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BHK21 FIBROBLAST AGGREGATION INHIBITED BY GLYCOPEPTIDES FROM THE CELL SURFACE

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SUMMARY

Glycopeptides were removed by trypsinization from the surface of baby hamster kidney cells (line BHK21-C_{13}), digested by pronase and separated into 2 fractions by exclusion chromatography. The addition of small amounts of either glycopeptide fraction to shaken suspensions of lightly trypsinized cells inhibited their rapid aggregation, but one fraction was more active than the other and in higher concentrations it was able to inhibit aggregation completely. After this fraction was purified by high-voltage electrophoresis one subfraction also inhibited aggregation. The effect of the glycopeptides increased following their pretreatment with neuraminidase, but preincubation with periodiate or galactose oxidase destroyed all activity. Galactose oxidase also inhibited cell aggregation directly. Similar glycopeptides from virus-transformed BHK21 cells, oligosaccharides and intact and desialysed human urinary glycoproteins had comparatively little or no effect on BHK21 cell aggregation. The results suggest terminal β-galactosides and possibly α-galactosides, and to some extent a particular substructure of cell surface heteroglycans are necessary for their inhibitory activity. The parent, plasma membrane glycoproteins might serve as adhesive binding sites in cell cohesion, but some evidence indicates cell surface sialyl- and galactosyltransferases may not ordinarily act as their complementary binding receptors.

INTRODUCTION

For the most part the ways in which animal cells adhere and the ways adhesion might regulate their morphogenetic and social behaviour remain largely unclear. We have used BHK21 cells to study cell adhesion because it is an easily grown line displaying social behaviour in culture which we think is important to similar cells *in vivo*. In addition we have noticed that at least some of the adhesive properties of cells in culture are probably also expressed during their aggregation in the artificial conditions of shaken cell suspension (Edwards, 1973; Edwards, Campbell, Robson & Vicker, 1975).

It is necessary to describe the aggregation of these cells in 2 ways. The molecular interactions between cell surfaces may be thought to form one dimension of their behaviour while another is determined by their metabolism and cytoplasmic-plasma membrane activities. The latter may govern the availability and distribution of adhesive surface. Following light trypsin dispersal of cultures cell aggregation proceeds rapidly, depending in part on the cell concentration and temperature (Edwards & Campbell, 1971), but requires at least glycolysis or electron transport (Edwards *et al.* 1975) and intact cytoplasmic microtubules (Waddell, Robson & Edwards, 1974). A requirement for protein synthesis during aggregation may be induced only by very heavy trypsin treatments (Edwards *et al.* 1975).

There are several observations which suggest the adhesive components of the plasma membrane include proteins and polyglycans, especially glycoproteins. Adhesive membrane may be identified with patches of glycoproteins on the cell surface (Edwards *et al.* 1975; Edwards, 1976). Trypsin treatment of intact cells decreases cell aggregation probably by removing adhesive plasma membrane elements (Edwards & Campbell, 1971; Edwards *et al.* 1975). Neuraminidase (NANase) treatment, on the other hand, increases aggregation in hamster BHK21 (Vicker & Edwards, 1972), rat 16C fibroblasts (Lloyd & Cook, 1974) and human HeLa (Deman, Bruyneel & Mareel, 1974) lines. While NANase exposes the sialic acid acceptors, β-galactosides, in isolated glycoproteins and glycolipids only glycoproteins may be involved in the NANase-stimulated increase in cell aggregation. Glycolipids in intact membranes are not attacked by the enzyme (Weinstein, Marsh, Glick & Warren, 1970; Barton & Rosenberg, 1973).

We have suggested that the aggregation of BHK21 cells could be caused by the binding of cell surface heteroglycans to receptors on the plasma membrane of neighbouring cells (Vicker & Edwards, 1972). Morell *et al.* (1971), and recently Balsamo & Lilien (1974), Allen & Minnikin (1975) and Merrell, Gottlieb & Glaser (1975), reported that certain homologous and heterologous glycoproteins and glycopeptides are able to bind to cell surfaces specifically. Some of these macromolecules, when added to cell suspensions, inhibited cell adhesion (Lloyd & Cook, 1974; Allen & Minnikin, 1975; Merrell *et al.* 1975). Presumably their effect was due to their competition with structurally similar heteroglycans on the cell surface.

In this study I have attempted to identify the role of some plasma membrane glycoproteins in cell aggregation. Glycopeptides were removed from the cell surface by trypsin treatment, digested thoroughly with pronase and separated into 2 fractions: A and B. Each fraction is a heterogeneous mixture of glycopeptides, but generally the polysaccharide segments are about 16 and 14 sugars long, respectively, in A and B. The sugar species include L-fucose, sialic acid, D-glucosamine, D-galactose and D-mannose (Warren, Fuhrer, Buck & Walborg, 1974). The effect of the fractions on aggregation was tested by adding them to shaken suspensions of lightly trypsinized cells before aggregation began. They were also variously pretreated with NANase, galactose oxidase or periodate in order to modify their structure. Similar preparations from virus-transformed BHK21 cells, oligosaccharides and human urinary glycoproteins were also tested.

