Effects of Nonionic Detergents on P-Glycoprotein Drug Binding and Reversal of Multidrug Resistance

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ABSTRACT

Multidrug-resistant cells are thought to maintain low intracellular cytotoxic drug concentrations through the active efflux of drugs across the cell membrane. It is presently believed that P-glycoprotein mediates this energy-dependent drug efflux by interacting directly with various lipophilic compounds. In this report, we have used [3H]azidopine in a photoaffinity labeling assay to study the effect of detergents and denaturing agents on P-glycoprotein drug binding in intact cells. Nonionic detergents such as Triton X-100 or Nonidet P-40 at very low concentrations were found to completely abolish azidopine photo-labeling to P-glycoprotein and are able to reverse the multidrug resistance phenotype. In contrast, high concentrations of the denaturing agent urea or the zwitterionic detergent 1-(3-cholamidopropyl)dimethylamino)-1-propanesulfonate did not inhibit azidopine photo-labeling to P-glycoprotein. A comparison between verapamil and Triton X-100 revealed that the latter was more effective in inhibiting azidopine photo-labeling to P-glycoprotein while verapamil was more effective in potentiating [3H]vinblastine accumulation in drug-resistant cells. Drug transport studies showed that [3H]Triton X-100 accumulated in both drug-sensitive and -resistant cells, and its accumulation was not modulated by excess vinblastine, verapamil, or colchicine. Taken together, these findings suggest that low concentrations of Triton X-100 reverse the multidrug resistance phenotype by inhibiting P-glycoprotein drug binding. In addition, it is also suggested that the site(s) of P-glycoprotein drug binding is localized to sequences found within the lipid bilayer.

INTRODUCTION

The overexpression of M, 170,000 phosphoglycoprotein (or P-glycoprotein) has been demonstrated in many drug-resistant tumor cell lines selected in vitro with lipophilic anti-cancer drugs (1, 2). Transfection studies of P-glycoprotein complementary DNA into drug-sensitive cells have confirmed its role in mediating the MDR phenotype (3, 4). Analysis of P-glycoprotein amino acid sequence predicted a tandem repeat of two domains each containing six transmembrane segments and one ATP binding site (5-7). The P-glycoprotein gene family of three genes in rodent and two in human (8) is part of a superfamily of membrane transport proteins which includes the bacterial periplasmic membrane transporters (9), the yeast STE6 membrane transporters (10), the cystic fibrosis transmembrane regulator (11), and the antigenic MHCI peptide transporter (12). The role of P-glycoprotein in mediating the drug efflux in MDR cells is presently accepted. More speculative is the role of P-glycoprotein in normal tissues and its native substrate. Based on its restricted tissue distribution such as in the adrenal cortex and in the epithelial cells of the large intestine, it has been suggested that P-glycoprotein could mediate the transport of hormones or cell metabolites in the adrenal gland and xenobiotics in the large intestine (13-15).

The apparent broad substrate specificity of P-glycoprotein drug efflux pump remains one of the most challenging questions relating to our understanding of P-glycoprotein-associated MDR. P-glycoprotein has been shown to bind a large number of lipophilic compounds with no apparent structural or functional similarities (16). Some of these compounds, which include the calcium channel blockers, have been shown to reverse the MDR phenotype and inhibit drug binding to P-glycoprotein (17). Thus it is thought that the mechanism of action of such reversing agents is via competition for the binding site(s) on P-glycoprotein. Early reports (18-20) on the physical-chemical properties of various compounds which are thought to reverse the MDR phenotype have indicated the presence of planar aromatic rings and a cationic charge. Although these structural moieties are found in some compounds which reverse the MDR phenotype, their role in P-glycoprotein drug binding or transport is presently not known nor is it known what the molecular interactions which mediate P-glycoprotein drug binding and transport are. In this study, it was of interest to evaluate the effect of denaturing agents and various detergents on P-glycoprotein drug binding. [3H]Azidopine, a dehydropyridine calcium channel blocker, was used in a photoaffinity labeling assay since it was previously shown to specifically bind to P-glycoprotein (21), and it contains a planar aromatic ring and a cationic charge. The results of this study demonstrate that nonionic detergents reverse the MDR phenotype by inhibiting P-glycoprotein drug binding. It is also suggested that hydrophobic interactions are likely to mediate P-glycoprotein drug binding, while the cationic charge associated with some lipophilic compounds may be important in drug transport.

