Edgar, D., Timpl, R., & Thoenen, H. (1984) *EMBO J. 3*, 1463–1468.
Engel, J. E., Odermatt, E., Engel, A., Madri, J., Furthmayr, H., Rhode, H., & Timpl, R. (1981) *J. Mol. Biol. 150*, 97–120.
Graf, J., Iwamoto, Y., Sasaki, M., Martin, G. R., Kleinman, H. K., Robey, F. A., & Yamada, Y. (1987) *Cell (Cambridge, Mass.) 48*, 989–996.
Humphries, M., Akiyama, S. K., Komoriya, A., Olden, K., & Yamada, K. (1986) *J. Cell Biol. 103*, 2637–2647.
Klebe, R. J. (1974) *Nature (London) 250*, 248–251.
Kleinman, H. K., Cannon, F. B., Laurie, G. W., Hassell, J. R., Aumailley, M., Terranova, V. P., Martin, G. R., & Dubois-Dalcq, M. (1985) *J. Cell. Biochem. 27*, 317–325.
Lesot, H., Kühl, U., & von der Mark, K. (1983) *EMBO J. 2*, 861–865.
Malinoff, H., & Wicha, M. S. (1983) *J. Cell Biol. 96*, 1475–1479.
Orkin, R. W., Gehron-Robey, P., McGoodwin, E. B., Martin, G. R., Valentine, T., & Swarm, R. (1977) *J. Exp. Med. 145*, 204.
Patthy, L., & Smith, E. L. (1975) *J. Biol. Chem. 250*, 557–564.
Pierschbacher, M. D., & Ruoslahti, E. (1984) *Nature (London) 309*, 30–33.
Rao, C. N., Margulies, I. M. K., Tralka, T. S., Terranova, V. P., Madri, J. A., & Liotta, L. (1982) *J. Biol. Chem. 257*, 9740–9744.
Rasheed, S., Nelson-Rees, W. A., Toth, E. H., Araskin, P., & Gardner, M. B. (1974) *Cancer (Philadelphia) 33*, 1027–1033.
Ruoslahti, E., & Pierschbacher, M. D. (1986) *Cell (Cambridge, Mass.) 40*, 517–518.
Sasaki, M., Kato, S., Kohno, K., Martin, G. R., & Yamada, Y. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 935–939.
Terranova, V. P., Aumailley, M., Sultan, L. H., Martin, G. R., & Kleinman, H. K. (1986) *J. Cell. Physiol. 127*, 473–479.
Timpl, R., & Dziadek, M. (1986) *Int. Rev. Exp. Pathol. 29*, 1–113.
Timpl, R., Rohde, H., Gehron Robey, P., Rennard, S. I., Foidart, J. M., & Martin, G. R. (1979) *J. Biol. Chem. 254*, 9933–9937.
Towbin, H., Staehilin, T., & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4350–4354.
Wewer, U. M., Liotta, L. A., Jaye, M., Ricca, G. A., Drohan, W. N., Claysmith, A. P., Rao, C. N., Wirth, P., Coligan, J. E., Albrechtsen, R., Mudryj, M., & Sobel, M. E. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 7137–7141.
Yamada, K. M., & Kennedy, D. W. (1985) *J. Cell. Biochem. 28*, 99–104.

Phosphorylation of the Multidrug Resistance Associated Glycoprotein†

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ABSTRACT: Drug-resistant cell lines derived from the mouse macrophage-like cell line J774.2 express the multidrug resistant phenotype which includes the overexpression of a membrane glycoprotein (130–140 kilodaltons). Phosphorylation of this resistant-specific glycoprotein (P-glycoprotein) in intact cells and in cell-free membrane fractions has been studied. The phosphorylated glycoprotein can be immunoprecipitated by a rabbit polyclonal antibody specific for the glycoprotein. Phosphorylation studies done with partially purified membrane fractions derived from colchicine-resistant cells indicated that (a) phosphorylation of the glycoprotein in 1 mM $MgCl_2$ was enhanced a minimum of 2-fold by 10 μM cAMP and (b) the purified catalytic subunit of the cAMP-dependent protein kinase (protein kinase A) phosphorylated partially purified glycoprotein that was not phosphorylated by [γ-^{32}P]ATP alone, suggesting that autophosphorylation was not involved. These results indicate that the glycoprotein is a phosphoprotein and that at least one of the kinases responsible for its phosphorylation is a membrane-associated protein kinase A. The state of phosphorylation of the glycoprotein, which is a major component of the multidrug resistance phenotype, may be related to the role of the glycoprotein in maintaining drug resistance.

