Journal of Cellular Biochemistry 20:113–126 (1982)

Ultrastructural and Biochemical Identification of Con A Receptors in the Desmosome

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Correlated ultrastructural and biochemical methods were used to identify and localize Concanavalin A (Con A) receptors in the desmosomes of bovine epidermis. Specific carbohydrate residues were labeled with ferritin-Con A in thin sections of tissue embedded in a hydrophilic resin. Quantitative mapping of ferritin distribution in labeled desmosomes revealed that Con A receptors are localized in the intercellular zone and concentrated along the desmosomal midline or central dense stratum. Labeling was almost entirely absent when sections were treated with ferritin-Con A in the presence of 0.1 M α-methyl mannoside, a hapten-inhibitor of Con A. "Whole" desmosomes and desmosomal intercellular regions (desmosomal "cores") were purified from bovine muzzle epidermis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis reveals a limited number of major desmosomal protein constituents. Certain of these are glycoproteins and are greatly enriched in the core fraction. Almost all the desmosomal glycoproteins are intensely labeled when electrophoretic gels of whole desmosome or core fractions are exposed to fluorescent Concanavalin A.

Key words: desmosome, macula adhaerens, cell junction, cell adhesion, Concanavalin A, glycoprotein, postembedding labeling, thin-section labeling, glycol methacrylate

Desmosomes are cell junctions particularly abundant in the stratified squamous epithelia of tissues such as skin and esophagus. Ultrastructural observations show that desmosomal elements mediate both cell-cell adhesion and the attachment of intracellular intermediate filament complexes to the plasma membrane [1–3]. Thus desmosomes are assumed to function in stabilizing the adhesions of cells in tissues.

Overton [4,5] and Wiseman and Strickler [6] have reported that when cells from different embryonic tissues capable of forming desmosomes cooperatively are com-

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Received April 15, 1982; accepted July 22, 1982.

0730-2312/82/2002-0113$04.00 © 1982 Alan R. Liss, Inc.

bined in vitro, cells which produce more desmosomes tend to sort out internally to those which produce fewer. According to earlier analyses on the adhesive behavior of cell populations, the more cohesive of two different cell populations mixed in vitro will tend to assume a position internal to the other [7,8]. Thus interactions among desmosomal components may guide certain adhesion-mediated morphogenetic phenomena during tissue assembly. Ultrastructural evidence indicates that desmosomes appear or disappear during certain normal morphogenetic processes in developing embryos. This has been observed during chick blastoderm expansion [9, 10] and teleost epiboly [11]. In addition, desmosome frequency is often altered in malignant tissue in the course of invasive and metastatic events [12–15], suggesting that perturbation of the desmosomal adhesion may contribute to abnormal cellular behavior in cancer.

Understanding how desmosomal components might mediate cellular recognition requires elucidation of the molecular mechanisms of desmosome-dependent adhesion. Previous studies have shown that fluorescent Concanavalin A (fl-Con A) will bind to cell surfaces on dispersed keratinocytes [6] and in frozen sections of epidermis [17–23]. Van Lis and Kalsbeek [23], using horseradish peroxidase-labeling for electron microscopy, showed low resolution localization of Con A receptors to intercellular regions of keratinocytes both within desmosomes and along nondesmosomal membranes.

Conventional ultrastructural studies reveal an electron-dense material, which has been referred to as the central (dense) stratum [24,25], in the intercellular region of the desmosome. Its sensitivity to proteases in at least some tissues [26,27] suggests that it is proteinaceous in nature. The central stratum is also labeled with various electron-microscopic histochemical stains for carbohydrates. These stains include ruthenium red [28], periodic acid-silver methenamine [29,30], and chromic acid-phosphotungstic acid [31]. We have recently isolated the intercellular regions of bovine epidermis and found them to consist almost entirely of a few glycoproteins [32].

Lectins conjugated to electron-dense markers are powerful histochemical tools for mapping carbohydrate residues at the ultrastructural level. However, this method has not been applied to the study of cell junctions due to the limited resolution of most markers and the inaccessibility of junctional components between cells to macromolecular probes. To overcome these problems we have developed a method for high-resolution labeling of carbohydrate residues with ferritin-lectin conjugates applied to thin sections of materials embedded in a hydrophilic resin [33]. In this paper we use the method to localize Con A receptors in desmosomes. We also demonstrate that nearly all the glycoproteins of purified bovine epidermal desmosomes bind fl-Con A after separation by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (SDS-PAGE). These results demonstrate that Con A-binding carbohydrates are components of the intercellular material of desmosomes and thus may participate in desmosomally mediated cellular adhesion.

MATERIALS AND METHODS

Fixation and Embedding

Fresh bovine muzzles were obtained from a local slaughterhouse and washed thoroughly in running tapwater. A 0.2-mm layer was removed from the internostril

region with an electrokeratotome (Storz Instrument Co, St. Louis, MO) and discarded. A second 0.2-mm layer, consisting primarily of live stratum spinosum, was then removed and used for the labeling procedure.