MATERIALS AND METHODS

Materials. [3H]Azidopine (75 mCi/mmol) was purchased from Amersham Biochemical Inc. (Mississauga, Ontario, Canada). [3H]Triton X-100 (5 mCi/mg) was purchased from Dupont NEN. Drug-sensitive human lymphoma cells (CEM) were a gift from Dr. W. Beck at St. Jude Children's Research Hospital, Memphis, Tennessee. The CEM/VLB100 line was established from CEM/VLB100 obtained from Dr. W. Beck. Nonradiola beled Triton X-100 and other nonionic detergents used in this study contained less than 1.0 µeq/ml peroxides and carbonyl (Pierce Chemical Co., Rockford, IL). All other chemicals used were of the highest grade available.

Cell Culture and Plasma Membrane Preparation. Drug-sensitive (CEM) and vinblastine-resistant (CEM/VLB100) cells were grown in suspension to 1 × 10⁶ cells/ml of αMEM media containing 10% fetal calf serum (Hyclone, Inc.). Drug-resistant cells (CEM/VLB100) were grown continuously in both drug-sensitive and -resistant media, and their accumulation was not modulated by excess vinblastine, verapamil, or colchicine. Taken together, these findings suggest that low concentrations of Triton X-100 reverse the multidrug resistance phenotype by inhibiting P-glycoprotein drug binding. In addition, it is also suggested that the site(s) of P-glycoprotein drug binding is localized to sequences found within the lipid bilayer.

Received 7/15/93; accepted 10/8/93.

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1 This work was supported by funds from the National Cancer Institute of Canada; Terry Fox Run and a Young Investigator Award from Bio-Mega/Boehringer Ingelhcim Inc.

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3 The abbreviations used are: MDR, multidrug resistance; αMEM, α-Eagle's minimal essential medium; PBS, phosphate-buffered saline; CHAPS, 1-(3-cholamidopropyl)dimethylamino)-1-propanesulfonate; TX100, Triton X-100; NP40, nonidet P-40; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; mAb, monoclonal antibody.

4 V. Ling, unpublished data.
the SS34 rotor. The supernatant from the above low speed spin was loaded onto a discontinuous sucrose gradient consisting of 16, 31, 45, and 60% sucrose in 10 mM Tris-HCl (pH 7.4). The plasma membrane-enriched fraction at the 16–31% sucrose interphase was collected and washed with 10 mM Tris-HCl (pH 7.4). Membrane fractions were stored at −85°C if not immediately used.

**Photolabeling Assay.** For [3H]azidopine photoaffinity labeling of P-glycoprotein in plasma membrane fractions, aliquots (20 µg) of plasma membrane from drug-sensitive (CEM) or -resistant (CEM/VLB<sup>1000</sup>) cells were incubated with [3H]azidopine (0.2 µM, final concentration) for 30 min in the dark prior to the addition of detergents or drugs. The incubation was continued for another 30–60 min and then UV-irradiated for 10 min at 254 nm on ice (Stratagene UV croslinker; Stratagene, La Jolla, CA). Azidopine photoaffinity labeling of intact cells was essentially as for plasma membrane; however, drug-sensitive (CEM) or -resistant (CEM/VLB<sup>1000</sup>) cells were washed three times in oMEM-Minus fetal calf serum and resuspended in 20 µl final volume of oMEM containing 0.2 µM [3H]azidopine. Cells were incubated with azidopine for 30 min at room temperature in the dark prior to the addition of detergents or MDR-reversing agents. After an additional 30–60 min incubation in the presence of drugs or detergents, cells were transferred to ice and UV-irradiated for 10 min. For azidopine photolabeling in the presence of detergents, the cell suspension was centrifuged after UV irradiation, and the supernatant was removed. Proteins or phospholipid present in this supernatant fraction were referred to as TX100- or CHAPS-soluble fractions at a given detergent concentration. The pellet fractions were referred to as TX100- or CHAPS-insoluble fractions at a given concentration of detergent. The pellet fractions from TX100- or CHAPS-insoluble samples were solubilized in 1% NP40 solution (20 mM Tris-HCl, pH 7.4, containing 1% NP40) and prepared for SDS-PAGE. It should be mentioned that photoaffinity labeling of CEM/VLB<sup>1000</sup> cells with [3H]azidopine in the presence of verapamil (a calcium channel blocker) were done with the above experiments to demonstrate the binding specificity of [3H]azidopine to P-glycoprotein (data not shown).

