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# MECHANISMS OF MULTIDRUG RESISTANCE IN HL60 CELLS

## ANALYSIS OF RESISTANCE ASSOCIATED MEMBRANE PROTEINS AND LEVELS OF *mdr* GENE EXPRESSION*

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**Abstract**—HL60 cells isolated for resistance to Adriamycin® do not contain P-glycoprotein, as determined with immunological probes. These cells, however, are multidrug resistant and defective in the cellular accumulation of drug. In view of these findings, we have examined in greater detail certain properties of the HL60/Adr cells and have compared these properties to an HL60 drug-resistant isolate (HL60/Vinc) which contains high levels of P-glycoprotein. The results of these studies demonstrated that verapamil induces a major increase in cellular drug accumulation in both HL60/Adr and HL60/Vinc isolates. An $^{125}$I-labeled photoaffinity analog of verapamil labeled P-glycoprotein contained in membranes of HL60/Vinc cells. In contrast, this agent did not label any protein selectively associated with drug resistance in membranes of the HL60/Adr isolate. The photoactive dihydropyridine calcium channel blocker [$^{3}$H]azidopine and [$^{125}$I]NASV, a photoaffinity analog of vinblastine, labelled P-glycoprotein in membranes from HL60/Vinc cells, whereas in experiments with the HL60/Adr isolate there was no detectable labeling of a drug resistance associated membrane protein. Additional studies have been carried out to analyze membrane proteins of HL60/Adr cells labeled with the photoaffinity agent 8-azido-$\alpha$-[$^{32}$P]ATP (AzATP$^{32}$). The results demonstrate that this agent labeled a resistance associated membrane protein of 190 kilodaltons (P190). P190 is essentially absent in membranes of drug-sensitive cells. Labeling of P190 with AzATP$^{32}$ in membranes of resistant cells was blocked completely when incubations were carried out in the presence of excess unlabeled ATP. Additional studies were carried out to analyze *mdr* gene amplification and expression in sensitive and resistant cells. Experiments carried out with human 5′,*mdr*1 (1.1 kb) and *mdr*3 (1.0 kb) cDNAs demonstrate that both of these sequences were highly amplified in the HL60/Vinc isolate. Only the *mrd*1 gene sequence however, was overexpressed. In contrast, there was no detectable amplification or overexpression of *mdr*1 or *mdr*3 sequences in HL60/Adr cells. The results of this study thus identify a new nucleotide binding protein which is overexpressed in membranes of HL60 cells isolated for resistance to Adriamycin®. P190, which exhibits properties distinct from P-glycoprotein, possibly functions in the energy-dependent drug efflux system contained in the HL60/Adr resistant isolate.

Multidrug resistance in a number of experimental cell lines appears to be related to the presence of a cell surface phosphoglycoprotein of 150–180 kilodaltons (P180) [1–4]. This protein, also referred to as the P-glycoprotein, is present in high levels in resistant cells as a result of an overexpression of the *mdr*1 gene [5–7]. Cells containing P180 are defective in cellular drug accumulation [8–10], and this probably occurs as a result of an enhanced energy-dependent drug efflux mechanism [11]. Recent studies have shown that P-glycoprotein can bind *Vinca* alkaloids [12, 13], calcium channel blockers [14] and ATP [15]. Evidence has also been obtained that P-glycoprotein has sequence homology with a number of bacterial transport proteins [16–18] and that transfection of sensitive cells with *mdr*1 DNA results in the formation of cells with a drug-resistant phenotype [19]. These results taken together strongly suggest that P-glycoprotein functions as a drug transporter which effluxes drug from resistant cells in an energy-dependent process.

Recently, we isolated and characterized Adriamycin® (doxorubicin hydrochloride)¶ resistant HL60 cells which exhibit properties distinct from a number of other experimental resistant cells lines [20–22]. Thus, we have shown that, although the HL60/Adr isolate is multidrug resistant and defective in the cellular accumulation of drug, these cells do not contain detectable levels of P-glycoprotein [21, 22]. Evidence for this is based on the finding that a monoclonal antibody (C219) against P-glycoprotein [23] does not react with any protein con-

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¶ Adriamycin is a registered trademark of Farmitalia Carlo Erba.

