Solubilization by Lysolecithin and Purification of the Plasma Membrane ATPase of the Yeast Schizosaccharomyces pombe*

Jean-Pierre Dufour and André Goffeau
From the Laboratoire d'Enzymologie, Université Catholique de Louvain, 1348 Louvain-la-Neuve, Belgium

Purified plasma membranes of Schizosaccharomyces pombe were obtained by precipitation at pH 5.2 of a crude particulate fraction, followed by differential centrifugations and isopycnic centrifugation in a discontinuous sucrose gradient. The specific activity of the Mg2+-requiring plasma membrane ATPase activity (EC 3.6.1.3) was enriched from 0.3 μmol min⁻¹ x mg⁻¹ of protein in the homogenate to 28 in the purified membranes. The optimal conditions for solubilization of the ATPase activity by lysolecithin were found to be: 2 mg/ml of lysolecithin, a lysolecithin to protein ratio of 8 at pH 7.5, and 15°C in the presence of 1 mM ATP and 1 mM ethylenediaminetetraacetic acid. A 6- to 7-fold purification of the solubilized ATPase activity was obtained by centrifugation of the lysolecithin extract in a sucrose gradient.

Part of the ATPase activity which was inactivated during the centrifugation in the sucrose gradient could be restored by addition of a micellar solution of 50 μg of lysolecithin/ml during the assay. Polycrystalline gel electrophoresis in the presence of sodium dodecyl sulfate of the purified enzyme showed only one band of M = 105,000 stained with Coomassie blue. Another ATPase component of apparent molecular weight lower than 10,000 was stained by periodic Schiff reagent but not colored by Coomassie blue. The purified enzyme was 85% inhibited by 50 μM N,N'-dicyclohexylcarbodiimide and 94% inhibited by 53 μg of Dio-9/ml.

The plasma membrane fraction purified by differential and isopycnic gradient centrifugation of a subcellular homogenate of the yeast Schizosaccharomyces pombe exhibits Mg2+-dependent adenosine triphosphatase activity (1). The properties of this plasma membrane-bound activity have been characterized and shown to be different in several aspects from those of the mitochondria-bound ATPase (1). In particular, the optimum pH of the plasma membrane ATPase is much lower than that of the mitochondrial enzyme, so that the ratio of the activities measured at pH 6.0 and pH 9.0 grossly reflect the relative proportion of plasma to mitochondrial membranes (1). A substantial amount of experimental data indicates that the plasma membrane-bound ATPase of S. pombe is involved in the cellular transport of ions and metabolites (2-4). Rather similar plasma membrane-bound ATPase activities have been identified in other fungi: Saccharomyces cerevisiae (5-9), Candida albicans (10), and Neurospora crassa (11-14). None of these plasma membrane ATPases have yet been purified.

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The samples were precipitated by 10% trichloroacetic acid for 30 min at 0°C and centrifuged twice for 2.5 min in an Eppendorf centrifuge, model 5417. The pellets were washed several times with ether, resuspended in 100 µl of 1% B-mercaptoethanol, 2% sodium dodecyl sulfate, 1% glycerol, 0.005% bromphenol blue, 27 mM HSO₄, pH 6.14, adjusted with Tris and heated at 100°C for 5 min. After electrophoresis, the gels were stained at room temperature with Coomasie blue and destained with methanol/acetic acid/water (5:1:5). Spectrophotometric scanning was carried out with the 580 to 650 nm filter of the Kipp and Zonen D22 densitometer.

RESULTS

Purification of Plasma Membranes of High ATPase Activity—One critical factor for successful solubilization of the plasma membrane ATPase is to use enriched plasma membrane fractions of high specific pH 6.0 ATPase activity. Such plasma membrane fractions of S. pombe with high ATPase activity were obtained by selective pH precipitation followed by centrifugation through a discontinuous sucrose gradient.

