Response of aggregating chick corneal cells to modifiers of N-linked oligosaccharides, endoglycosidase H and deoxymannojirimycin

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Summary

Chick corneal epithelium takes on its mature conformation between 11 and 16 days of incubation. Earlier work has shown that desmosome frequency increases during this period, reaching its highest rate at 15½ days. In the present report aggregation rates of cells from embryos of 11 days and those of 15½ days are compared. Younger cells, which form fewer desmosomes, aggregate at a more moderate rate than older cells. In addition, younger cells bind less concanavalin A (ConA) than older cells. To determine if increase in ConA binding could be related to these cellular responses, aggregating cells were exposed to endoglycosidase H (EndoH) and to deoxymannojirimycin. This treatment should permit comparison of the response of cells that have a normal complement of N-linked oligosaccharides with those that have reduced high-mannose or complex type sugars. The effectiveness of EndoH under the conditions used was confirmed by failure of treated glycoprotein after separation by SDS-PAGE and electrophoresis to bind ConA. Aggregation rates of both older and younger cells were unaffected, as measured by disappearance of single cells, though older cells formed somewhat smaller aggregates at the highest dosage used. Desmosome formation was markedly reduced in the presence of the enzyme, even in the absence of other changes in the fine structure. At the highest dose of the enzyme the fine structure of older but not younger cells showed indications of blockage of transport. Deoxymannojirimycin appears to cause a build-up of high-mannose groups, since treated cells showed increased incorporation of [3H]mannose. In addition, SDS-PAGE of an NP40-extracted cell fraction showed a similar distribution of proteins, but incorporation of [3H]mannose differed between inhibitor-treated cells and controls. There was no effect on cell fine structure and junctional frequency was normal. Thus N-linked sugars appear to be important in this system to permit re-formation of normal cell contacts, but there is no evidence that processing is required.

Key words: endoglycosidase H, deoxymannojirimycin, desmosomes, cell adhesion.

Introduction

Desmosome formation in developing chick corneal cells has been quantified in vivo and in culture (Overton, 1973; Overton & DeSalle, 1980). The corneal epithelium begins to take on its differentiated conformation after the tenth day of incubation, when there is a gradual increase in the number of desmosomes as the epithelium becomes increasingly stratified (Hay & Revel, 1969). Between the 15th and 16th days of incubation desmosome formation reaches its most rapid rate. At this time dissociated cells aggregate rapidly and desmosomes in these aggregates are numerous (Overton & DeSalle, 1980). In the present report corneal cells at 15½ and 11 days are compared. Cells aggregated at 11 days of incubation, which form few desmosomes, aggregate more slowly than older cells and bind less concanavalin A (ConA). In order to try and obtain evidence connecting rapid adhesion and/or formation of adhesive junctions with increased ConA binding, corneal cells at these two stages were examined using agents that modify N-linked oligosaccharides.

Asparagine-linked oligosaccharides of cell surface glycoproteins may be modified by using inhibitors of enzymic processing (Elbein, 1984, 1987). This ap-
proach can be used to demonstrate a role for glyco-
proteins in a number of complex cellular responses
(Ratner et al. 1986). In this report the importance of
N-linked oligosaccharides is investigated using a pro-
cessing inhibitor, and also using enzymic treatment.
Endoglycosidase H removes high-mannose groups
from glycoproteins by hydrolyising the chitobiose core
of high-mannose oligosaccharides (Tarentino & Mal-
ley, 1974). In addition, it has also been shown to
deglycosylate the oligosaccharides of the dolichol car-
ter (Chalifour & Spiro, 1984). Thus high-mannose
oligosaccharides as well as the complex sugars resulting
from processing of high-mannose groups should be
affected. Enzymic treatment has the disadvantage that
it may, though not necessarily, interfere with transport
(reviewed by Ratner et al. 1986). In the present case
only high doses cause structural changes similar to
those seen with accumulation of non-glycosylated pro-
teins in intracellular membrane organelles (Olden et al.
1985; Schwartz & Datema, 1982; Hickman et al. 1977;
Rothman, 1987). Deoxymannojirimycin inhibits pro-
cessing of high-mannose precursors by blocking ac-
civity of mannosidases IA and IB, which remove the
α-(1→2)-linked residues from the high-mannose in-
termediate (Elbein, 1984, 1987; Elbein et al. 1984;
Therefore, by comparing EndoH- and deoxymannojiri-
mycin-treated cells with controls, one should be able
to compare the responses of corneal cells to the full
complement of oligosaccharides, the reduction of both
high-mannose and complex types, or the reduction of
complex sugars only. Using this approach the re-
sponses of cell adhesion and junction formation in
corneal cells have been examined.