Selection of cultured mammalian tumor cells for resistance to the cytotoxic effects of natural products often results in the expression of a complex set of characteristics referred to as the multidrug resistant (mdr) phenotype. The characteristics of this phenotype include (a) cross resistance to functionally and structurally unrelated drugs, (b) a net decrease in drug accumulation, (c) overexpression of a multidrug resistance associated plasma membrane glycoprotein (MDRG;[1] also known as P-glycoprotein), and (d) amplification and expression of the gene(s) coding for the MDRG. The importance of the MDRG in maintaining the mdr phenotype is exemplified by resistant cells that revert to drug sensitivity in the absence of drug and no longer overexpress the MDRG (Roy & Horwitz, 1985). Recent studies suggest that the MDRG may function

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[1] Abbreviations: MDRG, multidrug resistance associated glycoprotein; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; WGA, wheat germ agglutinin; Hepes, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; EDTA, ethylenediaminetetraacetic acid; Tris, tris(hydroxymethyl)aminomethane.

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as an energy-dependent efflux pump that is responsible for maintaining the low level of drug associated with mdr cells [see Gerlach et al. (1986a) and Pastan and Gottesman (1987)]. Drug-sensitive cells express the mdr phenotype after being transfected with the cDNA coding for the MDRG (Gros et al., 1986a), again indicating the significance of the MDRG. In addition, photoaffinity vinblastine analogues bind to the MDRG (Safa et al., 1986; Cornwell et al., 1986). The gene coding for the MDRG has been fully (Gros et al., 1986b; Chen et al., 1986) or partially (Gerlach et al., 1986b) sequenced, and this information has made it possible to suggest that the MDRG has two nucleotide binding sites and 12 membrane-spanning domains. As further evidence that the MDRG may be acting as a drug-efflux pump, there is a striking sequence homology between the MDRG and bacterial transport proteins, particularly hemolysin B.

Although it is clear that the MDRG plays an important role in maintaining the mdr phenotype, little is understood concerning the induction of its overexpression or the regulation of its function. We have been investigating the expression of the MDRG in cell lines derived from the mouse macrophage-like cell J774.2 that have been selected for resistance to colchicine, vinblastine, or taxol (Roy & Horwitz, 1985; Lothstein & Horwitz, 1986). In this paper we have focused on the phosphorylation of the MDRG in both intact cells and membrane preparations. Previous phosphorylation studies with intact cells (Center, 1983, 1985; Roy & Horwitz, 1985) and with membrane preparations (Carlsen et al., 1977; Marsh & Center, 1985a) indicated that the MDRG was a phosphoprotein and suggested that this modification may influence drug accumulation. Recent research by Hamada et al. (1987) revealed that verapamil and trifluoperazine, drugs that can partially reverse drug resistance, and phorbol diester enhanced phosphorylation of the MDRG. In our studies, membrane fractions from colchicine-resistant cells, partially purified on discontinuous sucrose gradients, contain kinase activity that can phosphorylate the MDRG on addition of ATP. This phosphorylation is enhanced by cAMP, suggesting that at least part of the phosphorylation is the result of protein kinase A activity. Purification of the MDRG through an agarose-bound wheat germ agglutinin (WGA) column results in a protein that requires, in addition to ATP, the catalytic subunit of protein kinase A for phosphorylation, indicating that the MDRG is not capable of autophosphorylation. An understanding of the mechanism(s) involved in the phosphorylation of the MDRG is important for investigating the role of phosphorylation in the function of the MDRG.

Experimental Procedures

Cell Culture. Drug-resistant cell lines derived from the macrophage-like cell line J774.2 were isolated and maintained as described (Roy & Horwitz, 1985). Cell lines maintained in either 100 μM colchicine (J7.C1-100), 1 μM vinblastine (J7.V1-1), or 50 μM taxol (J7.T1-50) were 2500-fold, 1000-fold, and 830-fold resistant to the selecting drugs, respectively.

