Solubilization and Peptide 'Mapping' of a Large External Glycoprotein Fraction Labelled by Lactoperoxidase-Catalysed Iodination of Cultured Fibroblasts

RODERICK NAIRN and R. COLIN HUGHES

National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

A glycoprotein(s) of apparent molecular weight 220000–250000 can be labelled by lactoperoxidase-catalysed iodination of fibroblasts from a variety of species (Hynes, 1973; Hogg, 1974; Pearlstein & Waterfield, 1974; Yamada & Weston, 1974). The glycoprotein of hamster fibroblasts appears at the cell surface during interphase and is lost from the surface at mitosis (Hynes & Bye, 1974). Further, the glycoprotein is absent from the surface of virally transformed derivatives of hamster, chick, human and mouse fibroblasts (Hynes, 1974) and has been termed LETS (large external transformation-sensitive) glycoprotein (Hynes & Bye, 1974). There are exceptions to this correlation, however (Meager et al., 1975). At present no function has been defined for the LETS glycoprotein, although its external location, apparently in a fraction related to the cell coat or glycocalyx (Graham et al., 1975), and relativity to growth state of the cells suggest a possible involvement in cellular processes mediated via the cell surface.

To study further the structure and function of the LETS glycoprotein it is necessary to isolate the material in pure form. In this report we describe the solubilization characteristics of LETS glycoprotein and also show that extensive differences exist in the primary structures of the LETS glycoproteins obtained from chick or hamster fibroblasts.

Baby-hamster kidney BHK21 C13 cells, hamster NIL 8 cells, primary chick-embryo fibroblasts and primary human skin fibroblasts were grown at 37°C in Glasgow-modified minimal essential medium supplemented with 10% (v/v) foetal bovine serum, 10% (v/v) tryptose phosphate broth, 0.2% (w/v) NaHCO₃, penicillin (0.1 M-i.u./l) and streptomycin (0.1 g/l). Confluent monolayers of cells were labelled on 60mm Falcon plastic culture dishes with a mixture of lactoperoxidase (Sigma Chemical Co., London S.W.6, U.K.) glucose oxidase (type V, Sigma) and carrier-free Na¹²⁵I (The Radiochemical Centre, Amersham, Bucks., U.K.) as described by Meager et al. (1975).

After labelling, the cell sheets were washed with phosphate-buffered saline, pH 7.4, scraped off with a rubber policeman and finally dissolved in 1% sodium dodecyl sulphate/1% 2-mercaptoethanol by heating at 90°C for 5 min. Samples were analysed on sodium dodecyl sulphate/polyacrylamide gels by the discontinuous gel system described by Maizel (1971). After resolution the gels were sliced into 2mm slices and counted directly for radioactive iodine associated with protein bands.

As a routine the separations show a slow-moving major iodinated band (LETS glycoprotein) which is well separated from other faster-moving labelled bands.

The LETS glycoprotein of BHK cells is not extracted into solution by either 0.5% Nonidet (NP-40) (Fig. 1) or 1% sodium deoxycholate. Extractions were carried out for up to 45 min at 4°C. Notably, the other iodinated bands were readily solubilized under these conditions. These findings strongly suggest that the LETS glycoprotein is differently organized within the cell surface structure and is not released, as are the integral components, after extensive membrane disassembly with detergents. Similar behaviour has been described for non-integral membrane components such as spectrin (Steck, 1974). However, the LETS glycoprotein, unlike extrinsic components of the erythrocyte membrane, is also resistant to extraction with 0.2% EDTA (results not shown). Under these conditions essentially no iodinated components are made soluble. The LETS
Fig. 1. Extraction of $^{125}$I-labelled glycoproteins but not LETS glycoprotein by a non-ionic detergent

Labelled BHK cells (approx. $10^7$ cells) were treated for 15 min at 4°C with 0.5% Nonidet (NP40), 10mM-Tris/HCl, pH8.2. The solubilized components obtained after centrifugation at 10$^5$g-min and the residual pellet were analysed by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis. Migration is from left to right. The arrow indicates the 250000-molecular-weight (LETS) glycoprotein. (a) Solubilized components; (b) pellet.

glycoprotein and other iodinated components are completely solubilized by 8M-urea at 4°C.

To compare the structure of the LETS glycoprotein obtained from fibroblasts of different species, peptide 'maps' of the purified material were prepared as follows. The radioiodinated LETS glycoprotein was eluted from gel slices by Dounce homogenization in 0.1% sodium dodecyl sulphate in Tris/glycine buffer, pH8.3. The clear supernatant obtained after centrifugation, containing 60–80% of the total radioactivity, was dialysed extensively against water at 4°C, freeze-dried and dissolved in 0.2M-NH$_4$HCO$_3$ (1ml). Thermolysin (100μg, Sigma) or trypsin (100μg, Sigma) was added and digestion was for 16h at 37°C. The mixtures were freeze-dried, dissolved in water (0.2ml) and portions were analysed by paper electrophoresis followed by chromatography. After
Fig. 2. 'Fingerprints' of radioactive peptides from thermolysin digests of lactoperoxidase-iodinated glycoproteins from primary chick-embryo fibroblasts (a) and baby-hamster kidney (BHK) cells (b)

After the cells were labelled, sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was used to separate the major iodinated components. The slowest-moving band (apparent molecular weight 220000–250000) in either case was eluted and digested as described in the text. Electrophoresis in pyridine/acetic acid/water (1:10:1000, by vol.) at pH 3.5 was followed in the second dimension by chromatography in butanol/acetic acid/water (4:1:5, by vol.). Radiographs of the 125I-labelled peptides were then prepared as described.

resolution the positions of the radioactive peptides were detected by radioautography on Kodak X-Ray film (Kodak, London W.C.2, U.K.).

Clear differences were apparent in the 'fingerprints' of tyrosine- (and histidine-) containing peptides obtained from the labelled glycoprotein fraction of unrelated species. For example, Fig. 2 shows thermolysin 'fingerprints' of LETS glycoprotein from chick-embryo fibroblasts and BHK cells respectively. Equally pronounced differences were found between these cells and human fibroblasts (results not shown). These results indicate that considerable sequence differences exist in the regions of tyrosine or histidine residues of the glycoproteins from different species. Antigenic cross-reactions have been demonstrated between the LETS glycoprotein of different species (A. Vaheri, personal communication; Lindner et al., 1975), indicating that some homology is present, although some evidence to the contrary for human and chick glycoproteins has been reported (Ruoslahti & Vaheri, 1974). It is of interest to determine the exact degree of homology between LETS glycoproteins from fibroblasts of different species in view of the similar cellular location and properties of this component.


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