Tissues were fixed with 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) at 4°C for 1 hr and cut with razor blades into pieces smaller than 1 mm in diameter. For postembedding labeling, Pease's glycol methacrylate-urea-glutaraldehyde copolymer [34] was adopted as the embedding resin. The details of this method are reported elsewhere [33]. Briefly, after glutaraldehyde fixation *without* osmium postfixation, tissues were washed in distilled water and immersed successively in 10%, 25%, and 50% glutaraldehyde, all neutralized with NaOH, for 10 min, 30 min, and 4 hr, respectively. Samples were then transferred to the resin mixture. This was prepared by first adding 3 gm of urea to 10 ml of 50% glutaraldehyde at pH 5.5. The pH was then adjusted to 6.0 and the solution was added to an equal volume of glycol methacrylate (cat. #0227, Polyscience Co, Warrington, PA) containing 0.05% benzoin. The sample was infiltrated 1–3 days at room temperature on a test-tube rotator and transferred to fresh resin in Beem capsules. These were cured in a UV curing chamber (Ladd Industries, Burlington, VT) overnight at 4°C and, after removing the caps of the Beem capsules, for 2 days at room temperature. Finally, 5-mm pieces of resin, including the tissues, were sliced from the block, put into new Beem capsules without caps, and cured in the UV chamber overnight at room temperature.

Thin Sectioning

Immediately after curing, the blocks were glued to epoxy rods and trimmed. After equilibration to room humidity at least overnight, the blocks were coated with a thin film of silicone grease and sectioned with glass knives onto a water-filled trough.

Preparation of Ferritin-Con A Conjugate

Con A was coupled to ferritin by a modification of the glutaraldehyde coupling procedure of Nicolson and Singer [35]. The details of coupling and purification of the conjugate are reported elsewhere [33]. Briefly, 50 mg of ferritin (Miles Laboratories, Inc., Elkhart, IN) and 10 mg of Con A (Sigma Chemical Co., St. Louis, MO) were mixed in 0.6% glutaraldehyde for 6 hr at 4°C. Two successive chromatographic steps, affinity chromatography on Sephadex G-100 followed by gel filtration on a Sepharose 6B column, were used for the purification of active conjugate. Before use, the conjugates were centrifuged at 30,000 g for 15 min to remove precipitates, and diluted to an optical density of 0.9 at 330 nm.

Labeling of Ultrathin Sections

Ultrathin sections of tissue on grids were first placed for 30 min at room temperature in Dulbecco's phosphate-buffered saline, prepared without calcium and magnesium salts (CMF-PBS), to hydrate the resin. Then, to block nonspecific protein binding sites, grids were immersed in a solution of 0.4% bovine serum albumin (BSA) in PBS for 30 min. The grids were subsequently labeled with the ferritin-lectin conjugate. The effects of various incubation periods (15, 30, and 60 min) were examined. The grids were then washed with 0.3 M potassium phosphate buffer at pH 7.4 for 20 min and with distilled water for 10 min. Finally, the sections were stained in 1% aqueous uranyl acetate for 15 min. In some instances, grids were further

stained with lead citrate [36] for 5 min. The heavy metal staining caused no decrease in specific or nonspecific labeling by the ferritin-Con A. The addition of calcium or manganese salts (0.1 mM) to labeling buffers had no effect on ferritin-Con A labeling. Presumably sufficient amounts of these cofactors, essential for Con A binding [37], were present in the reagents used.

Quantitation of Con A-Receptor Distribution

Desmosomes sectioned at right angles to the cell membranes were selected for quantitative analysis. On photographs at × 330,000 final magnification, lines were drawn through the intracellular plaques parallel to and equidistant from the plasma membranes. Histograms were prepared by plotting the positions of ferritin molecules as a function of distance from these lines.

Effect of Section Thickness on Amount of Ferritin-Con A Binding

To ascertain the extent of penetration of the ferritin-lectin conjugate into the resin, we varied the thickness of the sections from about 60 nm (silver-gray) to 170 nm (purple) [38] and quantitated the amount of specific labeling. If the resin was freely permeable, then the thicker sections, containing more receptors, should bind more Con A.

Effects of High pH Treatment

To investigate whether carbohydrate-protein linkages in the embedded desmosomes were sensitve to high pH treatment, sections were incubated in 0.05N sodium hydroxide for 12 hr at room temperature and then labeled by the normal procedure. Control sections were incubated in distilled water for 12 hr. Histograms of ferritin distribution were obtained as before.

Resolution of Desmosomal Proteins by SDS-PAGE

Whole desmosomes, which contain both intercellular and cytoplasmic desmosomal elements, and desmosomal cores, which consist almost entirely of intercellular material sandwiched between plasma membranes, were isolated as previously described [32]. Preparations were electrophoresed on polyacrylamide gradient gels according to the general method of Laemmli [39]. To detect total protein, gels were stained for 12 hr in 0.1% Coomassie brilliant blue R in 50% methanol, 10% acetic acid in water, and destained in several changes of 5% methanol, 10% acetic acid.

Labeling of Proteins in Polyacrylamide Gels With Fluorescent Con A

All steps were carried out at room temperature, and gels were agitated in all solutions. Routinely after electrophoresis, gels were placed briefly (5–10 min) in Coomassie blue staining solution and then destained overnight with two changes of destaining solution. This light prestaining facilitated accurate slicing of gel lanes and did not interfere with subsequent binding of the fl-Con A. In some experiments, prior to labeling with fl-Con A, gels were not prestained but were fixed in 10% glutaraldehyde and subsequently treated with sodium borohydride to reduce excess aldehyde groups. However, simple overnight incubation in destaining solution effectively fixed proteins such that very little diffusion of proteins in gels occurred during the labeling procedure.