Table I shows the distribution of ATPase activities measured at pH 6.0 and pH 9.0 in the supernatant and the pellet of a low speed centrifugation after bringing a crude membrane fraction to the indicated pH. This procedure was inspired by the data of Fuhrman and Kramer (25) obtained with S. cerevisiae. As previously shown (1), the ATPase activity measured at pH 9.0 represents only the mitochondrial-bound activity, while that at pH 6.0 expresses not only the plasma membrane-bound activity but also about one-third of the mitochondrial activity maximally expressed at pH 9.0. It can be seen in Table I that treatment at pH 5.4 or lower, aggregates preferentially the mitochondrial-bound activity. From pH 5.4 and below the activity assayed at pH 6.0, in the supernatant, expresses essentially the plasma membrane-bound ATPase since the correction for residual mitochondrial activity amounts to less than 10% of the measured value. However appreciable inactivation of the plasma membrane-bound ATPase is observed at treatments below pH 5.0, as reflected by decreasing recoveries of the pH 6.0 ATPase. Treatment at pH 5.2 was chosen as the best compromise between yield and purification. Under these conditions, the ratio of pH 6.0 to pH 9.0 activity was raised to 8.9 and 59% of the total pH 6.0 activity was recovered in the supernatant. Fig. 1 shows that when the particular fraction obtained by centrifugation of the supernatant of a pH 5.2 treatment at

| pH       | Activity (%) | Recovery (supernatant + pellet) (%) |
|----------|--------------|-------------------------------------|
| 6.0      | 98           | 102                                 |
| 9.0      | 98           | 102                                 |
| Specific ATPase activity (µmol/min) | 7.5 | 7.0 | 6.5 | 6.0 | 5.5 | 5.0 | 4.8 |
| pH 6.0   | 1.3 98      | 100                                 |
| pH 9.0   | 1.5 98      | 100                                 |
proteins of known molecular weight (Fig. 2C).

mated for this peptide by comparison of its mobility to that of the component. A molecular weight of 100,000 ± 5,000 was estimated for this peptide from a gel pattern as described under "Materials and Methods." The plasma membrane ATPase assays were carried out in presence of ATP-regenerating mixture. The recoveries of ATPase and protein units were of 90 to 97% and 88 to 96%, respectively. The protein recovery was 91%.

The combination of the pH precipitation and centrifugation steps results in a 10-fold purification of the plasma membrane ATPase activity and a similar decrease of the mitochondrial one as shown in Table II. The plasma membrane ATPase specific activity attained 25.7 with a pH 6.0 to pH 9.0 ratio of 26.8. An appreciable increase of pH 6.0 ATPase specific activity is observed in the pellet obtained after the centrifugation at 45,000 × g of the 7,500 × g supernatant after pH 5.2 treatment. This indicates that the pH treatment releases a considerable amount of soluble proteins which were enclosed in the crude membrane fraction. These proteins which are present in the 7,500 × g supernatant but eliminated in the 45,000 × g supernatant do not contain plasma membrane ATPase activity.

The purification of the plasma membrane was monitored by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. It is apparent from the gel patterns illustrated in Fig. 2, A to B, that a slow moving band (indicated by the heavy arrow) which is a minor component of the crude membrane fraction becomes more and more prominent during purification of the plasma membrane. Since this is the major component which noticeably increases in parallel with the increase of pH 6.0 ATPase activity, it follows that plasma membrane ATPase activity is likely to be related to this component. A molecular weight of 100,000 ± 5,000 was estimated for this peptide by comparison of its mobility to that of proteins of known molecular weight (Fig. 2C).

