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Fractionation of Desmosomes and Comparison of the Polypeptide Composition of Desmosomes Prepared From Two Bovine Epithelial Tissues

Stephanie M. Jones, Jonathan C.R. Jones, and Robert D. Goldman

Department of Cell Biology and Anatomy, Northwestern University Medical School, Chicago, Illinois 60611

Desmosomes isolated from bovine tongue mucosa or muzzle epidermis appeared identical by ultrastructural analyses but had some differences in their polypeptide compositions as determined by SDS-PAGE. These preparations were extracted in 9 M urea, 10 mM Tris-HCl (pH 9), and 25 mM B-mercaptoethanol and then centrifuged at 240,000g for 30 min. The urea-soluble and insoluble fractions were analyzed by SDS-PAGE. The urea soluble fractions of both tongue and muzzle desmosomes were enriched in polypeptides of 240, 210, 81, and 75 kDa and also polypeptides (40 to 70 kDa) that were keratin-like, as determined by immunoblotting analyses with keratin antisera. The urea insoluble fraction of tongue desmosomes contained glycoproteins of 165, 160, 140, 110, and 100 kDa, while this fraction from muzzle contained glycoproteins of 165, 115, and 105 kDa. Ultrastructural examinations of insoluble pellets obtained from urea extracted tongue and muzzle desmosomes showed that most of the components at the cytoplasmic faces of the desmosomes were removed, while the membrane regions of the desmosomes resisted the treatment. The urea soluble proteins were dialyzed against 10 mM Tris-HCl (pH 7.6), and the resulting preparation was pelleted by centrifugation and examined by electron microscopy. Ultrastructural examination of this material revealed that it had assembled into a fibrillar meshwork, similar to the fibrillar region adjacent to the submembranous plaque of isolated desmosomes. Thus, treatment of isolated desmosomes with 9 M urea allowed the fractionation of membrane-associated desmosomal proteins from cytoplasmic desmosomal proteins. A comparison of these fractions from tongue and muzzle indicated that the polypeptide compositions of the desmosomes varied between tissues, especially with respect to the fractions enriched in either glycoproteins or keratin.

Key words: desmosomes, bovine epithelial tissue, fractionation, polypeptide composition

Desmosomes are specialized junctional regions of epithelial cell membranes and are thought to be important in cell-to-cell adhesion. They also serve as attachment

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sites for cytoskeletal elements, most notably intermediate filaments (IF) (for a review, see [1]). Desmosomes possess a characteristic morphology that appears to be very similar from tissue to tissue. They are symmetrical about an intermembrane space, which generally contains fine fibers that are associated with an electron-dense midline and the outer leaflets of the plasma membranes. On either side of the cytoplasmic leaflets of the membrane there is an electron dense "plaque." Furthermore, one or more layers of lesser electron density are located adjacent to the dense plaque. Keratin containing IF of epithelial cells form tightly packed bundles (tonofilaments), which appear to be bound to these latter desmosome-associated layers [1]. It has been suggested that desmosome-IF complexes probably contribute to the strength and integrity of a tissue by distributing the forces exerted in one cell or a small group of cells to the tissue as a whole [1].

Desmosomes have been isolated from several stratified squamous epithelial tissues for biochemical studies. Desmosomes prepared from muzzle epidermis are the best characterized with regard to protein composition [2–6]. They contain at least eight major polypeptides*, four of which are glycosylated (165, 115, 105, and 22 kDa [3,7]) and four of which are not glycosylated (240, 210, 81, and 75 kDa [3,5,8]). Desmosomes prepared from muzzle epidermis also contain polypeptides with apparent molecular weights of 40–70 kDa, which are thought to be keratins [7,9,10].

Several of the major polypeptides of muzzle desmosomes have been grouped into families based on immunological and biochemical similarities. The 240 kDa and 210 kDa polypeptides are thought to be closely related. They have similar tryptic peptide maps [5], and antibodies directed against them show immunological cross-reactivity [5,11]. Also, the 115 kDa and 105 kDa polypeptides are related immunologically to each other but not to other desmosomal proteins [2].

The 240 kDa and 210 kDa polypeptides are believed to be located in the plaque region of the desmosome [3,11–13], while the 165, 115, and 105 kDa polypeptides are believed to be membrane associated [7,14]. The 81 kDa polypeptide is now known to occur at the plaques of several types of adhering junctions in addition to the desmosome and has been named "plakoglobin" [15].

We have succeeded in separating the desmosomal proteins into a fraction enriched in the membrane-associated glycoproteins and a fraction containing desmosomal plaque components and keratin-like proteins. Furthermore, following fractionation of bovine muzzle epidermal desmosomes and bovine tongue mucosal desmosomes we show that there is heterogeneity in the desmosomal membrane-associated glycoproteins in two different but related bovine stratified squamous epithelial tissues.