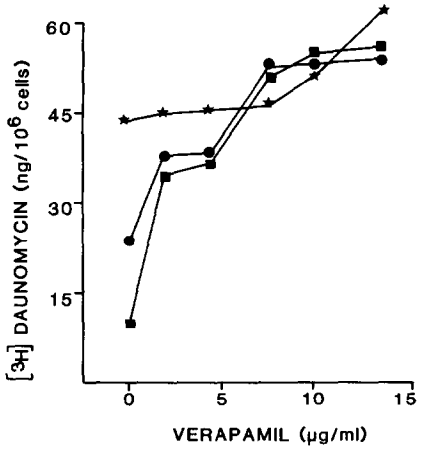


Fig. 1. Effect of verapamil on drug uptake in sensitive and resistant cells. Sensitive and resistant cells ($3 \times 10^5$/ml) in 2 ml of RPMI medium were preincubated in the absence or presence of various concentrations of verapamil, and the cellular uptake of [$^3$H]daunomycin (600 cpm/ng) was determined as described in Materials and Methods. Drug accumulation for $6 \times 10^5$ cells occurring in the absence or presence of 7.5 μg/ml verapamil was 26, 28 ng (HL60/S); 13, 32 ng (HL60/Adr); and 5, 31 ng (HL60/Vinc). The results of this figure represent single experimental points which were repeated in three different experiments. The results obtained in the three experiments were essentially identical. Key: (★—★) sensitive cells; (●—●) HL60/Adr; and (■—■) HL60/Vinc.

tained in the HL60/Adr isolate [21, 22]. Further analysis of this isolate reveals, however, that surface membranes contain a 150 kD protein (P150) which exists as a modified (phosphorylated) form of a protein contained in drug-sensitive cells [20, 21].

In the present study, we have further analyzed mechanisms of drug resistance in the HL60/Adr isolate. In these experiments we used photoaffinity analogs of vinblastine, calcium channel blockers, and ATP to label proteins of membranes from resistant cells. The results reveal the presence of a resistance associated ATP binding protein in membranes of HL60/Adr cells which has properties distinct from P-glycoprotein.

## MATERIALS AND METHODS

*Chemicals.* The photoactive dihydropyridine calcium channel blocker [$^3$H]azidopine (40 Ci/mmol), 2,6-dimethyl-[4-(2′-trifluoromethyl)phenyl]-1,4-dihydropyridine-3,5-dicarboxylic acid, ethyl, (*N*-4′-azido[3″,5″-$^3$H]benzoyl aminoethyl) diester and [$^3$H]vincristine (6.1 Ci/mmol) were purchased from the Amersham Corp. (Arlington Heights, IL). 8-Azido-α-[$^{32}$P]ATP (AzATP$^{32}$) (9.5 Ci/mmol) was purchased from ICN (Irvine, CA). *N*-(*p*-Azido[3-$^{125}$I]salicyl)-*N*′-(3-aminoethylvindesine) ([$^{125}$I]NASV) was synthesized as described previously [12, 24]. The radioactive photoactive verapamil analog, *N*-*p*-azido[3-$^{125}$I]salicyl-5-[(3,4-

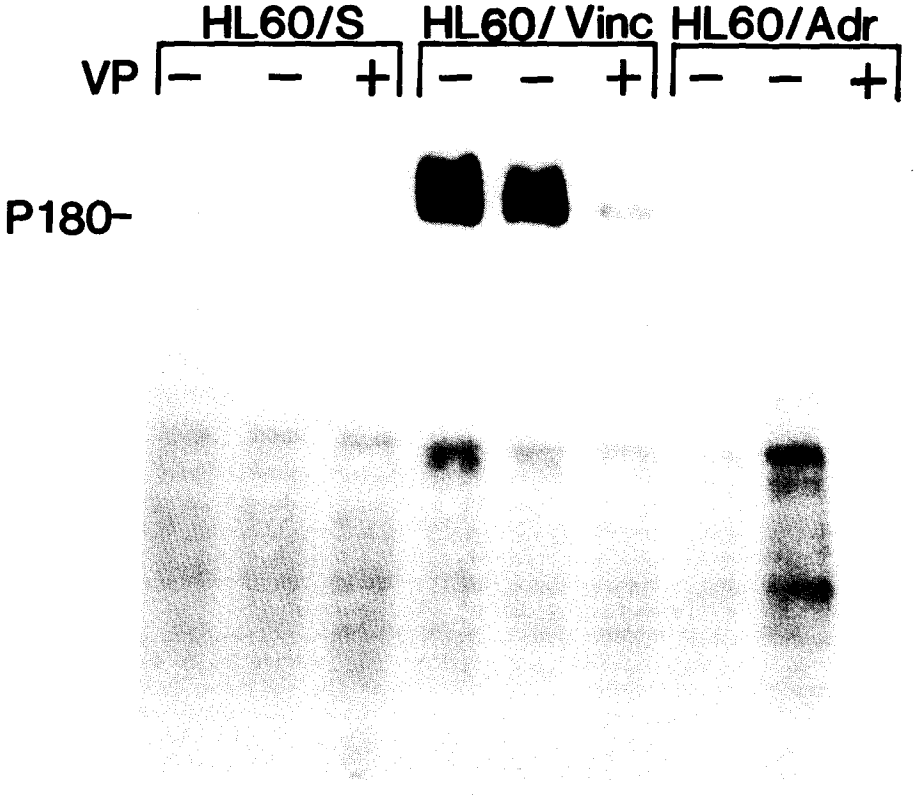


Fig. 2. [$^{125}$I]NASVP photolabeling of membranes of sensitive and resistant cells. Photoaffinity labeling of isolated membranes with $1 \times 10^{-8}$ M [$^{125}$I]NASVP was carried out in the absence (−) or presence (+) of 50 μM verapamil as described in Materials and Methods. Proteins labeled with [$^{125}$I]NASVP were detected after polyacrylamide gel electrophoresis [30] and autoradiography.