Solubilization of Plasma Membrane ATPase—After unsuccessful attempts with several nonionic detergents (Triton X-100, Brij 35, Lubrol WX) or bile salts (sodium cholate and sodium deoxycholate) which were found to inactivate the ATPase activity, lyssolecithin was finally used to solubilize the plasma membrane. The conditions of solubilization have, however, to be carefully controlled. Fig. 3A shows that, at a

| Fraction | Specific activity | Relative yield |
|----------|------------------|----------------|
| Homogenate | pH 6.0 | 0.3 | 100 |
| Selective pH 5.2 precipitation (7,500 × g supernatant) | pH 9.0 | 1.3 | 100 |
| High speed centrifugation (45,000 × g pellet) | pH 6.0 ATPase activity | 51 | 22 |
| Purified plasma membranes (isopycnic centrifugation on sucrose gradient) | Protein | 42 | 7 |

Fig. 2. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of crude and purified plasma membrane. Sodium dodecyl sulfate–polyacrylamide gels were carried out using the sulfate/borate discontinuous buffer system and stained by Coomassie blue as described under "Materials and Methods." The following applications were made: in A, 180 µg of crude plasma membrane (as defined in Table II); in B, 50 µg of purified plasma membrane (as defined in Table II); in C, standard protein (10 µg of β-galactosidase, 25 µg of bovine serum albumin, 10 µg of pyruvate kinase, 25 µg of cytochrome c with $M_c = 130,000, 68,000, 57,000, 45,000, \text{ and } 11,800, \text{ respectively}. The arrow indicates the band of $M_c = 100,000$ which is enriched during purification.
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Fig. 3. Parameters of the solubilization by lysolecithin of the plasma membrane ATPase activity. A, effect of concentration of lysolecithin. The specific activity of plasma membrane-bound ATPase was 12.4. Aliquots of 56 μl of plasma membrane fraction (613 μg of protein) were added to solubilization buffer containing the indicated concentration of lysolecithin at the constant lysolecithin/protein ratio of 7. These suspensions were incubated for 10 min at 15°C and centrifuged at 100,000 × g for 45 min at 15°C in the Spinco R-40 rotor. The supernatants and the pellets, each resuspended in 500 μl of solubilization buffer, were stored at 15 and 0°C, respectively. The yield of solubilization of ATPase activity (●) and of protein (■) are the ratio of ATPase units (or protein units) in the supernatant to those in the original purified membrane when the recoveries were less than 90% or higher than 110%. Otherwise, the ratio of ATPase units to those in the original purified membrane when the recoveries were between 90% and 110%. Otherwise, the ratio of ATPase units (or protein units) in the supernatant to the sum of the units recovered in the pellet and supernatant were used. The yield of ATPase solubilization is thus function of the inactivation or the stimulation of the ATPase activity as well as the efficiency of the extraction. The recoveries were determined as described under Table I. The same conventions were used in B and C. The recoveries of ATPase and protein units were of 91 to 110% and 89 to 108%, respectively. B, effects of the lysolecithin/protein ratio. The specific activity of plasma membrane-bound ATPase was 12.4. Aliquots of 85 μl of plasma membrane fraction (504 μg of protein for the first seven points) or 42 μl (249 μg of protein for the last seven points) were added to solubilization buffer at each of the indicated lysolecithin/protein ratio. The final concentration of lysolecithin was 0.5 mg/ml of solubilization buffer. The suspension was shaken 10 min at 15°C and centrifuged at 100,000 × g for 1 h at 15°C in the Spinco R-40 rotor. The supernatants and the pellets resuspended in 500 μl of solubilization buffer were stored at 15 and 0°C, respectively. The recoveries of ATPase and protein units were of 89 to 110% and 89 to 99%, respectively. C, effects of pH. The specific activity of plasma membrane-bound ATPase was 12.4. One milliliter of solubilization medium contained 213 μg of protein and 1491 pg of lysolecithin. The pH was adjusted with CH₃COOH or NaOH. Other conditions were as described under A. For protein units, the recoveries were of 91 to 101%. For the ATPase units, the recoveries were of 80, 91, 102, 101, 95, 62, 38, and 26% at pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, and 9.0 respectively.

