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DRUG-RESISTANT MUTANTS OF CHINESE HAMSTER OVARY CELLS POSSESS AN ALTERED CELL SURFACE CARBOHYDRATE COMPONENT

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Surface label experiments using the galactose oxidase-[^{3}H]-borohydride technique reveal that cells from drug-resistant Chinese hamster ovary clones possess a surface carbohydrate component of apparent molecular weight 165,000 which is absent from wild-type cells. The component may also be demonstrated by [^{14}C]glucosamine incorporation but not by [^{3}H]leucine incorporation or by the lactoperoxidase surface labeling reaction.

INTRODUCTION

Chinese hamster ovary (CHO) cells were selected for resistance to the drug colchicine (1). Clones resistant to the cytotoxic effects of colchicine were also cross-resistant to several other amphipathic drugs (1), such as puromycin and actinomycin D. The drug-resistant phenotype was well correlated with a reduced rate of drug permeation via a passive diffusion mechanism (1, 2, and S. Carlson, personal communication); however, other membrane transport systems including those for sugars and nucleosides were unaltered in the drug-resistant clones (3). Drug permeation in both parental lines and resistant mutants could be modulated by altering cellular ATP levels with metabolic inhibitors or with glucose (2), indicating active metabolic control of the drug-permeation mechanism.

The molecular basis of the drug-permeation system, as well as the alteration involved in the expression of the drug resistant phenotype, remains unknown. In this communication we wish to report on a specific alteration of the cell surface membrane which seems to be correlated with the phenomenon of drug resistance due to reduced drug permeability.

METHODS

Cells

Growth conditions and selection procedures for colchicine-resistant CHO cells have been described previously (1). The parental or wild-type line (WT) employed in these experiments was an auxotroph, AUXB1, described previously (1). Independent clones CH^RC4 and CH^RC5 were selected for a high degree of drug resistance. A revertant clone,

I8-31, was a drug-sensitive line selected from CH^RC4 by [^{3}H]leucine suicide in the presence of puromycin (4). As seen in Fig. 1, CH^RC4 (and also CH^RC5) cells were approximately 200-fold more resistant to the cytotoxic effects of colchicine than were WT cells (AUXB1), while I8–31 cells were about 5–8-fold more resistant thatn WT. For use in experiments, cells were maintained in logarithmic growth (doubling time = 18–22 hr) in suspension culture and were harvested when a density of about 5×10^5 cells/ml was achieved. In experiments involving metabolic labeling of cell membrane protein and carbohydrate moieties, either [^{14}C]glucosamine (0.3 μCi/ml) or [^{3}H]leucine (1–2 μCi/ml) was added to the cultures 36 hr prior to harvesting the cells.

Biochemical Techniques

Surface galactose and galactosamine residues of intact cells were labeled via the galactose oxidase-[^{3}H]-borohydride technique (5). Available cell surface tyrosine and histidine residues were labeled with ^{125}I utilizing the lactoperoxidase technique. Plasma membranes were isolated from surface-labeled cells, or from cells labeled by metabolic incorporation of radioactive precursors, utilizing the aqueous polymer separation system described by Brunette and Till (6). Details of the surface labeling and membrane isolation procedures have been described previously (7–9).

Cell membrane polypeptides and glycopeptides were resolved by electrophoresis in SDS containing polyacrylamide discs or slabs (10). The distributions of radioactivity in the gels were analyzed by slicing and counting (7) in the case of disc gels, or by autoradiography following impregnation of the gel with PPO (11) in the case of slabs. Autoradiograms were scanned in a Beckman Model Acta CII densitometer.

RESULTS

Drug-resistant cells (CH^RC4), WT cells (AUXB1), and revertant cells (I8–31) were

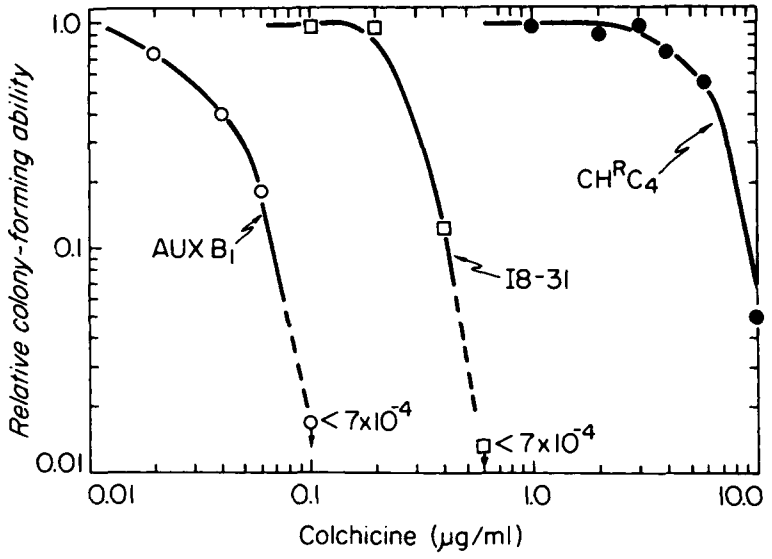

Fig. 1. Relative colony-forming ability of WT (AUXB1), drug-resistant mutant (CH^RC4), and revertant (I8–31) clones as a function of colchicine concentration.

