AN INVESTIGATION OF THE MOLECULAR COMPONENTS OF DESMOSOMES IN EPITHELIAL CELLS OF FIVE VERTEBRATES

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SUMMARY

We have shown previously, by fluorescent antibody staining, that desmosomal antigens are widely distributed in the tissues of vertebrate animals. Furthermore, we have demonstrated mutual desmosome formation between cells derived from man, cow, dog, chicken and frog. In this paper we have studied the components of desmosomes in a tissue or a cell line from each of these animals by immunoblotting with antibodies raised against the desmosomal components isolated from bovine nasal epithelium. Blotting was carried out on bovine nasal epithelial desmosomal cores, desmosome-enriched fractions derived from chicken and frog epidermis, nuclear matrix-intermediate filament scaffolds derived from Madin-Darby bovine and canine cells (MDBK and MDCK), and unextracted cultured human foreskin keratinocytes.

The results show that desmosomes from all these sources contain high molecular weight proteins (desmoplakins) of similar or identical molecular weights (250 000 and 215 000). Antibodies against the two lower molecular weight desmosomal proteins (83 000 and 75 000) always recognized one or two bands in very similar molecular weight regions of the gels. The desmosomal glycoproteins were found to be much more variable than the proteins: they vary between sources in molecular weight, heterogeneity and antibody cross-reactivity. For instance, antibody specific for a group of glycoprotein bands of 175 000, 169 000 and 164 000 (Mr) in bovine nasal epithelium recognizes three bands of 245 000, 230 000 and 210 000 in MDCK cells but only a single band of 190 000 in keratinocytes. In mammals, the 175 000–164 000 glycoproteins and the desmosomal adhesion molecules, the desmocollins (Mr 130 000 and 115 000 in cow's nose), are immunologically distinct. In chicken and frog, however, there are glycoproteins that react with both anti-175 000–164 000 and anti-desmocollin antibodies, but there are also distinct desmocollin bands. The significance of these results is discussed in relation to conservation of desmosomal components and adhesion mechanisms. It is suggested that adhesion may be performed by a well-conserved protein domain and that the variation between desmosomal glycoproteins from different sources may be due to differences in their carbohydrate composition.

INTRODUCTION

Desmosomes or maculae adhaerentes are adhesive intercellular junctions that occur in most vertebrate epithelia (Farquhar & Palade, 1963; Cowin, Mattey & Garrod, 1984a; Garrod & Cowin, 1985; McNutt & Weinstein, 1973; Overton, 1974; Staehelin, 1974), an exception being the pigmented retinal epithelium (Docherty, *Present address: Department of Pure and Applied Biology, Imperial College at Silwood Park, Ascot, Berks SL5 7PY, England.

Key words: desmosomes, cell adhesion, desmocollins, desmoplakins, epithelial cells, immunoblotting.
Edwards, Garrod & Mattey, 1984). They are also present in the intercalated discs of cardiac muscle, but are absent from skeletal muscle, connective tissue and other non-epithelial tissues. Ultrastructurally their most distinctive feature is a pair of dense, cytoplasmic plaques, 10–15 nm in thickness and adjacent to the inner leaflets of the opposed plasma membranes of adhering cells. The intercellular space, which contains the adhesive material, is 25–35 nm wide and is characterized by an electron-dense mid-line (Farquhar & Palade, 1963; Kelly, 1966; McNutt & Weinstein, 1973; Overton, 1974). Cytoplasmically, the plaques are joined to intermediate filaments (tonofilaments), which are usually composed of cytokeratin (Henderson & Weber, 1981). However, the intermediate filaments associated with desmosomes are composed of vimentin in the arachnoid (Kartenbeck, Schwechheimer, Moll & Franke, 1984) and may be desmin in the heart (Kartenbeck, Franke, Moser & Stoffels, 1983).

Skerrow & Matoltsy (1974a) isolated desmosomes from bovine nasal epithelium. Gorbsky & Steinberg (1981) have refined this technique in order to produce desmosomal cores, structures enriched in intercellular material but with reduced plaques and tonofilaments. These procedures have enabled the molecular composition of desmosomes to be studied.

The protein and glycoprotein components of bovine nasal desmosomes are listed in Table 1. The high molecular weight proteins, desmoplakins, have been localized to the cytoplasmic plaques (Franke et al. 1981). Gorbsky & Steinberg (1981) proposed that the glycoproteins are all localized in the intercellular space because they are enriched in desmosomal cores. However, Cowin, Mattey & Garrod (1984b) found that only antibodies to the 130 000 and 115 000 components bound to the surfaces of living Madin-Darby Bovine Kidney (MDBK) cells and keratinocytes (see also Watt, Mattey & Garrod, 1984). Furthermore, only Fab' fragments of antibodies directed against these glycoproteins inhibited desmosome formation in MDBK cells (Cowin et al. 1984b). It was concluded that these molecules constitute the adhesive or glue of the desmosome and they were therefore named desmocollins (δεσμός = link; κόλλα = glue). (In previous publications we have referred to desmosomal components by the molecular weights reported by Gorbsky & Steinberg (1981). Henceforth, however, we shall use the molecular weights of Franke et al. (1982) because these correspond more closely with the results obtained with our gel system (see Table 1).)