Preparation of Membrane Fractions. Membrane fractions were prepared by the method of Roy and Horwitz (1985). The final membrane fraction was resuspended in 20 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Hepes) buffer, pH 7.4, containing protease inhibitors (leupeptin, benzamidine, phenylmethanesulfonyl fluoride at 10 μg/mL each). Protein was estimated by the method of Lowry et al. (1951).

[^{32}P]P_i *Labeling of Intact Cells.* Cells growing in 100-mm tissue culture dishes at approximately 70% confluency were incubated for 2 h in 5 mL of phosphate-free Dulbecco's modified Eagle's medium (Gibco) containing 100 μCi/mL [^{32}P]P_i (carrier free, Amersham). The cells were washed 3 times with cold phosphate-buffered saline, and membrane fractions were prepared.

[^{32}P]P_i *Labeling of Membrane Fractions.* Membrane protein (30 μg) was incubated with 20 mM Hepes buffer, pH 7.4, 5 mM $MgCl_2$, 0.5 mM ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), and 20 μM [γ-^{32}P]ATP (2.5 Ci/mmol, Amersham) at 22 °C for 5 min. The reaction was stopped by the addition of an equal volume of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer. Phosphorylated membrane proteins were analyzed by SDS–PAGE. In other reactions, 4 μg of partially purified MDRG eluted from a WGA column was incubated at 22 °C for 5 min with 0.04–1 μg of the bovine catalytic subunit of protein kinase A (a kind gift from Dr. C. Rubin, Albert Einstein College of Medicine) in 20 mM Hepes buffer, pH 7.4, 5 mM $MgCl_2$, 0.5 mM EGTA, and 20 μM [γ-^{32}P]ATP (2.5 Ci/mmol). The reaction was stopped and analyzed as indicated above.

Lectin Chromatography. Membrane protein (500 μg) was extracted twice for 15 min at 4 °C with 0.1% Lubrol PX (Pierce) in buffer containing 20 mM Hepes, pH 7.4, 5 mM $MgCl_2$, and 10 mM ethylenediaminetetraacetic acid (EDTA). After centrifugation at 13500*g* for 10 min at 4 °C, the supernatant was collected and loaded on a 0.7-mL WGA (Vector Laboratories) column that had been equilibrated and washed at 4 °C with buffer A (20 mM Hepes, pH 7.4, 0.5 M NaCl, and 0.1% Lubrol PX). Bound material was eluted with buffer B (20 mM Hepes, pH 7.4, 0.5 M NaCl, 0.1% Lubrol PX, and 0.5 M *N*-acetylglucosamine) at 4 °C. An aliquot of each fraction was mixed with SDS–PAGE sample buffer, and the proteins were analyzed by SDS–PAGE.

Immunoprecipitation of the MDRG. Immunoprecipitation was performed essentially as done by Greenberger et al. (1987). The precipitated protein A–Sepharose immune complex was washed extensively with 0.1% Triton X-100, 0.03% SDS, 0.5 M NaCl, and 5 mg/mL bovine serum albumin in 50 mM Tris-HCl, pH 7.4. Rabbit polyclonal antiserum used in all experiments was prepared as described and was specific for the MDRGs (Zeheb et al., 1987).

SDS–PAGE and Immunoblotting. Electrophoresis was performed as described by Laemmli (1970) using a 7% running gel except that samples were not heated prior to SDS–PAGE. Gels were stained with silver by the method of Wray et al. (1981) and exposed to Kodak X-Omat film at −70 °C. Immunoblotting and identification of the MDRG were done as described (Zeheb et al., 1987). Molecular mass markers (Bio-Rad) were myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase *b* (93 kDa), bovine serum albumin (69 kDa), and ovalbumin (45 kDa).