METHODS

Cell culture and aggregation

BHK21/C_{13} cells and those transformed by Bryan strain Rous sarcoma virus (C_{13}-B_4) were freshly grown from frozen stocks and cultured in roller bottles. Each culture was discarded after about one month of use. For aggregation experiments cells were harvested by a 2–3 min treatment of 0·05 % Difco trypsin plus 5 mM EDTA, washed and prepared as before (Edwards & Campbell, 1971; Vicker & Edwards, 1972). Single-cell suspensions were prepared by Pasteur pipette trituration of cell pellets in an ice-cold, bicarbonate-free Hanks' medium containing salts, glucose and 25 mM Tricine buffer (HST, pH 7·4). Cell suspensions of 2 ml were placed in 10-ml stoppered flasks and shaken reciprocally in a 37 °C water bath to induce random cell

collisions. For measurement of the cell concentration samples were removed with a wide-bore 0·1-ml glass micropipette and diluted 200-fold in unbuffered, Ca^{2+}- and Mg^{2+}-free Hanks'. The samples were kept ice-cold and counted electronically within 30 min using a Coulter counter model Z_B with a 0·2-mm aperture and duplicate 2-ml counting volumes (Edwards & Campbell, 1971; Edwards, 1973). In all experiments aggregation was also monitored microscopically. The data are presented as N_t/N_0, where N represents the concentration of particles of all sizes (single cells and aggregates) at various times (t) after the beginning of shaker incubation. The fractional extent of aggregation $\phi = (N_t - N_0)/N_0$ as t approaches infinity (Vicker & Edwards, 1972).

Glycopeptide preparation

For each preparation several roller bottles, individually producing up to 10^8 cells, were grown 3 days in BHK21 medium with 10% calf serum plus 0·1 μCi/ml of either L-[^{3}H]fucose (4 Ci/mmol), L-[^{14}C]fucose (50 Ci/mol), or D-[^{14}C]glucosamine (6·3 Ci/mmol), which were obtained from New England Nuclear Corp. (Boston, Mass.). As the cultures reached confluency they were trypsinized and the supernatant fluid was treated with pronase, using a slight modification of the method of Buck, Glick & Warren (1970). Monolayers were washed twice with HST and incubated at 37 °C for 10 min in HST containing 0·3 mg/ml pure trypsin and 10 μg/ml DNAse, but trypsin inhibitor was not added. Nearly all cells appeared undamaged by the treatment and remained refractile when observed by phase-contrast microscopy. After pronase treatment of the cleared supernatant, urea was added to 4 M (pH 6·0) and the pronase was allowed to digest itself by further incubation for 5 days at 37 °C. Fractions A and B of glycopeptide were isolated as before by Sephadex G-50/water chromatography using a 2-m column (Warren, Fuhrer & Buck, 1972). The urea treatment eliminated all proteolytic activity which normally migrated with fraction A. Fractions were dialysed against water and freeze-dried or dissolved in HST and kept frozen at −20 °C for up to one year. Yields were too low to weigh accurately (of the order of μg) and therefore only a relative quantitative measure is used, according to which 1 Equivalent (Eq) corresponds to the amount of glycopeptide, in any class, produced from 10^6 cells in culture. Thus 10^9 cells would yield 10^3 Eq of A, B or their subfractions.

For high-voltage electrophoresis desialysed fraction A was streaked on Whatman 3 M paper and run for 90 min at 2000 V (25 mA) and 2 °C using a pyridine–acetate–water buffer (8·05/1·8/990, pH 5·6). Three subfractions migrated as follows: A_1, 1·0–13·2 cm; A_2, 13·3–25·9 cm; A_3, 26–36 cm (see also Warren *et al.* 1974). These areas were cut out, eluted with water and detoxified by Bio Gel P_2-water chromatography then freeze-dried.

Human urinary glycoproteins (fractions: DEAE I, F31, F33 and F34) were highly purified by DEAE chromatography and kindly donated by Dr Yves Goussault (Paris). Other oligosaccharides and enzymes were purchased from Sigma Chemical Co. (St Louis, Mo.).

Glycopeptide modification

Fractions A and B were desialysed by pretreatment with 2 units/ml of *Vibrio cholerae* NANase at 37 °C for 3 h (Warren *et al.* 1972). One unit of enzyme is the number of μg of NAN split from α_1-acid glycoprotein in 15 min at 37 °C in a solvent of normal saline containing 1 mg/ml of $CaCl_2$ and buffered to pH 5·5 with 0·05 M acetate. Electrophoretically purified and desialysed subfraction A_3 was also pretreated with 18 units/ml of fungal galactose oxidase (type I) in HST at 37 °C for 60 min. In order to treat cells with the enzyme it was necessary to add 300 units/ml of catalase which prevented damage due to peroxide formation. After the pretreatments of glycopeptide, enzyme activities were destroyed by immersion in a boiling water bath for 3 min. Galactose oxidase activity was checked using a Galactostat (Worthington Biochemical Corp., Freehold, New Jersey). One unit of enzyme (130 units/mg) causes a change in absorbance at 425 nm of 1/min at pH 6 and 25 °C in a peroxidase *o*-toluidine system of 3·4 ml.

G-50 purified fraction A was also incubated with 10 mM sodium periodate for 100 min at room temperature. Then 10 μl of 1·3 M glycerol were added and the mixtures were dialysed overnight against water before being freeze-dried.

