A 180-kDa plasma membrane protein of the yeast Saccharomyces cerevisiae was overexpressed by mutating the PDR1 or the PDR3 transcription factor gene. The protein is the membrane-bound ATP binding cassette transporter PDR5 (Balzi, E., Wang, M., Leterme, S., Van Dyck, L., and Goffeau, A. (1994) J. Biol. Chem. 269, 2206-2214). PDR5 was solubilized with n-dodecyl-β-D-maltoside and separated from the PMA1 plasma membrane H^+-ATPase by glycerol gradient centrifugation. The PDR5 protein hydrolyzes nucleoside diphosphates and triphosphates. This activity is sensitive to low concentrations of vanadate, of ouabain, and of a variety of hydrophobic compounds. Many of these properties liken PDR5 to the purified mammalian P-glycoprotein responsible for multidrug resistance.

Tumor cells can acquire resistance to a variety of cytotoxic drugs (Riordan et al., 1985). This multidrug resistance (MDR)^1 is frequently linked with increased expression of an integral membrane protein called P-glycoprotein or P170, which functions as an ATP-dependent efflux pump for drugs (reviewed, for instance, in Endicott and Ling (1989) and Gottesman and Pastan (1993)). The physiological substrates of the mammalian efflux pump are largely unknown. Sharma et al. (1992) have recently shown, however, that the P-glycoprotein encoded by the MDR1 gene is capable of transporting peptides. They have suggested that the P-glycoprotein could be involved in a new pathway for secretion of cellular proteins.

Significant sequence homologies have been reported between the mammalian P-glycoprotein and several transporters of the ABC superfamily (reviewed by Higgins (1992)), including bacterial permeases (Ames, 1986), the yeast STE6 transporter for secretion of the mating factor (Mc Grath and Varshavsky, 1989; Kuchler et al., 1989), the products of the pfmdr genes conferring resistance to anti-malaria drugs in Plasmodium falciparum (Foord et al., 1989; Wilson et al., 1989), and the human cystic fibrosis gene product cystic fibrosis transmembrane conductance factor (Riordan et al., 1989; Rommens et al., 1989; Kerem et al., 1989). Recently, two multidrug resistance genes, SNQ2 (Servos et al., 1993) and PDR5 (Balzi et al., 1994; Bissinger and Kuchler, 1994) were found to encode new yeast homologues of the mammalian P-glycoprotein.

First attempts to purify the P-glycoprotein resulted in low ATPase activity (Hamada and Tsuuro, 1988a, 1988b; Shimbuku et al., 1992), probably due to inhibition and/or denaturation during solubilization and purification (Al-Shawi and Senior, 1993). More recently, Sarkadi et al. (1992) have shown that drugs reported to interact with the isolated P-glycoprotein strongly stimulate ATPase activity in MDR1-containing membranes from baculovirus-infected Sf9 cells. Ambudkar et al. (1992) confirmed the stimulatory effects of drugs after reconstitution of the partially purified P-glycoprotein in proteoliposomes.