Results

Phosphorylation of Drug-Sensitive and -Resistant Intact Cells. Each of the resistant cell lines overexpressed a MDRG (130–140 kDa) that was barely detectable in the drug-sensitive parental cell line (Figure 1A). The MDRG can be metabolically labeled with [^{32}P]P_i (Figure 1B). Membrane fractions from J7.T1-50 overexpressed two MDRGs (Figure 1, lane 3), both of which were phosphorylated (Figure 1B,C). Antiserum specific for the MDRGs (Zeheb et al., 1987) immunoprecipitated the phosphorylated MDRGs from the different resistant cell lines but did not immunoprecipitate a similar protein from J774.2, the parental drug-sensitive cell line (Figure 1C). An immunoblot probed with the antiserum showed no reaction with J774.2 but clearly demonstrated two

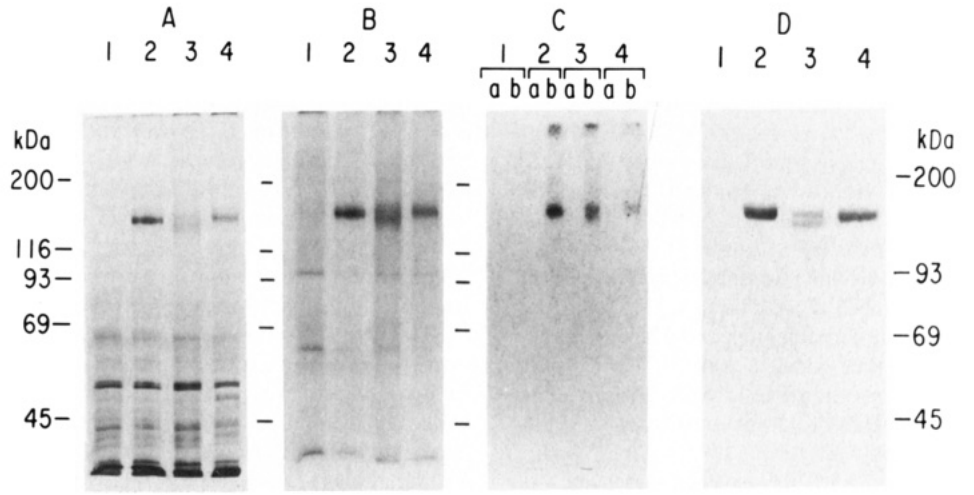

FIGURE 1: Identification of phosphorylated MDRGs in drug-resistant cells. Polypeptides from plasma membrane enriched subcellular fractions were resolved by SDS–PAGE. In (A), (B), and (C), cells were labeled for 2 h with [^{32}P]P_i. Lanes contain polypeptides from parental, drug-sensitive cells (1) or from cells selected for resistance to colchicine (2), taxol (3), or vinblastine (4). (A) Ten micrograms of protein was loaded in each lane, and the protein was visualized by silver staining. (B) Autoradiogram of the gel seen in (A) after exposure for 3 days. (C) Immunoprecipitates from 20 μg of membrane proteins were prepared with (a) preimmune serum or (b) immune serum. All precipitated material was loaded in the gel. An autoradiogram of the gel is shown. Exposure was for 15 days with an intensifying screen. (D) Fifty micrograms of material was loaded in each lane, and the material was resolved by SDS–PAGE, transferred to nitrocellulose, and probed with immune serum.

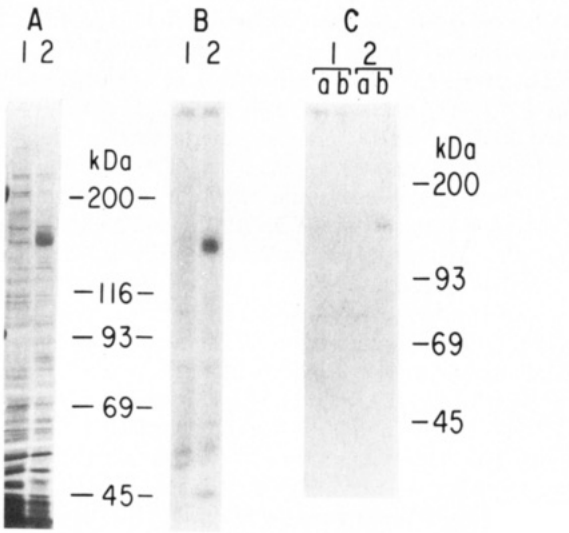

FIGURE 2: Identification of a phosphorylated MDRG prepared from colchicine-resistant cells in a membrane-enriched fraction labeled with [γ-^{32}P]ATP in vitro. Membrane fractions were phosphorylated as described under Experimental Procedures, and the polypeptides were resolved by SDS–PAGE. Lanes contain polypeptides from sensitive cells (1) or colchicine-resistant cells (2). (A) Twelve micrograms of protein was loaded in each lane, and the protein was visualized by silver staining. (B) Autoradiogram of gel seen in (A). Exposure was for 15 h with an intensifying screen. (C) Immunoprecipitates were prepared from 15 μg of ^{32}P-labeled membrane fractions with (a) preimmune or (b) immune serum. All precipitated material was loaded in the gel. Exposure was for 3 days with an intensifying screen.