Materials and methods

Cells and culture
Corneas were dissected from White Leghorn eggs incubated
at 38°C for 11, or 154 days (referred to here as 154, and
11-day corneas or corneal cells). Tissue was placed in collagenase
(200 units ml⁻¹; Sigma type VII) for 1 h at 37°C, after which the
epithelial sheet could be lifted off the stroma. Epithelial
sheets were dispersed into single cells by incubation in
calciu--magnesium-free Hanks' solution (CMF) with 0.5% EDTA
twice for 5 min at 37°C, then in 1·0% trypsin in CMF
with EDTA (Grand Island, 1:250). After rinsing three times
with Hanks' solution with trypsin inhibitor (Sigma, type IS,
1 mg ml⁻¹) and DNase (0·1 mg ml⁻¹, Worthington) cells were
 dissociated by gentle pipetting in culture medium.
Culture medium consisted of L-15 (Leibovitz) medium with
0.5% fetal calf serum, 100 U ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin,
and 2 mM-phenylmethylsulphonyl fluoride (PMSF), 5 µg ml⁻¹ leupeptin and 5 µg ml⁻¹ pepstatin (all from Sigma), then sonicated four times on ice at 15 s with
15-s rest periods using a Fisher Dismembrator model 150 at a
setting of 60%. The preparation was centrifuged 10 min at
10000 g and the pellet was washed twice in EDTA/
Tris·HCl. The pellet was then dispersed in 75 µl 1.0% NP40
with protease inhibitors and incubated 1 h at 4°C with
occasional mixing. Following incubation, the preparation was
centrifuged for 10 min at 10000 g and the supernatant
removed. The supernatant was assumed to contain NP40-
soluble proteins plus some possible insoluble material not
eliminated by the centrifugation schedule used. After
addition of an equal volume of 2xSDS sample buffer (2% SDS, 5% β-mercaptoethanol (BME) in Tris–glycine buffer)
preparations were loaded onto SDS–polyacrylamide gels
(Laemmli, 1970) prepared with 10% acrylamide and 0.6% bisacrylamide and run at 60 mA with Bio-Rad high molecular
weight markers (myosin, 200×10³ Mr; β-galactosidase, 116×10³ Mr; phosphorylase B, 92×10³ Mr; bovine serum
albumin, 66×10³ Mr; and ovalbumin, 45×10³ Mr). Gels were
stained with Coomassie Blue or silver (Wray et al. 1981).
To assay for distribution of [³H]mannose, destained gels were
embedded for fluorography and exposed to preflashed Kodak
XR5 X-ray film at ~70°C for 5–6 weeks (Laskey & Mills, 1975).

To assay for EndoH activity ConA–HRP (horseradish
peroxidase) binding to enzyme-treated ovalbumin on North-
een blots was used (Laskey & Mills, 1975). Gels were blotted
for 5 h at 70 V using the Hoeffer Electrotan transfer system with
Towbin buffer (Towbin et al. 1979). Nitrocellulose blots
were dried overnight, incubated for 30 min in 3% bovine

ppH 6·8 unless otherwise noted, and cells were cultured for
aggregation assays at a concentration of 1×10⁶ ml⁻¹ in 1 ml of
medium in 10 ml Erlenmeyer flasks on a rotary shaker at 37°C
and 75 rpm min⁻¹. To study aggregation cell counts were
made at each time point using 1·0 ml samples run in
tripletic. The number of single cells ml⁻¹ in suspension in
each sample was recorded and aggregates were ignored. Cell
counts were made using a haemocytometer.