**Table III**

Effects of ATP and EDTA on solubilization by lysolecithin of the plasma membrane ATPase activity

The specific activity of plasma membrane-bound ATPase was 9.5. The final concentration of lysolecithin was 1.5 mg/ml. The lysolecithin/protein ratio was 7. The pH was adjusted at pH 7.5 with CH₃COOH or NaOH. Other conditions were as described under Fig. 3A.

| Solubilization medium | Yield of solubilization | Recovery | ATPase activity |
|-----------------------|------------------------|----------|----------------|
|                      | %                      | %        | μmol × min⁻¹ × mg⁻¹ |
| 10 mM Tris           | 29                     | 57       | 90              |
| + EDTA 1 mM          | 45                     | 66       | 91              |
| + ATP 1 mM           | 45                     | 60       | 94              |
| + EDTA 1 mM + ATP 1 mM | 51                | 69       | 95              |

The temperature does also influence markedly the solubilization which is incomplete at 4°C and is optimal and more specific at 15°C (not shown). The presence of 1 mM EDTA or 1 mM ATP in the solubilization medium increases significantly the specific activity of the solubilized ATPase (Table III). These effects are not additional but combined additions of EDTA and ATP are slightly more favorable than ATP alone. The presence of 1 mM ATP does also exhibit protective effects against inactivation during the subsequent purification of the ATPase (not shown).

The purification of Plasma Membrane ATPase—As described above, the optimal solubilization of plasma membrane ATPase requires a high lysolecithin/protein ratio and therefore extensive dilution to maintain a relatively low concentration of lysolecithin. Concentration of the diluted lysolecithin extract was carried out by filtration through an Amicon CF 25 cone which retains molecules of molecular weight higher than 25,000. For volumes smaller than 3 ml, about 90% of the ATPase activity can be recovered by ultrafiltration at 15°C or below, but this yield is decreased to 70% when the filtration is carried out at 20°C. It is also decreased when larger volumes are concentrated. The concentrated extract loses about 50% of its activity in 2 days at 4°C and is completely inactivated when exposed to higher temperatures. Fortunately, the concentrated extract is stable during storage at −180°C for 2 days or longer and can be thawed and frozen four to six times with loss of only 30% of its initial activity.