The gels were removed from the destaining solution, rinsed in water, and sliced with a pizza cutter. They were then treated with three 30-min washes in 0.05 M

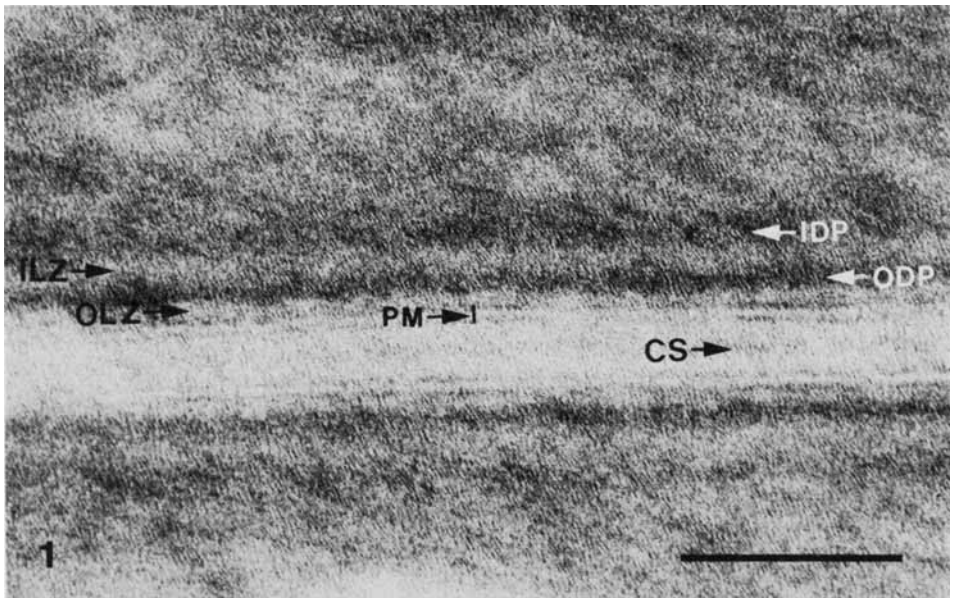

Fig. 1. Desmosome of bovine muzzle epidermis fixed in glutaraldehyde without osmium postfixation, embedded in Pease's resin, sectioned, and stained with uranyl acetate. Note stratification of intracellular plaque. Central dense stratum (CS), outer electron-lucent zone (OLZ), outer electron-dense plaque (ODP), inner electron-lucent zone (ILZ), inner electron-dense plaque (IDP) × 330,000. Bar = 0.1 μm.

phosphate, 0.15 M NaCl, 0.1 mM $CaCl_2$, 0.1 mM $MnCl_2$ adjusted to pH 7.4 (PBS+). The addition of the calcium and manganese salts to staining buffers proved essential for fl-Con A labeling in gels. Gel slices were transferred to large culture tubes and 0.5 mg of fl-Con A (Sigma Chemical Co, St. Louis, MO) in 10 ml of PBS+ was added. The tubes were wrapped in aluminum foil. All subsequent steps were carried out while avoiding exposure of the gel to light. As controls, some gels were incubated in fl-Con A in the presence of 0.2 M α-methyl mannoside. After an 18–24-hr incubation in the lectin solutions, gels were rinsed and washed 2 to 3 times in PBS+ for 30 min each. Fluorescent bands were visualized with an ultraviolet transilluminator (360 nm peak wavelength, Ultraviolet Products, Inc, San Gabriel, CA) and photographed on Polaroid type 57 film through a dark yellow gelatin filter and two 3-mm-thick glass plates.

RESULTS

Desmosomal Ultrastructure

As shown in Figure 1, a trilaminar plasma membrane image is observed in desmosomes embedded in the hydrophilic resin. Plasma membrane leaflets of the non-desmosomal areas are less clearly resolved. The intercellular region of the desmosome (25–37 nm) is filled with an electron-dense material, a portion of which forms the central stratum.

On the cytoplasmic side of the plasma membrane, four layers were clearly resolved. The most prominent is the outer electron-dense plaque (ODP) near the inner

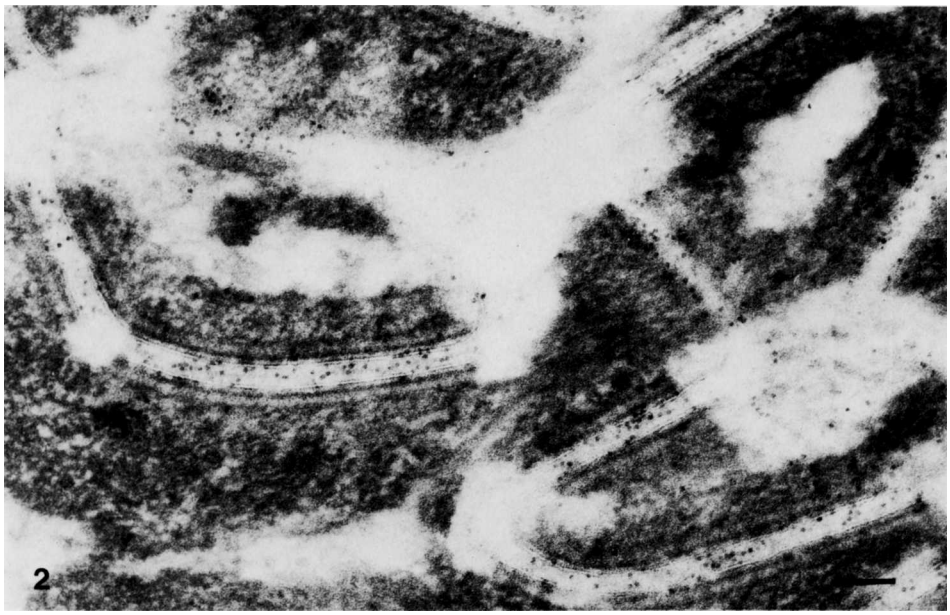

Fig. 2. Ferritin-Con A labeling of bovine epidermis. Ferritin cores are concentrated in the intercellular space of desmosomes. × 120,000. Bar = 0.1 μm.