Cell extraction and biochemical analysis
Cells harvested from 154-day embryos were dissociated and
placed in culture medium with and without the inhibitor
dMM at 1×10⁵ or 5×10⁵ cells ml⁻¹. Flasks were rotated at
125 revs min⁻¹ for 1 h, then, after addition of 100 µCi ml⁻¹ of
[³H]mannose, rotation was reduced to 75 revs min⁻¹ and cells
were allowed to aggregate for 6 h. To assay for mannose
incorporation by whole cells, aggregates were washed three
times in PBS (130 mM-NaCl, 2 mM-KCl, 8 mM-Na₂HPO₄,
1 mM-KH₂PO₄, 0·5 mM-MgCl₂, 1 mM-CaCl₂, pH 7·2), the
sample from each flask was solubilized in 1·0 ml 1·0% SDS
overnight, then transferred to scintillation tubes, 1·0 ml of
Triton-based scintillation fluid was added, and samples were
assayed for radioactivity using a Packard Tri-Carb liquid
scintillation counter. To assay for [³H]mannose incor-
poration of an NP40 fraction, cells labelled with [³H]mannose
as described above and washed in PBS were stored at ~70°C.
Samples of 5×10⁶ inhibitor-treated cells and an equal num-
ber of control cells were used for fractionation. Cells were
deroftered in ice, rinsed in cold 10 mM-EDTA in 10 mM-
Tris·HCl, pH 7.4, with 2 mM-phenylmethylsulphonyl
fluoride (PMSF), 5 µg ml⁻¹ leupeptin and 5 µg ml⁻¹ pepstatin (all
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were dried overnight, incubated for 30 min in 3% bovine
serum albumin in PBS, rinsed in 50 mM-Tris·HCl with 0.1 mM-MnCl₂, pH 7.4 (twice for 10 min), incubated in 0.1 mg·ml⁻¹ ConA-HRP (EY Laboratories for 1 h, then rinsed with PBS and reacted with 4-chloro-1-naphthol (Hawks, 1982; King et al. 1985).

**ConA labelling of aggregated cells**

Cells were aggregated for 6 h at 1×10⁶ cells·ml⁻¹, pH 6.8, rinsed with PBS with 0.1 mM-MnCl₂ (PBS-Mn), incubated in graded concentrations of N-[acetetyl-³H]-acetylated ConA in PBS-Mn for 30 min at 37°C and rinsed four times with PBS-Mn (Elbein et al. 1983). Samples of 1×10⁶ cells were solubilized overnight in 1-0% SDS, transferred to scintillation tubes, 1-0 ml of Triton-based scintillation fluid was added, and samples were assayed for radioactivity.

**Electron microscopy**

Cultured cells were fixed in Karnovsky's fixative (Karnovsky, 1965) for 30 min at room temperature, post-fixed for 1 h in 1% osmium tetroxide in the cold, dehydrated in a series of ethanol and embedded in Epon. Thin sections were stained with uranyl acetate and lead acetate, and viewed with a Hitachi HU 11A electron microscope. For a quantitative comparison of desmosome frequency, desmosomes were scored as the presence of parallel plaques. Possible type II adherens plaques (Volberg et al. 1986; O'Keefe et al. 1987; Drenckhahn & Franz, 1987) were not distinguished. Micrographs taken at ×10000 and viewed at ×70000 (total magnification) were used to score the number of desmosomes/nucleus (Overtoil, 1973). This method was used, since cell surfaces in many aggregates tended to be quite irregular.

**Results**

**Aggregation rate and ConA binding of older and younger cells**

When chick corneal cells are aggregated in L15 culture medium, adjusted routinely by us for these cells at pH 7.4, the 15½-day cells aggregate precipitously after a short lag and by 4 h less than 10% of the cells are still single (Fig. 1, circles). In contrast, the 11-day cells aggregate at a more moderate rate, also after a short lag, so that by 4 h about 40% are still single (Fig. 1, squares). This difference in aggregation rate was conspicuous and consistent. A difference was also seen with medium adjusted to pH 6.8, the pH at which most of the experiments described here were carried out. In addition to the difference in aggregation rate, there was a distinct difference in aggregate size, the older cells forming larger aggregates (not illustrated).

An attempt was made to correlate the difference in response of older and younger cells with some developmental change in the property of the cells using lectin binding. Older cells not only adhere more readily, but form numerous desmosomes, which have been shown to bind ConA at the intercellular region (Shida et al. 1982; Steinberg, 1987). Using ³H-acetylated ConA binding in this system as an assay, older and younger cells were compared after 6 h of aggregation. Older cells clearly showed specific binding (total binding minus binding in the presence of mannose) while younger cells showed little if any (Fig. 2). In order to investigate this difference in lectin binding and cellular responses further, aggregating cells were treated with EndoH and dMM.