constant lysolecithin/protein ratio of 7, solubilization is obtained between 0.25 and 2 mg of lysolecithin/ml. Under these experimental conditions lysolecithin is in micellar form since its critical micellar concentration is 9.1 μg/ml as determined by the three different methods mentioned under "Materials and Methods." Such treatment solubilizes about 65 to 70% of the ATPase activity and 75 to 80% of the proteins. In addition, lysolecithin protects the plasma membrane ATPase activity during solubilization since recoveries of 91 to 97% of ATPase activities were obtained. Fig. 3B demonstrates that the optimal ratio of 8 mg of lysolecithin/mg of protein solubilizes the highest amount of ATPase and protein units. The extraction of ATPase activity is clearly bimodal with a partial extraction at a ratio of 4 and a more complete extraction at a ratio of 8 mg of lysolecithin/mg of protein. The pH affected the solubilization markedly. As shown in Fig. 3C, alkalinization of the solubilization medium increases steadily the release of proteins. However the solubilization of the plasma membrane ATPase is optimal at pH 7.5 and ATPase activity is lost irreversibly below pH 6.0 and above the optimal pH of 7.5. Increasing the ionic strength, by increasing concentrations of buffer did not modify significantly the solubilization of the ATPase activity. Similarily, prolongation of the incubation time up to 30 min had little effect (not shown). The presence of 1 mM EDTA or 1 mM ATP in the solubilization medium increases significantly the specific activity of the solubilized ATPase (Table III). These effects are not additional but combined additions of EDTA and ATP are slightly more favorable than ATP alone. The presence of 1 mM ATP does also exhibit protective effects against inactivation during the subsequent purification of the ATPase (not shown).
Because of the relative instability of the enzyme, it was necessary to use purification methods which can be carried in less than 24 h. Purification of the plasma membrane ATPase by chromatography through DE52 (Whatman), DEAE-Sephaceous (Pharmacia), or DEAE-Bio-Gel (LKB) yielded variable results and at this stage, these procedures cannot be recommended. On the other hand, good results were obtained by centrifugation through a sucrose density gradient. As shown in Fig. 4, centrifugation through a linear 10 to 30% (w/w) sucrose gradient separates the ATPase activity from the bulk of contaminating proteins which are of lower sedimentation rate. Most of the ATPase activity put on the gradient was recovered between 1.070 and 1.078 g cm$^{-3}$ of sucrose. No mitochondrial activity was detected. The recovery of 28% of the ATPase activity put on the sucrose gradient (assayed in the absence of lysolecithin) indicated that the plasma membrane ATPase activity was partially inactivated during the centrifugation. However the addition of lysolecithin during the assay restored the activity as shown in Fig. 4. The stimulation factor by addition of lysolecithin during the assay is more pronounced after purification in the sucrose gradient than before. In the experiment of Fig. 4, the stimulation was 1.2-fold for the concentrated extract and 10-fold for the purified enzyme. The amount of lysolecithin required to give half-maximal activation was 6.5 μmol of lysolecithin × mg$^{-1}$ of protein for the purified enzyme (Fig. 5). It must also be pointed out that lysolecithin does not restore the activity lost during concentration and aging of the lysolecithin extract which amounts to 60 to 70% of the ATPase activity initially solubilized. The plasma membrane ATPase peak fraction of the sucrose gradient was analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Staining by Coomassie blue reveals the presence of only a single subunit as demonstrated in Fig. 6, showing a spectrophotometric scan and a photograph of a gel containing the purified ATPase. The molecular weight of the major subunit estimated by simultaneous electrophoresis of samples containing purified ATPase and marker proteins is 105,000. This molecular weight is identical to that which was predicted from the observation of the gel pattern during purification of the plasma membrane. In addition to this major band, a white opaque zone is observed slightly ahead of the tracking dye. No periodic acid-Schiff staining was observed for the protein band; however, the opaque zone of high mobility was intensely colored red. After staining by Coomassie blue, the relative area of the $M_r = 100,000$ peak was estimated in the plasma membrane and purified enzyme (Table IV). It can be concluded from this gross estimation that the $M_r = 100,000$ peak is enriched 6- to 7-fold during purification through the sucrose gradient. Concomitant loss of activity already mentioned is responsible of the much lower increase of specific activity which reaches 35 μmol × min$^{-1}$ × mg$^{-1}$ of protein in the purified enzyme (Table IV).

This loss of activity and the unexpected simplicity of the subunit composition of the purified plasma membrane ATPase raises the suspicion that unidentified ATPase components could have been lost during the purification. It is very difficult to completely rule out this possibility; however, from the data of Table V it can be definitively stated that neither the site

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**Fig. 4.** Distribution of solubilized membrane ATPase activity after centrifugation on a sucrose density gradient. The specific activity of plasma membrane-bound ATPase was 17.1. Aliquots of 1.6 ml of concentrated lysolecithin extract (1.33 mg of protein) were layered on a linear sucrose density gradient containing 10 to 30% sucrose (w/w) in 16 ml of solubilization buffer and centrifuged for 20 h at 82,000 × g at 4°C in a Spinco SW 27 rotor. The tube was punctured at the bottom and 34 fractions of 14 drops each were collected. For the ATPase assays carried out in absence (-- ---) and in presence (-----) of 50 μg of lysolecithin, the recoveries were 28 and 90%, respectively. For protein measurement (-----) the recovery was 96%.

**Fig. 5.** Restoration of ATPase activity by lysolecithin. Purified enzyme (1.3 μg of protein) was preincubated 10 min at 4°C in presence of indicated lysolecithin concentrations in a final volume of 500 μl of solubilization buffer.