RESULTS

Inhibition of cell aggregation by BHK21 glycopeptides

When the pronase-treated supernatant from sparsely grown BHK21 cells is chromatographed on Sephadex G-50, fraction A appears as a shoulder of B but is relatively less prominent when derived from confluent, slower-growing cultures (Buck, Glick & Warren, 1971). The fractionation is incomplete and some A is found in fraction B (Warren *et al.* 1974). NANase pretreatment of the cell or the isolated fractions

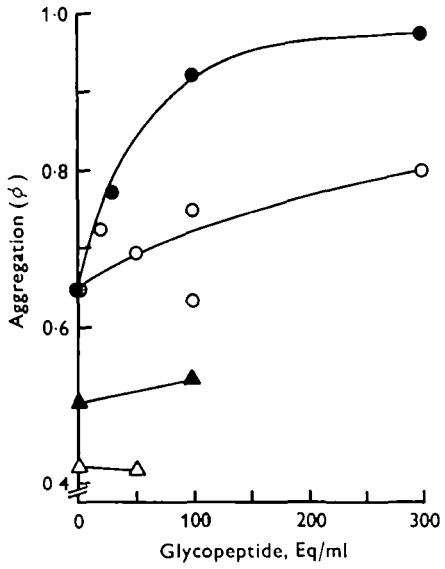

Fig. 1

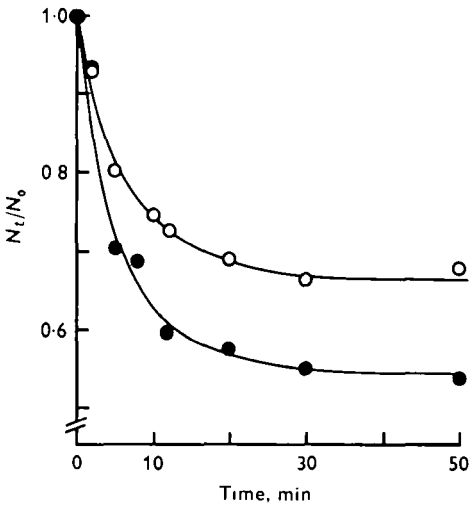

Fig. 2

Fig. 1. Effect of glycopeptides from BHK21 and C_{13}-B_4 on aggregation of BHK21 cells. Separate flasks were prepared at 2 °C containing 1·4 ml of HST with 1×10^6 single cells/ml and different concentrations of [^{3}H]fucose-labelled BHK21 fraction A (16·7 cpm/Eq) ●, fraction B (23·7 cpm/Eq) ○; or [^{14}C]glucosamine-labelled C_{13}-B_4 fraction A (28·7 cpm/Eq) ▲, fraction B (88·5 cpm/Eq) △. Flasks were then shaken reciprocally at 37 °C and the cell concentrations counted electronically. From each flask 4 samples of 0·1 ml were taken at t = 0 and at t = 30 min. The BHK21 material was obtained from one cell culture.

Fig. 2. Inhibition of BHK21 cell aggregation by an electrophoretically purified component of BHK21 fraction A. [^{3}H]fucose-labelled fraction A (34·8 cpm/Eq) was desialysed by NANase treatment and subfractionated by high-voltage electrophoresis on paper. Flasks contained $0{\cdot}7 \times 10^6$ cells/ml in 2 ml HST ●, or HST + 13·2 Eq/ml of fraction A_3 ○.

causes them to migrate less quickly and virtually as one peak during chromatography (Warren *et al.* 1972). The behaviour of the glycopeptides from C_{13}–B_4 cells is identical to that of sparse, fast-growing BHK21 and the A and B fractions of both lines co-chromatograph (Buck *et al.* 1970). One general difference between A and B is the presence of 2 additional sialic acid molecules on A. Both fractions act as sialic acid acceptors although fraction A must first be desialysed (Warren *et al.* 1972).

In spite of the close similarities between fraction A and B glycopeptides, BHK21 fraction A was capable of completely inhibiting BHK21 cell aggregation, while fraction B was much less active (Fig. 1). Five different preparations of A and B were each tested several times. On the basis of Eq the activity of fraction A varied from that shown for the one preparation in Fig. 1 more or less by a factor of 2. However, the low activity of B was often not seen, even though in equal Eq of A and B more glycopeptide by far is contained in the latter. Perhaps its variability results from the inclusion of some A during fractionation on the G-50 column. That the fractions did not disperse aggregates is a sensitive indication that no proteolytic activity was present

Table 1. *Effects of several oligosaccharides and intact and desialysed human urinary glycoproteins on BHK 21 aggregation*

Addition	Concentration, mg/ml	Aggregation: difference from control
Raffinose	59·5	−0·025
Stachyose	3·84	+0·045
Glycoproteins:		
DEAE I	2·61	+0·075
F 31	1·80	+0·055
F 31 + NANase	1·80	+0·050
F 33	1·78	+0·140
F 33 + NANase	1·78	+0·175
F 33 + NANase	5·00	+0·085
F 34	1·78	−0·040
F 34 + NANase	1·78	+0·115
F 34 + NANase	5·00	+0·090

Experimental conditions were as in Fig. 2. Suspensions with and without additions (controls) were compared after 30 min of aggregation ($\phi_{control} - \phi_{addition}$). + indicates inhibition of aggregation compared to the control. In some experiments 1·09 units/ml of NANase were added to control and glycoprotein-containing suspensions at $t = 0$.

and suggests they inhibit only the process of adhesive bond formation. In contrast to these results, fractions A and B from C_{13}-B_4 cells never inhibited BHK21 aggregation although several different preparations were tried. In their low aggregation in shaken suspensions C_{13}-B_4 cells resemble sparsely grown BHK21. It would be interesting to learn if the activity of the BHK21 fractions is decreased when they are prepared from cells grown at lower densities. So far extremely low yields of material have prevented tests of this idea.