*The nomenclature for the proteins that have been identified as desmosomal polypeptides varies with research groups. Although the series of names and numerical designations that have appeared in the literature have been correlated [14, 36], the use of the names remains confusing. For example, the 115 kDa and 105 kDa polypeptides of muzzle desmosomes are called band 4a and 4b [3], desmocollins [14], and desmogleins [36]. For clarity, in this paper the major polypeptides of the desmosome will be referred to according to their apparent molecular weights on SDS-PAGE. The high molecular weight polypeptides of the desmosomes, which have been named desmoplakins I and II [3,5], are referred to as the 240 kDa and 210 kDa polypeptides, respectively.

MATERIALS AND METHODS

Preparation of Desmosomes

Desmosomes were isolated according to a modification of the Skerrow and Matoltsy [16] procedure. Pieces (1 cm^2) of the epithelial and subjacent connective tissue layers of fresh bovine muzzle or tongue were treated with 6 mM Na^+K^+ phosphate (pH 7.0), 120 mM NaCl, 3 mM KCl, 20 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride (PMSF) at 4°C overnight. The connective tissue was removed, the epithelial layers were minced finely with scissors, and the tissue was then extracted with buffer containing 0.1 M citrate (pH 2.6), 0.1% NP 40, pepstatin and leupeptin (5 μg/ml each) and 1 mM PMSF for 30 min at room temperature. The resulting solution was sonicated [7] and subjected to centrifugation at 500g for 10 min to remove large fragments. The supernatant was then centrifuged at 13,000g for 15 min at 4°C. The resulting pellet was washed twice with buffer containing 0.1 M citrate (pH 2.6) and 1 mM PMSF and then centrifuged on a sucrose gradient [16]. Alternatively, desmosomes were prepared according to the Gorbsky and Steinberg [7] modification of the Skerrow and Matoltsy [16] procedure.

Partial Fractionation of Desmosome Preparations

Desmosomes were extracted in urea using a modification of a procedure originally developed by Franke et al [17]. Purified preparations of desmosomes were suspended in 9 M urea, 10 mM Tris-HCl (pH 9.0), 20 mM B-mercaptoethanol, and 0.1 mM PMSF at a concentration of 0.1–0.3 mg of protein per milliliter of buffer. The suspension was homogenized with a Potter-Elvejhem homogenizer (5–10 strokes) and was then stirred at room temperature for 30 min. Urea-resistant material was pelleted by centrifugation at 240,000g in a Beckman SW 50.1 rotor for 30 min at 15°C. No further solubilization of the desmosome preparation was observed following longer periods of extraction in the urea buffer described above (up to 24 hr).

Keratin Preparation

Keratin was obtained from either bovine muzzle epidermis or tongue mucosa prepared as described above. The tissue was extracted in 8 M urea, 50 mM Tris-HCl (pH 9), and 25 mM B-mercaptoethanol [18]. Keratin was also extracted from the tissues with 0.1 M citrate buffer (pH 2.6) [19]. Solubilized keratin was assembled into IF by dialysis against 100 volumes of 10 mM Tris-HCl, pH 7.6, at 4°C for 12–16 hr.

Gel Electrophoresis

Samples were solubilized in 8 M urea, 62 mM Tris-HCl (pH 6.8), 1% SDS, 1% B-mercaptoethanol and were subjected to electrophoresis on 7.5% acrylamide slab gels with 4.5% stacking gels according to Laemmli [20]. Gels were stained for protein with Coomassie Brillant Blue. Rabbit muscle myosin, B-galactosidase, phosphorylase b, bovine albumin, and ovalbumin were used as molecular weight markers (Sigma Chemical Co., St. Louis, MO). For the identification of glycoproteins, gels were treated with periodic acid-Schiff's reagent according to the procedure of Fairbanks [21]. For Western blot analysis, polypeptides subjected to SDS-PAGE were transferred to nitrocellulose in a Hoefer transphor apparatus (Hoefer Scientific Instruments, San Francisco, CA) [22].

Electron Microscopy

Pellets of isolated desmosomes or desmosomal fractions were fixed in 1% glutaraldehyde in 6 mM Na^+K^+ phosphate (pH 7.4), 171 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, and 0.5 mM $MgCl_2$ for 30 min, washed 1 hr in the same phosphate buffer, postfixed in 1% OsO_4 in phosphate buffer, rinsed in distilled water, and dehydrated and embedded as previously described [23]. Thin sections were cut with a diamond knife, mounted on uncoated copper grids, and stained with uranyl acetate and lead citrate. The thin sections were viewed in a JEOL 200 CX electron microscope (JEOL USA, Peabody, MA)) at an accelerating voltage of 80 kV.