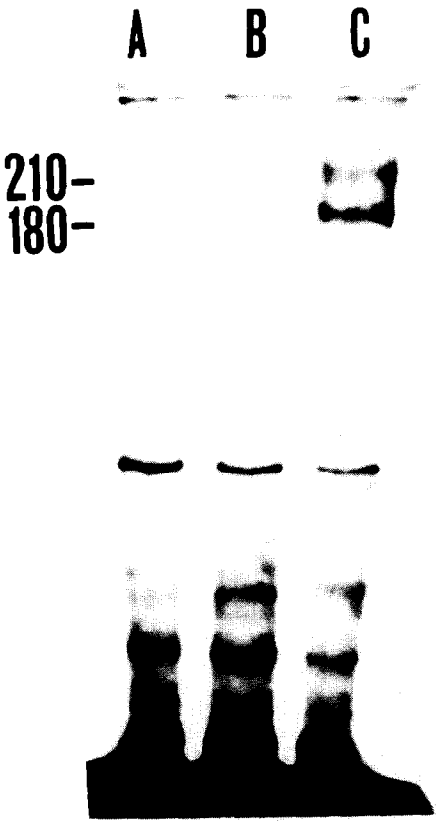


Fig. 3. [$^3$H]Azidopine photolabeling of membrane proteins of sensitive and resistant cells. [$^3$H]Azidopine was incubated with membranes isolated from sensitive and resistant cells. Membrane proteins (50 $\mu$g) were exposed to UV light as described in Materials and Methods. Proteins were separated by electrophoresis in a 7% polyacrylamide gel, and the radioactively labeled proteins were detected by fluorography. Key: Lane A, sensitive; lane B, HL60/Adr; and lane C, HL60/Vinc.

dimethoxyphenethyl)methylamino] - 2 - (3,4 - dimethoxyphenyl) - 2 - isopropylpentylamine ([$^{125}$I]NASVP), was prepared from (±)-5-[(3,4-dimethoxyphenethyl)methylamino] - 2 - 2(3,4 - dimethoxyphenyl)-2-isopropylpentylamine [25] and *N*-hydroxysuccinidyl-4-azido-[3-$^{125}$I]salicylate and purified by silica gel column chromatography by procedures similar to those previously described [12, 24]. A detailed characterization of [$^{125}$I]NASVP will be presented elsewhere*. [$^3$H]Daunomycin (4.0 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Verapamil was provided by Knoll Pharmaceutical (Wippany, NJ).

*Cells.* HL60 cells, a human acute myeloblastic leukemia cell line [26], were isolated for resistance to Adriamycin® (HL60/Adr) or vincristine (HL60/Vinc) as previously described [20–22]. The HL60/Adr isolate exhibits an 80- and 20-fold increase in resistance to Adriamycin® and vincristine respectively. The HL60/Vinc isolate exhibits a 140- and 15-fold increase in resistance to vincristine and Adriamycin® respectively. Revertants of the HL60/Vinc isolate were obtained by growing cells in the absence of drug for about 6 months. These cells exhibit a 20- and 8-fold increase in resistance to vincristine and Adriamycin® respectively. These cells contain about 4% of the P-glycoprotein of the parent isolate as determined by densitometric trace of an autoradiogram prepared after immunoblot analysis [27].

*Labeling membranes with* [$^3$H]*azidopine.* Membranes were prepared from HL60 sensitive and resistant cells [28] and suspended in 40 mM potassium phosphate buffer (pH 7.0). Labeling of cell membranes with [$^3$H]azidopine was carried out as described by Safa *et al.* [14]. Membranes containing 20 $\mu$g of protein [29] were incubated in a solution containing 40 mM potassium phosphate (pH 7.0), 10 $\mu$M $CaCl_2$, 4% dimethyl sulfoxide and 5 $\mu$Ci [$^3$H]azidopine in 50 $\mu$l for 30 min at 25°. At the end of the incubation period the solution was irradiated with a 15-W 302-nm UV lamp for 20 min at 25°. Labeled membrane proteins were electrophoresed in a sodium dodecyl sulfate (SDS) 7% polyacrylamide gel [30] and thereafter detected by fluorography using Enhance (New England Nuclear).

*Labeling membranes with* [$^{125}$I]*NASV or* [$^{125}$I]*NASVP.* Isolated membranes were labeled with the photoactive agents [$^{125}$I]NASV or [$^{125}$I]NASVP as previously described [12, 24]. After the labeling procedure, membrane proteins were separated by electrophoresis in an SDS–polyacrylamide gel [30].