Purification of BHK21 fraction A by high-voltage electrophoresis after NANase treatment produced 3 subfractions, one of which, A_3, contained most of the total radioactivity. The limited amount of A_1 and A_2 obtained was tested together in one experiment but it had no effect on aggregation. However, subfraction A_3 inhibited aggregation when added in low concentrations (Fig. 2).

Effects of heterologous glycoproteins and oligosaccharides

Several human urinary glycoproteins and plant carbohydrates had little or no effect on cell aggregation (Table 1), even though rather high concentrations were used (milligrammes as compared to submicrogramme/ml amounts of fraction A). The reducing end of both oligosaccharides is terminated with α-galactose. All glycoproteins except DEAE I contained sialic acid; but the addition of NANase to the flasks, while increasing aggregation (ϕ) slightly by +0·065 (confluent cultures were used, see Vicker & Edwards, 1972) did not induce any latent inhibitory activity in the glycoproteins. In similar experiments Lloyd & Cook (1974) found the NANase-stimulated portion of the aggregation of rat fibroblasts was inhibited by 1 mg/ml of desialysed bovine submaxillary mucin. It is not possible to draw a similar conclusion from the results in Table 1 because of the small effect of NANase.

Increase of BHK21 glycopeptide inhibitory activity

When fractions A and B from BHK21 cells were desialysed with NANase their inhibitory activity was increased (Fig. 3), although that of B only slightly. The effect of NANase on fraction A was not seen in every preparation and the cause of this

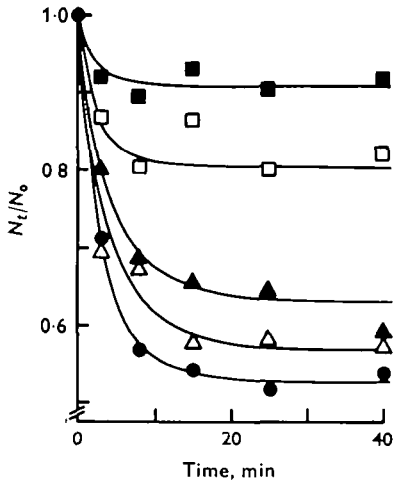

Fig. 3. Effect of NANase-pretreated BHK21 fraction A and B on BHK21 cell aggregation. The glycopeptides were pretreated with NANase and enzyme activity destroyed by incubation for 3 min in a boiling waterbath. Each flask contained $0{\cdot}5 \times 10^6$ cells/ml in 2 ml HST with either 20·9 Eq/ml fraction A ([^{14}C]fucose-labelled, 15·6 cpm/Eq) untreated □, or NANase-treated ■; or 24·1 Eq/ml fraction B ([^{3}H]fucose-labelled, 314 cpm/Eq) untreated △, or NANase-treated ▲; or no addition ●.

variation is unknown. Possibly overlong storage of some preparations increased the loss of sialic acid from its relatively labile position so as to preclude any additional effect of NANase. Variability might also arise in the substructure of cell surface heteroglycans due to growth at different culture densities.

The increase in fraction A and B activity after NANase treatment parallels the effect of the enzyme in increasing cell aggregation. This suggests that the newly exposed sialic acid acceptors, β-galactosyl and β-*N*-acetylgalactosaminyl groups, on both the cell surface (Roseman, 1970; Vicker & Edwards, 1972) and the isolated glycopeptides are important in cell aggregation. Since both fractions A and B and some of the urinary glycoproteins contain sialic acid, it might be that some sialic acid acceptors are more important than others. Lloyd & Cook (1974) have shown that the NANase-stimulated increase in rat fibroblast aggregation is reversed by the presence of some types of desialysed glycoproteins, but not others. However, the ability of fraction A to inhibit aggregation completely, when intact and without demonstrable ability to accept more sialic acid, indicates its activity might not rest on exposed sialic acid acceptors alone.

This point is also raised by the difference in aggregation between sparsely and densely grown cells. Cells harvested from cultures of growth density up to 5×10^4 cells/cm^2 show almost no aggregation (Edwards & Campbell, 1971; Vicker & Edwards, 1972; O'Neill, 1973). One explanation of this difference may be that increased growth density results in more incomplete, or degraded, cell surface polysaccharides which lack sialic acid and are therefore more adhesive. Pronase-treated glycopeptides from these cells would be found more frequently in fraction B during G-50 chromatography. But the low inhibitory activity of fraction B in comparison to that of A, from the same densely grown cells, argues against this possibility.

Inhibition of fraction A activity

The ability of BHK21 fraction A and of desialysed A to inhibit cell aggregation was destroyed after the glycopeptides were pretreated with periodate (Fig. 4). The loss of inhibitory activity is consistent with the sensitivity of galactosides to periodate, but other important terminal or internal carbohydrates of the glycopeptide might also have been attacked.

The activity of fraction A was also destroyed after it was pretreated with galactose oxidase (Fig. 5). This result is similar to those of Chesney, Harper & Colman (1972) and Vermylen, Donati, De Gaetano & Verstraete (1973), who found that treatment of collagen and blood factor VIII, respectively, with galactose oxidase destroyed the ability of these glycoproteins to adhere to platelets. On the other hand, the activity of a glycoprotein obtained from chick neural retina cells, which promotes cell clumping, was not affected by either NANase, periodate or galactose oxidase, although it was abolished by trypsin treatment (Hausman & Moscona, 1975).