Electron microscopy has shown that desmosomes from different vertebrate sources have similar ultrastructure (Overton, 1974). Immunofluorescence studies using specific polyclonal antisera against individual desmosomal components suggested that these components are highly conserved in vertebrate epithelia. Thus the epidermis of man, cow, rat, guinea pig, chick, lizard and frog stained with equal intensity for all antigens. However, the intensity of staining for the desmocollins was reduced in non-epidermal tissues in all these species, while staining for the desmosomal plaque constituents and the 175 000–164 000 glycoprotein was undiminished (Cowin & Garrod, 1983; Cowin et al. 1984a). This may indicate differences between desmocollins of epidermal and non-epidermal tissues within the same species.
Table 1. Components of bovine epithelial desmosomes

<table>
<thead>
<tr>
<th>Proteins</th>
<th>( M_r (\times 10^{-3}) )</th>
<th>Location</th>
<th>Name used in text</th>
</tr>
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<tr>
<td>Proteins</td>
<td>250</td>
<td>Cytoplasmic</td>
<td>Desmoplakin I</td>
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<tr>
<td></td>
<td>215</td>
<td>Plaque</td>
<td>Desmoplakin II</td>
</tr>
<tr>
<td></td>
<td>83</td>
<td>Cytoplasmic</td>
<td>83 000 protein</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>Cytoplasmic</td>
<td>75 000 protein</td>
</tr>
<tr>
<td>Glycoproteins</td>
<td>175–164</td>
<td>Disputed</td>
<td>175 000–164 000 glycoprotein</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>Cell surface</td>
<td>Desmocollin I</td>
</tr>
<tr>
<td></td>
<td>115</td>
<td>Cell surface</td>
<td>Desmocollin II</td>
</tr>
<tr>
<td></td>
<td>22</td>
<td>?</td>
<td></td>
</tr>
</tbody>
</table>

Based on the work of Franke et al. (1981), Gorbsky & Steinberg (1981) and Cowin & Garrod (1983).

* This protein has been called desmoplakin III by Guidice et al. (1984) but it clearly differs from desmoplakins I and II, and occurs in non-desmosomal locations. The glycoproteins have all been termed 'desmogleins' by Guidice et al. (1984), but they clearly differ from each other and may have different functions.

In order to test the functional conservation of desmosomal adhesion mechanisms, Mattey & Garrod (1985) have made binary combinations between five different cell types, HeLa (human cervical carcinoma cells), MDBK, MDCK (Madin-Darby Canine Kidney), chick embryonic corneal epithelial cells and frog (Rana pipiens) adult corneal epithelial cells. Immunofluorescent staining and electron microscopy showed that mutual desmosome formation took place in every combination. There was thus complete non-selectivity of desmosome formation. It was concluded that the adhesion-recognition domain of the desmocollin molecule is likely to be highly conserved between different tissues, and different vertebrate species.

Franke et al. (1982) and Mueller & Franke (1983) have shown that the desmoplakins have identical molecular weights and similar peptide maps in bovine nasal and tongue epithelia, and human oesophageal mucosa. Furthermore, desmoplakin I has the same molecular weight in bovine and human myocardial tissue although desmoplakin II is missing. Giudice, Cohen, Patel & Steinberg (1984) have shown desmoplakin I to be of the same molecular weight in bovine nasal epithelium, corneal epithelium and oesophageal epithelium, but desmoplakin II to be absent from the latter two. The 83 000 protein had the same molecular weight in all three tissues. (Giudice et al. (1984) refer to this protein as desmoplakin III and quote its molecular weight as 81 000.) This protein has now also been localized to the desmosomal plaques (Gorbsky et al. 1985) but also occurs in non-desmosomal locations (Cowin et al. 1984a; Docherty et al. 1984). The glycoproteins were found to have similar but not identical molecular weights in all tissues and, using four monoclonal antibodies, tissue-restricted antigenic determinants were demonstrated (Giudice et al. 1984).
The object of the present study was to compare the components of desmosomes from a wider range of tissues, cell lines and species, and in particular to cover the range dealt with in our fluorescent antibody and cell combination studies (Cowin & Garrod, 1983; Cowin et al. 1984a; Mattey & Garrod, 1985; Watt, Mattey & Garrod, 1984). We have therefore carried out immunoblotting with anti-desmosomal antibodies on frog and chicken skin, human keratinocytes, MDBK and MDCK cells.

