62K & 62K & 62K & 62K \\
\end{array}
\]

\[
\text{B. Anti-72K} \quad \begin{array}{cccc}
\text{1} & \text{2} & \text{3} & \text{4} \\
72K & 72K & 72K & 72K \\
\end{array}
\]

\[
\text{C. Anti-ATPase} \quad \begin{array}{cccc}
\text{1} & \text{2} & \text{3} & \text{4} \\
62K & 62K & 62K & 62K \\
\end{array}
\]

FIG. 1. Immunoblot analysis of three antibodies cross-reacted to plant and fungal proteins. Antibodies were produced against the M, 62,000 subunit cut out from SDS gels (A), the M, 62,000 subunit cut out from SDS gels (B), and gradient-purified ATPase fractions (C). Lanes: 1, gradient-purified maize tonoplast ATPase (-7 μg); 2, deoxycholate-washed maize tonoplasts (-20 μg); 3, Neurospora vacuolar membranes (-50 μg); 4, Neurospora gradient-purified vacuolar ATPase (-5 μg); 5, barley tonoplast-enriched membranes (-50 μg). Molecular mass standards are given on the left.
purified ATPase taken from the sucrose gradient (Fig. 1A, lanes 1 and 2). This antibody also cross-reacted with a M<sub>r</sub> 62,000 protein from barley membranes enriched in tonoplasts (lane 5) but did not recognize any Neurospora proteins when tested against vacuolar membranes or purified ATPase (lanes 3 and 4). Antibodies to the M<sub>r</sub> 72,000 protein specifically recognized that protein in maize tonoplast membranes and ATPase fractions (Fig. 1B). In addition to cross-reacting to the barley M<sub>r</sub> 72,000 protein (lane 5), the anti-72 kDa antibodies also recognized a polypeptide of similar M<sub>r</sub> in Neurospora vacuolar membranes and ATPase fractions as we have previously shown (5). Proteins in Neurospora vacuolar membranes are slightly displaced upward in this molecular weight range, apparently by the presence of lower M<sub>r</sub> glycoproteins (5).

Antibodies to the partially purified ATPase were produced by immunization against peak ATPase fractions taken from sucrose gradients. By the immunoblotting procedure, several polypeptides from gradient-purified ATPase fractions were recognized by this antiserum including the M<sub>r</sub> 72,000 protein (Fig. 1C).

The effect of the three different antibodies on tonoplast ATPase activity is shown in Fig. 2. Partially purified ATPase was preincubated with the indicated amounts of antibody, and then ATPase activity was monitored. Antibodies made against the gradient-purified ATPase and the M<sub>r</sub> 72,000 protein were both inhibitory, giving 50% inhibition of activity with approximately 15 and 45 µg of serum protein, respectively. Almost 90% inhibition was achieved at the highest concentrations used. The anti-62-kDa antibodies showed no effect on ATPase activity at the highest tested concentration. Inhibition of ATPase activity in intact tonoplast vesicles by the anti-ATPase and anti-72-kDa antibody required higher concentrations.

NBD-Cl Binding and Inhibition—To further characterize the subunit composition of the tonoplast ATPase, radiolabeled inhibitors of ATPase activity were utilized. NBD-Cl had been used to covalently bind the β subunit of the mitochondrial ATPase (29). NBD-Cl inhibited maize tonoplast ATPase activity as shown in Fig. 3. When the incubation with NBD-Cl was performed in the presence of 5 mM ATP, inactivation of the ATPase was partially prevented. In the concentration range of 35–70 µM NBD-Cl, used to label tonoplast proteins (Fig. 4), approximately 75–90% inhibition was obtained in the absence of ATP and 48–60% inhibition in the presence of ATP.

NBD-Cl labeling of maize tonoplast proteins. Deoxycholate-washed tonoplasts (TP) and gradient purified ATPase (TP ATPase) were incubated with [14C]NBD-Cl in the presence (+) or absence (−) of 5 mM MgATP, and were electrophoresed on a 10% SDS-polyacrylamide gel. The Coomassie stained gel is shown in A and the fluorograph in B.
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NBD-Cl in the fluorograph (Fig. 4B) was the M, 72,000 polypeptide. The M, 72,000 protein was also the only one to show marked protection from labeling by MgATP.

