A MEMBRANE GLYCOPROTEIN-CONTAINING FRACTION WHICH PROMOTES CELL AGGREGATION

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Received October 1,1975

SUMMARY

Glycoproteins have been extracted from 16C rat fibroblasts using acetone/lithium di-iodosalicylate/phenol purification. This fraction increases the aggregation of the fibroblasts *in vitro*.

INTRODUCTION

The way in which cells adhere is immensely important for many aspects of multicellularity but the mechanisms involved in the process are far from being fully appreciated. There is, though, a good deal of evidence (1) to suggest that such cell behaviour is governed by the sugar residues which are displayed in various forms at the cell's surface. These sugars occur in the form of glycolipids, glycosaminoglycans, and both carbohydrate-rich (e.g. glycophorin) and protein-rich (e.g. the anion transport protein of red cells) glycoproteins. It is not known which of these forms have important functions in cell interactions.

Using a model system for cell adhesion we previously indicated (3) the involvement of surface carbohydrates and, further, demonstrated that plasma membranes isolated from cultured rat fibroblasts stimulated the aggregation of these cells. In the present study, we have shown that by exploiting

techniques for extracting glycophorin from erythrocytes (2), an aggregation-promoting activity can be isolated from the plasma membranes of rat fibroblasts and so goes one step further towards characterising the carbohydrate-containing species responsible for the adhesion of cultured rat fibroblasts. This suggests that the active material is analogous to glycophorin, the major glycoprotein of the human red cell which bears many antigenic carbohydrate side-chains, making up about 60% of its weight.

MATERIALS AND METHODS

Rat dermal fibroblasts, a spontaneously-transformed, established line (16C), were maintained as previously described (3). Large scale cultures for membrane preparation were prepared in 1.6 l plastic spiral vessels (Sterilin Ltd., Richmond, Surrey) as described by Maroudas (4) using approx. 1×10^8 cells as inoculum. The cells were grown for up to 7 days in Dulbecco's modified Eagle's medium containing 10% (v/v) heat-inactivated calf-serum (Flow Laboratories) under 10% CO_2 in air. Plasma membranes were prepared by the method of Warley and Cook (5). In order to minimise the degradation of membrane constituents the cells were harvested by mechanical, rather than by enzymatic means. Glycoproteins were extracted with lithium di-iodosalicylate (LIS) used in the isolation of red cell glycoproteins (2) and which has subsequently also been employed with success by Merrell and Gottlieb (6) in solubilising cell recognition proteins from embryonic tissue cells. Membranes were first washed in an excess of acetone and then extracted (30 min. at 23°C) in this solvent and then solubilised in lithium di-iodosalicylate.

The mixture was extracted with phenol and the aqueous phase, containing the carbohydrate-rich species, was dialysed against water until material absorbing at 323 nm (LIS) had been removed. To desialyse this membrane fraction, it was treated with 0.1N H_2SO_4 for 60 min. at 80°C, neutralized, extensively dialysed against physiological saline and concentrated by reverse dialysis against polyethyleneglycol.

In a previous investigation (3), a model glycoprotein, desialysed bovine submaxillary gland mucin, was found to inhibit a neuraminidase-stimulated increase in 16C aggregation. Accordingly, our biological assay for the plasma membrane glycoprotein fraction was to observe whether it, too, affected the aggregation of neuraminidase-treated cell suspensions. The procedure was scaled-down in order to maximise the effect of small amounts of material isolated. Aliquots of a single-cell suspension (0.5 ml), at 2×10^6/ml, obtained by brief dissociation in trypsin/EDTA (3) were dispensed into flat-based, siliconized glass vials and after adding 5 units of Vibrio cholerae neuraminidase (Behringwerke), the suspension was made to 1 ml with the suspending buffer: Dulbecco's phosphate-buffered saline containing 5 μg/ml deoxyribonuclease (Sigma, Type 1). The cells were aggregated under standardized conditions by reciprocally shaking the vials for 30 min. at 100 strokes/min. at 37°C on a shaking water bath. The collision of adhesive cells results in the formation of compact, spheroidal aggregates. This process of aggregation was made quantitative by measuring the decrease caused by the formation of aggregates in the total number of cellular particules, using a Celloscope 401 electronic particle counter fitted with a 100 μm aperture tube. Aggregation was estimated as the reduction in particles counted after 30 minutes, expressed as a percentage of the initial cell count.