**Fig. 6.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of purified plasma membrane ATPase. Conditions were as described under Fig. 2. Fifteen micrograms of protein were applied on the gel.
TABLE IV

Purification of solubilized plasma membrane ATPase

| Fraction                        | Protein yield | ATPase units | Specific ATPase activity | Relative amount of $M_0 = 100,000$ protein |
|--------------------------------|---------------|--------------|--------------------------|------------------------------------------|
|                                 | %             | With lysolecithin | Without lysolecithin | With lysolecithin | Without lysolecithin | μmol × min⁻¹ | μmol × min⁻¹ × mg⁻¹ | %          |
| Purified membranes              | 100           | 56.9         | 12.1                     | 13                        |
| Lysolecithin extract            | 42.9          | 24.4         | 12.0                     | 13                        |
| Extract after ultrafiltration   | 2.8           | 12.6         | 0.5                      | 6                         |
| Purified enzyme                 |               | 4.7          | 35                       | 6                         |

TABLE V

Inhibition of plasma membrane ATPase activity by $N,N'$-dicyclohexylcarbodiimide and Dio-9

| Plasma membrane ATPase activity | No addition | $50 \mu M$ dicyclohexylcarbodiimide | $50 \mu M$ Dio-9/ ml |
|--------------------------------|-------------|-------------------------------------|---------------------|
| Plasma membrane                | 100         | 82                                  | 13                  |
| Lysolecithin extract           | 100         | 39                                  | 4                   |
| Purified enzyme                | 100         | 14                                  | 6                   |

These rather unusual conditions, lysolecithin solubilizes the ATPase instead of stripping the membrane as in other preparations of mitochondrial or sarcoplasmic ATPase yielding particulate ATPase - lysolecithin complexes (26, 27). From the results of Fig. 3B, it can be proposed that the plasma membrane ATPase is a transmembrane protein since it is less efficiently solubilized than other proteins which might be more superficially located on the plasma membrane.

Properties of the Purified Membrane ATPase—This paper is the first report of purification of a plasma membrane ATPase from a fungus. In a two-step purification, we have succeeded in purifying an active soluble plasma membrane ATPase of specific activity above $30 \mu mol × min⁻¹ × mg⁻¹$.

The purified enzyme is highly stable during storage at $-180°C$ but loses its original activity when stored unfrozen. In addition, considerable loss of ATPase activity is observed during the sucrose gradient centrifugation and might be explained as follows. The buoyant density of the lyssolecithin micelles (or lyssolecithin-lipid mixed micelles) is lower than that of the protein (or the protein-lyssolecithin complexes). The two types of complexes are therefore separated during centrifugation (29) and a subsequent reduction of the protection of ATPase by lyssolecithin follows. This loss of activity is not totally irreversible since addition of a micellar solution of lyssolecithin restores at least partly the ATPase activity of the purified enzyme. The number of $6.5 \mu M$ of lyssolecithin/mg of protein for half-maximal stimulation of the purified ATPase might be compared with those reported for the ATPase of beef heart submitochondrial particles which required 1.1 $\mu mol$ of lyssolecithin/mg of protein for half-maximal activation (29), whereas 4 $\mu mol$ of lyssolecithin/mg was required for the plasma membrane ATPase complex of Escherichia coli. The variation of the extent of stimulation that we observe from one experiment to another might be attributed to variable quantities of residual lipids or to variable state of inactivation of the enzyme.

As revealed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the single-peptide structure of the yeast plasma membrane ATPase permits clear assessment of the homogeneity of the purified enzyme. The structure of the yeast plasma membrane ATPase is so different from that of the mitochondrial, bacterial, or chloroplast ATPases that the possibility for existence of common subunits are very reduced, even though the mitochondria and plasma membrane-bound enzyme share some common properties such as sensitivity to Dio-9 or $N,N'$-dicyclohexylcarbodiimide (1). The fact that sensitivity to these two inhibitors is conserved and even increased in the purified plasma membrane ATPase suggests that the enzyme structure has not been seriously altered during purification. It, however, must be mentioned that the...

