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ALTERED CELL SURFACE GLYCOPROTEINS IN PHYTOHEMAGGLUTININ-RESISTANT MUTANTS OF CHINESE HAMSTER OVARY CELLS

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Summary

Purified membranes from surface-labelled phytohemagglutinin-resistant (Pha^R) and wild-type chinese hamster ovary cells have been analysed by sodium dodecyl sulphate gel electrophoresis. Gel patterns were compared for cells labelled via galactose oxidase and B^3H_4 or lactoperoxidase and radioactive iodide. The results suggest that Pha^R cells are altered in the carbohydrate portion of a number of their membrane glycoproteins.

Chinese hamster ovary (CHO) cells selected for resistance to the cytotoxicity of the phytohemagglutinin from *Phaseolus vulgaris* have been shown to behave as authentic somatic cell mutants [1]. Phytohemagglutinin-resistant (Pha^R) CHO clones which bind markedly less [^{125}I]phytohemagglutinin than "parental" or wild-type clones exhibit a high degree of cross resistance to the cytotoxicity of wheat germ agglutinin, *Ricinus communis* agglutinin and *Lens culinaris* agglutinin (Stanley, Caillibot and Siminovitch, manuscript in preparation), whereas they are 4—5 times more sensitive than wild-type clones to the cytotoxicity of concanavalin A [1]. These properties of Pha^R cells and the fact that the different lectins bind to specific carbohydrate configurations [2], suggested that the mutation to phytohemagglutinin resistance might be manifested in the carbohydrate moieties of surface membrane glycoproteins. In the experiments described here, we have compared the availability of surface galactose residues in neuraminidase-treated wild-type and Pha^R CHO cells by sodium dodecyl sulfate gel electrophoresis of purified membranes labelled via galactose oxidase and B^3H_4. Similarly, the availability

Abbreviation: GlcNAc, *N*-acetylglucosamine.

of surface tyrosine (and histidine) residues has been investigated following labelling of intact cells with lactoperoxidase and radioactive iodide.

The isolation characterisation and nomenclature of wild-type and Pha^R CHO cell lines have been described in detail elsewhere [1]. Briefly, Pro^- 5 is a proline-requiring CHO clone and $Pro^- Pha^R$ 1-1 is a clone selected in a single step from Pro^- cells plated in 12 μg/ml phytohemagglutinin.$Gat^- Pro^+2$ is a glycine-, adenosine- and thymidine-requiring CHO auxotroph reverted for the proline requirement and $Gat^- Pro^+2$ Pha^R 1 is a clone selected in a single step from $Gat^- Pro^+2$ cells plated in 15μg/ml phytohemagglutinin. Both wild-type and Pha^R clones retained their characteristic phenotypes during several months of continuous culture in the absence of specific selection conditions. Cells were grown in suspension culture in complete alpha medium [3] containing 10% foetal calf serum to between 0.5 and $1.0 \cdot 10^6$ cells/ml.

Prior to surface-labelling, the cells were washed 3 times in phosphate-buffered saline, pH 7.2. The procedures for surface labelling, membrane isolation and gel electrophoretic analysis have been described in detail in previous communications (Juliano and Behar-Bannelier, submitted for publication) [4]. Briefly, for surface-labelling of galactose residues, $1-3 \cdot 10^8$ washed cells were resuspended in 2ml phosphate-buffered saline and incubated 5 min at 37°C with 50 units of *Vibrio cholerae* neuramindase, obtained from Calbio Chem. La Jolla, Calif. The cells were washed once with approx. 50ml phosphate-buffered saline, resuspended in 2 ml phosphate-buffered saline and incubated 5 min at 37°C with 15 units galactose oxidase, obtained from Sigma Chemical Co., St. Louis Miss. Following another wash in approx. 50 ml cold phosphate-buffered saline, the cells were incubated in ice with approximately lmCi sodium boro[^{3}H]hydride, obtained from the Radiochemical Centre, Amersham, U.K. (700 Ci/mol, dissolved in cold 0.01M NaOH shortly before use). Unbound isotope was removed by washing 4 times in 50 ml phosphate-buffered saline 1% bovine serum albumin.
For surface-labelling of tryosine (and histidine) residues, $1-3 \cdot 10^8$ washed cells were resuspended in 2ml phosphate-buffered saline and incubated 5 min at 37°C with 40 μg lactoperoxidase, obtained from Calbio Chem. La Jolla, Calif. and 100—150 μCi carrier-free sodium radioiodide (^{125}I approx. 17Ci/mg; ^{131}I approx. 25Ci/mg) Radiochemical Centre, Amersham, U.K. Aliquots (50μl) of 10 mM H_2O_2 were added at the beginning of the incubation and twice subsequently. Unbound isotope was removed by 4 washes in phosphate-buffered saline 1% bovine serum albumin. Plasma membranes were prepared from a homogenate of labelled cells by centrifugation in an aqueous two-phase system [5]. Membranes prepared from neuraminidase-treated cells labelled with galactose oxidase and B^3H_4 possessed $1-2 \cdot 10^5$ cpm/mg protein and those from cells labelled with lactoperoxidase and radioactive iodine possessed $1-5 \cdot 10^5$ cpm/mg membrane protein. Purified membranes were solubilized in an equal volume of a solution containing 1% dodecyl sulfate and 0.01 M dithiothreitol, incubated 15 min at room temperature, boiled 1 min and analysed by dodecyl sulfate gel electrophoresis according to the method of Fairbanks et al. [6]. Gels were sliced and radioactivity counted as previously described [4].