leaflet of the plasma membrane. The ODP may correspond to some part of the dense plaque referred to by many previous workers though it is only about 6–9 nm wide, unlike the 12–15 nm wide density observed by Leloup et al [2] in the same tissue using conventional EPON sections. Between the ODP and the plasma membrane is the outer lucent zone (OLZ). Just inside the ODP are two more stratifications, namely, the inner electron-dense plaque (IDP) and the inner lucent zone (ILZ). The borders of these zones are not sharp, making it difficult to determine their exact widths. The tonofilaments insert into the IDP.

Con A Receptor Localization

As shown in Figure 2, ferritin-Con A was primarily localized in the desmosomal intercellular space. Nonjunctional plasma membrane labeled much more sparsely. Specific binding was almost entirely eliminated when ferritin-Con A was applied in the presence of 0.1 M α-methyl mannoside (Fig. 3a). Essentially no labeling was evident when unconjugated ferritin was used (Fig. 3b). Quantitative measurements revealed that the labeling index dropped from 66 ferritin molecules per μm of membrane to less than 3 when α-methyl mannoside was added or when free ferritin was used (Fig. 4).

The method for determining the distribution of label is described in Figure 5. The ferritin molecules are distributed unimodally around the central stratum (CS). In Figure 5, half of the ferritin cores lie close to the central stratum (within the central 20% of the span (0–100) between the center of the outer dense plaques).

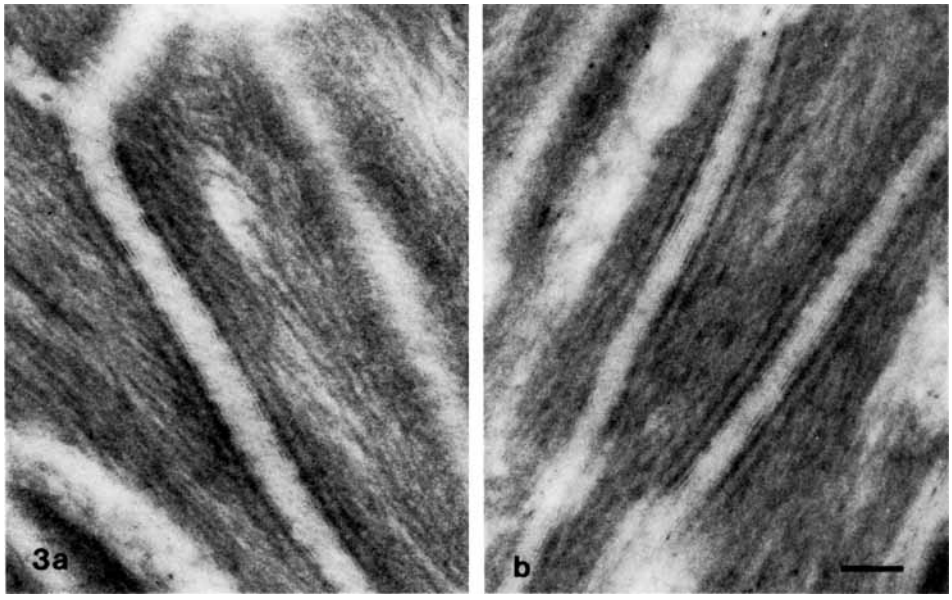

Fig. 3. a) Labeling of desmosome with ferritin-Con A in the presence of its hapten inhibitor, α-methyl mannoside. b) Labeling with unconjugated ferritin. Nonspecific binding is almost completely absent. × 120,000. Bar = 0.1 μm.

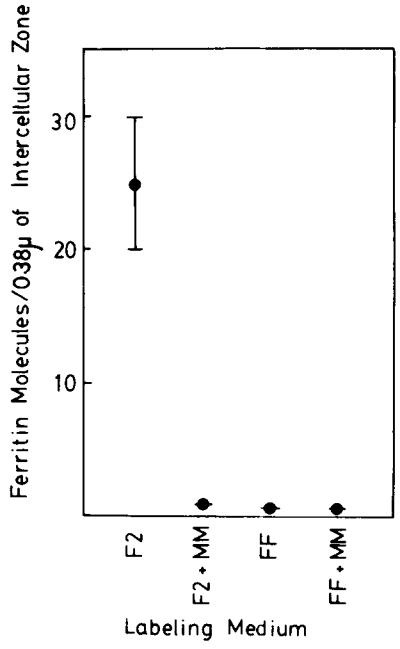

Fig. 4. Comparison of labeling counts of ferritin cores under various labeling conditions. Ferritin-Con A conjugate (F2), ferritin-Con A conjugate plus α-methyl mannoside (F2 + MM), free ferritin (FF), free ferritin plus α-methyl mannoside (FF + MM).