**Drug Accumulation.** Drug-sensitive and -resistant cells were washed three times in oMEM without fetal calf serum. Cells were then incubated in 1 µM [3H]vinblastine or [3H]Triton X-100 alone or in the presence of 50–100 µM of cold verapamil, TX100, or colchicine. Samples were removed after 15, 30, and 60 min incubation at 37°C. Cells were then transferred to ice and washed three times with ice-cold PBS. The cell pellets were solubilized in 1 N NaOH and allowed to incubate overnight. Equal volume of 1 N HCl was added and 100 µl of the mixture were spotted onto a Whatman filter disc. The amounts of radioactivity were measured by liquid scintillation spectrometer using Beckman LKB1219 Rackbeta counter. The results are expressed as cpm/1 × 10<sup>6</sup> cells.

**Gel Electrophoresis and Western Blotting.** Membrane protein fractions (~20 µg) or 1 × 10<sup>6</sup> cells were resolved on SDS-PAGE using the Fair-banks gel system with some modifications (24). Briefly, proteins were dissolved in 1/5 volume of 5× solubilization buffer I (2% SDS, 50 mM dithiothreitol, 1 mM EDTA, and 10 mM Tris-HCl, pH 8.0), and mixed with an equal volume of buffer II (2X buffer I and 9 M urea). Gel slabs containing the resolved membrane proteins or phospholipid present in this supernatant fraction were solubilized in 1% NP40 solution (20 mM Tris-HCl, pH 7.4, containing 1% NP40) and prepared for SDS-PAGE. The plasma membrane-enriched fraction at the 16–31% sucrose interphase was collected and washed with 10 mM Tris-HCl (pH 7.4). Membrane fractions were stored at −85°C if not immediately used. Dried SDS-PAGE gels were exposed to Kodak X-ray film at −70°C.

**Immunostaining of Cells.** Drug-sensitive or -resistant cells were incubated in the absence or the presence of TX100 (1–1000 µM) for 60 min at room temperature. MRK16, a mAb which recognizes an extracellular epitope of P-glycoprotein (Ref.26; 0.5 µg; a generous gift from Dr. T. Tsuruo at Institute of Applied Microbiology, University of Tokyo, Japan), was added to each cell suspension and reverse the MDR phenotype (21). Although the mechanism of MDR reversal by chemosensitizing agents is currently not understood, the specific interactions between azidopine and P-glycoprotein are likely to be important for its mechanism of action in the reversal of the MDR phenotype. To gain insight into the role of the different molecular interactions between P-glycoprotein and azidopine, it was of interest to examine the effect of various detergents and denaturing agents on azidopine photolabeling to P-glycoprotein in intact cells. Drug-sensitive (CEM) or -resistant (CEM/VLB<sup>1000</sup>) cells were incubated with [3H]azidopine in the presence or absence of detergents or denaturing agents prior to UV photolabeling (see “Materials and Methods”). The results in Fig. 1a, Lane 2 show specific photolabeling of azidopine to a M, 170,000 polypeptide (P-glycoprotein) in drug-resistant cells, while no M, 170,000 azidopine-photolabeled protein is seen in drug-sensitive cells (Fig. 1a, Lane 1). Fig. 1a, Lanes 3–5 show azidopine-photolabeled P-glycoprotein in the presence of 4 M urea, 1 mM TX100, or 1 mM SDS, respectively. The presence of urea did not affect azidopine photolabeling to P-glycoprotein (Fig. 1a, Lane 3). In contrast, both TX100 or SDS inhibited azidopine photolabeling to P-glycoprotein (Fig. 1a, Lanes 4 and 5, respectively). Triton X-100 was more effective than SDS in blocking azidopine-photolabeling to P-glycoprotein (Fig. 1a, Lane 4). It is of interest to note that Triton X-100 in the absence of detergents does not affect azidopine photolabeling to P-glycoprotein (Fig. 1a, Lane 1). Fig. 1a, Lanes 1 and 2, sensitive or resistant cells photolabeled to azidopine (Fig. 1a, Lane 3). In contrast, both TX100 or SDS inhibited azidopine photolabeling to P-glycoprotein (Fig. 1a, Lane 3).