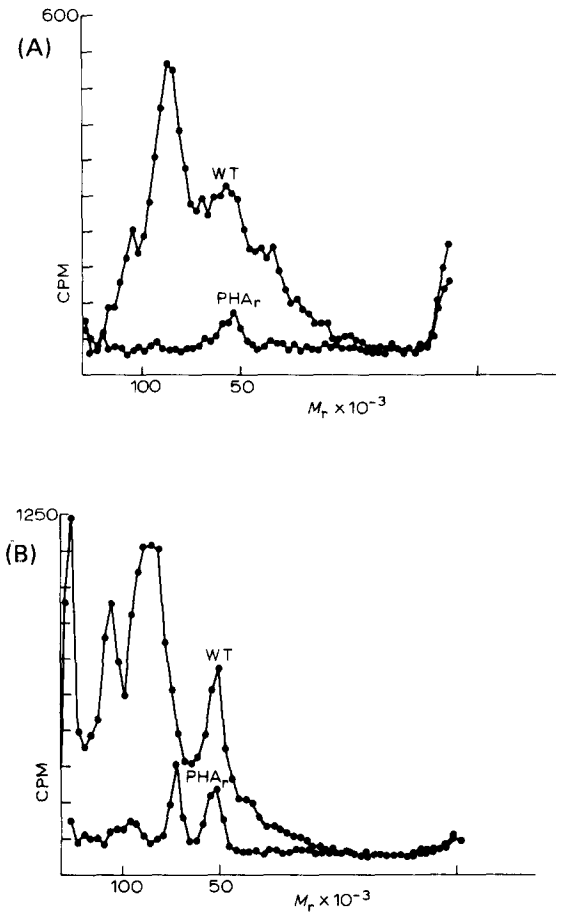

Fig.1. Dodecyl sulfate gel electrophoresis in 7.5% acrylamide of purified membranes from neuraminidase-treated wild-type and Pha^R CHO cells surface-labelled with galactose oxidase and B^{3}H$_4$. The ordinate is cpm of ^{3}H per fraction and the abscissa is a molecular weight scale ($M_r \cdot 10^{-3}$) obtained from measurements with marker proteins (chymotrypsin, ovalbumin, serum albumin, β-galactosidase) subjected to electrophoresis in parallel. It should be noted that glycoproteins migrate somewhat anomalously in dodecyl sulfate gels and therefore molecular weight estimates are at best approximate. (A) Clone Pro$^-$5 (wild-type) and clone Pro$^-$Pha^{R}1-1 (Pha^R). Equal amounts of radioactivity (26 000 cpm) were layered on each gel. Most of the label associated with Pha^R membranes migrated with the dye marker and is only partly visualized here. (B) Clone Gat$^-$Pro$^+$2 (wild type) and clone Gat$^-$Pro$^+$2 Pha^{R}1 (Pha^R). Equal amounts of protein (approx. 60μg) containing 31 720 cpm for wild type and 7200 cpm for Pha^R respectively were layered on each gel. The peaks co-originating with the dye marker are only partly visualized. The radioactivity at the origin probably represents an aggregation artefact.

Purified membranes from two independent Pha^R clones and from their respective parental wild-type clones were compared by dodecyl sulfate gel electrophoresis following labelling of the intact, neuraminidase-treated cells via galactose oxidase and B^{3}H$_4$ (Figs 1A and 1B). Although there were minor differences between the wild-type clones, they both exhibited a complex pattern of labelled glycoproteins including three broad peaks, ranging in apparent molecular weight from 50 - 140 · 10^3. By contrast, the gel patterns of the independent Pha^R clones differed markedly both from wild-type and from each other. The major glycoproteins labelled in the

Pro$^-$ Pha^R 1-1 clone migrated together as a broad peak (Fig. 1A) whereas the Gat$^-$ Pro$^+$2 Pha^{R}1 clone exhibited at least two major glycoprotein peaks (Fig. 1B). The amount of ^3H incorporated into glycoproteins of high molecular weight was dramatically reduced in both Pha^R clones. These labelling patterns were reproduced in several different experiments. A varying and often quite large proportion of the label in these gels migrated with the dye marker and presumably consisted of tritiated glycolipids and breakdown products of B^3H_4 [4]. Therefore, the degree of labelling of the wild-type and Pha^R glycoproteins was compared by weighing the area under the peaks, which migrated with an apparent molecular weight $> 20 \cdot 10^3$ (Figs 1A and 1B). In both cases the area under the Pha^R gel patterns was approx. 16% of that found for the wild-type clones. The results suggest that many of the galactose-containing moieties available for enzymic labelling in wild-type CHO cells are no longer available in their Pha^R derivatives. Also, the membranes of the independent Pha^R isolates appear to differ in their surface glycoproteins.