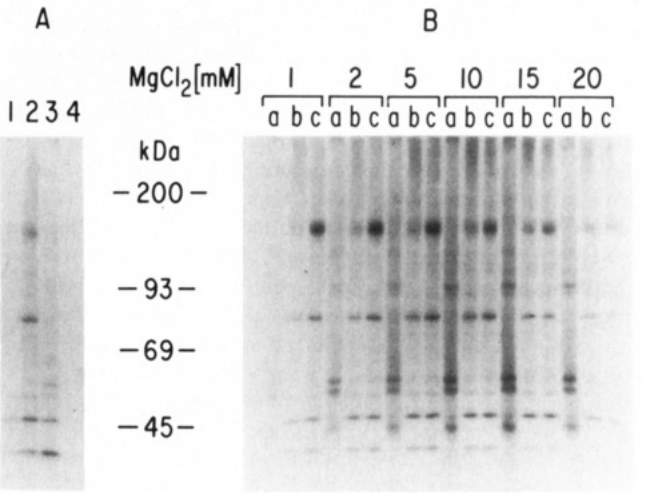

FIGURE 3: Effect of $MgCl_2$ and cAMP on phosphorylation of a membrane fraction prepared from colchicine-resistant cells. Polypeptides from plasma membrane enriched subcellular fractions were resolved by SDS–PAGE after labeling with [γ-^{32}P]ATP in vitro. (A) Twelve micrograms of protein was labeled in the absence of exogenous divalent cation (1) or in the presence of 5 mM $MgCl_2$ (2), $MnCl_2$ (3), or $CaCl_2$ (4). (B) Twelve micrograms of protein from drug-sensitive cells (a) or colchicine-resistant cells (b and c) was labeled in the presence of the indicated concentrations of $MgCl_2$ in either the absence (b) or presence (c) of 10 μM cAMP. Exposures were for 17 h. Autoradiograms of the gels are shown.

MDRGs in J7.T1-50 (Figure 1D).

Phosphorylation of Membrane Preparations from Resistant Cells. To study the phosphorylation of the MDRG, membrane fractions were isolated from the colchicine-resistant cell line J7.C1-100. The MDRG was visualized by silver staining and fluorography (Figure 2A,B). The major phosphorylated protein that was unique to the resistant cells was immunoprecipitated by antiserum specific for the MDRG (Figure 2C). To further characterize the in vitro phosphorylation reaction, membrane fractions were phosphorylated in the presence of divalent cations and cAMP. The results indicated that phosphorylation of the MDRG required Mg^{2+} (Figure 3A) and was enhanced a minimum of 2-fold by 10 μM cAMP when the reaction contained 1 or 2 mM $MgCl_2$. This enhancement diminished as the Mg^{2+} concentration was increased, and no effect of cAMP was seen at 20 mM $MgCl_2$ (Figure 3B, lanes b and c). Phosphorylation of the MDRGs present in membrane fractions prepared from vinblastine- and taxol-resistant cells was also enhanced by 10 μM cAMP in the presence of 1 mM $MgCl_2$. When membrane fractions from J7.T1-50 were phosphorylated in either the absence or presence of cAMP, only the upper MDRG band was labeled (Figure 4, lanes 3a and 3b). However, both MDRG bands were labeled when intact cells were incubated with [^{32}P]P_i (Figure 4, lane 5).