surface labeled under identical conditions using the galactose oxidase-[^{3}H]borohydride technique. Plasma membranes were isolated and analyzed by SDS gel electrophoresis and autoradiography. As seen in Fig. 2, the mutant cells (CH^RC4) possessed a distinct radiolabeled peak of apparent molecular weight 165,000 (Star) which was absent from WT cells and was markedly reduced in the revertant cells. The 165,000 MW peak was even more pronounced in CH^RC5 cells (data not shown). These results indicated the presence of an altered cell surface carbohydrate component in the drug-resistant mutant clones.

Metabolic incorporation of [^{14}C]glucosamine, followed by membrane isolation and SDS gel electrophoresis, revealed that the drug-resistant clone CH^RC4 possessed an augmented radiolabeled component of MW 165,000 (Fig. 3); other differences between the labeling patterns of WT and resistant cells were also observed. Double label experiments (data not shown) proved that the 165,000 MW peak visualized by [^{14}C]glucosamine incorporation coincided with the peak visualized by surface labeling with the galactose oxidase-[^{3}H]borohydride technique. Experiments employing metabolic incorporation of [^{3}H]leucine followed by membrane isolation and SDS gel analysis failed to reveal any

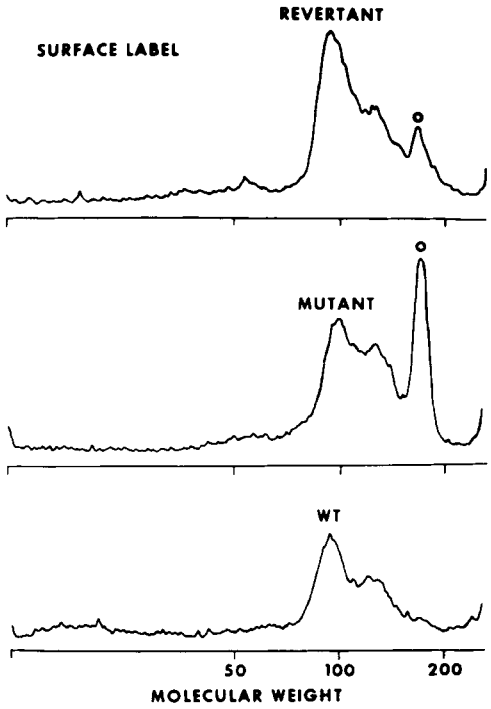

Fig. 2. Galactose oxidase-[^{3}H]borohydride surface labeling. Intact WT, drug-resistant mutant (CH^RC4) and revertant (I8–31) cells were surface labeled via the galactose oxidase technique including a neuraminidase pretreatment (5, 7). The cells were washed free of label and were subjected to subcellular fractionation. The plasma membrane fraction was dissolved in 3% SDS plus 1% mercaptoethanol, boiled, and analyzed by SDS slab gel electrophoresis (acrylamide concentration, 7.5%). Equal amounts of radioactivity were placed into each well. Molecular weight standards were run in parallel. After electrophoresis the gel was impregnated with PPO (11), dried, and used to prepare an autoradiogram. The autoradiographic image was scanned with a densitometer. Ordinate: arbitrary unit of density. Abscissa: molecular weight ($X\ 10^{-3}$).

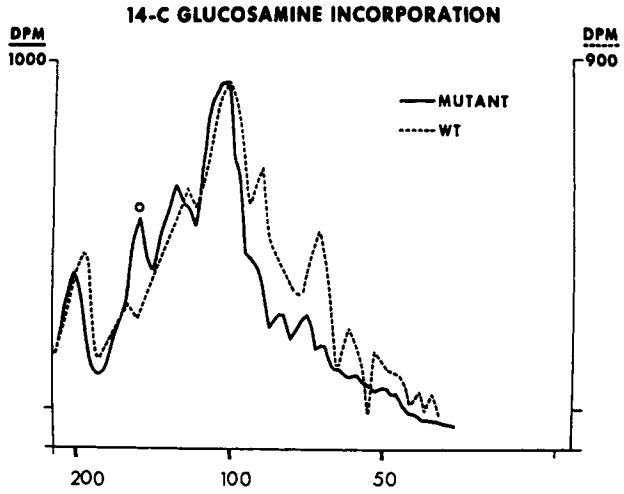

Fig. 3. Metabolic incorporation of [^{14}C]glucosamine. WT (AUXB1) and drug-resistant (CH^{R}C4) cells were labeled with [^{14}C]glucosamine. Plasma membranes were prepared, solubilized as given in Fig. 2, and analyzed on individual SDS disc gels. The gels were sliced and counted for radioactivity (7). Ordinate: dpm per gel slice. Abscissa: molecular weight (X 10^{-3}).

differences between the labeling patterns of WT and drug-resistant (CH^{R}C4) cells.

