Isolation of Cell Surface Glycoproteins from Normal and Transformed Cells by Affinity Chromatography on Plant Lectin Columns

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Carbohydrate-containing components of the cell surface have been implicated in many important biological functions, including cell–cell interactions, receptors for some hormones and viruses and immunological specificity (Kraemer, 1971). Further, studies on normal cells and their virally-transformed derivatives have shown some alterations in plasma-membrane glycoproteins (Warren et al., 1973) and increased agglutinability of transformed cells by plant lectins (Eckhart et al., 1971). Isolation of the glycoproteins from purified plasma membranes would permit a direct comparison of these proteins from normal and transformed cell, and, in addition, might make possible the analysis of their biological functions, as well as their organization in the cell surface membrane. The binding of lectins occurs through their interaction with saccharides, and, although each lectin has its own saccharide specificity, a wide diversity can be obtained from various plants and animals (Sharon & Lis, 1972). Recently a method was described for the isolation of lymphocyte plasma-membrane glycoproteins (Hayman & Crumpton, 1972) and virus glycoproteins (Hayman et al., 1973) by means of affinity chromatography of sodium deoxycholate-solubilized membrane on *Lens culinaris* lectin covalently attached to Sepharose. These reports suggest that sodium deoxycholate-solubilized membrane glycoproteins may be fractionated by affinity chromatography on immobilized lectins of different specificities. The present paper describes the application of this technique to the isolation and partial characterization of plasma-membrane glycoproteins from normal mouse (3T3), simian virus 40-transformed mouse (SV3T3), normal hamster (BHK) and polyoma virus-transformed hamster (PyBHk) fibroblast cell lines.

Cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% (v/v) calf serum. To facilitate removal of membrane-bound serum proteins, the medium was replaced with fresh medium minus calf serum 24 h before harvesting. Cells were harvested by scraping, washed with phosphate-buffered saline and plasma membrane prepared by the two-phase polymer procedure of Brunette & Till (1971). In some experiments cells were labelled for a 24 h period before harvesting with [14C]leucine (2 μ Ci/ml) in medium containing 10% dialysed calf serum and leucine at 10% of its normal concentration. The labelled normal or transformed cells were harvested and then mixed with an excess of unlabelled transformed cells before membrane preparation. The final plasma-membrane pellet was suspended in 2% sodium deoxycholate and immediately frozen at -70°C.

Plant lectins were prepared from *L. culinaris* by the method of Hayman & Crumpton (1972), and from *Ricinus communis* by the method of Nicolson et al. (1974). The lectin from *L. culinaris* (LcH) possesses a specificity for glucose- and mannose-related sugars, whereas the lectin from *R. Communis* (RCA11) exhibits a specificity for D-galactose-related sugars. Lectin–Sepharose 4B conjugates were prepared as described by Hayman & Crumpton (1972).

The frozen plasma-membrane solution was thawed and diluted with an equal volume of H2O before centrifugation at 100000 g for 1 h. The supernatant, the 'soluble' membrane components, was applied to the desired lectin column equilibrated with 1% sodium deoxycholate. After application of the solution the column was washed extensively with 1% sodium deoxycholate and the adsorbed glycoproteins were subsequently eluted with 0.3 M-α-methyl-D-mannose (LcH) or 0.3 M-D-galactose (RCA11) in 1% sodium deoxycholate. The eluted fractions were dialysed against 1% sodium deoxycholate before freezing and freeze-drying. Sodium deoxycholate was removed from the freeze-dried residues by extraction with ethanol at -20°C. The precipitated proteins were analysed by polyacrylamide-gel electrophoresis in sodium dodecyl sulphate as described by Studier (1972).
With all cell types investigated approx. 2% of the total membrane protein is specifically bound to LcH columns, and presumably represents the fraction of glycoproteins which contains carbohydrate moieties terminating in glucose- or mannose-related sugars. Slightly less of the total membrane protein is specifically bound to RCA\textsubscript{II} columns. This fraction presumably contains only those cell surface glycoproteins whose carbohydrate moieties terminate in D-galactose-related sugars. If an unretarded peak is rechromatographed on the same column, all of the protein is again unretarded. Likewise, if, after dialysis against 1% sodium deoxycholate, a specifically bound glycoprotein peak is rechromatographed on the same column, all of the applied material is bound, and again specifically eluted with the competitive sugar.

Sodium dodecyl sulphate-polyacrylamide-slab-gel-electrophoretic analysis of the glycoprotein bound to and specifically eluted from the lectin columns produces a pattern of bands which, in both cases, show essentially no resemblance to the banding pattern of the original plasma-membrane sample. Practically all of the major glycoproteins selectively bound to LcH or RCA\textsubscript{II} exhibit apparent molecular weights of greater than 60000, with particularly noticeable bands at approx. 85000, 130000, 160000, 180000, and 250000 mol.wt. Approx. 20–25 major glycoprotein bands are observed on such analysis of the LcH-bound glycoproteins whereas fewer major bands (15–20) are observed when the RCA\textsubscript{II}-bound glycoproteins are analysed. If the glycoproteins bound to LcH are dialysed against 1% sodium deoxycholate and then rechromatographed on RCA\textsubscript{II}, approximately two-thirds of the proteins are bound and specifically eluted with D-galactose. Sodium dodecyl sulphate-polyacrylamide-slab-gel-electrophoretic analysis of the unretarded and specifically bound peaks shows that each is a distinct subset of the original population of glycoproteins. Thus only a portion of the glycoproteins which have carbohydrate moieties terminating in glucose- or mannose-related sugar residues, also have carbohydrate moieties terminating in galactose-related residues. On the other hand, if RCA\textsubscript{II}-bound glycoproteins are similarly rechromatographed on a LcH column, most, if not all, of the glycoproteins are bound to the column and specifically eluted with α-methyl-D-mannose. Molecular-weight analysis of these glycoproteins, which presumably contain carbohydrate moieties which terminate, not only in glucose- and mannose-type sugars, but also moieties which terminate in galactose-type sugars, shows a pattern which is essentially identical with that of glycoproteins bound to only RCA\textsubscript{II}. Thus apparently most of the cell surface glycoproteins which bind to RCA\textsubscript{II} also bind to LcH. Comparisons between the cell surface glycoproteins of normal and transformed cells shows that practically all of the bands remain unaltered. However, a few changes are apparent; most noticeable are decreases in two bands (mol.wt. approx. 250000 and 85000) in transformed hamster cells and an increase in a band (mol.wt. approx. 125000) in SV-40-transformed mouse cells.

Sharon, N. & Lis, H. (1972) *Science* 177, 949–959