As with the effect of NANase there is a parallel effect of galactose oxidase in inhibiting fraction A activity and cell aggregation (Fig. 6). Five to ten times higher enzyme concentrations caused cell damage, but this was prevented by the inclusion of 300 units/ml of catalase in the cell suspension. The effect of the enzyme on cells is unlike that caused by trypsin, which also disperses previously formed aggregates (Edwards & Campbell, 1971). In any case, overnight incubations of galactose oxidase with Azocoll revealed no proteolytic activity. The results suggest that terminal α- or β-galactosyl or *N*-acetylgalactosaminyl groups on cell surface polyglycans or on

fraction A are indispensable for their activity in aggregation. However, until a purified form of this enzyme can be prepared and tested this conclusion must be regarded as tentative.

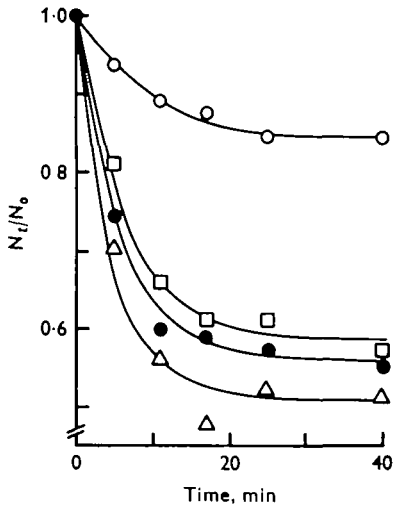

Fig. 4

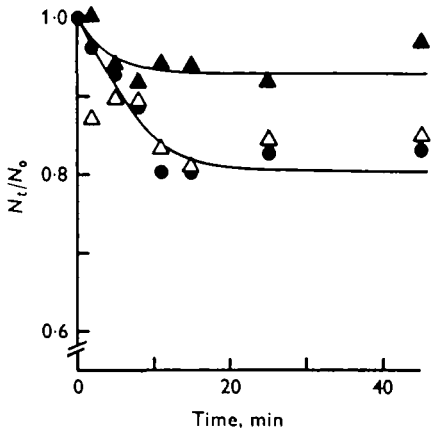

Fig. 5

Fig. 4. Effect of periodate-pretreated fraction A on BHK21 aggregation. Intact and desialysed BHK21 fraction A ([^{3}H]fucose-labelled, 34·8 cpm/Eq) was pretreated with periodate and added to the aggregation flasks. Each flask contained 2 ml of HST with $0{\cdot}6 \times 10^6$ cells/ml and 7·7 Eq/ml fraction A untreated ○, periodate-pretreated □, NANase and periodate-pretreated △, or no addition ●.

Fig. 5. Effect of galactose oxidase-pretreated subfraction A_3 on BHK21 cell aggregation. Electrophoretically purified BHK21 subfraction A_3 (from Fig. 2) was pretreated with galactose oxidase and the enzyme activity was then destroyed by incubation for 3 min in a boiling waterbath. Flasks contained 2 ml of HST with $0{\cdot}55 \times 10^6$ cells/ml and 9·7 Eq/ml of subfraction A_3: untreated ▲, enzyme-pretreated △, or no addition ●, The cells were harvested from a sparsely grown culture and therefore aggregated little.

DISCUSSION

It is likely that the cohesion of BHK21 cells is caused by molecules integral to the plasma membrane. Soluble molecules or cements (Humphreys, 1963) have not been demonstrated with this cell type (Edwards *et al.* 1975). In at least 4 ways the results suggest that BHK21 fraction A, and possibly B, are remnants of plasma membrane glycoproteins which normally cause cell cohesion. (1) It is possible to inhibit BHK21 aggregation to any degree by treatment with tryptic enzymes or pronase (Edwards & Campbell, 1971; Edwards *et al.* 1975; and our unpublished observations) which remove a number of large glycopeptides from the plasma membrane (Buck *et al.* 1970). These fragments originate from the cell surface because trypsin-labile, cytoplasmic glycoproteins are not disturbed by the treatment of intact cells (Soslau, Fuhrer, Nass & Warren, 1974). The tryptic fragments can bind to cell surfaces (Allen & Minnikin, 1975) and, when pronase-digested, some of the smaller glycopeptides

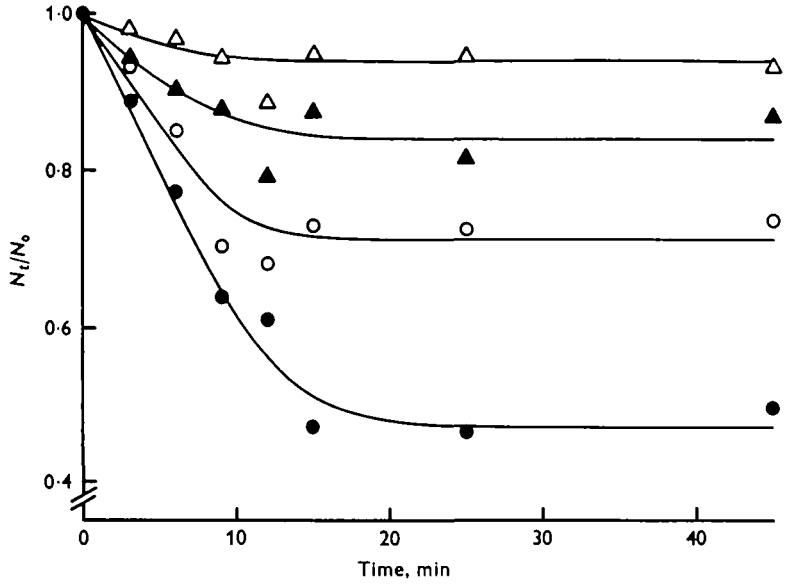

Fig. 6. Inhibition of BHK21 aggregation by the presence of galactose oxidase. 1×10^6 cells/ml were suspended in 4 ml of HST in each flask. HST containing galactose oxidase was added at $t = 0$ to the flasks which were then shaker incubated at 37 °C. The final enzyme concentration in units/ml was: no addition ●, 1·13 ○, 2·25 ▲, 5·62 △.