Antisera

Rabbit antisera used in these studies were directed against bovine prekeratin [24], the mouse keratin K1 subunit [25], and the mouse keratin K2 subunit [25]. A rabbit antiserum directed against the 240 kDa and 210 kDa proteins obtained from bovine muzzle desmosomes was provided by Drs. James Arnn and Andrew Staehelin [26,27]. A mouse monoclonal antibody that recognizes the 160/165 kDa desmosomal glycoproteins was used in this work. The preparation and characterization of this antibody have been described [28]. A rabbit autoimmune serum that recognizes the 140 kDa polypeptide of bovine tongue desmosomes was also used [29]. Antibody specific for the 140 kDa polypeptide was affinity purified by binding the antibody to nitrocellulose strips onto which the 140 kDa polypeptide had been transferred. The strips were washed, and the bound antibody eluted by extraction with sodium iodide according to the method of Olmsted [30]. The affinity purified antibody specific for the 140 kDa polypeptide has been characterized [29].

RESULTS

General Morphological and Biochemical Properties of Isolated Desmosomes

Fractions enriched in desmosomes obtained from bovine muzzle epidermis and tongue mucosa were examined by electron microscopy and analyzed by SDS-PAGE. When thin sections of the pellets of the preparations were observed, the desmosomes from the two tissues appeared to be morphologically very similar (Fig. 1). Furthermore, the morphology of isolated desmosomes was comparable to that seen in the work of others [16]. In both tongue and muzzle preparations, the regions adjacent to the dense desmosomal plaque appeared to be composed of a layer of fine fibrils (see Fig. 1). It has been previously noted that this region of fine fibrils may be obscured in desmosomes in situ by the approach of the more electron dense keratin-containing bundles of IF [16].

The enriched preparations of muzzle desmosomes were analyzed by SDS-PAGE (Fig. 2), and many of the major polypeptides resolved could be related to previously identified polypeptides [see below and 3,4,7,31]. Side-by-side comparisons indicated that the relative mobilities of most of the polypeptides of tongue and muzzle desmosomes are similar, but others are different (Fig. 2). The 240, 210, 81, and 75 kDa desmosomal proteins have the same apparent mobilities in tongue and muzzle desmosome preparations, but the glycoproteins (in the 100–170 kDa region) have slightly different apparent molecular weights in tongue and muzzle.

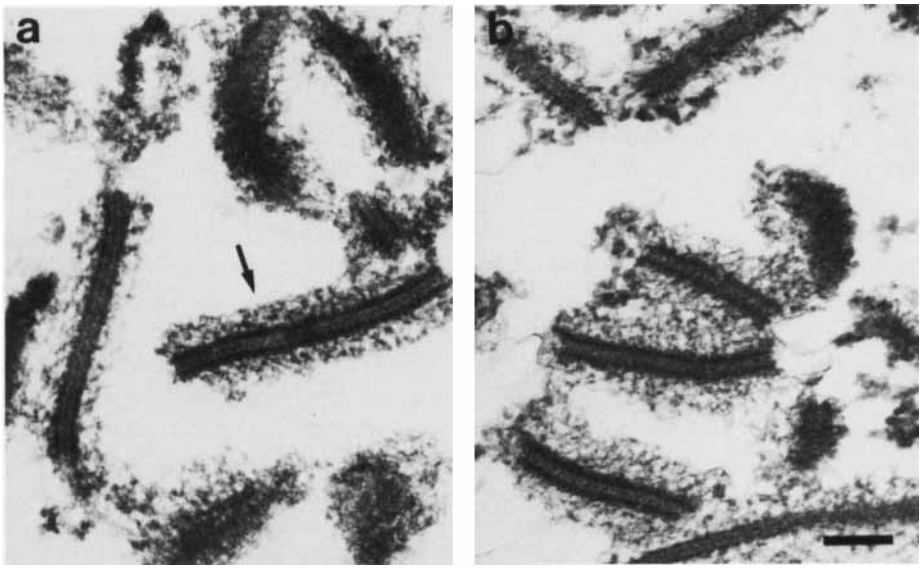

Fig. 1. Thin sections of enriched preparations of desmosomes from bovine tongue mucosa **(panel a)** and bovine muzzle epithelia **(panel b)** isolated by a modification of the method of Skerrow and Matoltsy [16]. The desmosomes in the preparations appear morphologically similar. The regions of the desmosomes, the intracellular material, the dense plaques adjacent to the plasma membrane, and fibrillar material adjacent to the plaque **(arrow, panel a)**, are easily identified. Bar represents 0.2 μm.

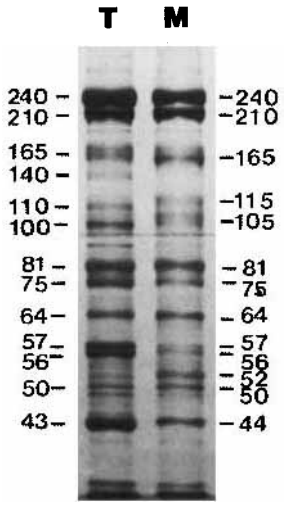

Fig. 2. The preparations of desmosomes from bovine tongue (labeled T) and bovine muzzle (labeled M) were analyzed by SDS-PAGE. Thirty micrograms of each preparation was loaded onto the gel. Apparent molecular weights of the major polypeptides in each preparation are indicated ($\times 10^{-3}$). Polypeptides with molecular weights of 240, 210, 81, and 75 kDa appear identical in the two preparations. The tongue preparation contains polypeptides that have molecular weights of 165, 110, and 100 kDa that are similar to the 165, 115, and 105 kDa glycoproteins of the muzzle preparation. A 140 kDa polypeptide appears to be present only in the tongue preparation.