*Labeling membranes with AzATP*$^{32}$. Membranes prepared from sensitive and resistant cells were suspended in 0.01 M Tris–HCl (pH 7.6)–0.125 M sucrose and stored at −70°. Labeling of membrane proteins with AzATP$^{32}$ was carried out essentially as described by Cornwell *et al.* [15]. Membranes (50 $\mu$g protein) [29] contained in 50 $\mu$l of 0.01 M Tris–HCl (pH 7.6)–1 mM $MgCl_2$ were placed in a microwell of a Falcon 3911 assay plate. The plate was placed on a bed of ice, and 5 $\mu$l of AzATP$^{32}$ (9.5 Ci/mmol) was added. After incubation for 1 min, the samples were irradiated at 366 nm for 8 min on ice at a distance of 8 cm. Thereafter, the membrane proteins were electrophoresed in an SDS–polyacrylamide gel [30], and the labeled proteins were detected by autoradiography.

*Effect of verapamil on cellular drug accumulation.* Sensitive or resistant cells ($3 \times 10^5$/ml) were incubated (37°) in 2 ml of complete RPMI medium in the absence or presence of various concentrations of verapamil. After a 10-min incubation period, [$^3$H]daunomycin was added to each dish and incubations were continued for an additional 30 min at 37°. At the end of the incubation period, the cells were poured over a glass fiber filter (GF/A) which had been maintained previously in 5% fetal calf serum. A zero time control was also obtained by mixing [$^3$H]daunomycin with cells and immediately pouring the mixture over the glass fiber filter. The filters were washed with 0.01 M phosphate buffer (pH 7.3)–0.15 M NaCl (PBS) and, after drying, radioactivity was determined.

* Felstad RL, Arnold ST and Safa AR, manuscript in preparation.

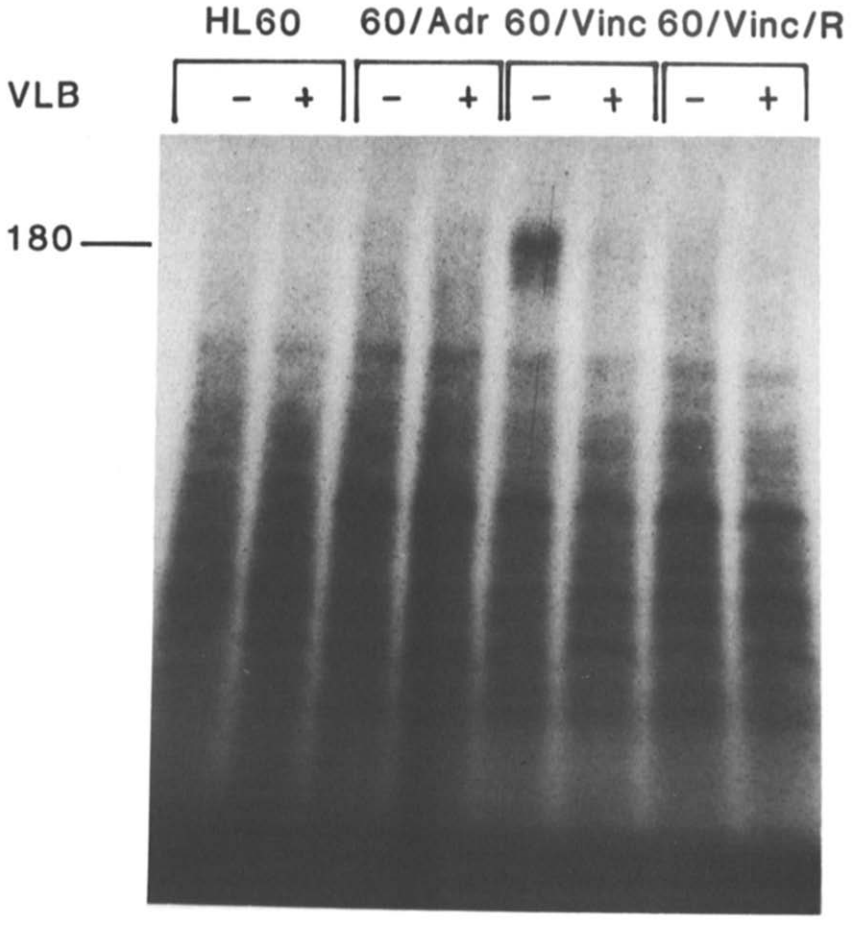


Fig. 4. [$^{125}$I]NASV photolabeling of membranes of sensitive and resistant cells. Photoaffinity labeling of isolated membranes (25 $\mu$g protein) was carried out in the absence (−) or presence (+) of 10 $\mu$M vinblastine. Labeled proteins were detected by autoradiography after SDS–polyacrylamide gel electrophoresis. 60/Vinc/R refers to the revertant of vincristine-resistant cells.