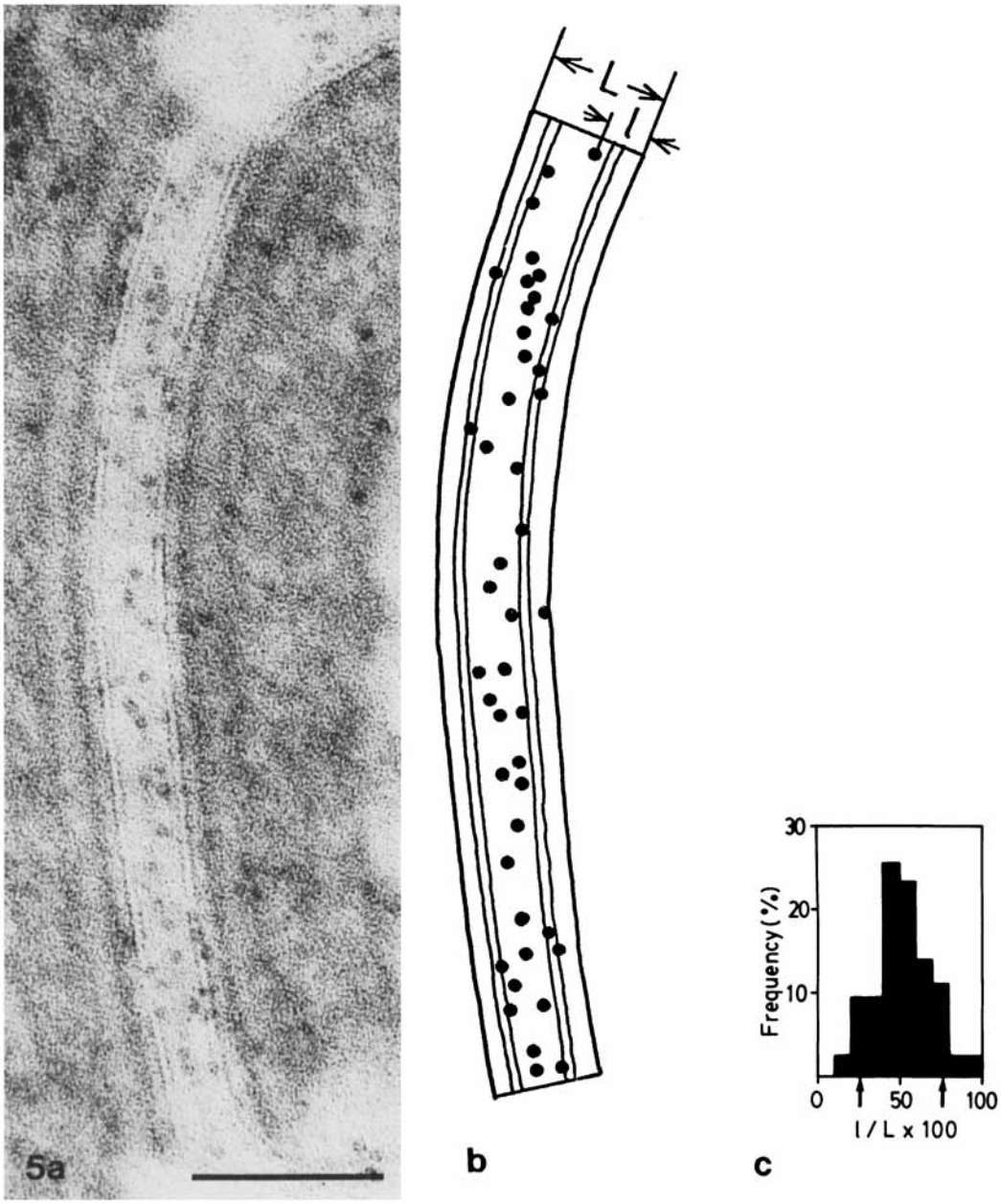

Fig. 5. Quantitation of ferritin distribution within desmosomes sectioned at right angles to the plane of the plasma membrane. a) Original micrograph × 300,000. Bar = 0.1 μm. b) Lines were drawn through the center of the ODPs. The distance (l) for each ferritin core was measured from one of the lines and calculated as a percentage of the total distance (L) between the lines. c) Histogram of ferritin core distribution. Arrows indicate position of the plasma membranes. Note clustering of label around the desmosomal midline.

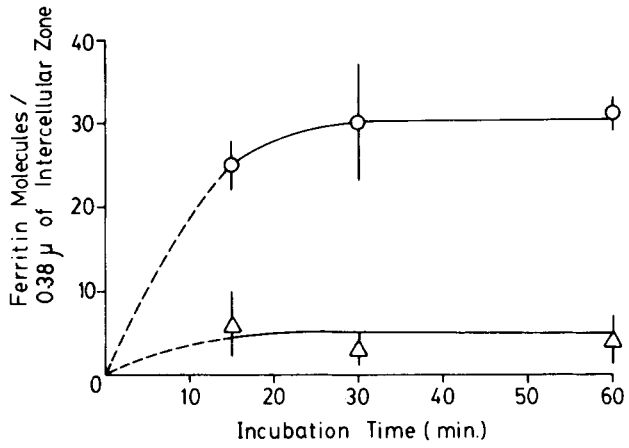

Fig. 6. Effect of duration of incubation of sections of epidermis with ferritin-Con A in the absence (○) and in the presence (△) of α-methyl mannoside. Both specific and nonspecific binding are saturated by 30 min.