inhibit the aggregation of lightly trypsinized cells in proportion to the amount of glycopeptide present. (2) The parallel effects of NANase and galactose oxidase on cell–cell and cell–glycopeptide interactions imply that both the cell surface molecules important in aggregation and the isolated glycopeptides have a similar sensitivity to the enzymes. (3) The destruction of inhibitory activity of the glycopeptide by periodate and galactose oxidase, and its enhancement by NANase, demonstrate the primary importance in aggregation of (*a*) carbohydrates, which are believed to project from the cell surface, and (*b*) their β-galactoside, or possibly α-galactoside, terminal groups. The effect of galactose oxidase indicates that terminal sugars other than galactosides are of little or no importance. However, it is possible that the heteroglycan substructure may also determine inhibitory activity, at least to some extent. This conclusion follows consideration of the difference in activity between fractions A and B, between BHK21 and C_{13}-B_4 fractions and the lack of effect of the other molecules tested. Thus, molecules closely related to BHK21 fractions A and B and others terminating with α- or β-galactoside groups, and sialic acid acceptors, are not generally capable of inhibiting BHK21 aggregation. (4) The ability of a small amount of BHK21 fraction A to inhibit aggregation completely suggests intercellular glycoprotein (or glycolipid) polysaccharide-receptor interactions might fully account for all aggregation measurable in the BHK21-shaken suspension system. The ability of fraction A is like that of hog gastric mucosa glycopeptides (Allen & Minnikin, 1975) and glycoprotein from neural retina cell plasma membrane (Merrell *et al.* 1975), but unlike that of modified bovine submaxillary mucin and fetuin, which inhibited only

the NANase-stimulated portion of rat fibroblast aggregation (Lloyd & Cook, 1974).

These results also support the suggestions that cell cohesion may be described in terms of chemical specificity in ligand-receptor interactions (see Roseman, 1970). In this sense cell aggregation inhibition occurs when the soluble fraction A, or B, competes with intact, A- or B-containing cell surface glycoproteins for binding sites on plasma membrane receptors. Several authors have proposed that cell surface glycosyltransferases, especially those for sialic acid and for galactose, may function directly as adhesive receptors by binding their specific substrate, or product, on the cell surface (Roseman, 1970; Barber & Jamieson, 1971*a*, *b*; Bosmann, 1971; Roth, McGuire & Roseman, 1971; Aronson, Tan & Peters, 1973; Lloyd & Cook, 1974). However, the incidence of sialyl- and galactosyltransferases on the surface of BHK21 cells does not support these arguments. The plasma membrane of sparsely grown cells contains a much higher amount of sialyltransferase specific for desialysed fraction A than does the membrane of confluent, slowly growing cells (Warren *et al.* 1972). Yet, in shaken suspension, cells from densely grown cultures aggregate far more than those sparsely grown (Edwards & Campbell, 1971; O'Neill, 1973). Furthermore, no galactosyltransferase activity has been detected on the surface of BHK21 cells (Deppert, Werchau & Walter, 1974).

These findings do not eliminate the possibility that sialyltransferase is involved as an adhesive receptor in the aggregation stimulated by NANase treatment of the cells. But neither transferase appears to be directly involved in the aggregation of lightly trypsinized cells or in the growth-density-determined differences in aggregation. They might effect aggregation indirectly by regulating the types of terminal sugars on cell surface heteroglycans. In these terms sialic acid would act to mask their acceptors, β-galactosides, and prevent their expression as adhesive sites (Vicker & Edwards, 1972; Lloyd & Cook, 1974).

Galactose-binding proteins have been isolated from plasma membranes and might also act as adhesive receptors in cellular slime mould aggregation (Rosen, Simpson, Rose & Barondes, 1974) and in liver cell membrane-glycoprotein adhesion (Hudgin *et al.* 1974). Recently, Aronson *et al.* (1973) suggested that the latter is a galactosyltransferase, in part, because lactalbumin inhibited the binding of desialysed fetuin to cell membranes. However, while lactalbumin is an inhibitor of galactosyltransferase, when glucose is used as an acceptor it has no effect on transferase activity towards glycoprotein acceptor (Roseman, 1970).

Apart from their possible binding to receptors, specifically structured polyglycans might also be capable of sticking to one another by hydrogen bonds formed between their glycosyl units (Roseman, 1970). Alternatively, calcium bridging between particular cell-surface sugars has been suggested as an intercellular binding mechanism (see Cook & Bugg, 1975). But while Ca^{2+} is necessary for many physiological functions related to cell adhesion, the cohesion of BHK21 cells is rapid and nearly unaffected by the absence of all divalent cations in the medium (Edwards *et al.* 1975).