**Protein Assay.** Plasma membrane proteins were measured using the Lowry protein assay (27).

**RESULTS**

The use of photoactive drug analogues in an UV photolabeling assay has led to the identification of a large group of compounds which interact with P-glycoprotein in isolated membrane from drug-resistant cells. Some of these compounds have been shown to reverse the MDR phenotype; however it is not clear if all drugs isolated by the above criteria do interact specifically with P-glycoprotein in intact cells and reverse the MDR phenotype. Azidopine, a calcium channel blocker, has been shown to bind P-glycoprotein and reverse the MDR phenotype (21). Although the mechanism of MDR reversal by chemosensitizing agents is currently not understood, the specific interactions between azidopine and P-glycoprotein are likely to be important for its mechanism of action in the reversal of the MDR phenotype. To gain insight into the role of the different molecular interactions between P-glycoprotein and azidopine, it was of interest to examine the effect of various detergents and denaturing agents on azidopine photolabeling to P-glycoprotein in intact cells. Drug-sensitive (CEM) or -resistant (CEM/VLB<sup>1000</sup>) cells were incubated with [3H]azidopine in the presence or absence of detergents or denaturing agents prior to UV photolabeling (see “Materials and Methods”). The results in Fig. 1a, Lane 2 show specific photolabeling of azidopine to a M, 170,000 polypeptide (P-glycoprotein) in drug-resistant cells, while no M, 170,000 azidopine-photolabeled protein is seen in drug-sensitive cells (Fig. 1a, Lane 1). Fig. 1a, Lanes 3–5 show azidopine-photolabeled P-glycoprotein in the presence of 4 M urea, 1 mM TX100, or 1 mM SDS, respectively. The presence of urea did not affect azidopine photolabeling to P-glycoprotein (Fig. 1a, Lane 3). In contrast, both TX100 or SDS inhibited azidopine photolabeling to P-glycoprotein (Fig. 1a, Lanes 4 and 5, respectively). Triton X-100 was more effective than SDS in blocking azidopine-photolabeling to P-glycoprotein (Fig. 1a, Lane 4). It is of interest to note that Triton X-100 in the absence of detergents does not affect azidopine photolabeling to P-glycoprotein (Fig. 1a, Lane 1). Fig. 1a, Lanes 1 and 2, sensitive or resistant cells photolabeled to azidopine (Fig. 1a, Lane 3). In contrast, both TX100 or SDS inhibited azidopine photolabeling to P-glycoprotein (Fig. 1a, Lane 3). In contrast, both TX100 or SDS inhibited azidopine photolabeling to P-glycoprotein (Fig. 1a, Lane 3). The positions of the molecular size markers are indicated to the left of 1a.
tive than SDS in inhibiting azidopine photolabeling to P-glycoprotein (Fig. 1a, Lane 4 versus 5). Differences between TX100 and SDS in the inhibition of azidopine photolabeling to P-glycoprotein may be due to differences in the critical micelle concentrations of these two detergents (1 mM and 10 mM for TX100 and SDS, respectively). Fig. 1a, Lane 5 also shows the presence of other azidopine-photolabeled polypeptides with $M_r$ of $\sim$50,000 to 80,000. To rule out the possibility that the azidopine-photolabeled polypeptides seen in Fig. 1a, Lane 5 represent cleavage products of P-glycoprotein and hence the loss of the azidopine-photolabeled P-glycoprotein signal, Western blot analysis was performed on identical samples as in Fig. 1a and probed with P-glycoprotein-specific monoclonal antibody (C219 mAb; Ref. 28). The results of the Western blot analysis (Fig. 1b) show a 170,000-molecular-weight band in drug-resistant cells in the absence (Fig. 1b, Lane 2) or the presence of 4 mM urea, 1 mM TX100, or 1 mM SDS, respectively (Fig. 1b, Lanes 3–5). P-glycoprotein appears intact in the presence of urea, TX100, or SDS as judged from the intensity of the protein band and the absence of low molecular weight C219 mAb-reactive cleavage peptides. Thus, the loss of the azidopine signal in the presence of TX100 or SDS is due to the inhibition of azidopine photolabeling to P-glycoprotein rather than proteolytic cleavage.