Dodecyl sulfate gel electrophoresis of membranes from wild-type and Pha^R cells labelled via lactoperoxidase iodination gave a different result (Fig. 2). In this case, Pha^R membranes exhibited a complex gel pattern which was very similar to that obtained from wild-type cells. However, many of the species labelled in Pha^R membranes migrated significantly faster than the corresponding species (of similar size) from wild-type membranes. This shift of approx. 5% in molecular weight is significant since both types of membranes were electrophoresed in the same gel. The result suggests that Pha^R and wild-type CHO cells possess similar classes of proteins and glycoproteins at the cell periphery,but that in Pha^R cells, many of the labelled species are altered so that they migrate more rapidly in dodecyl sulfate gels.

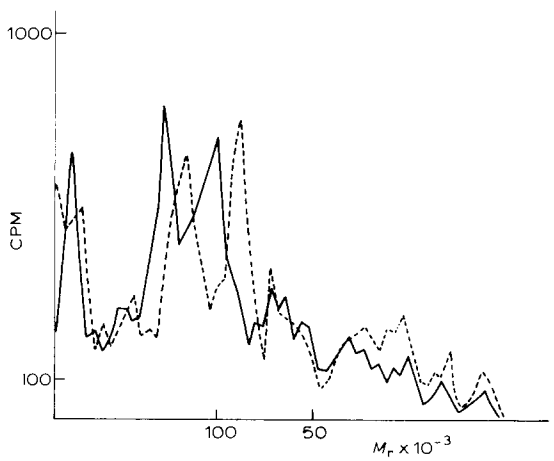

Fig.2. Dodecyl sulfate gel electrophoresis in 5.6% acrylamide of purified membranes from CHO cells labelled by lactoperoxidase and radioactive iodide. Cells of clone Pro$^-$5 (wild-type ——) were labelled with ^{131}I and cells of clone Pro$^-$Pha^{R}1-1 (Pha^R, - - - -) were labelled with ^{125}I. Equal amounts of radioactivity (approx. 16 000 cpm) were mixed and analysed by dodecyl sulfate gel electrophoresis in the same gel. Ordinate; cpm of ^{125}I, (- - - -) corrected for overlap of ^{131}I using a standard curve. There was no overlap of ^{125}I in the ^{131}I channel, (——). Abscissa; $M_r \cdot 10^{-3}$ obtained as described for Fig.1.

These surface-labelling experiments have provided futher information on the nature of the alteration(s) at the plasma membrane of Pha^R CHO cells. The results of galactose oxidase-B^3H_4 surface-labelling clearly show that Pha^R cells possess few available ultimate and penultimate surface galactose residues compared with wild-type CHO cells. The glycoproteins which do label in Pha^R membranes may represent a subset of wild-type glycoproteins which are not affected by the mutation to phytohemagglutinin-resistance. These membrane mutants therefore provide unique biological material for experiments designed to analyze the surface architecture of mammalian cells.

By contrast, Pha^R and wild-type cells label to a similar extent by lactoperoxidase iodination, but many of the species from Pha^R membranes appear to migrate with a decreased molecular weight. There is evidence that the major surface components in CHO cells which label via the galactose method are also those which label via the lactoperoxidase method (Juliano and Behar-Bannelier, submitted for publication). Therefore, glycoproteins and proteins in wild-type membranes are also available for enzymic iodination in Pha^R cells but that the glycoproteins in Pha^R cells, being significantly less glycosylated, migrate more rapidly in dodecyl sulfate gels. This interpretation is supported by recent experiments in our laboratory which show that both Pha^R clones Pro^- Pha^R 1-1 and Gat^- Pro^+2 Pha^R 1 possess very little activity ($\leqslant 5\%$ that present in wild-type cell extracts) of a specific glycosyl transferase which transfers *N*-acetylglucosamine (GlcNAc) to terminal α-mannose residues (Stanley, Narasimhan, Schachter and Siminovitch, manuscript in preparation). If the phytohemagglutinin receptor in CHO cells is similar to that characterized from human red blood cells [7] a predicted consequence of this enzyme lesion would be that cell glycoproteins containing the α-mannose-GlcNAc sequence would be incompletely glycosylated. It has recently been shown that *Ricinus communis*-resistant CHO cells also possess very low activity for the GlcNAc-transferase, absent in Pha^R cells and that glycoproteins in crude membrane extracts from these cells appear to migrate with a decreased molecular weight in dodecyl sulfate gels [8].

The availability of membrane mutants will greatly facilitate the study of membrane structure and function in mammalian cells. It is of interest that the independently isolated Pha^R clones Pro^- Pha^R 1-1 and Gat^- Pro^+2-Pha^R 1 appear to differ in the exposure of their surface glycoproteins (Fig. 1), despite both having lost the activity of the same specific glycosyl transferase. Further comparisons such as these are now possible since a number of phenotypically distinct lectin-resistant cells have recently been isolated in our laboratory (Stanley, Caillibot and Siminovitch, manuscript in preparation).

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