A ^{32}P-labeled membrane fraction was used to develop a partial purification of the MDRG, which was necessary in order to determine if the MDRG was phosphorylated by an exogenous kinase or autophosphorylated. Membrane fractions

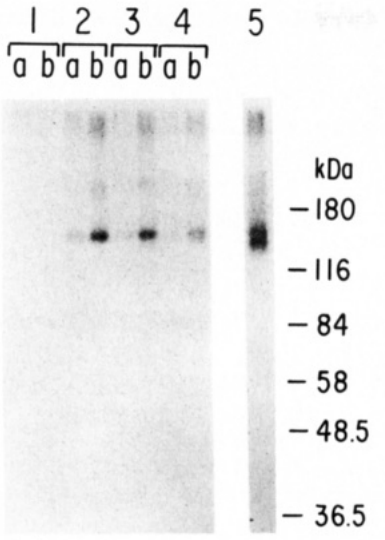

FIGURE 4: Effect of cAMP on phosphorylation of membrane fractions prepared from drug-resistant cell lines. Membrane fractions, 30 (1 and 2), 60 (3) and 17 μg (4), were phosphorylated with [γ-^{32}P]ATP in vitro in the absence (a) or presence (b) of 10 μM cAMP and immunoprecipitated. Lanes contain all immunoprecipitated material from parental, drug-sensitive cells (1) or from cells selected for resistance to either colchicine (2), taxol (3 and 5), or vinblastine (4). In (5) 27 μg of a membrane fraction prepared from taxol-resistant cells that had been labeled with [^{32}P]P_i for 2 h (see Figure 1) was immunoprecipitated. An autoradiogram of the gel is shown. Exposure was for 7 days with an intensifying screen.

prepared from J7.C1-100 were phosphorylated and extracted with 0.1% Lubrol PX. The MDRG could also be phosphorylated after extraction in Lubrol (not shown). The MDRG bound efficiently to a WGA column and was eluted with 0.5 M *N*-acetylglucosamine, resulting in a fraction enriched in the MDRG. The MDRG was detected in fractions 8 and 9 by silver staining (Figure 5A), by fluorography (Figure 5B), and by a specific antiserum (Figure 5C).

Phosphorylation of the MDRG by the Catalytic Subunit of Protein Kinase A. Results demonstrating an enhancement of phosphorylation by cAMP (Figures 3 and 4) support the idea that protein kinase A may be involved in the phosphorylation of the MDRG. Unlabeled, partially purified MDRG from a WGA column (a fraction similar to the one shown in lane 9 in Figure 5A) was phosphorylated with a purified bovine brain catalytic subunit from protein kinase A. The results demonstrated that the MDRG was phosphorylated by [γ-^{32}P]ATP in the presence of the catalytic subunit (Figure 6, lane 2) but not in its absence (Figure 6, lane 1).

Discussion

A significant part of the mdr phenotype relates to the diminished accumulation of drug in resistant cells and to the presence of the MDRG. There is growing evidence that the MDRG acts as an efflux pump to maintain a reduced level of drug within the resistant cell and that it plays a pivotal role in maintaining the mdr phenotype [see Pastan and Gottesman (1987) and Gerlach et al. (1986a)]. However, there is little data on the regulation of the function of the MDRG.

It is well-known that different types of regulation may result from the covalent modification of proteins, specifically membrane receptors, by phosphorylation [see Sibley et al. (1987)]. Studies in our laboratory with mdr cell lines derived from the murine cell line J774.2 indicated that the MDRG present in the membranes of the resistant cells was phosphorylated after incubation of intact cells with [^{32}P]P_i. Phosphoamino acid analysis of the MDRG indicated that both threonine and serine, but not tyrosine, were phosphorylated (Roy & Horwitz, 1985). In this paper, phosphorylation of partially purified membrane fractions derived from colchicine-resistant cells has been examined. Phosphorylation, in the absence of an exogenous kinase, was enhanced at least 2-fold by the addition of cAMP in the presence of 1 or 2 mM Mg^{2+}. As the concentration of Mg^{2+} was increased to 10 mM Mg^{2+}, the effect of cAMP diminished. This observation may suggest that a cAMP-independent kinase phosphorylates the MDRG at higher Mg^{2+} concentrations. It is interesting to note that, in taxol-resistant cells, both MDRGs are phosphorylated in intact cells but only the upper band is phosphorylated in cell-free membrane preparations. This may be the result of an altered conformation of the lower band in cell-free preparations that results in its being a poor substrate for phosphorylation. Alternatively, the upper and lower MDRG bands may be phosphorylated by two distinct enzymes in intact cells, and only one of the enzymes retains its activity in membrane preparations or the lower band may be more susceptible to phosphatase activity. Recent studies from our laboratory (Greenberger et al., 1987) have determined that the two MDRGs observed in the taxol-resistant cells are derived from two individual precursors and therefore their sites of phosphorylation may be distinct.