**DCCD Binding and Inhibition**—The presence of a particular polypeptide (M, ~ 8,000) that is involved in proton conductance distinguishes F,E,F-type ATPases from E,E,E-type ATPases. [14C]DCCD has been used to identify the proton channel of both types of ATPases (23, 30), since DCCD is thought to inhibit H+-ATPases by blocking proton conductance (30). Tonoplast ATPase activity was also inhibited by DCCD. Fifty percent inactivation of ATPase was measured at a concentration less than 10 μM DCCD (Fig. 3). [14C]DCCD was used to compare labeling of maize mitochondrial and tonoplast proteins. Fig. 5 shows that [14C]DCCD-labeled a polypeptide in mitochondrial membranes at a M, of approximately 8,000, expected for the F,F proteolipid. Binding to this subunit was prevented by venturicidin, as has been previously demonstrated (11). Another protein of M, ~ 25,000 was also labeled in mitochondrial membranes. This may be explained by the presence of several other mitochondrial proteins, such as subunits of cytochrome oxidase and cytochrome b6, that are sensitive to DCCD (30).

In tonoplast membranes, [14C]DCCD also labeled a low molecular weight polypeptide, which differed from the mitochondrial proteolipid by having twice the relative mobility on the gel (M, 16,000) and by not showing sensitivity to venturicidin. The same polypeptide was also identified as the major [14C] DCCD binding protein in purified tonoplast ATPase fractions from the sucrose gradient. The low level of binding to the M, 72,000 and 62,000 proteins in the gradient fraction may be nonspecific binding due to the method of directly loading the sample on the gel or may represent specific binding as has been reported for the β subunit of the mitochondrial ATPase (31). When mitochondrial and tonoplast membranes were treated the same way (i.e. without dilution and pelleting) these subunits also showed preferential labeling.

**DISCUSSION**

Studies on partially purified vacuolar membrane ATPases from plants and fungi have provided evidence for an enzyme that differs kinetically and in subunit composition from F,F-type and E,E,E-type ATPases (4–12). Although the complete subunit composition of the vacuolar ATPase is not known, purification from several species reveals substantial agreement on the major polypeptides (Table I). In all species examined so far, two major polypeptides, M, 66,000–89,000 and M, 54,000–64,000, have been detected as well as a smaller DCCD-binding polypeptide of M, 13,000–19,500. In Hevea (10), and *Saccharomyces cerevisiae* (8) other bands also co-purify with ATPase activity, as indicated in Table I. The apparent subunit composition of the animal chromaffin granule ATPase I bears a striking resemblance to that of the vacuolar membrane ATPases. These data are consistent with a growing consensus that the vacuolar membrane ATPase represents a new class of proton-pumping ATPases associated with a variety of single membrane-bound acidic organelles in eukaryotes (e.g. Refs. 5, 6, 11, 32, and 33). The functional size of the tonoplast ATPase has been measured by radiation inactivation and shown to be large, approximately 400 kDa for the maize enzyme (4) and 520 kDa for the ATPase of *Neurospora* (5). A dichloromethane-solubilized vacuolar ATPase of *Hevea* has a molecular mass of about 200 kDa, determined by gel filtration (10).

The presence of multiple subunits, including a low M, polypeptide that binds [14C]DCCD, is reminiscent of F,F-type ATPases. The soluble F,F portion is composed of 5 or more subunits with molecular weights ranging between 7,500 and 62,000 Da (34). The catalytic polypeptide is thought to be the β subunit of M, ~ 55,000. The membrane-bound F,F portion contains the M, 8,000 proton channel that binds [14C]DCCD and 2 other subunits. In contrast, the proton-translocating E,E,F ATPase found on plant and fungal plasma membranes consists of a single type of polypeptide of M, 100,000. This subunit functions catalytically and also binds [14C] DCCD (23). With respect to sensitivity to inhibitors, the tonoplast ATPase appears to more closely resemble the F,F,F-type than the E,E,F-type enzyme (11, 32). The tonoplast and F,F,F-type ATPases are not inhibited by vanadate, suggesting that they do not form phosphorylated intermediates characteristic of the reaction cycle of E,E,E-type enzymes (4, 7, 11, 32). In comparisons of the effectiveness of a large variety of inhibitors on all three types of ATPases in *Neurospora* (11) and between the mitochondrial and tonoplast ATPases in oat roots (32), it was demonstrated that many inhibitors had strikingly similar effects on the mitochondrial and tonoplast enzymes. However, the tonoplast ATPase is resistant to the mitochondrial inhibitor, azide (4, 7, 9, 32).