Other explanations for the effects of glycopeptides on cell aggregation might also be advanced. Aggregation inhibition caused by the binding of fraction A to the cell surface does not seem to depend on the acquisition of additional negatively charged

sialic acid groups. While these would tend to repel similarly charged cell surfaces (Curtis, 1967), the increase in fraction A activity after NANase pretreatment suggests the underlying β-galactoside groups are more important for inhibition. The adsorption of some glycopeptides to the cell surface might induce a general physiological response affecting aggregation, but this possibility has not been tested. McDonough & Lilien (1975) have examined the activity of a glycoprotein from neural retina cells which is released during culture for 48 h without serum. They found glycopeptides produced by pronase digestion of the protein adhered specifically to neural retina cells and caused a general inhibition of lectin-induced antigen cap formation. However, patch formation was not affected. Terminal *N*-acetylgalactosamine groups on the glycopeptide were required for its activity (Balsamo & Lilien, 1974). Nevertheless, these glycopeptide fragments have no known effect on neural retina cell aggregation. Trypsin digestion of the parent glycoprotein destroyed its ability to increase the clumping of neural retina cells while galactose oxidase had no effect (Hausman & Moscona, 1975).

Since BHK21 cells are able to adhere to a variety of other cell types (Walther, Öhman & Roseman, 1973) and since one species of glycopeptide inhibits the adhesion of both BHK21 and HeLa cells (Allen & Minnikin, 1975) the chemical or structural specificity of adhesive, cell surface molecules may be shared. Therefore fraction A ought to inhibit HeLa cell aggregation and possibly that of other cell types as well. But it seems unlikely that cell social interactions important, for example, in migration, metabolic cooperation and patterning in culture and in tissues are determined only through the adhesion of their surfaces. Physiological reactions by cells to adhesion may have led to its control perhaps through the distribution, organization and metabolic turnover of adhesive plasma membrane elements (Waddell *et al.* 1974; Edwards *et al.* 1975), as well as to the specific structuring of cell surface heteroglycans and their receptors. The ability of cells to regulate their surface properties might determine cell adhesion and social behaviour in ways quite unlike that possible by binding between cell surface ligands and receptors (see Steinberg, 1964, for example). The introduction of fraction A or similar molecules to cell cultures may help discriminate between cell interactions of 3 sorts: those determined by co-operation between adhesive cell surface molecules and other plasma membrane or cytoplasmic elements; those occurring with only the involvement of adhesive cell surface; and those requiring no intercellular cohesion.

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REFERENCES

ALLEN, A. & MINNIKIN, S. M. (1975). The binding of the mucoprotein from gastric mucus to cells in tissue culture and the inhibition of cell adhesion. *J. Cell Sci.* **17**, 617–631.

ARONSON, N., TAN, L. Y. & PETERS, B. P. (1973). Galactosyl transferase – the liver plasma membrane binding-site for asialo-glycoproteins. *Biochem. biophys. Res. Commun.* **53**, 112–118.

BALSAMO, J. & LILIEN, J. (1974). Embryonic cell aggregation: kinetics and specificity of binding of enhancing factors. *Proc. natn. Acad. Sci. U.S.A.* **71**, 727–731.

BALSAMO, J. & LILIEN, J. (1975). The binding of tissue-specific adhesive molecules to the cell surface. A molecular basis for specificity. *Biochemistry, N.Y.* **14**, 167–171.

BARBER, A. J. & JAMIESON, G. A. (1971*a*). Platelet collagen adhesion: characterization of collagen glucosyltransferase of plasma membranes of human blood platelets. *Biochim. biophys. Acta* **252**, 533–545.

BARBER, A. J. & JAMIESON, G. A. (1971*b*). Characterization of membrane-bound collagen galactosyltransferase of human blood platelets. *Biochim. biophys. Acta* **252**, 546–552.

BARTON, N. W. & ROSENBERG, A. (1973). Action of *Vibrio cholerae* neuraminidase (sialidase) upon the surface of intact cells and their sialolipid components. *J. biol. Chem.* **248**, 7353–7358.

BOSMANN, H. B. (1971). Platelet adhesiveness and aggregation: the collagen:glycosyl, polypeptide:*N*-acetylgalactosaminyl and glycoprotein:galactosyl transferases of human platelets. *Biochem. biophys. Res. Commun.* **43**, 1118–1124.

BUCK, C. A., GLICK, M. C. & WARREN, L. (1970). A comparative study of glycoproteins from the surface of control and Rous sarcoma virus transformed hamster cells. *Biochemistry, N.Y.* **9**, 4567–4576.

BUCK, C. A., GLICK, M. C. & WARREN, L. (1971). Effect of growth on the glycoproteins from the surface of control and Rous sarcoma virus transformed hamster cells. *Biochemistry, N.Y.* **10**, 2176–2180.

CHESNEY, C. M., HARPER, E. & COLMAN, R. W. (1972). Critical role of the carbohydrate side chains of collagen in platelet aggregation. *J. clin. Invest.* **51**, 2693–2701.

COOK, W. J. & BUGG, C. E. (1975). Calcium-carbohydrate bridges composed of uncharged sugars, structure of a hydrated calcium bromide complex of α-fucose. *Biochim. biophys. Acta* **389**, 428–435.

CURTIS, A. S. G. (1967). *The Cell Surface: Its Molecular Role in Morphogenesis.* New York: Academic Press.

DEMAN, J. J., BRUYNEEL, E. A. & MAREEL, M. M. (1974). A study on the mechanism of intercellular adhesion, effects of neuraminidase, calcium and trypsin on the aggregation of suspended HeLa cells. *J. Cell Biol.* **60**, 641–652.

DEPPERT, W., WERCHAU, H. & WALTER, G. (1974). Differentiation between intracellular and cell surface glycosyl transferases: galactosyl transferase activity in intact cells and in cell homogenate. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3068–3072.