Fractionation of Desmosomes

Partial fractionation of the isolated desmosomes obtained from both tissues was achieved by extraction with 9 M urea at pH 9. Subsequent centrifugation of this solution yielded an insoluble (pelletable) fraction and a soluble (supernatant) fraction. For all desmosome preparations studied, 20% of the total protein was pelletable after treatment with urea, and 80% of the protein was in the supernatant.

Electron micrographs of the insoluble pellet indicated that the material in this fraction appeared to correspond to residual membrane portions of the isolated desmosomes (Fig. 3b,d). When the urea insoluble fraction of muzzle desmosomes was analyzed by SDS-PAGE, it was seen to be enriched in the 165, 115, and 105 kDa polypeptides (Fig. 4). The corresponding fraction of tongue desmosomes was enriched in polypeptides that have apparent molecular weights of 165, 160, 140, 110, and 100 kDa. Each of these polypeptides was stained by the periodic acid-Schiff's reagent (Fig. 5), and thus, these polypeptides probably represent a fraction of the glycoprotein components of muzzle and tongue desmosomes. Desmosomes from tongue or muzzle that were isolated according to a second method of preparation [7] also yielded a glycoprotein-enriched urea-resistant fraction (Fig. 6) following urea extraction.

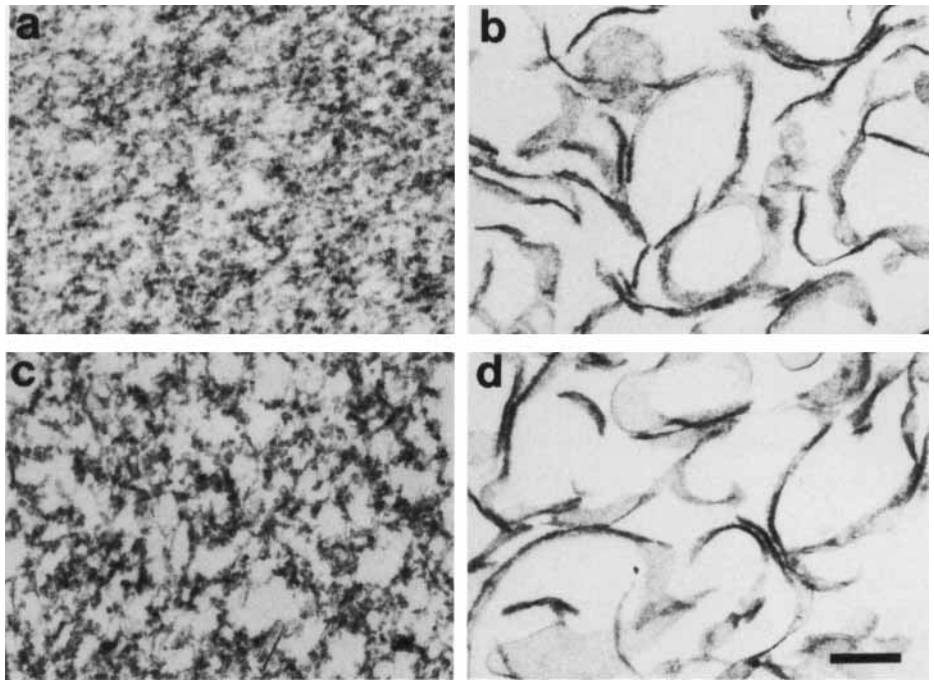

Fig. 3. Electron micrographs of thin sections of material in the urea resistant and urea soluble fractions of tongue and muzzle desmosome preparations. **Panels a and c** are micrographs of the renatured urea soluble fractions from tongue and muzzle preparations, respectively. **Panels b and d** show the urea insoluble fractions of tongue and muzzle. Note that while the urea resistant material appears to correspond to membrane regions of the desmosome, the renatured urea soluble material appears to resemble the fibrous region adjacent to the desmosome plaque (Fig. 1a). Bar represents 0.2 μm.