The recent identification of yeast genes sharing homology with the mammalian drug resistance genes is of great interest given the possibilities offered by yeast as a tool for genetic and molecular manipulations. The identified genes are STE6 (Kuchler et al., 1989; Mc Grath et al., 1989), ADP1 (Purnelle et al., 1991), SNQ2 (Servos et al., 1993), and PDR5 (Balzi et al., 1994; Bissinger and Kuchler, 1994). In the yeast Saccharomyces cerevisiae, there exists a phenotype resembling the mammalian multidrug resistance phenotype and known as pleiotropic drug resistance (PDR) (reviewed by Balzi and Goffeau (1991)). Underlying this phenotype, a complex genetic network is now emerging. First, there is the PDR1 gene. This gene encodes a transcription factor. Mutations in PDR1 lead to multidrug resistance, whereas disruption of the gene causes hypersensitivity (Balzi et al., 1987). Then there is the PDR3 gene, which encodes a homologue of PDR1 (Delaveau et al., 1992). When mutated, PDR3 also confers multidrug resistance (Subik et al., 1986). A second class of PDR genes confers resistance when the wild-type alleles are amplified (Leppert et al., 1990). Among these, the PDR5 gene, whose amplification leads to pleiotropic drug resistance and whose disruption causes hypersensitivity to drugs (Leppert et al., 1990), has been proposed as a putative target of the PDR1 gene product (Balzi and Goffeau, 1991). Investigators have reported a genetic interdependence of the PDR1 and PDR5 genes as well as overexpression of PDR5 mRNA in pdr1 mutants (Meyers et al., 1992). PDR4, PDR7, and PDR9, whose encoded products are still unknown, also influence the steady-state level of the PDR5 transcript (Dexter et al., 1994). The PDR5 gene has recently been shown to encode an MDR-like protein which shares homologies with ATP-dependent transporters at its nucleotide-binding sites. Furthermore, an abnormally high amount of PDR5 protein is found in the plasma membranes of pdr1 mutants, indicating that it is overexpressed (Balzi et al., 1994).

In this report we have exploited the remarkable amplification of the PDR5 protein in the plasma membranes of regulatory pdr1 mutant strains (Balzi et al., 1994). We have solubilized the overexpressed PDR5 plasma membrane protein and

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1 To whom correspondence and reprint requests should be addressed. Tel.: 32-10-47-36-14; Fax: 32-10-47-38-72.

2 The abbreviations used are: MDR, multidrug resistance; ABC, ATP binding cassette; P170, P-glycoprotein; PDR, pleiotropic drug resistance; PMA, plasma membrane ATPase; MES, 2-(N-morpholino)ethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis; CHAPS, 3-[3-cholamidopropyl]dimethylammonio-1-propanesulfonic acid; MES, 4-morpholineethanesulfonic acid. Genes are in italics, and proteins are in capitals (set roman).
separated it from its major contaminant, the plasma membrane H+-ATPase (PMA1), by glycerol gradient centrifugation. We show that its nucleoside triphosphatase properties closely resemble those of the mammalian P glycoprotein.

**MATERIALS AND METHODS**

**Chemicals—**Thesit, n-dodecyl-β-D-maltoside, Triton X-100, Genapol, CHAPS, UTP, GTP, CTP, ADP, phosphoenolpyruvate, pyruvate kinase, isocitrate dehydrogenase (from Boehringer; disodium salt, grade II), bovine serum albumin, lysolectin, sodium deoxycholate, n-octyl-β-D-glucopyranoside, verapamil, doxorubicin, colchicine, N-ethylmaleimide, and tetracycline were from Sigma; Tween 20 and 80 were from Merck; erythrosine B and oligomycin were from Janssen Chimica, molecular mass markers (range 53,000-212,000 Da); pepstatin, leupeptin, and E64 were purchased from Boehringer; ATP (disodium salt, grade II), bovine serum albumin, lysolecithin, sodium deoxycholate, Triton X-100, Genapol, n-octyl-β-D-glucopyranoside, verapamil, doxorubicin, colchicine, N-ethylmaleimide, and tetracycline were from Sigma; Tween 20 and 80 were from Merck; erythrosine B and oligomycin were from Janssen Chimica, molecular mass markers (range 53,000-212,000 Da); pepstatin, leupeptin, and E64 were purchased from Boehringer; ATP (disodium salt, grade II), bovine serum albumin, lysolecithin, sodium deoxycholate, Triton X-100, Genapol, n-octyl-β-D-glucopyranoside, verapamil, doxorubicin, colchicine, N-ethylmaleimide, and tetracycline were from Sigma; Tween 20 and 80 were from Merck; erythrosine B and oligomycin were from Janssen Chimica, molecular mass markers (range 53,000-212,000 Da); Pepstatin, Leupeptin, and E64 were purchased from Boehringer. Janssen Chimica. All other reagents were of analytical grade.