MATERIALS AND METHODS

Preparation of desmosome-enriched fraction from Rana pipiens skin

The skins of 10 adult Rana pipiens (Xenopus Ltd) were cut into 1 mm³ pieces and washed with 150 mM-NaCl, 200 mM-acetate buffer (pH 6) at 4°C. The pieces were then incubated in 10 ml of the same buffer containing 10 mM-phenylmethylsulphonyl fluoride (PMSF), 0·2 mg ml⁻¹ leupeptin, 0·01 mg ml⁻¹ pepstatin A and 50 mg ml⁻¹ bovine testicular hyaluronidase (Sigma) for 1 h at 37°C. After a wash in citric acid/sodium citrate buffer, pH 2.6 (CASC) at 4°C, the pieces were stirred in 500 ml of CASC containing 0·05 % NP40, 5 µg ml⁻¹ each of pepstatin A and leupeptin (CASC A) (Gorbsky & Steinberg, 1981) for 3 h at 4°C. The homogenate was passed through eight layers of gauze and centrifuged for 30 min at 20,000 g (radius average) at 4°C. The pellet was dispersed in CASC containing 0·01 % NP40 plus the protease inhibitors (CASC B) by 10 strokes of the loose pestle of a Dounce type homogenizer. The suspension was then spun at 20,000 g (radius average) for 30 min at 4°C. The upper white layer of the triaminar pellet was dispersed and the procedure repeated. The final layer was divided between two 14-ml discontinuous sucrose gradients (0 %, 40 %, 50 %, 55 % and 60 %) (Skerrow & Matoltsy, 1974b) in CASC B and two continuous 14-ml metrizamide gradients (10 % to 50 %) in CASC B (Gorbsky & Steinberg, 1981) and spun for 3 h at 180,000 g (radius average) at 4°C. Each band was collected with a pipette, dispersed and pelleted at 20,000 g (radius average) in CASC B. The final pellets were processed for electron microscopy and polyacrylamide gel electrophoresis.

Preparation of a desmosome-enriched fraction from chicken skin

Two chickens were plucked and skinned, and the skin was chopped into small pieces. A similar procedure to that for the frog was used except that the hyaluronidase treatment was omitted, the NP40 concentration was maintained at 0·05 % throughout, the preparation was made up to 1 % with Triton X-100 before the first spin and the metrizamide gradient was 10 % to 60 %. (The Triton X-100 was included to disperse excess lipidic material; see Results.)

Preparation of cytoskeletal desmosome-enriched fractions from MDBK and MDCK cells

Confluent 'high passage' (Richardson, Scalera & Simmons, 1981) MDCK or MDBK (approx. 10⁶ cells in each case, cultured for 2 weeks in modified Eagle’s MEM plus 10 % foetal calf serum) cells (Flow Laboratories) were treated with 50 mM-NaCl, 300 mM-sucrose, 10 mM-PIPES (pH 6·8), 3 mM-MgCl₂, 0·5 % Triton X-100, 1·2 mM-PMSF, 0·1 mg ml⁻¹ DNase and 0·1 mg ml⁻¹ RNase for 20 min at 20°C. Ammonium sulphate was then added to a final concentration of 250 mM and incubation continued for 5 min at 20°C (so-called cytoskeletal or CSK buffers). This procedure is a slightly shortened version of that used by Fey, Wan & Penman (1984) to isolate the nuclear matrix–intermediate filament scaffold (NM-IF).

Keratinocytes

Stratified newborn human foreskin keratinocytes (second passage), which had been cultured for 3–4 weeks (Rheinwald & Green, 1975), were kindly supplied by Dr F. Watt and Mr I. Kill of the Kennedy Institute of Rheumatology, Hammersmith.
Polyacrylamide gel electrophoresis (PAGE)

Frog and chicken desmosome-enriched fractions were first dialysed overnight against phosphate-buffered saline (PBS) containing 0.1% sodium dodecyl sulphate (SDS) and 5 mM-mercaptoethanol. An equal volume of solubilizing buffer (Laemmli, 1970) was then added and the samples boiled for 1–2 min. MDBK and MDCK nuclear matrix–intermediate scaffolds, and whole keratinocytes were dissolved in hot sample buffer. All samples were spun at 10,000 g for 20 min on a microcentrifuge and filtered through a 0.22 μm Millipore membrane before loading onto a 10% (Laemmli, 1970) polyacrylamide gel (1 mm × 140 mm × 140 mm). Dansylhydrazine staining of gels for glycoproteins was performed according to the method of Eckhardt, Hayes & Goldstein (1976).

Immunoblotting

After polyacrylamide gel electrophoresis a reference strip that included the molecular weight markers was cut from the gel and stained into Coomassie Brilliant Blue R, followed by destaining. The rest of the gel was renatured according to the method of Bowen, Steinberg, Laemmli & Weintraub (1980). The gel was then pre-shrunken in transfer buffer (Towbin, Staehelin & Gordon, 1979) and the proteins were transferred onto nitrocellulose for 36 h at 37°C. The excess binding capacity of the nitrocellulose replicas was then blocked by incubating for 1 h at 40°C in 3% ovalbumin, 150 mM-NaCl, 10 mM-Tris·HCl (pH 7.4), followed by 30 min at 37°C in 0.25% gelatin, 0.25% Tween 20 (Blake, Johnston, Russel-Jones & Gotschlich, 1984), 150 mM-NaCl, 50 mM-Tris HCl, pH 7.4 (buffer A).