It is interesting that the denaturing agent urea, which does not disrupt the lipid bilayer, had no effect on azidopine photolabeling to P-glycoprotein, while TX100 at 1 mM concentration completely abolished azidopine photolabeling to P-glycoprotein. These results suggest that the intactness of the lipid bilayer may be essential for P-glycoprotein drug binding. Alternatively, the effect of TX100 on azidopine photolabeling to P-glycoprotein may be independent of the lipid bilayer. To differentiate between these two possibilities, azidopine photolabeling to P-glycoprotein in intact cells was examined in the presence of low molar concentrations of TX100 which do not disrupt the lipid bilayer. Intact MDR cells were incubated with azidopine for 30 min prior to the addition of TX100 (1 µM to 1000 µM) and then further incubated for another 60 min prior to UV photolabeling. The photolabeled material was centrifuged at high speed to separate the TX100-soluble and -insoluble fractions at a given TX100 concentration (see "Materials and Methods"). The results in Fig. 2a, Lanes 2–5) show a decrease in azidopine-photolabeled P-glycoprotein in the TX100-insoluble fractions. TX100 at 10 µM final concentration completely abolished azidopine photolabeling to P-glycoprotein (Fig. 2a, Lane 3). It should be pointed out that at low concentrations of TX100 (1 µM to 100 µM) no disruption of the cell membrane was detected after 2 h incubation as determined by a dye exclusion assay using trypan blue (cell viability > 90%). At 1000 µM TX100, the cell viability was decreased to 15% (data not shown).

Earlier reports (29–31) on the effect of low concentrations of TX100 on the preferential extraction of some transmembrane proteins or phospholipid from endoplasmic reticulum or retinal lipid bilayer prompted us to verify if the selective extraction of P-glycoprotein or phospholipid into the TX100-soluble fractions was responsible for the observed reduction in azidopine-photolabeled P-glycoprotein seen in the TX100-insoluble fractions. Fig. 2b shows the TX100-soluble fractions after azidopine photolabeling of intact cells. No azidopine-photolabeled P-glycoprotein was detected at any concentration of TX100 in the TX100-soluble fractions (Fig. 2b, Lanes 1–5). The absence of azidopine-photolabeled P-glycoprotein in the TX100-soluble fractions was further confirmed by Western blot analysis using identical samples as in Fig. 2a and b. Western blot analysis of TX100-soluble fractions demonstrated the presence of P-glycoprotein only at 1000 µM of TX100 (Fig. 2d, Lane 5). In contrast, TX100-insoluble fractions contained equivalent amounts of P-glycoprotein except at 1000 µM of TX100 (Fig. 2c, Lanes 1–4). Similar results were also obtained when NP40 was used instead of TX100 (data not shown). These results demonstrate that the inhibition of azidopine photolabeling to P-glycoprotein in the presence of TX100 or NP40 is not due to the disruption of the cell membrane.

To determine if the presence of hydrophobic molecules which may perturb the lipid bilayer could explain the TX100 or NP40 effect,
azidopine photolabeling to P-glycoprotein was examined in the presence of increasing concentrations (0.01 mM to 10 mM) of the zwitterionic detergent CHAPS. Again, CHAPS-soluble and -insoluble fractions were examined after [3H]azidopine photolabeling to P-glycoprotein in intact cells. The results in Fig. 3a show that CHAPS at 1 mM final concentration did not inhibit azidopine photolabeling to P-glycoprotein. The reduction in azidopine photolabeling to P-glycoprotein in the presence of 10 mM CHAPS was due to the solubilization of the plasma membrane and the extraction of P-glycoprotein into the CHAPS-soluble fraction (Fig. 3b, Lane 5). Surprisingly, P-glycoprotein in mixed micelles of phospholipid and CHAPS was still capable of specifically binding to azidopine (Fig. 3b, Lane 5). Western blot analysis of identical samples as in Fig. 3, a and b probed with C219 mAb confirmed the presence of P-glycoprotein in CHAPS-insoluble fractions at up to 1 mM CHAPS (Fig. 3c, Lanes 1—4) and the solubilization of P-glycoprotein into the CHAPS-soluble fraction at 10 mM CHAPS (Fig. 3d, Lane 5). These results indicate that the perturbation of the lipid bilayer by a hydrophobic detergent does not inhibit P-glycoprotein-azidopine interactions.