**Experiments with EndoH**

EndoH has a pH optimum (5-6) that is too low for cell culture, so it was necessary to find an intermediate pH at which cellular responses would be characteristic and the enzyme would still be active. At pH 6.8 cells

**Fig. 1.** Aggregation of corneal cells under standard conditions, pH 7.4. Symbols, mean values for three flasks; bars, standard errors; (○) 15½-day cells; (□) 11-day cells.

**Fig. 2.** Binding of N-[acetetyl-³H]-acetylated ConA to corneal cells (total binding). (○) 15½-day cells; (□) 15½-day cells incubated with ConA and 2 mM-mannose; (△) 11-day cells; (△) 11-day cells incubated with ConA and 2 mM-mannose. The amount of ConA bound after 5 min was the same as that at 30 min.

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aggregated, though with a slightly longer lag, and desmosomes formed rapidly in the aggregates. To test for enzyme activity at this pH, ovalbumen was incubated with EndoH, run on a polyacrylamide gel, blotted and stained for ConA binding (Fig. 3). Under these conditions ConA binding of the enzyme-treated glycoprotein was markedly reduced.

Cells were treated with EndoH at a dosage of 12.5 mIU and 2.0 mIU during aggregation. There was no apparent effect on aggregation rate as measured by disappearance of single cells even at the higher dosage with either 15i- or 11-day cells (Figs 4, 5). Aggregates at 6 h are very irregular in outline so that aggregate size is hard to quantify; however, visual inspection indicated that at 12.5 mIU of EndoH, the 15i-day cells formed somewhat smaller, less rounded aggregates (Fig. 6). There appeared to be no difference in the aggregate size of 11-day cells even at the higher dose (Fig. 6).

Desmosome frequency was compared in 15i-day cell aggregates at 6 h of aggregation (Table 1). In the presence of EndoH these junctions were much less common, their frequency being reduced by two thirds even at the lower dose. At 2 mIU, other aspects of cell fine structure showed little or no change (Fig. 7). In these cells both Golgi bodies and endoplasmic reticulum are prominent. In some cases the endoplasmic reticulum of treated cells appeared to be more abundant than that of controls, but this was not generally characteristic. At the higher EndoH dose a marked

Fig. 3. Effect of EndoH on ovalbumen. Ovalbumen was incubated in 0.1 M-phosphate buffer and 2 mM-PMSF with or without 12.5 mIU of EndoH for 3 h at 37°C, boiled for 2 min in sample buffer and loaded at 1 μg per lane on a polyacrylamide gel, blotted and stained with ConA–HRP. Lane 1, ovalbumen; lane 2, ovalbumen with EndoH; lane 3, molecular markers. Of the molecular markers, only ovalbumen binds ConA heavily.

Fig. 4. Corneal cells at 15i days, aggregated in the presence of 12.5 mIU of EndoH. (○) EndoH; (●) controls.

Fig. 5. Corneal cells at 11 days, aggregated in the presence of 12.5 mIU of EndoH. (○) EndoH; (●) controls.
Table 1. Desmosome formation in treated aggregates

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Desmosomes/nucleus Mean</th>
<th>S.E.</th>
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<tbody>
<tr>
<td>Corneas at 15i days</td>
<td></td>
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<tr>
<td>2 mIU EndoH</td>
<td>0.64</td>
<td>0.05</td>
</tr>
<tr>
<td>2 mIU EndoH with 5 mg ml⁻¹ leupeptin</td>
<td>0.72</td>
<td>0.10</td>
</tr>
<tr>
<td>12.5 mIU EndoH</td>
<td>0.30</td>
<td>0.08</td>
</tr>
<tr>
<td>Enzyme control</td>
<td>1.75</td>
<td>0.37</td>
</tr>
<tr>
<td>1 mM-deoxymannojirimycin</td>
<td>1.49</td>
<td>0.15</td>
</tr>
<tr>
<td>4 mM-deoxymannojirimycin</td>
<td>1.79</td>
<td>0.37</td>
</tr>
<tr>
<td>Inhibitor control</td>
<td>1.76</td>
<td>0.33</td>
</tr>
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</table>

* Mean represents counts of three to six samples of 18 micrographs each. When EndoH samples are compared with controls, P < 0.01.