The nitrocellulose was cut into strips and reacted for 4 h at room temperature with antiserum, diluted 1/50 in buffer A, in sealed polythene bags. The strips were then washed in two changes of 150 mM-NaCl, 50 mM-Tris·HCl (pH 7.4), 0.02% Triton X-100, 0.02% sodium lauryl sarcosine (buffer B) followed by a wash in the same buffer containing 400 mM-NaCl for 30 min. The strips were then blocked in buffer A for 1 h at room temperature. The strips were then washed in seven changes of buffer B for 24 h including two washes at 37°C and two washes in buffer B with 400 mM-NaCl.

The strips were then briefly washed in water, dried and autoradiographed through a sheet of aluminium foil at −70°C using Fuji RX X-ray film. Each blot was repeated a minimum of three times.

Anti-desmosomal antibodies

The antibodies used were raised in guinea pigs against the bovine desmosomal desmoplakins I and II, the 175–164K triplet, desmocollin I, desmocollin IF and the 83/75K proteins (Cowin & Garrod, 1983). The anti-desmoplakin and 83/75K antibodies showed cross-reactivity with cytokeratins and were therefore pre-absorbed with bovine muzzle keratin (Matoltsy, 1965) overnight at 4°C with shaking before use. Immunoblotting on isolated desmosomal cores, prepared using the method of Gorbsky & Steinberg (1981), shows the specificity of the antisera (Fig. 1). As shown previously (Cohen, Gorbsky & Steinberg, 1983; Cowin & Garrod, 1983) desmocollins are immunologically related. The anti-desmocollin II antibody possessed anti-175–164K activity and will therefore be called anti-desmocollin II/175–164K antibody. We believe this reactivity with the 175–164K antigen to be due to contamination of the desmocollin II antigen used for immunization with breakdown products of 175–164K, which comigrated with desmocollin II on preparative polyacrylamide gels. Details of the antibodies and how we refer to them are set out in Table 2.

Electron microscopy

Small pieces of tissue or desmosome-enriched pellets were fixed in 2.5% glutaraldehyde in 100 mM-cacodylate buffer containing 1 mM-CaCl₂ for 2 h at room temperature. Specimens were washed, post-fixed in 1% osmium tetroxide for 1 h, dehydrated through an ethanol series and embedded in Spurr resin. Thin sections were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate, and examined using a Philips 300 electron microscope.
RESULTS

Frog skin desmosomes

*R. pipiens* skin was chosen as a source of desmosomes because the frog was the lowest animal in the evolutionary scale whose epidermal desmosomes stained equally brightly with all the antisera raised from bovine desmosomal components (Cowin & Garrod, 1983; Cowin *et al.* 1984a). Furthermore, the skin could be easily removed from the animal, and frog skin desmosomes are stable to calcium removal (Borysenko & Revel, 1973), an important characteristic in view of the fact that the extraction buffer contains citrate, which chelates calcium. The appearance of desmosomes in intact frog skin is shown in Fig. 2A.

Frog skin yielded approximately 1 mg of desmosome-enriched material per 5–10 g wet weight of adult *R. pipiens* skin. The mucus layer covering the skin was the major contaminant of this preparation but could be largely removed by preincubation with hyaluronidase. Frog desmosomes had a slightly lower density than those of bovine nose, collecting mainly at the 50%/55% sucrose gradient interface rather than the 55%/60% interface (Skerrow & Matoltsy, 1974a). The metrizamide gradient treatment (Gorbsky & Steinberg, 1981), which enriches the glycoprotein components of bovine desmosomes, gave a broader band that was also slightly higher on

![Image](image-url)
the metrizamide gradient. Electron micrographs of the frog desmosome-enriched fraction collecting at the 50%/55% sucrose interface showed reasonable morphological preservation, although midline structures, sometimes discernible in intact specimens, were not evident (Fig. 3A, B). Micrographs of the metrizamide fraction showed extensive loss of cytoplasmic material leaving small knob-like protrusions (Fig. 3D, arrowheads). Periodically spaced cross-bridges were also frequently observed (Fig. 3C, arrows).

**Table 2. Anti-desmosomal antibodies**

<table>
<thead>
<tr>
<th>Immunizing antigen</th>
<th>Specificity by blotting on bovine desmosomal cores</th>
<th>Name of antibody used in text</th>
</tr>
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<tbody>
<tr>
<td>Desmoplakins I and II</td>
<td>Desmoplakins I and II*</td>
<td>Anti-desmoplakin</td>
</tr>
<tr>
<td>83 000 and 75 000 proteins</td>
<td>83 000 and 75 000 proteins*</td>
<td>Anti-83/75K</td>
</tr>
<tr>
<td>175 000–164 000 glycoprotein</td>
<td>175 000–164 000 glycoprotein</td>
<td>Anti-175–164K</td>
</tr>
<tr>
<td>Desmocollin I</td>
<td>Desmocollins I and II</td>
<td>Anti-desmocollin I</td>
</tr>
<tr>
<td>Desmocollin II</td>
<td>Desmocollins I and II and 175 000–164 000 glycoprotein</td>
<td>Anti-desmocollin II</td>
</tr>
<tr>
<td>175 000–164 000 glycoprotein</td>
<td>175–164K</td>
<td></td>
</tr>
</tbody>
</table>

*After absorption with bovine muzzle cytokeratin.