*DNA hybridization.* Probes for hybridization studies included a human 5′,1.1 kb *mdr*1 cDNA [31] and a 5′, 1.0 kb *mdr*3 cDNA [31, 32] provided by Dr P. Borst. Before use, the cDNA probes were excised from their vectors and purified by agarose gel electrophoresis. Radioactive labeling of the probes was carried out with the random primer method [33] using [$\alpha$-$^{32}$P]dCTP (3000 Ci/mmol). The DNA was labeled to a specific activity of $10^9$ cpm/$\mu$g. For hybridization studies, DNA (10 $\mu$g) purified [34] from sensitive and resistant cells was digested with either *Eco*RI or *Bam*HI, and the fragments were separated by electrophoresis in a 0.7% agarose gel. The separated fragments were denatured and transferred to Gene Screen which was washed two times in 2 × 0.01 M sodium citrate, 0.15 M sodium chloride (SSC) and thereafter baked for 2 hr at 80°. Hybridization with a radioactively labeled probe ($10^7$ cpm) was carried out for 16 hr at 65° in a solution containing 0.1% SDS–0.05 M phosphate buffer (pH 7.0)–1 M NaCl–10% dextran sulfate-salmon sperm DNA (300 $\mu$g/ml). After hybridization, the paper was washed twice for 5 min in 2 × SSC at 25°, twice for 30 min in 2 × SSC–1% SDS, and twice for 30 min in 0.1 SSC at 65°.

*RNA hybridization.* Cytoplasmic RNA (10 $\mu$g) [35] was treated for 5 min at 80° and electrophoresed in a 1.2% agarose gel containing 2.2 M formaldehyde. The RNA was transferred to Gene Screen, and hybridizations were carried out as described above for DNA hybridization. Equal loading of RNA samples was determined after ethidium bromide staining of the gels. Following hybridization and autoradiography, the RNA transferred to Gene Screen was stained in 0.5% methylene blue. All experiments were conducted under conditions of equal loading and transfer of the RNA samples.

## RESULTS

*Effect of verapamil on drug accumulation in resistant cells.* Previous studies have demonstrated that verapamil induces a major increase in cellular drug accumulation in a number of multidrug resistant cell lines which contain P-glycoprotein [36]. In view of these findings, we carried out studies to examine the effect of verapamil on drug uptake in the P-glycoprotein negative HL60/Adr isolate. In these studies, sensitive and resistant cells were incubated in the presence of various concentrations of verapamil,

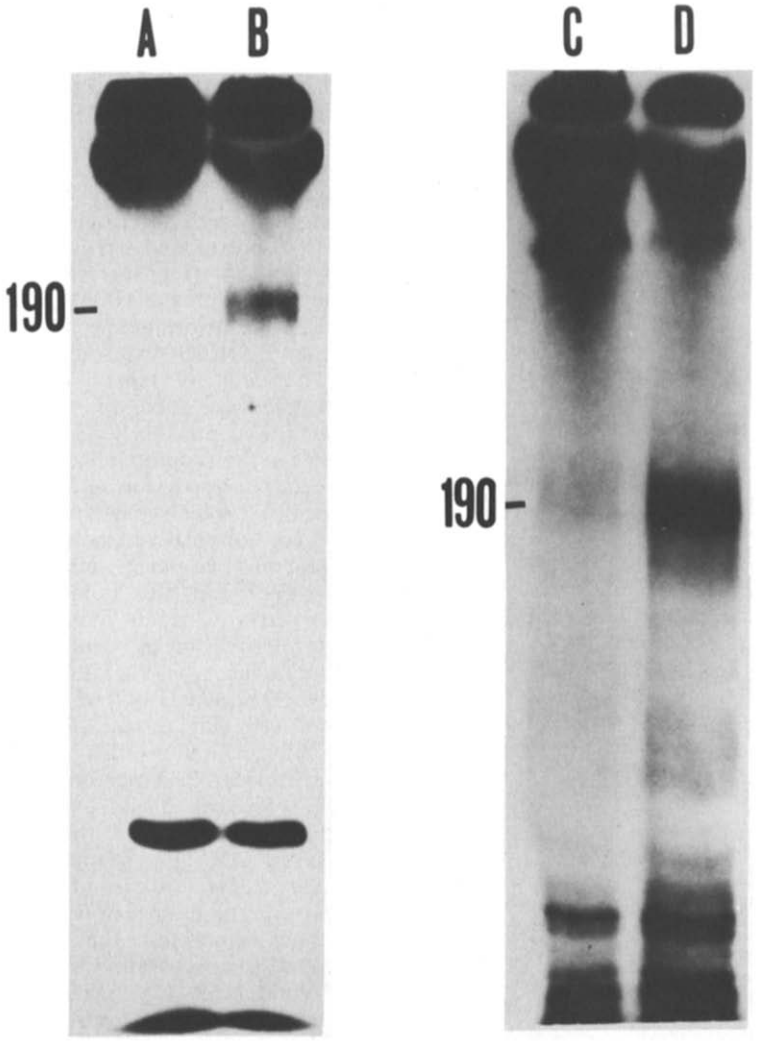


Fig. 5. AzATP$^{32}$ photolabeling of membranes of sensitive and resistant cells. Membranes were incubated under standard conditions with AzATP$^{32}$ for 1 min as described in Materials and Methods. Labeled proteins were analyzed after polyacrylamide gel electrophoresis in an SDS 7% (lanes A and B) or 5% (lanes C and D) polyacrylamide gel. Key: lanes A and C, HL60-sensitive; and lanes B and D, HL60/Adr.