Experiments with dMM
To evaluate the effectiveness of dMM, 15½-day corneal cells were incubated for 1 h in the presence of the inhibitor, then labelled for 6 h during aggregation with [³H]mannose at 100 μCi ml⁻¹. Blocking the processing of high-mannose oligosaccharides can result, in some cells, in an accumulation of high-mannose precursors (Elbein et al. 1983; Elbein, 1984).

In labelled corneal cells, isotope incorporation was greater in experimental samples (Table 2). In addition there was a change in distribution of the isotope. The NP40 fractions of treated and control cells were compared using SDS–PAGE and fluorography. The gels were loaded with equal counts and the distribution of isotope showed a pattern of incorporation in dMM-treated preparations that was distinct from that of controls (Fig. 10). No corresponding differences were seen in peptides after staining with silver or Coomassie Blue.

To assay for the effects of dMM on aggregation, cultures were incubated for 1 h in the presence of the drug at 125 revs min⁻¹ to inhibit aggregation while the cells became acclimatized, then at 75 revs min⁻¹ to allow aggregation to proceed. With both 15½-day and 11-day cells, no effects on aggregation were observed, as measured by the disappearance of single cells (Figs 11, 12), and no difference in aggregate size was noted.

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Fig. 7. EndoH-treated 15½-day cells (2 mIU) at 6 h of aggregation. G, Golgi bodies; and er, endoplasmic reticulum are prominent; n, nucleus. ×26,000.

Fig. 8. EndoH-treated 15½-day cells at 6 h (12.5 mIU); v, characteristic vacuole indicating blockage of transport; er, endoplasmic reticulum; arrow, association between er and vacuole. ×26,000.

Fig. 9. EndoH-treated 11½-day cells at 6 h (12.5 mIU). ×26,000. The er is sparse in contrast to Fig. 7 and there is no evidence of blockage of transport.

Table 2. [³H]mannose incorporation into dMM-treated aggregating 15½-day corneal cells*

<table>
<thead>
<tr>
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<th>Disintegrations min⁻¹</th>
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<tbody>
<tr>
<td>Control cultures</td>
<td></td>
</tr>
<tr>
<td>30.072</td>
<td>85,799</td>
</tr>
<tr>
<td>31.093</td>
<td>97,553</td>
</tr>
<tr>
<td>31.033</td>
<td>92,287</td>
</tr>
<tr>
<td>Cultures with 4 mM-dMM</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>30.072</td>
<td>85,799</td>
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<td>97,553</td>
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<tr>
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<td>92,287</td>
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</table>

*Samples consisted of 5 x 10⁶ 15½-day cells incubated for 1 h in dMM, then labelled for 6 h.

Formation of desmosomes during aggregation also appeared to be unaffected by the presence of dMM (Table 1).

The fine structure of dMM-treated cells, like controls, revealed numerous desmosomes (not illustrated) and both endoplasmic reticulum and Golgi bodies were common in the cytoplasm. In summary, during exposure to the dMM used in this study, both 11½-day and 15½-day cells formed aggregates, initiated typical cell contacts and appeared to respond to culture conditions in the same manner as controls, despite differences in [³H]mannose incorporation.

Discussion

The corneal epithelium of the chick becomes stratified between days 10 and 16 of incubation (Hay & Revel, 1969). In the mature condition, five or more strata of cells are present, so that the cell population has become composed of a large percentage of cells with no strict basal or apical organization, which adhere to each other at desmosome sites. Desmosomes appear to be the dominant junctions in many cells at later stages. At 15½ days of incubation desmosome frequency is increasing at its maximum rate (Overton, 1973). Eleven- and 15½-day cells are compared here with regard to cell surface changes. Dissociated epithelial cells will aggregate at a more rapid rate in culture in older stages. Older aggregating cells, which form more desmosomes, will also bind more ConA. This is consistent with what is known about desmosomal components.

Although terminology for desmosomal glycoproteins varies, recent reports (Steinberg et al. 1987; Franke et al. 1987; Mettay et al. 1986; Penn et al. 1987; Jones et al. 1986) are in general agreement that at least three major types occur, termed by Steinberg et al. (1987) desmogleins (DG1a,b,c, of average molecular mass 150×10³; DG11a,b, 97–118×10³; and DG11I,
Fig. 10. A. Gel of [3H]mannose-labelled 154-day cell NP40 fraction with silver staining. Lane 1, control; lane 2, dMM at 4 mM; arrows, molecular markers (Mx10^{-3}). B. Fluorogram of the same preparation. Lane 1, control; lane 2, DMM. Lanes were loaded with ∼54 000 counts and exposed for 5 weeks. Some heavily labelled bands in the experimental lane are only lightly or faintly labelled in controls (filled arrows) and the reverse (open arrows).