Fig. 2. Electron micrographs of frog and chicken skin desmosomes. A. High power, showing desmosomes in frog skin. ×135 000. B. High power, showing desmosomes in chicken skin. ×120 000. Both A and B show the characteristic appearance of desmosomes: parallel plasma membranes, dense intercellular material and very dense cytoplasmic plaques. Bars, 0.1 μm.
Polyacrylamide slab gels of the different sucrose gradient fractions and the metrizamide fraction are shown in Fig. 4 (lanes A–D). The 50–55% sucrose fraction, shown by electron microscopy to be rich in desmosomes (Fig. 3) is shown in lane c, though in fact lane b has similar bands. The electrophoresis patterns are broadly similar to those obtained for bovine desmosomal preparations (Drochmans et al. 1978; Skerrow & Matoltsy, 1976).

Bands marked with arrows are enriched in metrizamide preparations. Two of these, at 170 000 and 140 000 $M_r$, are glycoproteins as revealed by dansylhydrazine staining (Fig. 4, lanes F–H). Both of these bands may be doublets. Bands labelled with arrowheads are reduced in metrizamide preparations. The two high molecular weight ones are probably desmoplakins (see below). The two upper bands of the group below 67 000 $M_r$ blot for cytokeratin (data not shown).

Immunoblotting with antibodies to bovine desmoplakins and 83 000/75 000 proteins revealed bands with molecular weights similar to those of bovine desmosomes (Fig. 5, lanes A,E). The frog glycoprotein bands, which have molecular weights different from those in bovine desmosomes, showed a surprising pattern of reactivity with anti-bovine antibodies. Both the anti-175–164K and the anti-desmocollin I antibodies reacted with the frog 170 000 glycoprotein. In bovine desmosomes the 175 000–164 000 glycoprotein and the desmocollins are immunologically distinct (Cohen et al. 1983; Cowin & Garrod, 1983). Furthermore, the anti-desmocollin II/175–164K antibody reacted mainly with the frog 170 000 band and only slightly with the 140 000 band. This may suggest that epitopes characterizing bovine desmocollin II are either nearly absent from frog or, present in the frog 170 000 glycoprotein.

**Chicken skin desmosomes**

Fig. 2b shows an electron micrograph of desmosomes in chicken skin and Fig. 6 shows isolated chicken skin desmosomes. Initial attempts to obtain these were thwarted by the large quantities of fat that exuded from the skin. This was overcome by including an extraction with 1% Triton X-100 and maintaining the NP40 concentration at 0.05% throughout the procedure. Furthermore, the preparation coagulated on the sucrose gradient, giving streaky, diffuse bands and a random distribution of aggregates throughout the gradient. The metrizamide gradient, however, produced a sharp band at about 50% metrizamide. Instead of appearing like desmosomal cores, the desmosomes in this band retained their plaques despite the metrizamide treatment (Fig. 6). The preparation appeared to be contaminated by large amounts of electron-dense material. Nevertheless the pattern of bands seen on a polyacrylamide gel of the metrizamide fraction (Fig. 7) was broadly similar to that seen in desmosomal fractions from other sources. Dansylhydrazine staining of the metrizamide fraction showed four prominent bands at 210 000, 190 000, 115 000 and 105 000 $M_r$. The 190 000 $M_r$ band appeared to be a non-desmosomal contaminant since it did not blot with any of the desmosomal antibodies (see below). Alternatively, it may be a unique component of chicken desmosomes.
Fig. 3. Electron micrographs of the frog desmosome-enriched fraction from the 50%/55% boundary of the discontinuous sucrose density gradient and from the metrizamide gradient. A. Low power, showing general appearance of the preparation from the sucrose gradient. Note that the desmosomes sometimes remain associated in zigzag chains. ×17 000. B. High power of isolated desmosome, showing that the structure is essentially preserved although midline structures and the electron-lucent plasma membranes are not visible (compare with Fig. 2A). Dense material in bottom right-hand corner of photograph may represent contaminants. ×114 000. C. Intermediate power of desmosomal cores from the metrizamide gradient. The desmosomes have been largely stripped of electron-dense plaque material. In the intercellular space periodically arranged cross-bridges between the plasma membranes are sometimes discernible (arrows). The preparation contains granular electron-dense contaminating material. ×57 000. D. High-power photographs, showing knob-like protruberances on the cytoplasmic face of the plasma membranes (arrowheads). ×125 000.
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Fig. 4. Polyacrylamide gel electrophoresis of fractions from sucrose density gradient and metrizamide gradient centrifugation of frog skin desmosomes: A–D, stained with Coomassie Blue; E–H, stained with dansylhydrazine. Lanes A, E, 0–40% sucrose; lanes B, F, 40–50% sucrose; lanes C, G, 50–55% sucrose; lanes D, H, from metrizamide gradient. Lanes C, G relate to the preparation shown in Fig. 3A, B and lanes D, H to that shown in Fig. 3C, D. The arrows indicate bands that are enriched on the metrizamide gradient preparations, while the arrowheads indicate bands that are diminished. The two glycoprotein bands at 170 000 and 140 000 are greatly enriched on metrizamide (lane H).