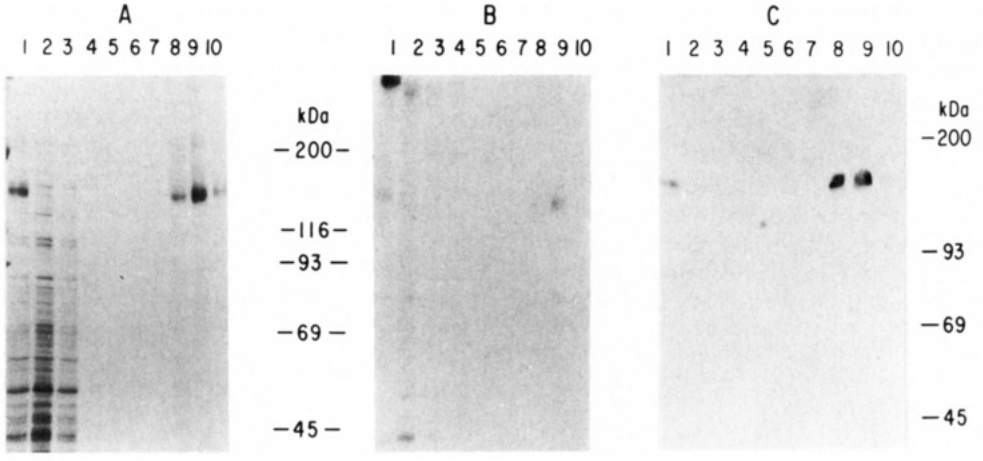

FIGURE 5: Enrichment for the phosphorylated MDRG by lectin chromatography. A plasma membrane enriched subcellular fraction from colchicine-resistant cells was phosphorylated with [γ-^{32}P]ATP and extracted with 0.1% Lubrol PX as described under Experimental Procedures. The extracted material (lane 1) was loaded on a WGA column, washed (lanes 2–7), and eluted with 0.5 M *N*-acetylglucosamine (lanes 8–10). Each fraction was resolved by SDS–PAGE. (A) Column profile observed by silver staining. (B) Autoradiogram of gel seen in (A). Exposure was for 6 days with an intensifying screen. (C) A similar gel as seen in (A) transferred to nitrocellulose and probed with immune serum.

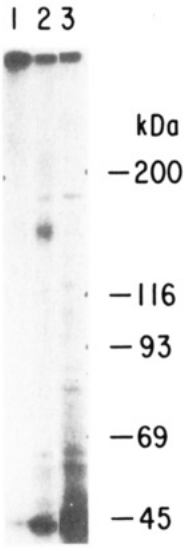

FIGURE 6: Phosphorylation of the MDRG from colchicine-resistant cells after enrichment by lectin chromatography. Four micrograms of protein eluted from the WGA column (fraction 9, Figure 5) was incubated with either [γ-^{32}P]ATP (1) or [γ-^{32}P]ATP plus 1.0 μg of the catalytic subunit of the cAMP-dependent protein kinase (2) as described under Experimental Procedures. In (3), [γ-^{32}P]ATP was incubated with the catalytic subunit in the absence of MDRG. Each incubation was resolved by SDS–PAGE. An autoradiogram of gel is shown. Exposure was for 10 h with an intensifying screen.

After partial purification of the MDRG on a WGA column, phosphorylation of the MDRG requires the addition of an exogenous kinase, thereby indicating that the MDRG is incapable of autophosphorylation under the conditions of our experiments. The MDRG is a substrate for the purified catalytic subunit of protein kinase A. A membrane-associated cAMP-dependent protein kinase has been described in human erythrocytes (Rubin, 1979).

