ATP-DEPENDENT PROTON PUMPING CATALYZED BY THE PURIFIED YEAST PLASMA MEMBRANE ATPASE RECONSTITUTED INTO PHOSPHOLIPID VESICLES

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Plasma membrane ATPases have recently been isolated from yeasts and fungi. These enzymes, characterized by a similar structure and catalytic properties, are likely to be proton pumps which can build a proton-motive force across the cellular membrane for the active uptake of metabolites. Until now, little has been known about this class of ATPase, especially with regard to its proton translocation mechanisms and its coupling to the catalytic reactions. Lipid vesicles containing phospholipids and a purified ATPase could help in elucidating these properties.

MATERIALS AND METHODS

The *Schizosaccharomyces pombe* plasma membrane ATPase has been solubilized by egg lysophosphatidylcholine and purified to near homogeneity (1). This enzyme is the only ATPase of its class that has been fully characterized in its purified form (2, 3), and it is obtained in an inactive oligomeric form consisting of a single type of peptide (M_r = 100,000). However, reactivation is achieved simply by mixing the enzyme with lipid micelles or vesicles (4).

RESULTS AND DISCUSSION

In this study we have undertaken a reconstitution of the enzyme by incorporating the solubilized protein into preformed lipid vesicles of dimyristoylphosphatidylcholine (DMPC) and asolectin (soy bean phospholipids). By using ^{35}S-labeled enzyme, we have shown that binding of the enzyme to DMPC microvesicles parallels the reactivation of the ATP hydrolysis activity and that the maximum activation is reached at the peptide to vesicle ratio of one. Thus, formation of a lipoproteic complex is required for the enzyme activity. These findings also suggest that reactivation of the enzyme is a result of the dissociation of enzyme aggregate and that the minimal size of an active ATPase is the monomeric unit. Moreover, binding of the enzyme to DMPC microvesicles (26-nm mean diameter)

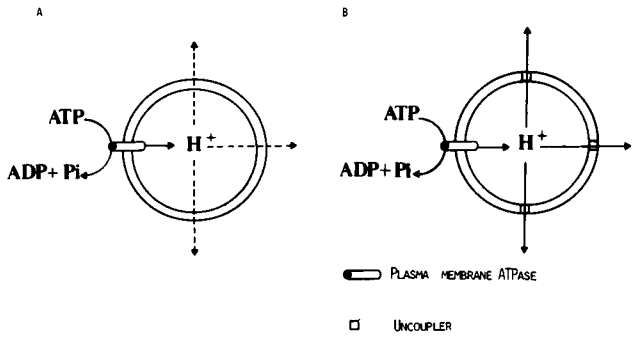

FIGURE 1 Effect of uncoupler on the ATPase activity and response of a ΔpH sensitive probe (quenching of fluorescence of acridine) in a reconstituted system. *A*, in the absence of uncoupler ($\Delta\psi \neq 0$, $\Delta pH \neq 0$, lower ATPase activity, quenching of acridine fluorescence); *B*, in the presence of uncoupler ($\Delta\psi = 0$, $\Delta pH = 0$, maximum ATPase activity, recovery of acridine fluorescence).

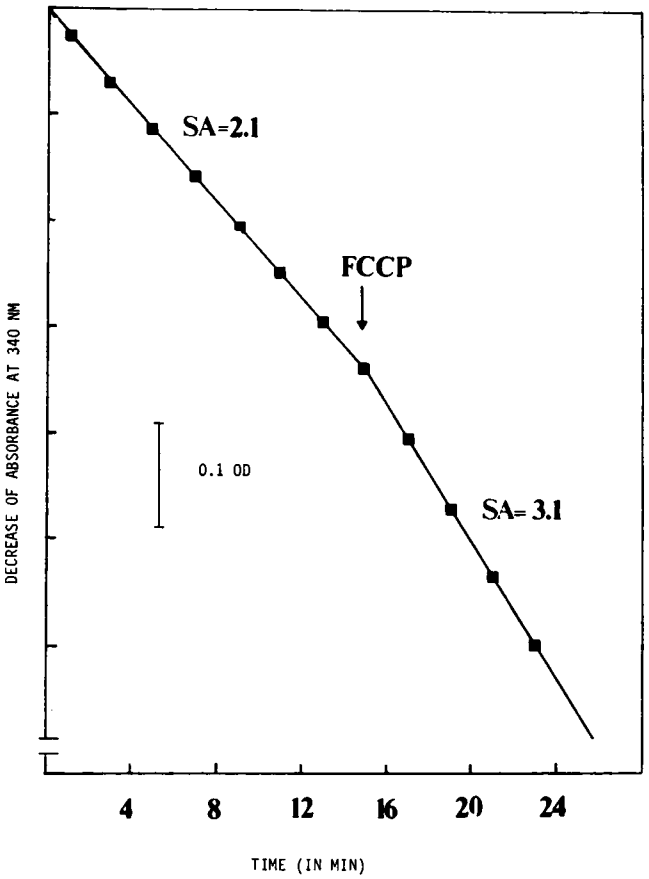

FIGURE 2 Stimulation of ATPase activity by FCCP (carbonyl cyanide *p*-trifluoromethyoxyphenylhydrazone). ATPase activity was measured spectrophotometrically at 340 nm by coupling the production of ADP to the oxidation of NADH via the pyruvate kinase and lactic dehydrogenase reactions. The reaction mixture contained in a volume of 1 ml at pH 6.0 and 30°C, 6 mM $MgCl_2$, 6 mM Tris-ATP, 10 mM MES/Tris, 0.45 mM NADH, 1.15 mM phosphoenolpyruvate, 1.2 unit of lactic dehydrogenase, 25 units of pyruvate kinase and 3 mg of phospholipid containing 3.3 μg of protein. FCCP was added at a final concentration of 5 μM. 50% stimulation of ATPase activity was observed.