Immunoblotting with anti-desmoplakin gave bands with molecular weights similar to the bovine proteins (Fig. 8). Anti-83/75K recognized a broad band in the same region of the gel as the corresponding bovine antigens. The glycoprotein antibodies, however, gave patterns similar to those found in the frog. Both the anti-175–164K antibody and the anti-desmocollin I antibody blotted a doublet at 210 000–205 000 $M_r$, a cross-reaction similar to that found in frog but never encountered in the cow. (This doublet was not clearly resolved by dansylhydrazine staining.) The anti-desmocollin I antibody blotted a further band at 115 000 $M_r$. The anti-desmocollin II/175–164K antibody blotted the high molecular weight doublet, the 115 000 band, plus an additional desmocollin band at 105 000, which appears to be immunologically distinct.

MDCK cells

Preparation of the NM-IF scaffold, which removes 95% of the cellular proteins (Fey et al. 1984), was necessary to provide enrichment of desmosomal antigens sufficient for blotting. The desmoplakins had the same molecular weights in MDCK
Fig. 5. Immunoblot of frog skin desmosomal core-enriched fraction with anti-desmosomal antibodies. A Coomassie-stained gel from which the nitrocellulose replicas were made is shown in Fig. 4D. A, anti-desmoplakin; B, anti-175–164K; C, anti-desmocollin I; D, anti-desmocollin II/17S-164K; E, anti-83/75K; F, guinea-pig pre-injection serum.

Fig. 6. Electron micrographs of desmosome-enriched fraction from chicken skin obtained by metrizamide gradient centrifugation. A, C. High power, showing well-preserved desmosome structure (compare with Fig. 2a). Much plaque material is still attached although less well organized. A, ×95 000; C, ×145 000. B. Intermediate power, showing desmosomes, a membrane vesicle and granular contaminating material. ×38 000.
cells as in the cow while the anti-83/75K antibody recognized a band of approximately $M_r$ 83,000 (Fig. 9). Fey et al. (1984), using an antibody to whole bovine desmosomes, report immunoblotting bands at 240,000, 210,000 (presumably the desmoplakins), at 56,000 (probably a cytokeratin) and also at 150,000 (a band not observed in this study). The glycoprotein bands showed minor differences from those of the cow. The anti-175–164K antibody blotted four bands at 245,000, 230,000, 210,000 and 200,000. The latter band was variable in intensity between preparations and may represent a degradation product. Both the anti-desmocollin I and the anti-desmocollin II/175–164K antibodies, blotted bands at 135,000 and 120,000 but the latter antibody did not react with the higher molecular weight triplet as it did in the cow.

 MDBK cells

The NM-IF scaffold of MDBK cells again showed desmoplakins with molecular weights similar to those found in other cases (Fig. 10). The anti-83/75K antibody revealed a band with a molecular weight of about 83,000. The anti-175–164K antibody identified a single band of molecular weight 205,000. The anti-desmocollin I antibody recognizes three (possibly four) bands of 175,000 (135,000), 130,000 and 120,000. This is the greatest amount of heterogeneity that we have yet found among

![Fig. 7. Polyacrylamide gel electrophoresis of the metrizamide gradient desmosome-enriched fraction from chicken skin. A. Coomassie Blue; B, dansylhydrazine, the latter showing four prominent glycoprotein bands at 210,000, 190,000, 115,000 and 105,000 (arrows and arrowhead). Arrows show bands blotting with anti-desmosomal antibodies.](image-url)
Desmosomal components from five vertebrates

Fig. 8. Immunoblot of desmosome-enriched fraction from chicken skin with anti-desmosomal antibodies. A Coomassie-stained gel from which the nitrocellulose replicas were made is shown in Fig. 7A. A, Anti-desmoplakin; B, anti-175–164K; C, anti-desmocollin I; D, anti-desmocollin II/175–164K; E, anti-83/75K; F, guinea-pig pre-injection serum.

desmocollin antigens. MDBK cells were not blotted with the anti-desmocollin II/175–164K antibody because we have none left.

Human foreskin keratinocytes

Human keratinocytes grown in medium with 1.8 mM-calcium (Rheinwald & Green, 1975) for 2–3 weeks stratify forming ten to fifteen cell layers. Such cultures contained enough desmosomal antigens for blotting if the cells were directly solubilized without prior enrichment.