Effects of Incubation Time, Section Thickness, High pH

After a 30-min incubation with ferritin-Con A, specific labeling was maximal and did not increase with longer exposure of the sections to the probe (Fig. 6). Likewise, labeling in the presence of 0.1 M α-methyl mannoside was not altered by increasing the incubation period.

Varying the thickness of the sections caused no change in the amount of ferritin-Con A bound. Therefore we conclude that binding of the probe occurs only near the surface of the sections.

The distribution profile of Con A-receptors was not affected by 12-hr preincubation of the sections in 0.05 N NaOH. The markers were again clustered around the CS. However, a 12-hr preincubation in either 0.05 N NaOH or distilled water did nearly double the total amount of ferritin-Con A bound.

Fluorescent Con A Labeling of Desmosomal Proteins in Polyacrylamide Gels

In gels of both whole desmosomes and desmosomal cores, fluorescent Con A labeled the series of bands between 97,000 and 150,000 molecular weight (Fig. 7). This labeling was absent when the fluorescent lectin was applied in the presence of 0.2 M α-methyl mannoside. These Con A-binding proteins are the predominant components in desmosomal cores and label intensely with the periodic acid-dansyl hydrazine stain for nonspecific carbohydrate in electrophoretic gels (see Fig. 4 in [32]).

DISCUSSION

Con A receptors in desmosomes of bovine muzzle epidermis are distinctly labeled when ferritin-Con A conjugates are applied to thin sections of tissue embedded in Pease's resin by our technique. These receptors are essentially restricted to the desmosomal intercellular space and are concentrated along the central stratum. Bind-

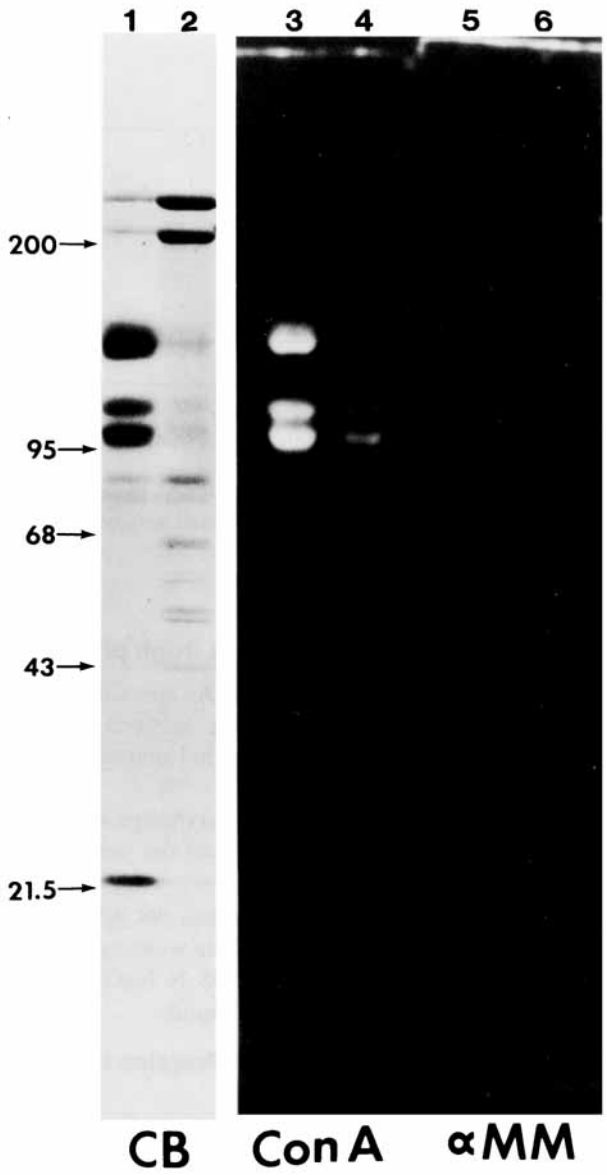

Fig. 7. Desmosomal cores (lanes 1, 3, and 5) and whole desmosomes (lanes 2, 4, and 6) run on 5–20% polyacrylamide gels in the presence of SDS and β-mercaptoethanol. Staining for total protein with Coomassie blue (CB); staining with fluorescent Con A (Con A); staining with fluorescent Con A in the presence of 0.2 M α-methyl mannoside (αMM).

ing of the ferritin-Con A probes is specific since it is abolished by labeling in the presence of α-methyl mannoside. Maximal labeling of the receptors in the thin sections occurred by 30 min and lengthening of the labeling period brought no increase in binding. However, overnight preincubation in distilled water did somewhat enhance penetration of the sections by affinity probes. This may be due to