**Cells and Culture Conditions—** The *S. cerevisiae* strains listed in Table 1 were grown in 10% glucose, 2% yeast extract, pH 4.5, and harvested in the exponential growth phase.

**Isolation of Plasma Membranes—**Plasma membranes were isolated from the particulate fraction pelleted at 15,000 g for 40 min by selective precipitation at pH 5.2 as described by Goffeau and Dufour (1988).

**Solubilization of PDR5—**DR19-T8 membranes containing about 5 mg protein were suspended in 1 ml of buffer containing 10 mM Tris adjusted to pH 7.5 with HCl (for the PDR5 gene sequence analysis of this band was determined by microsequencing. The amino-terminal sequence of PDR5 was determined with an Applied Biosystem sequencer (model 477A) equipped with an on-line amino-acid-derivative analyzer (model 120A).

**RESULTS**

**Table 1** Yeast strains used in this study

<table>
<thead>
<tr>
<th>Strains</th>
<th>Genotype</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL125-2B</td>
<td>MATa, PDR1, PDR5, his1 parental of DR19-T8 and DR19-T7</td>
<td>Balzi et al. (1987)</td>
</tr>
<tr>
<td>DR19-T8</td>
<td>MATa, pdr1-3, PDR5, his1</td>
<td>Guérineau et al. (1974)</td>
</tr>
<tr>
<td>RW2802</td>
<td>MATa, PDR1, PDR5, leu2, met5, ura3-53, mak71, KRE1</td>
<td>Meyers et al. (1992)</td>
</tr>
<tr>
<td>JG436</td>
<td>RW2802 disrupted by PDR5::Tn5</td>
<td>Balzi et al. (1987)</td>
</tr>
<tr>
<td>US50-15C</td>
<td>MATa, pdr1-3, ura3, his1</td>
<td>Balzi et al. (1987)</td>
</tr>
<tr>
<td>DI-3/3</td>
<td>US50-18C disrupted by pdr1Δ1::URA3</td>
<td>Meyers et al. (1992)</td>
</tr>
<tr>
<td>JG936-DR19-T8</td>
<td>MATa, pdr1-3, PDR5::Tn5, met5, ura3</td>
<td>Guérineau et al. (1974)</td>
</tr>
<tr>
<td>DR19-T7</td>
<td>MATa, pdr3-3, his1</td>
<td>Balzi et al. (1987)</td>
</tr>
</tbody>
</table>

**Analysis of PDR5 in Plasma Membranes from Drug-resistant and -sensitive Cells—**Plasma membranes were isolated from drug-sensitive and multidrug-resistant cells and analyzed by SDS-PAGE. Fig. 1 shows that a 160-kDa protein is abnormally abundant in the plasma membranes of drug-resistant *pdr1-3* and *pdr3-2* mutants. The amino terminus of the major component of this band was determined by microsequencing. The obtained P-E-A-K-L sequence matches that deduced from the PDR5 gene sequence (Balzi et al., 1994), without the NHL-terminal methionine. Its apparent molecular mass (160 kDa) is slightly lower than predicted from the gene sequence (170 kDa). The NH2 terminus of another protein band, located between PDR5 and PMA1 (the 100-kDa subunit of the plasma membrane H+-ATPase), was also sequenced. It matches the terminus of the gp125 glycoprotein encoded by the GAS1 gene (Nuoffer et al., 1991; Vai et al., 1991).