Fig. 11. Corneal cells at 154 days aggregated in the presence of 4 mM-dMM. (C) dMM; (■) controls.

Fig. 12. Corneal cells at 11 days aggregated in the presence of 4 mM-dMM. (C) dMM; (■) controls.

22×10^3). Although some of these molecules have been characterized biochemically, it seems probable that not all are known (Steinberg et al. 1987; Franke et al. 1987). DG1 and DG11 have been localized by immunolabelling and shown to extend into the intercellular space (Steinberg et al. 1987), and it is this externally exposed region that is glycosylated and binds ConA (Shida et al. 1982).

Carbohydrate analysis (Kapprell et al. 1985) of bovine desmosomes indicates a particularly high content of mannose in band 4 peptides (Steinberg’s DGII). Pulse–chase studies of tissue culture cells combined with immunoprecipitation indicated that N-linked sugars are characteristic, each peptide was estimated as having two to four such chains (Penn et al. 1987).

In this study we have exposed aggregating corneal cells to agents that modify N-linked oligosaccharides, to look for possible effects on adhesiveness and/or desmosome formation. EndoH at high doses (12.5 mlU ml^{-1}) affects aggregation in older cells, but not in younger cells, by reducing aggregate size. However, in neither younger nor older cells does it change the rate of disappearance of single cells during aggregation, though aggregate size is reduced in older cells. Aggregation, which results in re-formation of the original types of cell surface contacts, is complex, involving a number of junctions and possibly non-junctional adhesions (Garrod, 1986). Results of EndoH treatment suggest that later stages in the aggregation process are more sensitive and that older

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cells, which have higher ConA binding, are likewise more subject to this treatment. EndoH markedly reduces the frequency of desmosome formation in treated aggregates.

At the pH at which cells were cultured, EndoH has the expected effect of reducing glycosylation and the fine structure of treated older cells at high doses shows characteristic signs of blockage of transport, i.e. enlarged membrane-bound vesicles associated with the ER containing dense granular deposits (Olden et al. 1985; Schwartz & Datema, 1982; Rothman, 1987). Younger cells, in which the ER is scant, show no blockage. However, even at low dosage, where no signs of transport blockage are evident in fine structure of older cells, desmosome formation is still inhibited by the enzyme. Glycosylation is clearly required for transport in some instances, but not in all (Rothman, 1987).

It has been pointed out that differences in response to tunicamycin may occur with differences in growth media, type, age or growth phase of cells (Elbein, 1987). In earlier experiments with corneal cells, in which tunicamycin was used to reduce glycosylation, cells cultured with foetal calf serum formed desmosomes at the normal rate when exposed to the drug in the presence of leupeptin (Overton, 1982). Tunicamycin can also promote desmosome formation in Ca\(^{2+}\)-depleted tissue culture cells (Mattey et al. 1987).

When aggregating corneal cells are treated with dMM no effect on cell aggregation or aggregate size was observed in either younger or older cells, and no effect on desmosome formation was seen in older aggregating cells. However, the inhibitor did have a pronounced effect on \(^3\)Hmannose incorporation, which increased two to three times. This is consistent with previous reports, and the expectation that blockage of processing may cause accumulation of precursor molecules (Elbein et al. 1983). In addition, SDS–PAGE of an NP40 fraction of corneal cells, which should include membrane proteins, showed a similar array of proteins but differences in \(^3\)Hmannose incorporation between treated and control cultures. Thus the drug had an expected effect on sugar incorporation, but caused no change in cell adhesion or junction formation. These findings imply, but of course do not demonstrate, that though glycosylation seems required for desmosome formation processing is less critical. In the experiments described here desmosomes were scored as parallel plaques early in aggregation. It has been shown that desmosomes may vary in their resistance to splitting by experimental treatment and that this resistance increases with time (Mattey et al. 1987). In this study, we may be looking at only an early part of a more extensive complex process.

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References


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