The anti-desmoplakin antibody again behaved in a similar manner to that of the cow (Fig. 11). The anti-83/75K antibody again recognized a broad band in the region of 75,000–85,000. The anti-glycoprotein antibodies also gave a very similar pattern, showing three bands for the anti-desmocollin I antibody and the anti-desmocollin II/175–164K antibody, with molecular weights of 130,000, 135,000 and 150,000, slightly higher than those seen in the bovine nose. The anti-175–164K antibody blotted only a single band at 190,000, which was also recognised by the anti-desmocollin II/175–164K antibody. The 190,000 component is therefore not heterogeneous, unlike the corresponding antigen of the cow.
DISCUSSION

The results of this study are summarized diagrammatically in Fig. 12. It should be stressed that the cells and tissues involved, as well as being from different species, provide examples of stratified epithelia, simple epithelia and cells cultured in vitro.

One of the most striking features of our results is the similarity between the protein constituents of desmosomes from different sources. The desmoplakins are represented in every case by two bands of approximately 250 000 and 215 000 $M_r$. While this result suggests remarkable evolutionary conservation of desmoplakins, we cannot exclude the existence of slight variations, the precise nature of which must await peptide mapping and amino acid sequencing.

The anti-83/75K antibody always blotted bands in similar regions of the gel. However, only in frog and bovine epidermis were two bands clearly distinguishable. This may indicate either that our procedure is insufficiently sensitive to resolve and/or detect both bands in every case, or that only one or other of the antigens is present in chicken epidermis, MDBK cells, MDCK cells and human keratinocytes. A solution to this dilemma must await preparation of monospecific antibodies against the 83 000 and 75 000 proteins or blotting two-dimensional gels. It is already known that these are unrelated proteins that differ greatly in charge (Franke et al. 1983). It

Fig. 9. Immunoblot of NM-IF from MDCK cells with anti-desmosomal antibodies. A. Anti-desmoplakin; B. anti-175–164K; C. anti-desmocollin I; D. anti-desmocollin 11/175–164K; E. anti-83/75K; F. guinea-pig pre-injection serum; G. Coomassie-stained gel from which nitrocellulose replicas were made.
Fig. 10. Immunoblot of NM-IF from MDBK cells with anti-desmosomal antibodies. 
A. anti-desmoplakin; B, anti-175–164K; C, anti-desmocollin I; D, anti-83/75K;  
e, guinea-pig pre-injection serum; F, Coomassie-stained gel from which nitrocellulose  
replicas were made.

should also be noted that one or both of these proteins occurs in non-desmosomal  
locations, for example in pigmented retinal epithelial cells of the chick embryo  
(Docherty et al. 1984) and the pillar cells of the trout pseudobranch (Cowin et al.  
1984).

The desmosomal glycoproteins show a more complex pattern than the proteins:  
they vary between cell types in (1) molecular weight (2) heterogeneity and (3)  
patterns of antibody reactivity. With respect to molecular weight, glycoproteins  
behave anomalously during SDS/polyacrylamide gel electrophoresis when com-  
pared with unglycosylated proteins (Bretscher, 1971; Segrest & Jackson, 1972),  
so large differences in electrophoretic mobility may be due to small variations in,  
for example, sialic acid content (Segrest, Jackson, Andrews & Marchesi, 1971).  
The commonly encountered multiple-band appearance of glycoproteins resolved  
on SDS/polyacrylamide gels has generally been attributed to carbohydrate  
heterogeneity (Sharon & Lis, 1982). This is the case for the bovine desmosomal  
glycoproteins: the 175 000–164 000 component can be resolved as a triplet and  
desmocollin I as a doublet (Cohen et al. 1983; Mueller & Franke, 1983). The  
differences in the number and separation of the desmosomal glycoprotein bands  
seen in the different species, especially apparent in the bands identified by the anti-  
175–164K antibody, may be due to such heterogeneity.
Fig. 11. Immunoblot of stratified human foreskin keratinocytes with anti-desmosomal antibodies. A, Anti-desmoplakin; B, anti-175–164K; C, anti-desmocollin I; D, anti-desmocollin II/175–164K; E, anti-83/75K; F, guinea-pig pre-injection serum; G, Coomassie-stained gel from which nitrocellulose replicas were made.