Fig. 1 shows that all the multidrug-resistant strains mutated in either the *PDR1* gene of chromosome VII (Fig. 1, lanes 2 and 5) or the *PDR3* gene of chromosome II (Fig. 1, lane 8) overproduce the PDR5 protein band to a similar extent. When the mutated allele *pdr1-3* is replaced with a disrupted *pdr1Δ1* gene yielding hypersensitive strain D1-3-3 (Balzi et al., 1987), the PDR5 band is no longer found (Fig. 1, lane 6). These results confirm, at protein level, the previous mRNA level observations showing that the *pdr1-3* mutant overexpresses the PDR5 gene (Meyers et al., 1992). This phenomenon is also caused by the *pdr3-2* mutation. After disruption of the PDR5 gene by Tn5 transposon insertion, the 160-kDa band decreases in both the *pdr1-3* mutant (Fig. 1, lane 7) and the wild-type *PDR1* strain (Fig. 1, lane 4).

**Location of PDR5 in the Plasma Membrane-enriched Fraction—**The protein compositions of different subcellular fractions obtained during purification of *pdr1-3* mutant plasma membranes were analyzed by SDS-PAGE (Fig. 2). PDR5 could barely be detected in crude membranes (lane 3), but the plasma membrane fraction was considerably enriched in the protein (lane 4). In the latter fraction, the PDR5 band was as pronounced as the 100-kDa band of the PMA1 H+-ATPase, estimated to constitute 10–15% of the plasma membrane protein (Serrano, 1978).

**ATPase and UTPase Activities in Plasma Membranes—**ATPase activity profiles at different pH values in plasma membrane preparations from the drug-resistant *pdr1-3* mutant and its drug-sensitive parental strain are different. ATPase activity is highest at pH 6.0, the optimal pH for PMA1 (Dufour and Goebel, 1980), in the parental strain plasma membranes, whereas a shoulder is observed at pH 7.5 with *pdr1-3* plasma membranes. The high specificity of yeast PMA1 for ATP (Borst-Pauwels and Peters, 1977) is confirmed by the flat pH profile obtained for UTPase activity with plasma membranes from the parental strain. This contrasts with the profile of the *pdr1-3* mutant, which displays high UTPase activity between pH 6.0 and 7.0.
and pH 7.5. Table II shows the ATPase and UTPase activities, measured at both pH 6.0 and pH 7.5, of plasma membranes from other pdr drug resistance mutants and their drug-sensitive parental strains. ATPase activity at pH 6.0 is about the same in all nine drug-resistant or drug-sensitive strains analyzed. However, the ratio of the ATPase activity at pH 7.5 to that measured at pH 6.0 was considerably higher for the pdrl-3 and pdr3-2 strains, which overexpress PDR5, than for the corresponding parental strains. UTPase activity, whether measured at pH 6.0 or pH 7.5, is high in all the PDR5-overexpressing drug-resistant mutants and low in all the drug-sensitive strains, parental or disrupted. The UTPase activity measured at pH 7.5 is 10 or 11 times higher than in the parental strains in membranes derived from pdrl-3 mutants DRI9-T8 and US50-18C and seven times higher in the membranes of a pdrl-3 mutant. Disruption of the PDR5 or PDR1 gene as in JG436 and JG365-4C leads to a marked drop in the measured UTPase activity.

Solubilization of PDR5—Plasma membranes from PDR5-enriched pdrl-3 cells were treated with different detergents in the presence of glycerol. Among them, Triton X-100, Thesit, Genapol, Tween20 and 80, CHAPS, and sodium deoxycholate did not solubilize the 160-kDa band, whereas n-dodecyl-β-D-maltoside, n-octyl-β-D-glucopyranoside, and zwittergents 3-10, 13-12, and 1-14 were effective. From all tested detergents, n-dodecyl-β-D-maltoside gave the highest and most stable PDR5 UTPase activity at pH 7.5. Solubilization at different protein concentrations (from 1 to 4.4 mg/ml) and at different n-dodecyl-β-D-maltoside/protein ratios (from 0 to 8) was tested. The best solubilization of the PDR5 band from pdrl-3 plasma membranes containing 4.4 mg of protein/ml was obtained at a detergent/protein ratio of 0.75 (w/w).