and the pattern of cellular accumulation of [$^3$H]daunomycin was determined. Previous studies have shown that the HL60/Adr isolate exhibits similar levels of resistance to Adriamycin® and daunomycin [20]. Analysis of drug-sensitive cells showed that verapamil at relatively high concentrations enhanced drug uptake only slightly (Fig. 1). In contrast to sensitive cells, verapamil at relatively low concentrations induced a major increase in drug accumulation in both the HL60/Adr and HL60/Vinc isolates (Fig. 1). In the presence of verapamil, drug uptake levels in the resistant isolates were comparable to levels obtained with sensitive cells. Additional studies demonstrated that verapamil (5 μg/ml) potentiated the cytotoxic effects of Adriamycin® in HL60/Adr cells and brought about a 7-fold decrease in levels of drug resistance (not shown).

*Labeling membrane proteins with a photoactive analog of verapamil,* [$^{125}$I]*NASVP.* Membranes isolated from sensitive and resistant cells were treated with [$^{125}$I]NASVP as described in Materials and Methods. Labeled proteins were analyzed after polyacrylamide gel electrophoresis. Membranes from HL60/Vinc cells contained a 180 kilodalton (P180) protein which was highly reactive with [$^{125}$I]NASVP (Fig. 2). Labeling of this protein was greatly reduced when unlabeled verapamil was included in the reaction mixture (Fig. 2). P180 labeled in the HL60/Vinc membrane corresponded exactly to a protein found in this isolate which is reactive with a monoclonal antibody against P-glycoprotein [21, 22]. Despite the ability of verapamil to induce an increase in drug uptake in HL60/Adr cells, there was no detectable resistance specific membrane protein labeled by [$^{125}$I]NASVP in this isolate (Fig. 2).

*Azidopine labeling of membrane proteins.* Safa *et al.* [14] have shown that the photoactive calcium channel blocker [$^3$H]azidopine labels P-glycoprotein

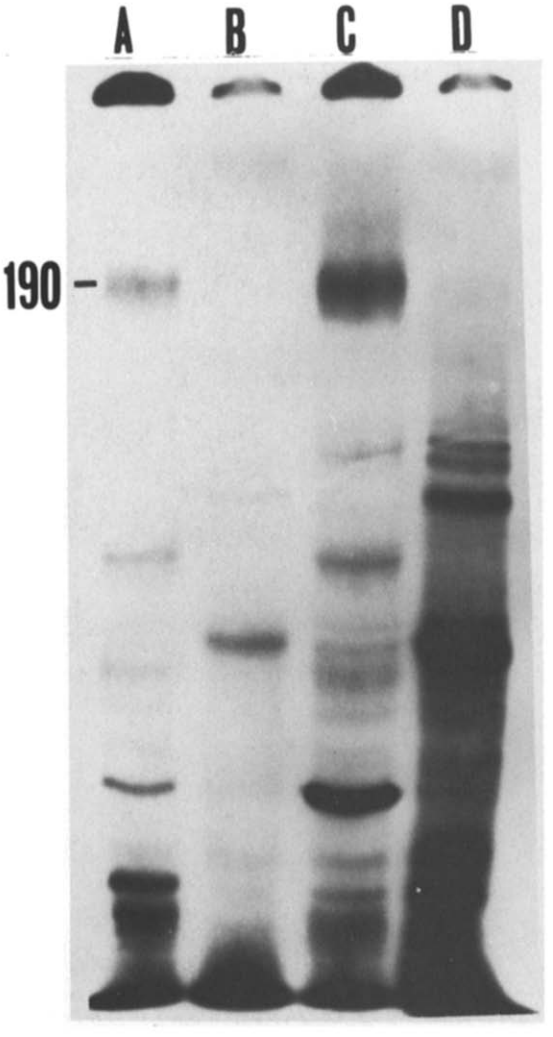


Fig. 6. Effect of ATP on AzidoATP$^{32}$ labeling of proteins in isolated membranes. Membranes from HL60/Adr cells were incubated in the absence (lanes A and B) or presence (lanes C and D) of 0.1% NP40 for 2 min at room temperature. ATP (0.5 mM) was added to membranes incubated in the absence or presence of detergent and incubations were continued for 5 min on ice. The membrane proteins were labeled with AzATP$^{32}$ as described in Materials and Methods. Electrophoresis of proteins was carried out in an SDS–7% polyacrylamide gel. Key: lanes A and C, labeling in the absence of ATP; and lanes B and D, labeling in the presence of ATP.

in the surface membrane of Chinese hamster lung cells isolated for resistance to actinomycin D. In agreement with these findings, [$^3$H]azidopine labeled two proteins of 180 and 210 kD contained in membranes of the HL60/Vinc isolate (Fig. 3). Both P180 and P210 have been shown to be reactive with monoclonal antibody against P-glycoprotein [21]. In contrast, [$^3$H]azidopine did not label any resistance associated membrane protein in the HL60/Adr isolate (Fig. 3).