EDWARDS, J. G. (1973). Intercellular adhesion. In *New Techniques in Biophysics and Cell Biology* (ed. R. Pain & B. J. Smith), pp. 1–27. London: Wiley.

EDWARDS, J. G. (1976). Cell adhesion. In *Mammalian Cell Membranes*, vol. 4 (ed. G. A. Jamieson & D. M. Robinson) (in Press). London: Butterworths.

EDWARDS, J. G. & CAMPBELL, J. A. (1971). The aggregation of trypsinized BHK21 cells. *J. Cell Sci.* **8**, 53–71.

EDWARDS, J. G., CAMPBELL, J. A., ROBSON, R. T. & VICKER, M. G. (1975). Trypsinized BHK21 cells aggregate in the presence of metabolic inhibitors and in the absence of divalent cations. *J. Cell Sci.* **19**, 653–667.

HAUSMAN, R. E. & MOSCONA, A. A. (1975). Purification and characterization of the retina-specific cell-aggregating factor. *Proc. natn. Acad. Sci. U.S.A.* **72**, 916–920.

HUDGIN, R. L., PRICER, W. E. Jr., ASHWELL, G., STOCKERT, R. J. & MORELL, A. G. (1974). The isolation and properties of a rabbit liver binding protein specific for asialoglycoproteins. *J. biol. Chem.* **249**, 5536–5543.

HUMPHREYS, T. (1963). Chemical dissolution and *in vitro* reconstruction of sponge cell adhesions. I. Isolation and functional demonstration of the components involved. *Devl Biol.* **8**, 27–47.

LLOYD, C. W. & COOK, G. M. W. (1974). On the mechanism of increased aggregation by neuraminidase of 16C malignant rat dermal fibroblasts *in vitro*. *J. Cell Sci.* **15**, 575–590.

MCDONOUGH, J. & LILIEN, J. (1975). Inhibition of mobility of cell-surface receptors by factors which mediate specific cell–cell interactions. *Nature, Lond.* **256**, 416–417.

MERRELL, R., GOTTLIEB, D. I. & GLASER, L. (1975). Embryonal cell surface recognition, extraction of an active plasma membrane component. *J. biol. Chem.* **250**, 5655–5659.

MORELL, A. G., GREGORIADIS, G., SCHEINBERG, I. H., HICKMAN, J. & ASHWELL, G. (1971). The role of sialic acid in determining the survival of glycoproteins in the circulation. *J. biol. Chem.* **246**, 1461–1467.

O'NEILL, C. H. (1973). Growth induction by serum or polyoma virus inhibits the aggregation of trypsinised suspensions of BHK21 tissue culture fibroblasts. *Expl Cell Res.* **81**, 31–39.

ROSEMAN, S. (1970). The synthesis of complex carbohydrates by multiglycosyltransferase systems and their potential function in intercellular adhesion. *Chem. Phys. Lipids* **5**, 270–297.

ROSEN, S. D., SIMPSON, D. L., ROSE, J. E. & BARONDES, L. H. (1974). Carbohydrate-binding protein from *Polysphondylium pallidum* implicated in intercellular adhesion. *Nature, Lond.* **252**, 128, 149–151.

ROTH, S., MCGUIRE, E. J. & ROSEMAN, S. (1971). Evidence for cell-surface glycosyltransferases, their potential role in cellular recognition. *J. Cell Biol.* **51**, 536–547.

SOSLAU, G., FUHRER, J. P., NASS, M. M. K. & WARREN, L. (1974). The effect of ethidium bromide on the membrane glycopeptides in control and virus-transformed cells. *J. biol. Chem.* **249**, 3014–3020.

STEINBERG, M. S. (1964). The problem of adhesive selectivity in cellular interactions. In *Cellular Membranes in Development* (ed. M. Locke), pp. 321–366. New York: Academic Press.

VERMYLEN, J., DONATI, M. B., DE GAETANO, G. & VERSTRAETE, M. (1973). Aggregation of human platelets by bovine or human factor. VIII. Role of carbohydrate side chains. *Nature, Lond.* **244**, 167–168.

VICKER, M. G. & EDWARDS, J. G. (1972). The effect of neuraminidase on the aggregation of BHK21 cells and BHK21 cells transformed by polyoma virus. *J. Cell Sci.* **10**, 759–768.

WADDELL, A. W., ROBSON, R. T. & EDWARDS, J. G. (1974). Colchicine and vinblastine inhibit fibroblast aggregation. *Nature, Lond.* **248**, 239–241.

WALTHER, B. T., ÖHMAN, R. & ROSEMAN, S. (1973). A quantitative assay for intercellular adhesion. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1569–1573.

WARREN, L., FUHRER, J. P. & BUCK, C. A. (1972). Surface glycoproteins of normal and transformed cells: a difference determined by sialic acid and a growth-dependent sialyl transferase. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1838–1842.

WARREN, L., FUHRER, J. P., BUCK, C. A. & WALBORG, E. F. JR. (1974). Membrane glycoproteins in normal and virus transformed cells. In *Membrane Transformations in Neoplasia*, Miami Winter Symposia, vol. 8, pp. 1–26. New York and London: Academic Press.

WEINSTEIN, D. B., MARSH, J. B., GLICK, M. C. & WARREN, L. (1970). Membranes of animal cells. VI. The glycolipids of the L cell and its surface membrane. *J. biol. Chem.* **245**, 3928–3937.

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