Solubilization led to the loss of UTPase activity in the supernatant. Partial reactivation was achieved, however, by adding asolectin, lyssolecithin, and bovine serum albumin as described under "Materials and Methods." The solubilized ATPase activity measured at pH 6.0 reached 30% of the membrane-bound activity measured prior to solubilization. Recovery after solubilization of ATPase or UTPase activity at pH 7.5 was 15–20% after addition of lipids.

It has to be noted that addition of protease inhibitors (peptatin, 10 μg; leupeptin, 20 μg; and E64, 10 μg) during solubilization significantly increased the stability of PDR5 activity at 4 °C.

Partial Purification of PDR5—After solubilization with n-dodecyl-β-maltoside, the extract obtained from the pdrl-3 plasma membranes was fractionated on a continuous 15–40% glycerol gradient. Fig. 3 (upper panel) shows the separation of the 100-kDa PMA1 band and the 160-kDa PDR5 band. Fractions 26–32 containing 18–21% glycerol are enriched in PDR5 (band detected by Coomassie Blue staining after SDS-PAGE), whereas the PMA1 protein is found in the heaviest fractions (fractions 17–24). The peak of ATPase activity measured at pH 6.0 is found in fractions 17–24, where UTPase activity is low (Fig. 3, lower panel). UTPase activity measured at pH 7.5 peaks in PDR5-enriched fractions 28–32.

Characterization of the Activity of the Partially Purified PDR5 NTPase—Fig. 4 shows the pH dependence of the ATPase activity of the PMA1- and PDR5-enriched fractions from the glycerol gradient (fractions 17–23 and 27–31, respectively) and that of the PDR5 UTPase activity in fractions 27 through 31. PDR5 ATPase activity peaked over a broad pH range extending from 5.5 to 8.0 as observed with the mammalian P-glycoprotein (Hamada and Tsuruo, 1988b; Al-Shawi and Senior, 1993). PDR5 UTPase activity peaked in a much narrower range, near pH 7.0. The optimal pH of 5.8 found for the PMA1 ATPase is in agreement with reported values (Dufour and Goffeau, 1980; Borst-Pauwels and Peters, 1977). There is no interference between the PDR5 UTPase activity measured at pH 7.5 and the activity of PMA1. After purification on the glycerol gradient, the PDR5 ATPase and UTPase activities are very low: about 0.05 μmol of Pi·min⁻¹·mg⁻¹. The NTPase activity can be stimulated some 10-fold, however, by addition of lipids to the assay medium.

Eadie-Hofstee plots allowed estimations of $K_M$ and $V_{max}$ for PDR5 NTPase activity. Plasma membrane-bound PDR5 exhibits a $K_M$ of 0.5 mm for MgATP and a $V_{max}$ of 2.5 μmol of P₃·min⁻¹·mg⁻¹, whereas the corresponding parameters for the purified enzyme are 0.25 mm MgATP and 0.25 μmol P₃·min⁻¹·mg⁻¹, respectively. Plasma membrane-bound PDR5 exhibits a $K_M$ of 0.35 mm for MgUTP and a $V_{max}$ of 2.8 μmol of P₃·min⁻¹·mg⁻¹. The values for the purified enzyme are 1 mm MgUTP and 0.2 μmol of P₃·min⁻¹·mg⁻¹, respectively. The $K_M$ of PDR5 for MgATP in yeast membranes is similar to that obtained for either the human (Hamada and Tsuruo, 1988b; Sarkadi et al., 1992) or the mammalian P-glycoprotein (Doige et
Yeast PDR5 Multidrug NTPase