This study was undertaken in an effort to further characterize the subunits and to identify the catalytic site of the maize tonoplast ATPase using immunological and inhibitor binding methods. Previously we have shown that the tonoplast ATPase represents a distinct immunological class based on cross-reactivity studies (5). Antibodies to the *Neurospora* plasma membrane ATPase and the β subunit of the *E. coli* F,F,F ATPase do not cross-react with the vacuolar membrane ATPase of *Neurospora*, and the antibody to the maize M, 72,000 tonoplast polypeptide does not recognize mitochondrial or plasma membrane polypeptides (5). Uchida et al. (7) have shown that antisera to the F,F ATPase of *S. cerevisiae* inhibits mitochondrial but not the vacuolar membrane ATPase. In the present study, we have shown that antibodies to the maize M, 72,000 and M, 62,000 polypeptides both cross-reacted with barley, while only the antibody to the maize M, 72,000 subunit cross-reacted with *Neurospora* (Fig. 1). This indicates that the larger subunit is more highly conserved. In a study on the immunological properties of F,F,F ATPases, Rott and Nelson (26) demonstrated that only the catalytic subunit (β) of mitochondrial, chloroplast, and *E. coli* ATPases

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**FIG. 5.** [14C]DCCD labeling of maize proteins. Mitochondrial membranes (Mito), deoxycholate-washed tonoplasts (TP), and gradient-purified tonoplast ATPase (TP ATPase) were incubated with [14C]DCCD in the presence (+) or absence (−) of 5 μg/ml venturicidin. Following electrophoresis on an 8% acrylamide gel (A), radioactive bands were detected by fluorography (B).
consistently cross-reacted to antibodies made to the ρ subunit from a different species. Antibodies made to α or γ subunits cross-reacted weakly or not at all to subunits from other species.

The importance of the M, 72,000 protein for the ATPase was also evident from measuring antibody effects on activity (Fig. 2). ATPase activity was inhibited only by the two antibodies that recognized the M, 72,000 protein in immunoblots: the antiserum made against gradient-purified ATPase fractions and the 72-kDa polypeptide. Thus, the immunological data support the M, 72,000 polypeptide as the catalytic subunit.

Studies with radioactively labeled inhibitors are consistent with this conclusion. NBD-CI reacts exclusively with the catalytic site since Ferguson et al. (29) have found that NBD-CI reacts exclusively with the subunit of the mitochondrial ATPase. NBD-CI inhibited the tonoplast ATPase in an ATP-protectable manner (Fig. 3). Fig. 4 shows that M, 72,000 primarily labeled the M, 72,000 polypeptide in maize tonoplast membranes. The M, 72,000 polypeptide was also the only band to show significant protection from labeling by ATP. Similar results have been obtained for the vacuolar ATPase of Neurospora (5). Manolson et al. (6) reported that the radioactively labeled substrate analog [3H]-[3-(O)-benzoyl]benzoyl ATP preferentially labeled the 57-kDa polypeptide of beet tonoplast ATPase, rather than the 67-kDa polypeptide. However, Manolson et al. (6) concluded that the binding site on the 57-kDa subunit is probably a regulatory site since BzATP was not a simple competitive inhibitor of the ATPase. In contrast, we have observed that M, 57-kDa azido-ATP binds to both the 72- and 62-kDa polypeptides of maize tonoplast ATPase, suggesting that both catalytic and regulatory sites are being labeled. By analogy to the F, ATPase, which has as many as three nucleotide-binding sites on the α and β subunits (34, 35), nucleotide-binding sites may be present on both of the major subunits of the tonoplast ATPase. Since other evidence strongly supports the 72-kDa polypeptide as the catalytic subunit, we concur with Manolson et al. (6) that the 62-kDa polypeptide probably represents a regulatory subunit.

In conclusion, further characterization of the subunit composition of the maize tonoplast ATPase has provided evidence for the oligomeric nature of the enzyme. Results from the immunological and inhibitor binding studies have suggested that the M, 72,000 polypeptide is the catalytic subunit of the ATPase. The M, 62,000 protein may be a regulatory subunit with nucleotide-binding sites, while the M, 16,000 polypeptide that binds DCCD is most likely the proton channel of the ATPase. Similarities between F,F,-type ATPases and the vacuolar ATPases from plants and fungi are evident from this and other studies (11, 32). As previously noted (5), the F,F,-ATPase that most closely resembles the tonoplast ATPase is one from the anaerobic bacterium, Clostridium pasteurianum (36). This enzyme has a simpler subunit composition (M, 66,000, 58,000, 43,000, and 15,000) than that found in mitochondria, chloroplasts, and aerobic bacteria (36). The possibility that this enzyme is a primitive form from which more complex F,F,-type ATPases has evolved has been proposed (33).