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below the phase transition temperature of the phospholipid triggers a formation of unilamellar macrovesicles (95-nm mean Diam). Studies of this fusion process together with characterization of the enzyme in these micro- and macrovesicles allow us to assess the effect of lipid structures on the enzyme-substrate interactions (5, 6).

The energy-transducing activity has been reconstituted from the purified enzyme and asolectin by a modified freeze-thaw procedure (7). After this step, all the enzyme binds to the phospholipid vesicles as judged by a gel chromatography on a Sepharose CL-2B. If the enzyme is an electrogenic proton pump, hydrolysis of ATP should accompany a rapid buildup of a $\Delta\psi$ (electric potential) and a ΔpH across the membrane, and this process would retard further ATP hydrolysis by the enzyme (Fig. 1*A*). A pH-sensitive fluorescence probe should also sense the movement of protons (e.g. quenching). On the other hand, if a proton ionophore is present, both $\Delta\psi$ and ΔpH dissipate, and a stimulation of ATPase activity and recovery of the probe fluorescence should be observed (Fig. 1*B*). As illustrated in Fig. 2, addition of uncoupler stimulates the ATPase activity by 50%. Moreover, ATP-dependent proton translocation monitored fluorometrically following quenching of a highly sensitive fluorescent acridine dye is completely abolished by the uncoupler (Fig. 3). A comparison of reconstitution into micellar and vesicular phospholipid shows that fluorescence quenching is observed only for systems where vectorial movement of protons can take place. Fluorescence quenching as well as the ATPase activity is specific for ATP. The fluorescence quenching is completely abolished by DCCD, an inhibitor of the plasma membrane ATPase activity. Moreover, our results indicate that the rate of proton translocation is directly dependent on the rate of the ATP hydrolysis. Absence of quenching in the presence of NH_4^+ (ΔpH = 0) indicates that the acridine dye responds primarily to the transmembrane ΔpH, not to $\Delta\psi$. The fluorescence quenching is also stimulated by valinomycin in the presence of K^+. These results are consistent with the proposal that the yeast plasma membrane ATPase is an electrogenic proton pump. Whether or not the movement of protons is accompanied by a movement of other ions (anions or cations) is presently under investigation.

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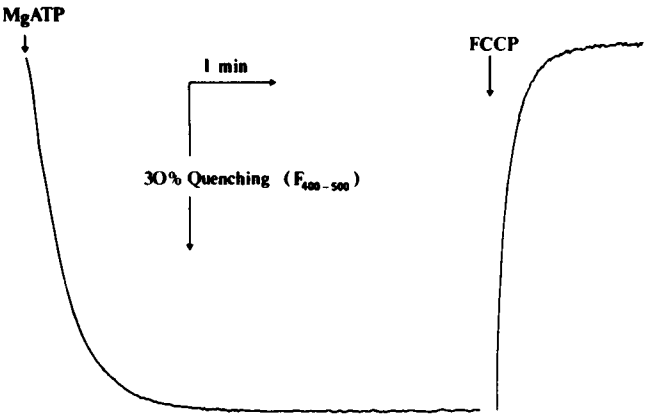

FIGURE 3 Fluorescence assay of ATP-dependent proton translocation. Proton influx was monitored following quenching of a fluorescent acridine dye (9-amino-6-chloro-2-methoxy-acridine). The assay medium contained in a final volume of 1.5 ml at pH 6.0 and 30°C, 6 mM MgATP (Sigma Chemical Corp., St. Louis, MO), 10 mM MES/Tris, 300 mM KCl, 1.6 μM acridine dye and 4.3 mg phospholipid vesicles containing 6.6 μg of protein. All assay components except acridine dye and MgATP were added first and allowed to incubate for 4 min. The acridine dye was then added followed by addition of MgATP. The excitation and emission wavelengths were, respectively, 400 and 500 nm. FCCP was added at a final concentration of 4.3 μM. Addition of MgATP activated the proton pump, quenched fluorescence. FCCP dissipated ΔpH and recovered the fluorescence intensity.

REFERENCES

1. Dufour, J.-P., and A. Goffeau. 1978. Solubilization by lysolecithin and purification of the plasma membrane ATPase of the yeast *Schizosaccharomyces pombe*. *J. Biol. Chem.* 253:7026–7032.
2. Dufour, J.-P., and A. Goffeau. 1980. Molecular and kinetic properties of the purified plasma membrane ATPase of the yeast *Schizosaccharomyces pombe*. *Eur. J. Biochem.* 105:145–154.
3. Dufour, J.-P., M. Boutry, and A. Goffeau. 1980. Plasma membrane ATPase of yeast: comparative inhibition studies of the purified and membrane-bound enzymes. *J. Biol. Chem.* 255:5735–5741.
4. Dufour, J.-P., and A. Goffeau. 1980. Phospholipid reactivation of the purified plasma membrane ATPase of yeast. *J. Biol. Chem.* 255:10591–10598.
5. Dufour, J.-P., and T. Y. Tsong. 1981. Plasma membrane ATPase of yeast: activation and interaction with dimyristoylphosphatidylcholine. *J. Biol. Chem.* 256:1801–1808.
6. Dufour, J.-P., R. Nunnally, L. Buhle, Jr., and T. Y. Tsong. 1981. A comparative study of an ATPase-trigger-fused lipid vesicle and other vesicle forms of dimyristoylphosphatidylcholine. *Biochemistry*. In press.
7. Kasahara, M., and P. C. Hinckle. 1977. Reconstitution and purification of the D-glucose transporter from human erythrocytes. *J. Biol. Chem.* 252:7384–7396.