**TABLE II**

**NTPase activities in plasma membranes of pdr mutant and parental strains**

<table>
<thead>
<tr>
<th>Strains</th>
<th>IL125-2B WT</th>
<th>DR19-T8 pdr-1-3</th>
<th>DR19-T7 pdr-1-2</th>
<th>RW2802 WT</th>
<th>JG436 PDR5::Tn5</th>
<th>U550-18C pdr-1-3</th>
<th>JG365-5C pdr-1-3 PDR5::Tn5</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTPase activity (in pmol Pi·min⁻¹·mg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATPase, pH 6.0</td>
<td>1.4</td>
<td>2.1</td>
<td>2.9</td>
<td>2.7</td>
<td>2.7</td>
<td>2.7</td>
<td>2.4</td>
</tr>
<tr>
<td>ATPase, pH 7.5</td>
<td>0.4</td>
<td>1.5</td>
<td>1.3</td>
<td>1.1</td>
<td>0.8</td>
<td>2.1</td>
<td>1.2</td>
</tr>
<tr>
<td>UTPase, pH 6.0</td>
<td>0.1</td>
<td>1.2</td>
<td>1.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>UTPase, pH 7.5</td>
<td>0.1</td>
<td>1.0</td>
<td>0.8</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
</tr>
<tr>
<td>UTPase pH 7.5 enrichment in pdr mutants (M/P)</td>
<td>1</td>
<td>10</td>
<td>8</td>
<td>1</td>
<td>0.7</td>
<td>11</td>
<td>0.09</td>
</tr>
</tbody>
</table>

* Compared with the JG436 strain.
* Compared to the DR19-T8 strain.

**Fig. 3.** Separation of PDR5 from PMA1 by centrifugation through a continuous glycerol gradient. The detergent extract of pdr-1-3 membranes (3.5–4 mg of protein) was layered onto a continuous 15–40% glycerol gradient and centrifuged as described under “Materials and Methods.” The gradient was fractionated into 35 fractions of 1 ml each. **Upper panel.** Coomassie Blue-stained SDS-PAGE analysis of purified PMAl and PDR5 fractions from the pooled fractions 17 through 34 corresponding to glycerol concentrations of 30–16% (w/v). Each lane contained 50 μl of the fraction. **Lower panel,** distribution of ATPase and UTPase activities through the glycerol gradient. NTPase assays were carried out at 35 °C in the presence of lyssolecithin and asolectin. The colorimetric method used is reported under “Materials and Methods.”

al., 1992; Al-Shawi and Senior, 1993).

**Fig. 5.** Nucleoside phosphatase activities of PDR5 and PMA1. Partially purified enzymes of fractions 17–23 and 27–31 of the glycerol gradient described in Fig. 5 were assayed at 35 °C in buffers containing 6 mM magnesium nucleotide and 59 mM Tris·HCl, pH 7.5, in the presence of lipids as described under “Materials and Methods.”

**Fig. 4.** Effect of pH on ATPase and UTPase activities. Partially purified PMAl and PDR5 fractions from the pooled fractions 17–23 and 27–31 of the glycerol gradient of Fig. 6 were tested for ATPase and UTPase activity at different pH values in the presence of 6 mM MgNTP and 59 mM MES (pH values from 5.0 to 7.0) or Tris (pH values from 7.5 to 9.0). The pH was adjusted with NaOH or HCl.

**Fig. 6A** shows the effect of verapamil on membrane-bound PDR5 UT-Pase activity at pH 7.5. In tumor cells, verapamil reverses the MDR phenotype, probably by competitive inhibition of drug transport (reviewed, for instance, in Endicott and Ling (1989) and Gottesman and Pastan (1993)). In plasma membranes from the pdr1-3 mutant, verapamil caused a 1.3-fold stimulation of UTPase activity at pH 7.5 when used at concentrations between 10⁻⁹ and 10⁻⁸ M. Inactivation occurred at verapamil concentrations above 10⁻⁸ M. Verapamil stimulation was lost upon solubilization of PDR5. The extent of verapamil stimulation of UTPase activity in pdr-1-3 plasma membrane fragments (which are probably largely nonvesiculated) is lesser than that reported for P-glycoprotein ATPase activity in vesicular systems (Horio et al., 1988; Ambudkar et al., 1992; Sarkadi et al., 1992, Al-Shawi and Senior, 1993). No stimulatory effect was observed in the 10⁻⁶ to 10⁻³ M concentration range of colchicine and doxorubicin, which are also thought to be transported by the P-glycoprotein (reviewed by Gottesman and Pastan (1993)). Cycloheximide enhanced 1.2-fold the UTPase activity at pH 7.5 in the pdr1-3 plasma membranes in the 10⁻⁶ to 10⁻³ M concentration range tested. Actinomycin D at 0.15 mM half-inhibited the UTPase activity at pH 7.5.