[$^{125}$I]*NASV labeling of membrane proteins.* Previous studies have demonstrated that P-glycoprotein contained in isolated membranes from drug-resistant cells can be labeled with the photoactive analogue of vinblastine, [$^{125}$I]NASV [12, 13]. Similar experiments with membranes from HL60/Vinc cells show that [$^{125}$I]NASV labeled several proteins including a major component of 180 kD (Fig. 4). In the presence of excess non-radioactive vinblastine, the labeling of P180 was inhibited completely (Fig. 4). In addition, membranes from cells which had reverted to drug sensitivity with a concomitant loss of P-glycoprotein [27] also exhibited a reduced labeling of P180 with [$^{125}$I]NASV (Fig. 4). In contrast to the results obtained with the HL60/Vinc cells, there was no resistance associated protein labeled in membranes from the HL60/Adr isolate (Fig. 4).

*Labeling membrane proteins with AzATP$^{32}$.* AzATP$^{32}$ is a photoaffinity labeling agent which has been used previously to detect nucleotide binding sites in P-glycoprotein [15]. We have thus used this agent to search for resistance specific nucleotide binding proteins contained in membranes of the HL60/Adr isolate. In these experiments, membranes from sensitive and resistant cells were incubated with AzATP$^{32}$ and, after treatment with a UV light, the labeled proteins were identified after polyacrylamide gel electrophoresis. The results demonstrate that membranes from the HL60/Adr isolate contained a 190 kD protein (P190) which was highly labeled with AzATP$^{32}$ (Fig. 5, lanes B and D). Under identical labeling conditions, this protein was present in only very low levels in membranes of drug-sensitive cells (Fig. 5, lanes A and C). Additional studies were carried out to analyze the effect of unlabeled ATP on the AzATP$^{32}$ labeling of P190. Competition experiments were carried out with membranes incubated in the absence or presence of 0.1% NP40. The results demonstrate that labeling of P190 in either membrane preparation was inhibited completely by 0.5 mM ATP (Fig. 6, lanes B and D). GTP at a concentration of 0.5 mM also effectively inhibited AzATP$^{32}$ labeling of P190 (not shown). The results obtained thus far strongly suggest that, although P-glycoprotein has binding sites for ATP, drug and calcium channel blockers, P190 is distinct and contains active sites only for nucleotide binding.

*Analysis of* mdr *gene amplification.* DNA from sensitive and resistant cells was digested with either *Eco*RI or *Bam*HI, and the fragments were separated by agarose gel electrophoresis and hybridized with radioactively labeled cDNA probes as described in Materials and Methods. Studies with the *mdr*1 probe show that this sequence was highly amplified in the HL60/Vinc isolate (Fig. 7A, lanes 1 and 4). In contrast, the level of *mdr*1 in HL60/Adr cells (Fig. 7A, lanes 3 and 6) was essentially identical to that occurring in drug-sensitive cells (Fig. 7A, lanes 2 and 5). Additional studies show that *mdr*3 sequences were also highly amplified in the HL60/Vinc isolate (Fig. 7B, lanes 1 and 4), whereas the level of this sequence is sensitive (Fig. 7B, lanes 2 and 5) and HL60/Adr cells (Fig. 7B, lanes 3 and 6) was essentially the same.

*Analysis of* mdr *gene expression in resistant cells.* *mdr* mRNA synthesized in sensitive and resistant cells was analyzed in Northern blots using the *mdr*1 and *mdr*3 cDNA probes. The results show that *mdr*1 sequences were overexpressed in the HL60/Vinc