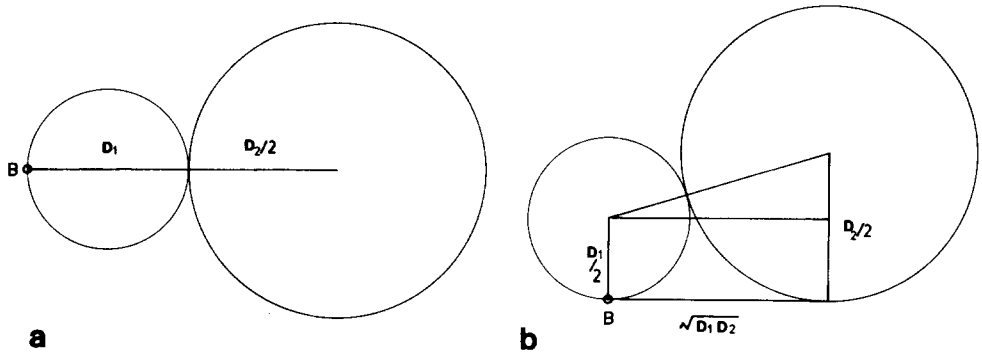

Fig. 8. Schematic representation of ferritin-Con A conjugates showing maximal distances of Con A-binding site (B) from ferritin core if section were freely permeable (a) or if Con A could bind only to receptors at the resin surface (b). Results from experiments where section thickness was varied indicate that b represents the more likely configuration for binding. Diameter of Con A = D_1; diameter of ferritin = D_2.

swelling of the resin since such pretreatment also increased slightly the observed width of the desmosomal intercellular zone.

Experiments in which section thickness was varied indicated that binding occurred primarily on or near the section surface. This might mean that the resin itself is permeable only to a very limited depth or that desmosomal components themselves are occluding those potential Con A receptors in the intercellular space away from the surface of the section. If penetration of the resin by macromolecular probes were possible, then the greatest distance of the electron-dense ferritin core from the binding site of the lectin would be

$$\frac{D_2}{2}+D_1$$

where D_1 and D_2 are the diameters of Con A and ferritin molecules, respectively. Assuming a diameter of 8.4 nm for Con A [40] and 12 nm for ferritin [41,42], the maximal distance would be 14.4 nm (Fig. 8a). However, if binding occurs only with receptors exposed at the section surface and no significant penetration of the resin by the probes occurs, then the maximal distance of the iron core from the receptor would be

$$\sqrt{D_1D_2}$$

or only 10nm (Fig. 8b). Nearly all ferritin-Con A molecules on the sections lie either within the intercellular space or within 10 nm of the outer leaflet of the plasma membrane, suggesting that the latter situation prevails.

Irimura et al [43] have divided lectins into three classes depending on their tendency to bind the major subgroups of carbohydrate chains on proteins. Lectins in the first class bind preferentially to moieties common in 0-linked sugar chains. Lectins

in the second class, which includes Con A, prefer the sugars common in N-linked oligosaccharides. The third class of lectins binds equally well to components with either sugar linkage. If their analysis is correct, alkaline treatment, which preferentially cleaves the more labile 0-linkage [44], would not be expected to affect Con A binding. The fact that ferritin-Con A binding was not altered by this treatment provides some evidence that the desmosomal carbohydrates may indeed be N-linked.

To corroborate the ultrastructural data, we have identified the Con A-binding glycoproteins of epidermal desmosomes on electrophoretic gels. These comprise a group of proteins which can be resolved into several bands, from 97,000 to 150,000 molecular weight. Data from studies with monoclonal antibodies against these proteins suggest that these bands represent minor variations of only two distinct major desmosomal glycoproteins [45].

Through use of a similar strategy, a series of Con A-reactive glycoproteins have been identified in the synaptic junctions of nerve cells. Labeling cellular fractions containing synaptic junctions with ferritin-Con A before embedding revealed that the probe binds primarily to the plasma membrane overlying the postsynaptic density [46, 47]. In this preembedding technique, labeling occurred only where complementary membranes of the junctions had separated, suggesting that the probe could not penetrate into intact junctions. ^{125}I-Con A applied to SDS-gels of isolated synaptic junctions labeled a series of high molecular weight bands [48,49]. Tryptic peptide maps revealed that some of these glycoproteins are very similar in their amino acid sequences [49].

The function of the carbohydrate moieties in desmosomes remains to be elucidated. Many workers have implicated cell surface glycoproteins in adhesion-related activities of sponges [50], slime molds [51, 52] and avian and mammalian cells [53, 54]. Overton [55] has recently reported that succinyl-Con A, a nonagglutinating form of the lectin, inhibits desmosome formation in aggregating chick corneal epithelium cells. Thus desmosomal sugars may participate in the adhesive mechanics of desmosomes and might contribute to cellular recognition phenomena involved in tissue assembly.

Using a method for affinity labeling of specific constituents of plastic-embedded and sectioned tissues, we have localized the Con A receptors of desmosomes to the intercellular region. We have identified these receptors as a set of specific glycoproteins among the electrophoretically separated proteins of isolated desmosomal cores. These two sets of experiments permit correlation of the biochemical properties of isolated macromolecules with their precise ultrastructural distribution within intact tissue. Similar correlated ultrastructural and biochemical mapping using these methods with monoclonal antibodies to desmosomes, probes which we have recently developed [45], should greatly increase both sensitivity and resolution. The techniques described above should prove generally useful in elucidating the structure, assembly, and function of cellular components both inside cells and in occluded positions on their surfaces.

ACKNOWLEDGMENTS

We thank Dorothy Spero for her assistance and excellent care of the electron microscope. This study was supported by grant 1 RO GM 26047 from the National Institute of General Medical Sciences and utilized the central equipment facilities of

the Department of Biology, supported by the Whitehall Foundation. G.G. was the recipient of a predoctoral fellowship from the National Science Foundation.