RESULTS AND DISCUSSION

When examined by sodium dodecyl sulphate gel electrophoresis, the plasma membrane fraction solubilised by acetone/LIS extraction, gave two major bands staining for both carbohydrate and protein. This suggests a predominantly glycoprotein nature for the material although up to three extra weakly-staining bands were detected with Coomassie brilliant blue stain for protein. This glycoprotein-rich fraction, when desialysed, has a marked effect on the aggregation of 16C fibroblasts. Neuraminidase is known to increase the aggregation of these cells and the addition of desialysed mucin inhibits this increase (3). But unlike the mucin, desialysed membrane glycoproteins potentiate the stimulatory effect of the enzyme. As Table 1 illustrates, the presence of these membrane glycoproteins causes an increase in the aggregability of 16C fibroblasts. Not only does the glycoprotein fraction affect the quantity of cell particles which adhere to one another, but microscopic examination confirms that it induces the formation of many large cell aggregates (stable upon dilution) with relatively few single cells; the aggregates are visibly larger than produced in controls.

What these studies demonstrate is that a plasma membrane glycoprotein fraction of the carbohydrate-rich variety, partitioning with the aqueous phase against phenol, increases the adhesion of rat dermal fibroblasts in

	Control	NANase	NANase plus glycoproteins
A. % Aggregation	51	71	82
Promotion	-	20	31
B. % Aggregation	47	57.5	72
Promotion	-	10.5	25

TABLE 1: Promoting effect of asialomembrane glycoproteins on cell aggregation. A and B are separate preparations extracted from membranes derived from 7 and 12 ml of packed cells respectively. Neuraminidase was at a concentration of 5 units/10^6 cells. Aggregation occurred over 30 minutes and the figures are averaged from duplicate samples, each counted three times.

suspension. For some time it has been considered that glycoproteins are involved in cellular interactions (1) and it is likely, therefore, that the effect we have observed here mirrors the function of the glycoproteins in situ.

How membrane glycoproteins actually cause cells to adhere is an open question but it clearly involves some form of cell-cell cross linking. It is noteworthy that desialysed mucin was previously shown (3) to inhibit the aggregation of neuraminidase-treated cells which contrasts completely with the behaviour of the membrane glycoproteins in stimulating cell aggregation. Desialysed mucin mainly bears monosaccharide side-chains and although it may bind to the cell surface it could be unable to bridge cells and instead block access to adhesive sites. Due perhaps to a combination of differences in shape, size and receptor density, the carbohydrate-rich glycoproteins are, as these results demonstrate, better able to cross-link cells and underlines the point that glycoproteins of the mucin type may be poor models for membrane glycoprotein function.

It has recently been demonstrated (7) that acetone/LIS-extracted glycoproteins from regions of embryonic chick brains inhibit cell aggregation. At first sight it is perhaps surprising therefore that we have obtained a fraction with aggregation-promoting activity. But it is worth bearing in mind that the chick extracts, unlike those of cultured rat fibroblasts, were obtained from the membranes of heavily trypsinized cells - a process which undoubtedly removed carbohydrate in the form of glycopeptides from the surface glycoproteins. Apart from differences in cell-type, it is quite possible that different biological effects may be produced according to whether the membrane glycoproteins are intact or degraded.

The model system we are investigating is of a defined cell type which has the advantage that it can be suspended for adhesive assay using mild procedures. The aggregation of these cells is rapid but, importantly, is characterized

by the formation of specialised intercellular junctions (Lloyd, Smith, Judge and Rees, unpublished) well within the assay period. If, as seems likely, the same mechanisms of adhesion are common to many biological events, these studies suggest that major, carbohydrate-rich membrane glycoproteins can be implicated in the adhesive process.

ACKNOWLEDGEMENTS

C.W.L. was supported by a Beit Memorial Research Fellowship. G.M.W.C. is a member of the External Staff of the Medical Research Council. We thank Prof. D. A. Rees for reading the manuscript.

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