**Fig. 6B** illustrates the inhibitory effects of the yeast ATPase
inhibitors vanadate (Willsky, 1979) and erythrosine B (Wach and Gräber, 1991) on plasma membrane-bound PDR5 UTPase activity at pH 7.5. Fifty percent inhibition was achieved with 3 μM vanadate or 15 μM erythrosine B. These values are in the same range as required to inhibit the ATPase activity of PMA1 at pH 6.0 in wild-type plasma membranes (1 μM vanadate or 3 μM, data not shown). For P-glycoprotein, half-maximal inhibition has been reported for vanadate concentrations ranging from 1.5 to 12 μM (Horio et al., 1988; Doige et al., 1992; Ambudkar et al., 1992; Sarkadi et al., 1992, Al-Shawi and Senior, 1993).

Mitochondrial ATPase inhibitors were also tested. Sodium azide had no effect on PDR5 activity, as reported earlier for P-glycoprotein ATPase activity (Ambudkar et al., 1992; Al-Shawi and Senior, 1993) and for drug transport by the P-glycoprotein (Horio et al., 1988). However, oligomycin and venturicidin did inhibit PDR5 ATPase activity, with 50% inhibition occurring at quite low concentrations: 0.07 μg/ml for both inhibitors (Table III). PDR5 is thus about 10 times more sensitive to oligomycin than yeast mitochondrial ATPase activity tested in vitro (Colson et al., 1974). Sarkadi et al. (1992) and Al-Shawi and Senior (1993) have shown that oligomycin and venturicidin inhibit the mammalian P-glycoprotein ATPase activity with half-maximal inhibition at 0.4 μg/ml. Curiously, although vanadate inhibits over 80% of the PDR5 UTPase, pH 7.5, activity, several other inhibitors, including erythrosine B (Fig. 6B) and oligomycin, inhibit only 50–60% of the activity.

Miconazole and Dio-9, known to inhibit yeast plasma membrane ATPase activity (Dufour et al., 1980), as well as fluonilid, which also inhibits PMA1 (data not shown), were also found to inhibit PDR5 ATPase activity. No inhibition of pdr1-3 plasma membrane PDR5 UTPase activity was found at N-ethylmaleimide concentrations between 10^{-6} and 10^{-2} M (Table III).

Low concentrations of Triton X-100 or CHAPS also drastically inhibited the UTPase activity of plasma membranes at pH 7.5. Fifty percent inhibition occurred at 0.004% (w/v) Triton X-100 or 0.05% (w/v) CHAPS. In contrast and as reported by Goffau and Dufour (1988), Triton X-100 (in the concentration range 0.004–0.06%) strongly stimulated PMA1 activity at pH 6.0 in the parental plasma membranes, whereas CHAPS had no effect. Ethanol and dimethyl sulphoxide, used to solubilize hydrophobic drugs, can be added to the assay medium at concentrations up to 8% (v/v) without causing any PDR5 activity inhibition.