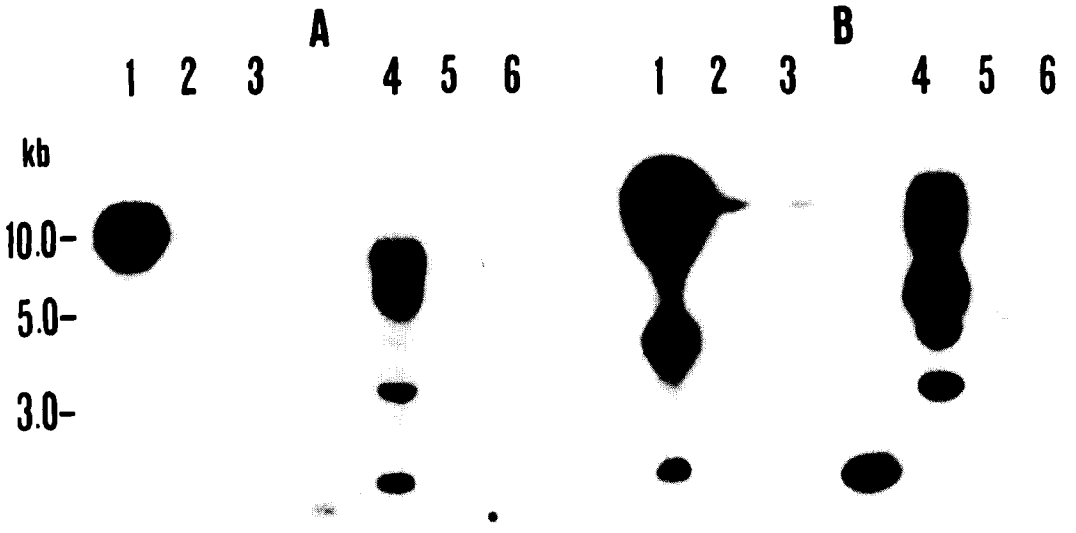


Fig. 7. DNA blot analysis of *mdr* sequences in sensitive and resistant cells. DNA was isolated from sensitive and resistant cells and digested with either *Bam*HI (lanes 1–3) or *Eco*RI (lanes 4–6). DNA fragments were separated by agarose gel electrophoresis, and DNA hybridization was carried out with radioactively labeled *mdr*1 (panel A) or *mdr*3 (panel B) as described in Materials and Methods. Key: lanes 1 and 4, HL60/Vinc DNA; lanes 2 and 5, HL60/sensitive; lanes 3 and 6 HL60/Adr DNA. Spot located below letters A and B represents a fragment of the DNA ladder which reacted with the probe.

isolate resulting in the synthesis of a major 4.3 kb and a minor 2.0 kb mRNA (Fig. 8A, lane 1). The 4.3 kb mRNA corresponded closely to mRNA species overexpressed in other multidrug resistant isolates [31, 37, 38]. A 2.2 kb mRNA has also been found to be expressed in some drug-resistant cell lines [7, 37]. The nature of this RNA is not known. Despite the amplification of *mdr*3 DNA in HL60/Vinc cells, there was no detectable overexpression of this sequence (Fig. 8B). Similar results using other drug-resistant isolates have been obtained by Van der Bliek *et al.* [38]. In contrast to the results obtained with HL60/Vinc cells, there was no detectable expression of *mdr*1 or *mdr*3 in either drug-sensitive cells (Fig. 8A and B, lane 2) or the HL60/Adr isolate (Fig. 8A and B, lane 3). Identical results were obtained in three other experiments using different preparations of isolated RNA.

## DISCUSSION

HL60 cells isolated for resistance to Adriamycin® (HL60/Adr) are multidrug resistant and defective in the cellular accumulation of drug [20, 27]. Reduced


Fig. 8. RNA blot analysis of *mdr* gene expression in sensitive and resistant cells. Cytoplasmic RNA was isolated from HL60/Vinc (lane 1), HL60/sensitive (lane 2) and HL60/Adr (lane 3). The mRNA was analyzed using the radioactively labeled cDNA probes (*mdr*1) (panel A) and *mdr*3 (panel B) as described in Materials and Methods.

drug levels appear to be related to an enhanced efflux system [20] which is energy dependent.* Despite this phenotype, HL60/Adr cells do not contain detectable levels of P-glycoprotein as determined with a monoclonal antibody against this protein [21, 22, 27]. Recently, we found that antiserum against synthetic peptides which correspond to various regions of the deduced sequence of P-glycoprotein [17] react with this protein in HL60/Vinc cells. In contrast, there was no reactivity of the antisera with any protein in sensitive or HL60/Adr cells.* In agreement with these findings, the present study also demonstrates that neither *mdr*1 nor *mdr*3 was overexpressed in the HL60/Adr isolate. Overexpression of *mdr*1, however, was readily detected in HL60/Vinc cells. These results taken together strongly suggest that genes coding for P-glycoprotein do not contribute to drug resistance in HL60/Adr cells. It is also interesting to note that other resistant cell lines have been isolated which are resistant to anthracyclines and *Vinca* alkaloids but do not contain detectable levels of P-glycoprotein [39, 40]. In the present study, we identified a 190 kD membrane ATP binding protein which was present in HL60/Adr cells but essentially absent in sensitive cells. This protein, thus, has certain similarities to P-glycoprotein which is also capable of binding ATP [15]. These two proteins, however, are distinct in other properties in that P-glycoprotein was labeled with the photoactive agents [$^{125}$I]NASV, [$^{125}$I]NASVP, and [$^{3}$H]azidopine, whereas there was no detectable labeling of P190 with these radioactive materials. At the present time the mechanistic implications of these results for drug resistance are not known. The results raise the possibility, however, that multiple mechanisms may contribute to resistance in isolates which exhibit reduced cellular accumulation of drug.

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