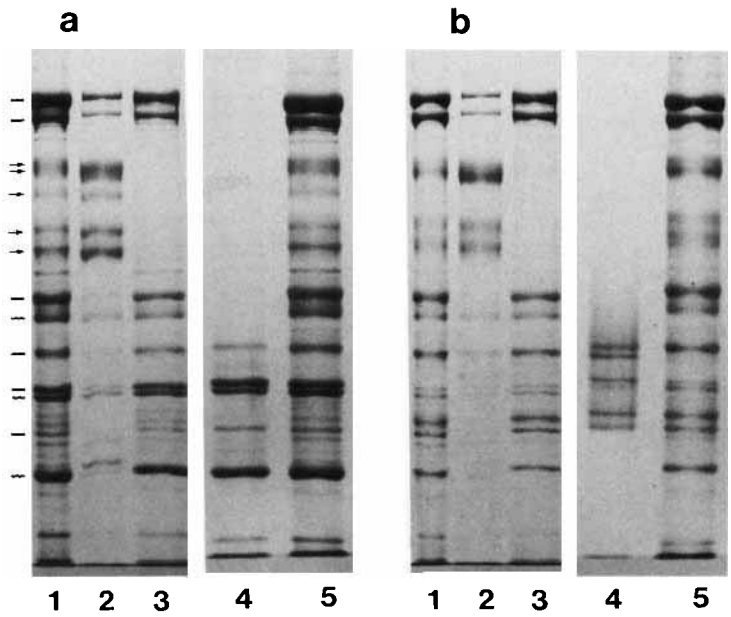

Fig. 4. SDS-PAGE analysis of fractions of desmosomes prepared from tongue (**panel a**) and muzzle (**panel b**). **Panel a: Lane 1**, the enriched tongue desmosome preparation (20 μg); **lane 2**, the urea-insoluble fraction of the tongue desmosome preparation (12 μg). This fraction is enriched in polypeptides of 165, 160, 140, 110, and 100 kDa (indicated by arrows, **lane 1**); **lane 3**, the urea soluble fraction (16 μg), which is enriched in polypeptides of 240, 210, 81, and 75 kDa and the lower molecular weight keratin-like proteins of 65, 57, 56, 50, and 43 kDa. These bands are indicated by lines (**lane 1**); **lane 4**, keratin prepared by extraction of the tongue mucosa with 8 M urea in buffer (7.5 μg) (see Materials and Methods); **lane 5**, the desmosome preparation (30 μg). **Panel b: Lane 1**, the enriched muzzle desmosome preparation (17 μg); **lane 2**, the urea insoluble fraction of the muzzle desmosome preparation (10 μg). This fraction is enriched in polypeptides of 165, 115, and 105 kDa (indicated by arrows, **lane 1**). **Lane 3**, the urea insoluble fraction of muzzle desmosome (15 μg), which contains polypeptides of 240, 210, 81, and 75 kDa as well as keratin-like proteins of 65, 57, 56, 52, and 75 kDa (**lines, lane 1**). **Lane 4**, keratin prepared by extraction of muzzle epidermis with 8 M urea in buffer (5 μg); **Lane 5**, the muzzle desmosome preparation (25 μg).

A side-by-side comparison of the glycoprotein components of the tongue and muzzle desmosomal urea insoluble fraction that were separated (Fig. 6) by SDS-PAGE revealed that the most obvious difference is that the tongue preparation is enriched in a 140 kDa polypeptide. This glycoprotein has been observed to be present in the tongue desmosomes, but a comparable polypeptide was apparently absent in the muzzle desmosomes [32]. In further support of this finding, an affinity purified antibody directed against the 140 kDa polypeptide reacted in Western blots of tongue desmosomal fractions but did not react in muzzle desmosome preparations (Fig. 7).

Minor differences were also seen in the electrophoretic mobility of the PAS-positive polypeptides in the tongue and muzzle urea insoluble fractions. Specifically, there were bands of 115 kDa and 105 kDa in muzzle, while in tongue the closest bands had approximate molecular weights of 110 kDa and 100 kDa. A monoclonal antibody preparation recognized both the 165 kDa glycoprotein of muzzle desmosomes and the 160–165 kDa glycoprotein of tongue desmosomes (Fig. 8).