Studies done a number of years ago with colchicine-resistant Chinese hamster ovary cells (Carlsen et al., 1977) showed no phosphorylation in intact cells, although phosphorylation occurred in cell-free preparations. No effect of cAMP could be demonstrated at that time. Phosphorylation of intact cells has also been reported in adriamycin-resistant hamster cells by Center (1985) and Marsh and Center (1985b) and in human cells (K562/ADM) by Hamada et al. (1987). In the latter case phosphorylation was enhanced by verapamil, trifluoperazine, and phorbol diester. An increased phosphorylation after incubation of the cells with phorbol ester suggested that protein kinase C may be involved in phosphorylation. Marsh and Center (1985a) have also demonstrated that the MDRG can be phosphorylated in cell-free membrane preparations.

This paper is the first to implicate an effect of cAMP on the phosphorylation of the MDRG and to demonstrate that the MDRG can be a substrate for protein kinase A. Although the effects of phosphorylation on the function of the MDRG are not understood, it is known that phosphorylation of receptors can regulate their function and distribution [see Sibley et al. (1987)]. It will be of interest to determine whether modulation of phosphorylation by cAMP will alter the function of the MDRG.

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Registry No. cAMP, 60-92-4; protein kinase, 9026-43-1; colchicine, 64-86-8; vinblastine, 865-21-4; taxol, 33069-62-4.

REFERENCES

Carlsen, S. A., Till, J. E., & Ling, V. (1977) *Biochim. Biophys. Acta 467*, 238–250.

Center, M. S. (1983) *Biochem. Biophys. Res. Commun. 115*, 159–166.

Center, M. S. (1985) *Biochem. Pharmacol. 34*, 1471–1476.

Chen, C-J., Chin, J. E., Ueda, K., Clark, D. P., Pastan, I., Gottesman, M. M., & Roninson, I. B. (1986) *Cell (Cambridge, Mass.) 47*, 381–389.

Cornwell, M. M., Safa, A. R., Felsted, R. L., Gottesman, M. M., & Pastan, I. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 3847–3850.

Gerlach, J. H., Kartner, N., Bell, D. R., & Ling, V. (1986a) *Cancer Surveys 5*, 25–46.

Gerlach, J. H., Endicott, J. A., Juranka, P. F., Henderson, G., Sarangi, F., Deuchars, K. L., & Ling, V. (1986b) *Nature (London) 324*, 485–489.

Greenberger, L., Williams, S. S., & Horwitz, S. B. (1987) *J. Biol. Chem. 262*, 13685–13689.

Gros, P., Ben Neriah, Y., Croop, J. M., & Housman, D. E. (1986a) *Nature (London) 323*, 728–731.

Gros, P., Croop, J., & Housman, D. (1986b) *Cell (Cambridge, Mass.) 47*, 371–380.

Hamada, H., Hagiwara, K.-I., Nakajima, T., & Tsuruo, T. (1987) *Cancer Res. 47*, 2860–2865.

Laemmli, U. K. (1970) *Nature (London) 227*, 680–685.

Lothstein, L., & Horwitz, S. B. (1986) *J. Cell. Physiol. 127*, 253–260.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951) *J. Biol. Chem. 193*, 265–275.

Marsh, W., & Center, M. S. (1985a) *Biochem. Pharmacol. 34*, 4180–4184.

Marsh, W., & Center, M. S. (1985b) *Cancer Res. 45*, 6088–6092.

Pastan, I., & Gottesman, M. (1987) *New Engl. J. Med. 316*, 1388–1393.

Roy, S. N., & Horwitz, S. B. (1985) *Cancer Res. 45*, 3856–3863.

Rubin, C. S. (1979) *J. Biol. Chem. 254*, 12439–12449.

Safa, A. R., Glover, C. J., Meyers, M. B., Biedler, J. L., & Felsted, R. L. (1986) *J. Biol. Chem. 261*, 6137–6140.

Sibley, D. R., Benovic, J. L., Caron, M. G., & Lefkowitz, R. J. (1987) *Cell (Cambridge, Mass.) 48*, 913–922.

Wray, W., Boulikas, T., Wray, V. P., & Hancock, R. (1981) *Anal. Biochem. 118*, 197–203.

Zeheb, R., Beittenmiller, H. F., & Horwitz, S. B. (1987) *Biochem. Biophys. Res. Commun. 143*, 732–739.