REFERENCES

1. Kelly DE: J Cell Biol 28:51, 1966.
2. Leloup R, Laurent L, Ronveaux MF, Drochmans P, Wanson JC: Biol Cellulaire 34:137, 1979.
3. Skerrow CJ: Invest Cell Pathol 1:23, 1978.
4. Overton J: Dev Biol 55:103, 1977.
5. Overton J: J Theor Biol 65:787, 1977.
6. Wiseman LL, Strickler J: J Cell Sci 49:217, 1981.
7. Steinberg MS: Science 141:401, 1963.
8. Steinberg MS: In Curtis ASG (ed): "Cell-Cell Recognition." Cambridge: Cambridge University Press, 1978, p 25.
9. Bellairs R: J Embryol Exp Morphol 11:201, 1963.
10. Overton J: Dev Biol 4:532, 1962.
11. Lentz TL, Trinkaus JP: J Cell Biol 48:455, 1971.
12. Alroy J, Pauli BU, Weinstein RS: Cancer 47:104, 1981.
13. McNutt NS, Hershberg BA, Weinstein RS: J Cell Biol 51:805, 1971.
14. Weinstein RS, Merk FB, Alroy J: Adv Cancer Res 24:23, 1976.
15. Weinstein R, Zel G, Merk FB: In Schultz J, Block RE (eds): "Membrane Transformations in Neoplasia." New York: Academic Press, 1974, p 127.
16. Gommans JM, Van den Hurk JJMA: Br J Dermatol 104:641, 1981.
17. Hashimoto K, King CE, Yamanishi Y, Beachey EH, Maeyens E: J Invest Dermatol 62:423, 1974.
18. Holt PJA, Anglin JH, Nordquist RE: Br J Dermatol 100:237, 1979.
19. Imamura S, Takigawa M, Ofuji S: Acta Derm Venereol (Stockh) 59:113, 1979.
20. Nieland ML: J Invest Dermatol 60:61, 1973.
21. Nishikawa T, Harada T, Hatano H, Ogawa H, Miyazaki H: Acta Derm Venereol (Stockh) 55:21, 1975.
22. Nishikawa T, Harada T, Hatano H, Ogawa H, Taneda A. Miyazaki H: Acta Derm Venereol (Stockh) 55:309, 1975.
23. Van Lis JMJ, Kalsbeek GL: Br J Dermatol 92:27, 1975.
24. McNutt N, Weinstein S: Prog Biophys Mol Biol 26:47, 1973.
25. Staehelin LA: Int Rev Cytol 39:191, 1974.
26. Borysenko JZ, Revel JP: Am J Anat 137:403, 1973.
27. Overton J: J Exp Zool 168:203, 1968.
28. Luft JH: Anat Rec 71:369, 1971.
29. Hopwood D, Logan KR, Milne G: Histochemistry 54:67, 1977.
30. Rambourg A, Leblond, CP: J Cell Biol 32:27, 1967.
31. Rambourg A: Int Rev Cytol 31:57,1971.
32. Gorbsky G, Steinberg MS: J Cell Biol 90:243, 1981.
33. Shida H, Shida M, Gorbsky G, Steinberg MS: J Histochem Cytochem 30:1153,1982.
34. Pease DC: J Ultrastruct Res 45:124, 1973.
35. Nicolson GL, Singer SJ: J Cell Biol 60:236, 1974.
36. Reynolds EF: J Cell Biol 17:208, 1963.
37. Liener, IE: In Bittinger H., Schnebli HP (eds): "Concanavalin A as a Tool." London: John Wiley and Sons, 1976, p 17.
38. Peachey LD: J Biophys Biochem Cytol 4:233, 1958.
39. Laemmli UK: Nature (Lond) 227:680, 1970.
40. Reeke GN, Becker JW, Cunningham BA, Wang JL, Yahara I, Edelman GM. In Chowdnury TK, Weiss AK (eds): "Concanavalin A." New York and London: Plenum Press, 1974, p 13.
41. Fischbach FA, Anderegg JW: J Mol Biol 14:458, 1965.
42. Harrison PM: J Mol biol 6:404, 1963.
43. Irimura T, Nakajima M, Hirano H, Osawa T: Biochem Biophys Acta 413:192, 1975.
44. Sharon N: "Complex Carbohydrates. Their Chemistry, Biosynthesis and Functions." Reading, Massachusetts: Addison-Wesley Publishing Co. Inc., 1975.
45. Cohen S, Gorbsky G, Steinberg MS: J Biol Chem (in press).

46. Cotman CW, Taylor D: J Cell Biol 62:236, 1974
47. Kelly P, Cotman CW, Gentry C, Nicolson GL: J Cell Biol 71:487, 1976.
48. Rostas JAP, Cotman CW: Anal Biochem 80:366, 1977.
49. Mena EE, Cotman CW: Science 216:422, 1982.
50. Henkart P, Humphreys S, Humphreys T: Biochemistry 12:3045, 1973.
51. Muller K, Gerisch G: Nature (Lond) 274:445, 1978.
52. Rosen SD, Chang C-M, Barondes SH: Dev Biol 61:202, 1977.
53. Rutishauser U, Thiery JP, Brackenbury R, Sela BA, Edelman GM: Proc Natl Acad Sci USA 73:577, 1976.
54. Takeichi M: J Cell Biol 75:464, 1977.
55. Overton J: J Cell Biol 87:92a, 1980.