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REFERENCES

1. Wagner, G. J. (1982) in Recent Advances in Phytochemistry (Creasy, L., and Hrazdina, G., eds) pp. 1-45, Plenum Publishing Corp., New York
2. Sze, H. (1985) Annu. Rev. Plant Physiol. 36, 175-208
3. Chanson, A. & Taiz, L. (1985) Plant Physiol. 78, 232-240
4. Mandala, S. & Taiz, L. (1986) Plant Physiol. 82, 397-399
5. Bowman, E. J., Mandala, S., Taiz, L. & Bowman, B. J. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 48-52
6. Mandala, S. M., Rea, P. A. & Poole, R. J. (1985) J. Biol. Chem. 260, 12737-12749
7. Uchida, E., Ohsumi, Y. & Anraku, Y. (1985) J. Biol. Chem. 260, 12737-12749
8. Lichko, L. P. & Okorokov, L. A. (1985) FEBS Lett. 187, 349-353
9. Tognoli, L. (1985) Eur. J. Biochem. 146, 581-588
10. Martin, B., Preissner, J. & Komor, E. (1985) Eur. J. Biochem. 151, 131-140
11. Bowman, E. J. (1983) J. Biol. Chem. 258, 15238-15244
12. Randall, S. K. & Sze, H. (1986) J. Biol. Chem. 261, 1364-1371
13. Ohkuma, S., Moriymama, Y. & Takano, T. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2768-2772
14. Cidon, S. & Nelson, N. (1983) J. Biol. Chem. 258, 2892-2898
15. Glickman, J., Cropen, K., Kelly, S. & Al-Awqati, Q. (1983) J. Cell Biol. 97, 1303-1308
16. Forgg, M., Cantley, L., Wiedernmann, B., Althiels, L. & Branton, D. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 1300-1303
17. Xie, X.-S., Stone, D. K. & Racker, E. (1984) J. Biol. Chem. 259, 11676-11678
18. Russell, T. & Holz, R. W. (1981) J. Biol. Chem. 256, 5950-5953
19. Van Dyke, R. W., Hornick, C. A., Belcher, J. & Schorschmidt, B.
Immunological and Binding Studies on Maize Tonoplast ATPase

20. Percy, J. M., Pryde, J. G. & Apps, D. K. (1985) Biochem. J. 231, 557–564
21. Mandala, S. & Taiz, L. (1985) Plant Physiol. 78, 104–109
22. Fiske, C. H. & Subbarow, Y. (1925) J. Biol. Chem. 66, 375–400
23. Sussman, M. R. & Slayman, C. W. (1983) J. Biol. Chem. 258, 1839–1843
24. Vaitukaitis, J., Robbin, J. B., Nieschlag, E. & Ross, G. T. (1971) J. Clin. Endocrinol. Metab. 33, 988–991
25. Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) Biochemistry 10, 2606–2617
26. Rott, R. & Nelson, N. (1981) J. Biol. Chem. 256, 9224–9228
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
28. DuPont, F. M. & Harkman, W. J. (1985) Plant Physiol. 77, 857–862
29. Ferguson, S. J., Lloyd, W. J. & Radda, G. K. (1975) Eur. J. Biochem. 54, 127–133
30. Solioz, M. (1984) Trends Biochem. Sci. 9, 309–312
31. Khananshvili, D. & Gromet-Elhanan, Z. (1983) J. Biol. Chem. 258, 3720–3725
32. Wang, Y. & Sze, H. (1985) J. Biol. Chem. 260, 10434–10443
33. Maloney, P. C. & Wilson, T. H. (1985) Bioscience 35, 43–48
34. Khananshvili, D. & Gromet-Elhanan, Z. (1985) Proc. Natl. Acad. Sci. U. S. A. 82, 1886–1890
35. Amzel, L. M. & Pedersen, P. L. (1983) Annu. Rev. Biochem. 52, 801–824
36. Clarke, D. J., Fuller, F. M. & Morris, J. G. (1979) Eur. J. Biochem. 98, 597–612