Antibody binding studies had previously demonstrated that non-ionic detergents (e.g., TX100 or NP-40) inhibited MRK16 mAb binding to P-glycoprotein (32). We have recently mapped the epitope sequence of MRK16 mAb to a discontinuous extracellular epitope in P-glycoprotein (33). Based on the localization of the discontinuous epitope sequence of MRK16 mAb, it may be possible that TX100 inhibits azidopine photolabeling to P-glycoprotein by perturbing the native conformation of P-glycoprotein which in turn affects MRK16 mAb binding. To determine if TX100 had a measurable effect on the native conformation of P-glycoprotein, the ability of MRK16 mAb to bind P-glycoprotein in intact CEM/VLB1000 cells was measured in the absence or the presence of 1–100 µM of TX100. Fig. 4 shows the results of such an enzyme-linked immunoabsorbent assay signal (Fig. 4). Although higher concentrations of TX100 may lead to structural changes in P-glycoprotein or the antibody which could result in the reported (32) effect of nonionic detergents on MRK16 mAb binding to P-glycoprotein, the results in Fig. 4 argue against large conformational changes in P-glycoprotein structure at 100 µM TX100. The increased binding of MRK16 mAb to P-glycoprotein in the presence of 1 µM TX100 was consistently seen and may be due to the unmasking of its epitope.

Given that TX100 at low concentrations inhibits azidopine photolabeling to P-glycoprotein, it was of interest to compare its capacity to

![Image](cancerres.aacrjournals.org)
potentiate the drug uptake to other MDR-reversing agents such as verapamil. Fig. 5 shows the results of an azidopine photolabeling of intact MDR cells in the absence or the presence of increasing molar concentrations of verapamil or TX100. Triton X-100 at 10 μM final concentration was more effective in reducing azidopine photolabeling to P-glycoprotein than verapamil at similar molar concentrations (Fig 5, Lane 5 versus 7). These results indicate that TX100 may be more effective than verapamil in potentiating the accumulation of cytotoxic compounds in MDR cells and as such may be a better MDR-reversing agent than verapamil. To address this possibility, [3H]vinblastine accumulation in CEM or CEM/VLB1000 cells was examined in the absence or the presence of 100 μM verapamil, TX100, or colchicine after 15 to 60 min at 37°C (see “Materials and Methods”). The results in Fig. 6a show that both verapamil and TX100 but not colchicine potentiated the accumulation of [3H]vinblastine in drug-resistant cells. However, TX100 was less effective in potentiating [3H]vinblastine accumulation in CEM/VLB1000 cells than verapamil. These results are in contrast with the above azidopine photolabeling data in Fig. 5 which show that TX100 is more effective than verapamil in inhibiting azidopine photolabeling to P-glycoprotein. Thus inhibition of azidopine photolabeling to P-glycoprotein with various lipophilic drugs may not always reflect the true capacity of a given compound to potentiate the accumulation of cytotoxic drugs in MDR cells and to reverse the MDR phenotype.

To determine if TX100 is a substrate for the P-glycoprotein drug efflux pump, CEM or CEM/VLB1000 cells were incubated with 1 μM [3H]TX100 for 15 to 60 min at 37°C in the absence or presence of 100 μM of verapamil, vinblastine, or colchicine. Both drug-sensitive and -resistant cells accumulated large amounts of [3H]TX100 (Fig. 6b). In addition, the presence of excess (100 μM) vinblastine, verapamil, or colchicine did not modulate the accumulation of [3H]TX100 in drug-sensitive or -resistant cells.

**DISCUSSION**

In this study, we have demonstrated that low concentrations of nonionic detergents such as TX100 or NP40 which do not disrupt the lipid bilayer or extract transmembrane proteins inhibit azidopine photolabeling to P-glycoprotein in intact MDR cells. In contrast, high concentrations of the denaturing agent urea which disrupts hydrophobic interactions and hydrogen bonds (34) but does not integrate within the lipid bilayer or extract transmembrane proteins had no effect on azidopine photolabeling to P-glycoprotein. These results suggest that P-glycoprotein-azidopine interactions are likely to be localized to sequences in P-glycoprotein which are found within the lipid bilayer or are inaccessible to the denaturing agent urea. Consistent with these observations is an earlier report (35) demonstrating a direct energy transfer from doxorubicin or rhodamine 123 to a photolabile membrane probe (5-125I-iodonaphthalene-1-azide) which cross-links to P-glycoprotein. Proteolytic mapping studies of photoaffinity labeled P-glycoprotein have led to the identification of two photoaffinity-labeled peptides which were suggested to encode transmembrane sequences 6 and 12 of P-glycoprotein (36, 37). Moreover, it was recently shown that mutations in P-glycoprotein predicted transmembrane sequences (TM 6 or TM 11) altered the cross-resistance profile of MDR transfectant cells towards some cytotoxic drugs (38, 39). Thus, although it remains unclear if some or all of the predicted transmembrane sequences in P-glycoprotein encode the drug-binding site(s), these results are in agreement with our observations in this study which...
suggest that P-glycoprotein-drug binding is likely to be mediated via sequences found in the lipid bilayer.