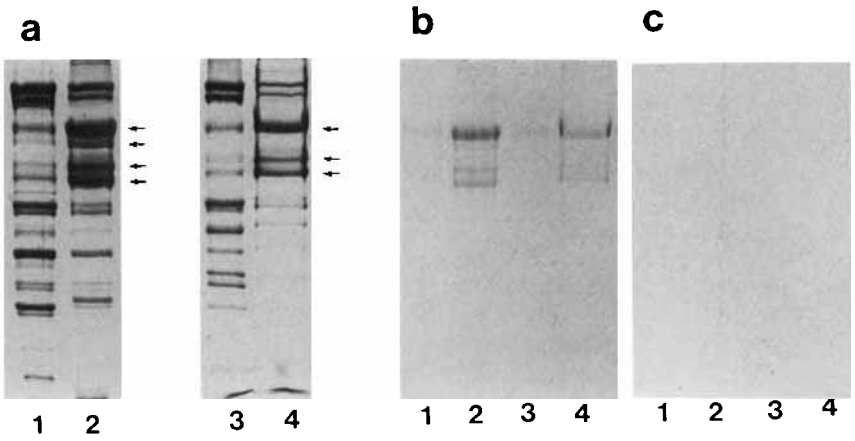

Fig. 5. Polypeptides present in the urea insoluble fraction of the desmosome preparations were identified as glycoproteins based on periodic acid-Schiff's reagent staining for carbohydrate. **Panels a–c** show gels of desmosomal preparations and urea insoluble fraction: **lane 1**, tongue desmosomes; **lane 2**, the urea insoluble fraction of a tongue desmosome preparation. The major polypeptides (indicated by arrows) have apparent molecular weights of 160–165, 140, 110, and 100 kDa; **lane 3**, muzzle desmosomes; **lane 4**, the urea insoluble fraction of muzzle desmosomes. The major polypeptides (indicated by arrows) have apparent molecular weights of 165, 115, and 105 kDa. **Panel a** was stained for protein with Coomassie Blue. **Panel b** was stained for glycoproteins with the periodic acid-Schiff's reagent. Each of the major polypeptides in the tongue and muzzle urea insoluble fraction of the desmosome preparation contains carbohydrate. In **panel c**, the periodate treatment step was omitted as a control.

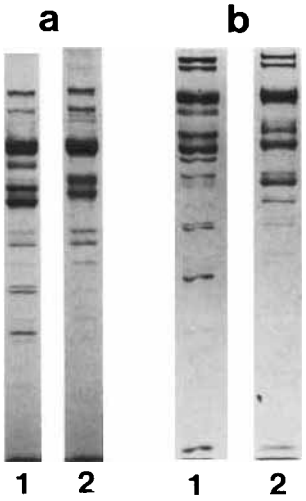

Fig. 6. **Panel a** shows the SDS-PAGE profile of the urea insoluble fraction from bovine tongue (**lane 1**) and bovine muzzle (**lane 2**) desmosome preparations isolated according to a modification of the method of Skerrow and Matoltsy [16]. **Panel b** shows the SDS-PAGE profile of the urea insoluble fractions from bovine tongue (**lane 1**) and bovine muzzle (**lane 2**) preparations isolated according to a second procedure [7]. Note that the polypeptide compositions of the tongue and muzzle fractions are similar.

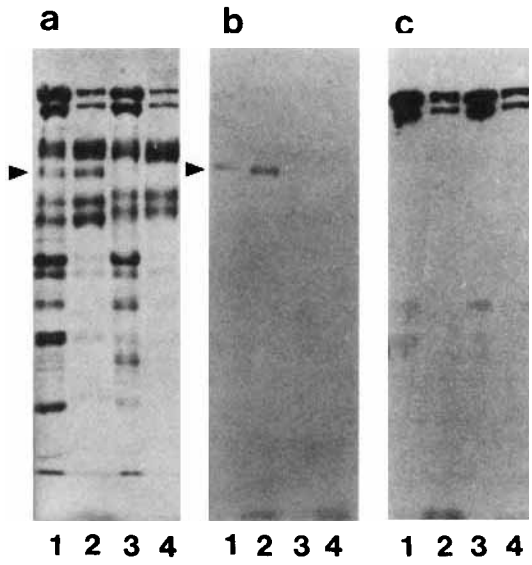

Fig. 7. The urea insoluble fractions of the desmosome preparations and the whole desmosome preparations were subjected to SDS-PAGE and electrophoretically transferred to nitrocellulose. **Panels a–c: lane 1**, tongue desmosome preparation; **lane 2**, the urea insoluble fraction of tongue; **lane 3**, muzzle desmosome preparation; **lane 4**, urea insoluble fraction from muzzle. **Panel a** shows the amido black stain of the transferred protein. **Panel b** shows an immunoblot that was reacted with an affinity purified rabbit antibody against the 140 kDa polypeptide of tongue desmosomes. Note that while this polypeptide is present in tongue desmosomes and is enriched in the urea insoluble fraction of tongue desmosomes it is apparently absent in the muzzle desmosome preparation and urea insoluble fraction. **Panel c** shows an immunoblot that was reacted with a rabbit polyclonal antiserum against the desmoplakins as a control. This antiserum recognized 240 kDa and 210 kDa polypeptides in each of the fractions.

The Keratin-Like Proteins Associated With Desmosomes

There were keratin-like polypeptides associated with the isolated desmosome, and these were identified by immunoreactivity with polyclonal antibodies directed against bovine prekeratin and mouse keratin subunits (Fig. 9) and by comparative gel electrophoresis with keratin prepared by conventional urea extraction of tissue preparations (Fig. 4). It can be noted that the keratins present in a desmosome preparation were found in the same number and amounts relative to that obtained from the whole tissue. However, there were differences in the subset of keratins expressed in tongue and muzzle. While some of the keratin polypeptides appeared to be present in both tissues, others appeared to be present only in tongue or muzzle.