The tyrosine and histidine residues of the surface polypeptides of intact WT and CH^{R}C4 cells were labeled with ^{125}I via the lactoperoxidase technique and the isolated membranes were analyzed by SDS disc gel electrophoresis. As seen in Fig. 4, no substantial differences in the labeling patterns were observed. In particular there is no indication of a major labeled peak corresponding to the 165,000 MW component visualized by surface labeling with the galactose oxidase technique.

CONCLUSIONS

Surface label experiments with the galactose oxidase-[^{3}H]-borohydride technique clearly revealed a radiolabeled component of the cell surface of drug-resistant CHO cells which was absent from WT cells. Since this component could also be detected by metabolic incorporation of [^{14}C]glucosamine, the cell surface differences between WT and resistant cells involve a biosynthetic alteration and not simply a conformational change leading to increased reactivity with the surface label reagent. However, the possibility of changes in membrane conformation as a consequence of altered biosynthetic activity in the drug-resistant cells remains open.

It is not yet clear if the novel component present on the surface of drug-resistant CHO cells represents an entirely new membrane glycoprotein, or a polypeptide which is shared by WT and resistant cells but which is more heavily glycosylated in the resistant clones. Another possibility is that the surface-labeled component is a mucopolysaccharide or a pure carbohydrate, rather than a typical glycoprotein; however, experiments with [$^{35}SO_4$=] incorporation (data not shown) rule out the possibility that the 165,000 MW component is a sulfated mucopolysaccharide. Experiments with [^{3}H]leucine incorporation, although failing to reveal any substantial differences in the labeling patterns of WT and

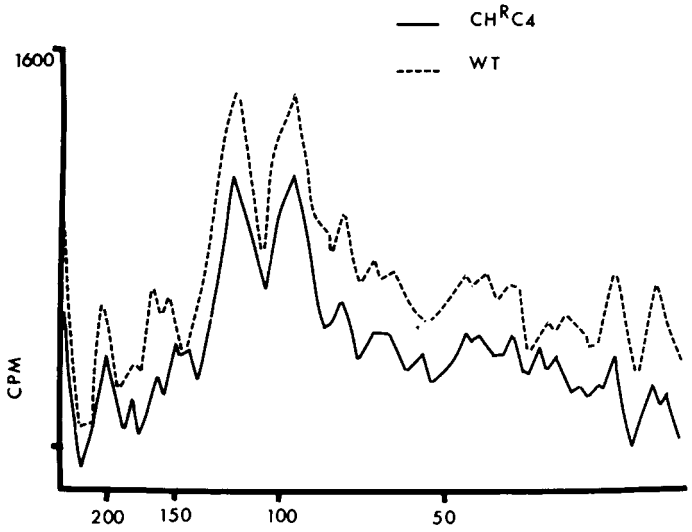

Fig. 4. Lactoperoxidase-[^{125}I] surface labeling. Intact WT and mutant (CH^{R}C4) cells were labeled by the lactoperoxidase technique (7). Plasma membranes were prepared and analyzed by SDS disc gel electrophoresis (5.6% acrylamide). Equal amounts of radioactivity were layered on individual gels. Following electrophoresis the gels were sliced and counted. The region corresponding to a molecular weight of 160,000–170,000 contains only minor peaks. Ordinate: cpm/slice. Abscissa: molecular weight (X 10^{-3}).

resistant cells, do not rule out the possibility of a novel glycoprotein in the resistant cells. A heavily glycosylated glycopeptide may represent a substantial portion of the total membrane carbohydrate and thus be readily visualized by [^{14}C]glucosamine incorporation or by the galactose oxidase technique, and yet such a glycopeptide may comprise only a small portion of the total protein in the membrane fraction and thus not be easily discriminated by metabolic labeling with [^{3}H]amino acids.

Although the 165,000 MW component found in drug-resistant CHO cells is highly reactive with the galactose oxidase-[^{3}H]borohydride surface label technique, it is not readily detected by lactoperoxidase labeling. This contrasts with the case of the 90,000–100,000 MW component seen in both WT and mutant cells which is readily labeled by both surface reagents (8). The unreactive state of the 165,000 MW component implies the lack of available tyrosine, which may be due either to the composition of the molecule or to a shielding effect of the carbohydrate side chains. A precedent for this type of behavior is the case of the sialoglycoprotein of the equine red cell which is clearly a surface component but which is unreactive with lactoperoxidase (12) in the intact cell.

Present studies have established a qualitative correlation between the presence of an altered cell surface component and a drug-resistant phenotype which is due to a reduced rate of drug permeation. In this study only five clones, namely, resistant clones CH^{R}C4 and CH^{R}C5, their parent WT clones, and the revertant I8–31, were examined. In order firmly to establish firmly the correlation, be necessary to examine a large number of other independent clones which exhibit various degrees of drug resistance. Moreover, it is important to establish firmly the chemical nature of the altered cell surface component. Studies on both of these problems are now under way.

ACKNOWLEDGMENTS

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