**DISCUSSION**

We report here the solubilization and partial purification of the PDR5 gene product which belongs to a new class of yeast plasma membrane ATPase. The PDR5 gene, identified several years ago as conferring resistance to cycloheximide, sulfomethyluron, and other drugs upon amplification on a multicopy plasmid (Leppert et al., 1990), has recently been sequenced and shown to encode a new ABC-type protein (Balzi et al., 1994; Bisssinger and Kuchler, 1994). The expression of PDR5, located on chromosome XV, is controlled by the transcription factor PDR1, located on chromosome VII (Balzi and Coffeau, 1991; Meyers et al., 1992). Mutant alleles of PDR1 such as pdr1-3 and pdr1-6 dramatically increase expression of the PDR5 gene product (Balzi et al., 1994). In the present work, we have taken advantage of this remarkable feature. A 160-kDa protein was detected in high quantity in the plasma membrane-enriched fraction of pdr1-3 mutant cells. The 160-kDa protein disappears when the pdr1-3 allele is replaced with a disrupted PDR1 gene, as already observed by Balzi et al. (1994). Overexpression of the 160-kDa protein also occurs in the pdr3-2 mutant, whose mutation affects another transcription regulator and has been mapped to chromosome II (Delaveau et al., 1992). That the 160-kDa protein is the PDR5 gene product is shown by amino acid sequencing of its NH₂ terminus which matches the sequence predicted by gene sequencing (Balzi et al., 1994; Bisssinger and Kuchler, 1994). This is confirmed by the fact that disruption of the PDR5 gene in a pdr1-3 mutant leads to the nearly complete disappearance of the multiple fpazy 160-kDa band, which must thus contain several post-translational modifications of the PDR5 protein.

The highly PDR5-enriched plasma membrane fraction obtained from pdr1-3 mutant cells was used for solubilization and
partial purification of the protein. After solubilization with n-dodecyl-β-D-maltoside, the 160-kDa PDR5 protein was separated from the 100-kDa PMA1 plasma-membrane H+-ATPase protein by centrifugation on a 15–40% glycerol gradient. After this purification step, the UTase activity measured at pH 7.5 peaked exactly with the 160-kDa protein, whereas the two peaks of ATPase activity measured at pH 6.0 coincided with the 100-kDa PMA1 protein and the 160-kDa PDR5 protein. Little UTase activity at pH 7.5 was observed in wild-type plasma membranes.

In pdr1-3 plasma membranes, the measured activity can be almost entirely attributed to the 160-kDa PDR5 protein. The UTase activity measured at pH 7.5 in pdr1-3 plasma membranes amounts to 1 μmol of P, min⁻¹·mg⁻¹. If PDR5 accounted for approximately 20% of the total plasma membrane protein in the pdr1-3 mutant, the specific activity of a perfectly purified PDR5 UTase should be about 5 μmol of P, min⁻¹·mg⁻¹ of PDR5 protein. Instead, we obtained 0.15 μmol of P, min⁻¹·mg⁻¹ in the glycerol gradient peak. It follows that during solubilization and purification only about 5% of the activity at pH 7.5 was preserved. This was due to the high sensitivity of the UTase activity to detergents. Partial reactivation was achieved, however, by addition of asolectin during the assay, a procedure which increased the PDR5 UTase activity by 10-fold. Similarly, Doige et al. (1993) have recently shown that P-glycoprotein ATPase activity requires specific lipids including asolectin.

In many other respects the properties of yeast PDR5 NTase resemble those of the mammalian P-glycoprotein, which has recently been solubilized and partially purified (reviewed by Gottesman and Pastan (1993)). PDR5 hydrolyzes CTP, GTP, and ADP but not AMP. This broad nucleotide specificity, also shown by the mammalian P-glycoprotein (Hamada et al., 1991), and by bacterial permeases like the histidine transporter (Bishop et al., 1989), may be related to the particular NTP-binding site of the ABC proteins (Higgins, 1992).

PDR5 is inhibited by vanadate with 50% inactivation occurring at 3 μM. Several authors have reported that other ABC-type membrane-associated ATPases are also inhibited by vanadate (see Doige et al., 1992), which in P-type ATPases has been related to the particular NTP-binding site of the ABC proteins by their structure and mechanism.

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REFERENCES

Yeast PDR5 Multidrug NTPase