Desmosomal Proteins Solubilized by the Urea Treatment

Treatment of the isolated desmosome with 9 M urea at pH 9 released most of the 240, 210, 81, and 75 kDa polypeptides of the desmosome as well as the putative keratin polypeptides. As described above, the examination of the urea insoluble pellets by thin section electron microscopy showed that the layer of fibrous material adjacent to the desmosomal plaque and most of the dense plaque region was removed (Fig. 3). Because the urea soluble material was likely to contain proteins corresponding to these structures, attempts were made to reassemble the desmosomal proteins solubilized by the urea treatment. The urea soluble fractions of the tongue or muzzle

Fig. 8. Tongue and muzzle desmosome preparations were subjected to SDS-PAGE and electrophoretically transferred to nitrocellulose. **Panel a** shows the amido black stain of the transferred tongue desmosomal preparation and **Panel b** shows an immunoblot of this preparation that was reacted with a monoclonal antibody that recognized the 160-165 kDa desmosomal glycoprotein. **Panel c** shows the amido black stain of the transferred muzzle desmosomal preparation and **panel d** shows an immunoblot that was reacted with the monclonal preparation.

preparations were dialyzed against 10 mM Tris-HCl (pH 7.6), 25 mM B-mercaptoethanol, and 1 mM PMSF. These conditions were selected because they support the assembly of keratin polypeptides into IF (see Keratin Preparations in Methods). The dialysate was centrifuged to collect the pelletable material, the resulting pellet was embedded in plastic, and thin-sections were prepared for electron microscopy. Negligible amounts of protein remained in the supernatant as determined by protein estimation and gel electrophoresis. Ultrastructural examination of the pelletable material showed that this material (Fig. 3) had a fibrillar organization that resembled the material adjacent to the dense plaques of the isolated desmosomes (Fig. 1). No obvious intermediate filaments were evident.

DISCUSSION

The results presented in this paper describe a fractionation procedure for desmosomes from two bovine epithelial tissues. We treated the isolated desmosomes with 9 M urea at Ph 9 and have recovered a glycoprotein-enriched urea resistant fraction and a urea soluble fraction (Figs. 3,4). This relatively simple method for fractionation of desmosomal polypeptides is highly reproducible. We have used it for desmosomes isolated from two different tissues in at least ten preparations. Similar results were obtained from desmosome preparations isolated by two variations of the citric acid procedure described by Skerrow and Matoltsy [16] (see Methods).

The polypeptide composition of the urea resistant fraction of desmosomes reported here was similar to that of the intercellular regions of muzzle desmosomes prepared by metrizamide gradient centrifugation, which have been described by Gorbsky and Steinberg [7]. Specifically, these structures contain major polypeptides of 150, 115, and 110 kDa [7], which appear to correspond to the 165, 115, and 105

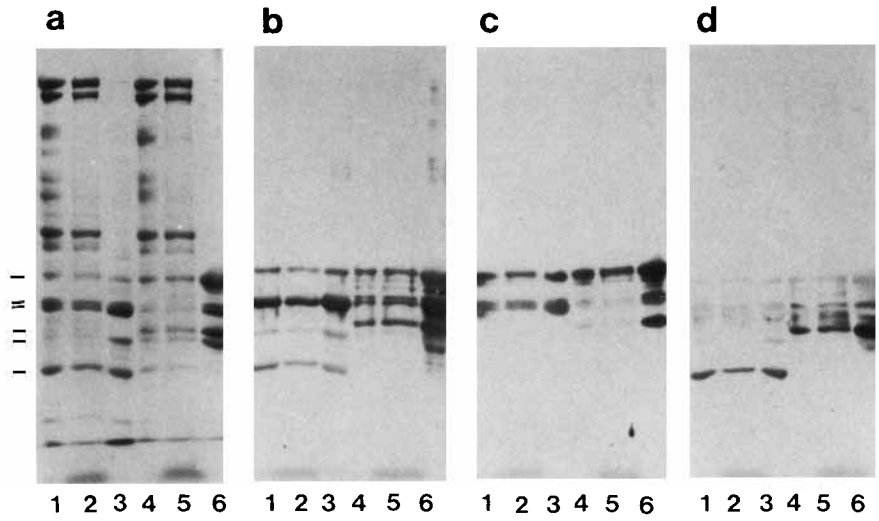

Fig. 9. Desmosome preparations, the urea soluble fractions of the desmosome preparations, and the keratin preparations were subjected to SDS-PAGE and electrophoretically transferred to nitrocellulose. **Panel a** shows the transfer stained for protein with amido black. **Lane 1**, tongue desmosomes; **lane 2**, the urea soluble fraction of tongue desmosomes; **lane 3**, keratin extracted from the tongue mucosa with 8 M urea. **Lane 4**, muzzle desmosomes; **lane 5**, the urea soluble fraction of muzzle desmosomes; **lane 6**, keratin extracted from muzzle epithelium with 8 M urea. Bars at left correspond to apparent molecular weights of the major tongue or muzzle keratin proteins (top to bottom): 64, 57, 56, 52, 50, and 43 kDa. **Panel b** shows a transfer that was reacted with a polyclonal rabbit antiserum against bovine hoof prekeratin. **Panel c** shows a transfer that was reacted with polyclonal rabbit antiserum against mouse keratin K1 (the basic keratin subunit) and **panel d** shows a transfer that was reacted with polyclonal rabbit antiserum against mouse keratin K2 (the acidic keratin subunit). While differences in the keratin expressed in tongue and muzzle epithelia are evident, the keratins present in each desmosome fraction are similar to those present in the corresponding tissue.