DISCUSSION

Purification and Solubilization of the Plasma Membrane—Once solubilized, the plasma membrane ATPase loses activity during the purification procedure. It is therefore essential to start from plasma membrane fractions as purified as possible. It is of special importance to reduce the mitochondrial contamination. This is achieved quickly and conveniently by the procedure which is reported above. This plasma membrane preparation, which has proven adequate for extraction of the ATPase, contains more than 20 components which can be distinguished by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (see Fig. 2A). Such a preparation could therefore be of further use for identification and purification of other plasma membrane-bound components. It also provides plasma membrane vesicles which could be used to study transport functions and, for instance, to verify whether indeed the plasma membrane ATPase is an electrogenic proton pump maintaining a cellular membrane potential as previously proposed (4).

The main problem encountered during this study has been to extract the plasma membrane ATPase with minimal inactivation of the enzyme activity. This was achieved with lyssolecithin. The ATPase extraction, however, is not selective since the specific activity of the lyssolecithin extract is slightly lower than that of the native membranes.

The low concentrations of lyssolecithin to protein ratio required for optimal solubilization cause an extensive dilution of the plasma membrane fraction. Under...

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Purified membranes (4.72 mg of protein with specific activity of 17.1 in presence of ATP-regenerating mixture) were solubilized by lyssolecithin at 0.8 mg/ml of solubilization buffer at a lyssolecithin/protein ratio of 5. Ultrafiltration was done as described under "Materials and Methods." Centrifugation and other conditions were as described under Fig. 4. When indicated, 50 mg of lyssolecithin/ml were added in the ATPase assay medium. An aliquot of the "extract after ultrafiltration" was kept at 4°C and tested simultaneously with the purified enzyme. For the purified enzyme, Fractions 15 and 16 of the sucrose gradient (as shown in Fig. 4) were collected. The relative amount of $M_0 = 100,000$ protein (%) was determined by planimetry of the gel area corresponding to the $M_0 = 100,000$ peak compared to the total area of the gel components stained by Coomassie blue.

The low concentrations of lyssolecithin and the high lyssolecithin to protein ratio of 5. Ultrafiltration was done as described under "Materials and Methods." Centrifugation and other conditions were as described under Fig. 4. When indicated, 50 mg of lyssolecithin/ml were added in the ATPase assay medium. An aliquot of the "extract after ultrafiltration" was kept at 4°C and tested simultaneously with the purified enzyme. For the purified enzyme, Fractions 15 and 16 of the sucrose gradient (as shown in Fig. 4) were collected. The relative amount of $M_0 = 100,000$ protein (%) was determined by planimetry of the gel area corresponding to the $M_0 = 100,000$ peak compared to the total area of the gel components stained by Coomassie blue.

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purified plasma-membrane ATPase contains a component of high electrophoretic mobility which is stained by periodic acid-Schiff but not by Coomassie blue. This might indicate glycolipidic, glycoprotein, or lipidic material. Since the mitochondrial ATPase proteolipid responsible for binding of N,N'-dicyclohexylcarbodiimide exhibits similar properties (30, 31), it cannot be excluded that the plasma-membrane ATPase unidentified component is similar to the mitochondrial proteolipid.

It is of interest to compare the yeast plasma membrane ATPase with transport ATPases from other sources. The mammalian cell ATPase has a major component of $M_r = 90,000$ to $100,000$; it has also a smaller peptide with unknown functions and its sensitivity to ouabain inhibition and stimulation by (Na$^+$ + K$^+$) seems not to be shared by the yeast enzyme (1). The ATPase from the sarcoplasmic reticulum also is composed of only one $M_r = 100,000$ peptide which is solubilized by neutral detergents, but it clearly differs from the yeast enzyme in its stimulation by Ca$^{2+}$ (1).

The availability of a purified and active plasma membrane ATPase of *S. pombe* obtained by the simple and reproducible purification procedure presented here, should now permit further molecular characterization of the purified enzyme as well as reconstitution studies into artificial membranes. It might be expected that the possibilities of physiological and genetic manipulations offered by yeast cells are going to be of great advantage in the study of the structure and function of this plasma membrane enzyme.

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