Fig. 12. Diagram summarizing the results of immunoblotting experiments with anti-desmosomal antibodies. The desmosomal components for each tissue or cell line are shown. Frog, *R. pipiens* epidermis; chicken, chicken epidermis; cow, bovine nasal epithelium; man, cultured human foreskin keratinocytes. Black, anti-desmoplakin; white, anti-175–164K; narrow cross-hatching, anti-desmocollin I; broad cross-hatching, anti-desmocollin II/175–164K; criss-crossing, anti-83/75K. Broken lines indicate suspected bands, which, in the case of the cow, have been found in many previous experiments but are not necessarily clear from the present data. This diagram does not accurately represent the molecular weights of individual bands, so readers are requested to refer to the previous figures, which show the actual blots.
In bovine nasal epithelium and the other mammalian cell types studied here, the 175,000–164,000 glycoprotein and the desmocollins are immunologically distinct (Cohen et al. 1983; Cowin & Garrod, 1983). In frog and chicken, however, anti-175–164K and anti-desmocollin I antibodies react with common glycoprotein bands (170,000 in frog and 210,000–205,000 doublet in chicken). Thus antigenic determinants that are on distinct glycoproteins in mammals are shared by the same glycoprotein in these lower vertebrates. Furthermore, the anti-desmocollin I antibody reacts with a 115,000 glycoprotein band but not with a 105,000 glycoprotein band in chicken. The latter band is recognized by the anti-desmocollin II/175–164K antibody but not by the anti-175–164K antibody alone. This probably indicates the presence of two immunologically distinct desmocollins in chicken, a situation not encountered so far in mammals. In the context of the desmocollins, however, we should note that Parrish & Garrod (unpublished observations) have demonstrated that these are immunologically distinct between the basal and suprabasal cells of human and bovine epidermis.

It should be pointed out that the glycoproteins of cultured cells may differ from those in tissues from which they were derived and also change with passage number. Koulu et al. (1984) report that an antibody (RpDG.I-1) raised against the bovine 175,000–164,000 glycoprotein detects a component in human skin that has a molecular weight 10,000 greater than its counterpart in bovine muzzle. This band seems to be broader and to have a slightly lower molecular weight than the band we have found in cultured human keratinocytes.

The molecular weights of fucose-labelled glycopeptides from cultured rabbit keratinocytes have been shown to increase with passage number (van Erp et al. 1984). The human keratinocytes used here had been passaged twice, the molecular weights given for the desmosomal glycoproteins may not, therefore, be the same as those found in human skin. Differences in labelling of cell surface proteins have also been demonstrated between high- and low-passage MDCK cells (Richardson et al. 1981). Our MDCK cells are morphologically similar to the former.

What is the significance of the variability of desmosomal glycoproteins from different sources? It has been shown by Mattey & Garrod (1985) that mutual desmosome formation takes place between all binary combinations of HeLa cells (human), MDCK cells, MDBK cells, chicken embryonic corneal epithelial cells and frog adult corneal epithelial cells. This is interpreted to mean that the adhesion–recognition domains of desmocollin molecules are likely to be conserved between different tissues and different species. Adhesion is after all a fundamental property of desmosomes and a fundamental requirement of those tissues that possess them, so it is perhaps not surprising that the adhesion mechanism, once developed, should be conserved by evolution. We have argued that the adhesive differences between epithelial tissues are to be sought in terms of the quantity, distribution and stability of similar adhesion mechanisms (Garrod, 1985; Mattey & Garrod, 1985). A striking illustration of such differences is to be found by comparison of cells of simple and stratified epithelia. The former have relatively few desmosomes confined to their lateral surfaces whereas the latter have many desmosomes all over their surfaces.
Moreover, modulation or rearrangement of desmosomal components to produce these different patterns have been demonstrated by studying MDBK cells and human keratinocytes in culture (Cowin et al. 1984b; Watt et al. 1984). Also, the desmosomes of these two cell types differ in resistance to disruption by calcium removal. The crucial question is how quantity, distribution and stability are controlled.

Present evidence suggests that the biological activity (adhesion in this case) of many glycoproteins is due to their protein rather than their carbohydrate moieties, which are thought to be important for control as sorting signals for glycoprotein routing, metabolic stability and cellular differentiation (Olden, Parent & White, 1982; Warren, Buck & Tuszynski, 1978). This is probably also true for desmosomal glycoproteins as desmosomes can form in the absence of N-linked carbohydrates (King & Tabiowo, 1981; Overton, 1982) and our own unpublished results. If the differences in desmosomal glycoproteins of different species reside in their carbohydrate moieties, the biologically active protein portion being well conserved, then this may reflect differences in carbohydrate-mediated control mechanisms. Alternatively, the adhesion domain alone may be well conserved, the rest of the polypeptide showing divergence. We are now investigating carbohydrate control mechanisms in desmosome assembly.

This work was supported by the Cancer Research Campaign. We thank Dr Derek Mattey for advice and help with electron microscopy.

REFERENCES


Desmosomal components from five vertebrates


*(Received 25 June 1985 – Accepted 20 September 1985)*

**Note added in proof**

A recent paper by P. Cowin, H.-P. Kapprell & W. W. Franke (*J. Cell Biol.* **101**, 1442–1454 (1985)) using monoclonal antibodies suggests that the lower molecular weight desmoplakin band of MDCK cells is not desmoplakin II but a breakdown product of desmoplakin I. That study did not include MDCK cells. They also suggest that the presence of desmoplakins I and II is characteristic of stratified epithelia whereas the presence of desmoplakin I only is characteristic of simple epithelia. This view conflicts with that of Guidice *et al.* (1984), who did not find desmoplakin II in bovine corneal and oesophageal epithelia.