kDa polypeptides present in our urea insoluble muzzle desmosome fractions. Previously, Franke et al [17] described an isolation procedure that uses buffer containing 9 M-urea or 5 M guanidine hydrochloride to obtain desmosomal membrane fractions from crude desmosome-tonofilament preparations. However, they reported that their urea resistant fractions contained major proteins of 250, 215, and 68 kDa and that the desmosomal glycoproteins were preferentially lost. More recently, Blaschuk et al [33] have used buffers containing 6 M guanidine hydrochloride to obtain desmosomal membranes from muzzle epidermis and have found these fractions to be enriched in the desmosomal glycoproteins.

The comparative analyses of urea resistant fractions of the desmosome preparations from bovine muzzle epidermis and tongue mucosa have resulted in the finding that there are differences in glycoprotein content between desmosomes obtained from the different epithelial tissues (see Figs. 5,6). The molecular bases for the minor differences in relative mobility are unknown at the present time. However, numerous possibilities exist to explain such differences, including the possibility that the polypeptides differ in primary sequence or their degree of glycosylation or phosphorylation. However, the apparent mobilities of the desmosomal glycoproteins on SDS-PAGE are consistent from preparation to preparation and are essentially identical in desmosomes prepared by two methods.

There is some controversy in the literature regarding the nature of desmosomal glycoproteins in different tissues and species. Desmosomal proteins have been reported to be highly conserved, as determined by immunological crossreactivity over a wide range of tissues and species [31]. Recently, Schmelz et al [34,35] have reported that two monoclonal antibodies recognize a single 165 kDa desmosomal polypeptide in various bovine tissues and in preparations from human and rat tissues or cell lines. On the other hand, differences in the reactivity of desmosomal glycoproteins from different tissues with monoclonal antibodies have been described [36]. However, the protein compositions of the desmosomal fractions studied were not shown in these latter studies [36]. More recently, variations in the apparent molecular weights, the number of bands resolved on SDS-PAGE, and immunoreactivity of glycoproteins present in desmosomes from five vertebrate species have been reported [37]. The determination of the details of similarities and differences in desmosomal glycoproteins from different tissues or species and the possible functional significance of such similarities and differences will require further investigation.

Another, and perhaps more significant difference between the glycoprotein compositions that we have observed in the tongue and muzzle urea insoluble fractions is that the tongue fraction contained a major 140 kDa polypeptide that was not dectectable in the muzzle fraction. Our finding that this polypeptide is enriched in the tongue desmosomal glycoprotein fraction but not in comparable muzzle fractions supports the previous observation of Jones et al [32] that human pemphigus antibodies recognize this 140 kDa desmosome-associated protein in tongue desmosomes but show little or no reactivity with muzzle desmosome preparations.

Differences in the glycoprotein fractions of bovine muzzle and tongue desmosomes are of interest because the desmosomal glycoproteins have been implicated in cell-to-cell adhesion. Cowin et al [14] have recently suggested that the 115 kDa and 100 kDa desmosomal glycoproteins are located on the cell surface and are involved in mediating cell-to-cell adhesion. The 140 kDa glycoprotein present in bovine tongue desmosome preparations also appears to mediate cell-to-cell adhesion. Jones et al [29] have found that the 140 kDa antigen is located along regions of cell-to-cell adhesion including desmosomes, and they have observed that an antibody directed against the 140 kDa molecule disrupts cell-to-cell contact in areas containing desmosomes. These results suggest that the heterogeneity of the desmosomal glycoproteins may be related to the tissue specificity of desmosomes. Such differences are potentially important in the establishment of specific cell-to-cell adhesion and tissue formation and stabilization [34].

The urea soluble fraction of desmosomes is also of interest. This fraction contains most of the 240, 210, 81, and 75 kDa polypeptides of the desmosome, as well as the lower molecular weight keratin-like proteins. When this fraction was dialyzed to remove the urea, renatured desmosomal and keratin-like proteins formed a meshwork that was ultrastructurally similar to the fibrillar regions of the isolated desmosome to which IF appear to attach in situ. Although a detailed ultrastructural comparison between the isolated desmosome and the urea soluble fraction cannot be made from the thin-sectioned material, the fact that the urea soluble fraction contained primarily desmosomal plaque and cytosplasmic proteins is consistent with the idea that one or more of these components form the fibrillar material in the desmosome. Unfortunately, we cannot rule out the possibility that the meshwork observed was the result of pelleting nonspecifically precipitated, denatured proteins that were unable to

refold after treatment with urea. Further fractionation of polypeptides of the urea soluble material should allow a more rigorous investigation of specific protein-protein interactions of the desmosomal components.

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