Earlier reports (40–42) on the effect of nontoxic concentrations of membrane active agents (e.g., TX100 or Tween 80) had demonstrated that nonionic detergents potentiated the accumulation of cytotoxic drugs in resistant cells. However, it was not clear from those studies how nonionic detergents at concentrations which do affect the permeability of cell membrane potentiated the accumulation of cytotoxic drugs in MDR cells. Our results suggest that TX100 or NP40 would likely increase cytotoxic drug accumulation in MDR cells by inhibiting P-glycoprotein drug binding. Studies on the interaction of TX100 with the cell membrane have indicated that at a low detergent:membrane ratio (10^{-5} M), TX100 becomes incorporated into the bilayer in the form of monomers (43). Thus, it may be speculated that TX100 monomers which can integrate within the cell membrane compete for the drug binding site(s) on P-glycoprotein or nonspecifically disrupt the hydrophobic interactions between azidopine and P-glycoprotein.

In view of the latter possibility, it is interesting to note that high concentrations of the zwitterionic detergent CHAPS which can integrate within the lipid bilayer did not inhibit azidopine photolabeling to P-glycoprotein even at concentrations which led to the extraction of P-glycoprotein into mixed micelles of CHAPS and phospholipid. The fact that the disruption of the lipid bilayer does not completely inhibit azidopine photolabeling to P-glycoprotein does not exclude the role of the phospholipid environment in P-glycoprotein structure or function but supports a direct interaction between azidopine and P-glycoprotein.

However, given that both detergents (CHAPS and TX100) can disrupt hydrophobic interactions, the observed differences between TX100 and CHAPS in inhibiting azidopine photolabeling to P-glycoprotein may be due to differences in the binding affinities of these detergents to hydrophobic sequences in proteins. Alternatively, the presence of a planar aromatic ring predicted from the physical-chemical analysis of the MDR reversing agents (18–20) in TX100 or NP40 but not in CHAPS may explain these differences. Taken together, our results suggest that TX100 inhibits azidopine binding to P-glycoprotein by interacting with sequences at the drug-binding site(s) or disrupts the hydrophobic interactions between P-glycoprotein and azidopine.

A comparison between TX100 and verapamil demonstrated that TX100 is more effective than verapamil in inhibiting azidopine photolabeling to P-glycoprotein but is less efficient in potentiating the accumulation of vinblastine in drug-resistant cells. Drug accumulation studies using [3H]TX100 have demonstrated that TX100 is not effluxed from resistant cells (Fig. 6b). These results indicate that TX100 and verapamil may potentiate the cytotoxic drug accumulation via different mechanisms. Consistent with earlier reports (44, 45), these results suggest that the capacity of some compounds to inhibit azidopine photolabeling to P-glycoprotein may not always correlate with their ability to reverse the MDR phenotype. Although neither mechanism of drug reversal is currently understood, it is conceivable that the observed differences between verapamil and TX100 in inhibiting azidopine photolabeling to P-glycoprotein and the potentiation of cytotoxic drug accumulation may be related to the ability of verapamil but not TX100 to serve as a substrate for the P-glycoprotein efflux pump. Consistent with these observations are the recent reports which demonstrated that progesterone which binds to P-glycoprotein (46) and potentiates the accumulation of cytotoxic drugs in resistant cells (44) is not a substrate for the P-glycoprotein drug efflux pump (47).

**ACKNOWLEDGMENTS**

The authors thank Drs. M. E. Scott and G. Matlashewski for their critical reading of the manuscript.
P-GLYCOPROTEIN-DRUG INTERACTIONS

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