ENZYMATIC DIGESTION OF DESMOSOME AND HEMIDESMOSOME PLAQUES PERFORMED ON ULTRATHIN SECTIONS

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INTRODUCTION

The fine structure of desmosomes is well established (1–3), and some information about their chemical composition is available (4–6). Recently techniques were developed for performing enzymatic digestions on osmium tetroxide–fixed, alcohol- or acetone-dehydrated and epoxy-embedded tissues (7–10). These methods can determine with considerable specificity the organic components of many cell structures (8–10). Protease digestion, for example, permits chemical discrimination among certain morphological components of desmosomes and hemidesmosomes.

MATERIALS AND METHODS

Small intestine from the glass catfish *Kryptopterus bicirrhus* and tadpole tail skin (*Rana pipiens*) were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.3, for 1 hr then postfixed in 1% osmium tetroxide for 30 min. The tissue was dehydrated in acetone and embedded in an Epon-Araldite mixture (11). Silver sections, cut on a Porter-Blum MT-1 ultramicrotome (Ivan Sorval, Inc., Norwalk, Conn.), were picked up on uncoated 300 mesh copper grids. To facilitate their total immersion during subsequent processing, the grids were mounted in Locke's staining rings (12).

The thin sections were oxidized in 5% H_2O_2 for 10 min at 23°C, and the grids were then thoroughly washed in distilled water. This was followed by incubation at 37°C in a 0.3% protease solution (Worthington) at pH 6.8 for 50 min. Control sections were incubated for the same length of time in distilled water at the same temperature and pH. The grids were washed thoroughly and then stained for 30 min in a saturated solution of uranyl acetate in 70% ethanol, 100% methanol 1:1 (v/v), and then in the stock solution of lead citrate (13). Sections were examined and photographed in an RCA EMU-3F electron microscope.

OBSERVATIONS

The fine structure of the desmosome in the catfish small intestine (Fig. 1) is in all respects similar to that in the amphibian epidermis (Fig. 4). In both cases there is a highly electron-opaque plaque on either side of the attachment zone and an intercellular contact zone of varying density in the interval between the apposed unit membranes. This is the characteristic fine structure of the macula adherens or typical desmosome (1, 2). The association of cytoplasmic tonofilaments with an electron-opaque, amorphous material above the plaque is particularly well illustrated in the hemidesmosome on the basal surface of the amphibian epidermis (Figs. 7, 8).

Little change is detected in the fine structure of the desmosomes and hemidesmosomes in peroxide-treated sections incubated in water (Figs. 2, 5, 8). There is, however, some over-all loss of density in these sections. After incubation in protease the desmosome plaques are completely removed (Figs. 3, 6), and the electron-opaque plaques of the hemidesmosomes become electron lucent (Fig. 9). The cytoplasmic tonofilaments associated with the plaques of the desmosomes and hemidesmosomes (Figs. 6, 9) and the intercellular components of the desmosome (Figs. 3, 10) are not affected by protease digestion. Fig. 10 clearly demonstrates that the plaque of the desmosome is specifically digested by the protease while the

adjacent unit membrane and the intercellular contact zone are retained.

Enzymatic digestions with other enzymes (pepsin, chymotrypsin, and trypsin) were carried out. Pepsin and chymotrypsin attack the electron-opaque plaques, rendering them electron lucent, but trypsin has no observable effect on the desmosome or hemidesmosome plaques.

When thin sections containing desmosomes or hemidesmosomes are exposed to prolonged enzymatic extraction, the plastic surrounding the digested material weakens, allowing the electron beam to produce holes in this region. The production of wide holes obscures the effects of specific enzyme digestion, and thus the digestion times utilized must allow for the removal of specific macromolecules without appreciably weakening the plastic embedment.

DISCUSSION

The removal of the desmosome plaque with protease suggests that the composition of the plaque is different from that of the tonofilaments, the intercellular material present between the apposed unit membranes, and the membranes themselves. Such a distinction between the plaques and the intercellular contact material is not surprising since Kelly (3), Benedetti and Emmelot

FIGURE 1 This untreated thin section of catfish small intestine, stained with uranyl acetate and lead citrate, shows the normal structure of the desmosome including the electron-opaque plaques (*p*) and the intercellular contact substance (*i*). × 53,000.

FIGURE 2 This thin section from catfish small intestine was oxidized in 5% H_2O_2 for 10 min at 23°C, incubated in distilled water pH 6.8 for 50 min at 37°C, and then stained with uranyl acetate and lead citrate. The dense plaques of the desmosome (*p*) and the intercellular contact substance (*i*) are unaffected by the treatment. × 53,000.

FIGURE 3 This thin section from catfish small intestine was oxidized in 5% H_2O_2 for 10 min at 23°C, then incubated in 0.3% protease pH 6.8 for 50 min at 37°C, and stained with uranyl acetate and lead citrate. The plaques of the desmosomes now appear as clear areas (*ca*), but the intercellular contact substance (*i*) is unaffected by the treatment. × 53,000.

FIGURE 4 In this untreated section of amphibian epidermis, stained with uranyl acetate and lead citrate, the dense plaques of the desmosome (*p*), intercellular contact substance (*i*), and tonofilaments (*f*) are evident. × 53,000.

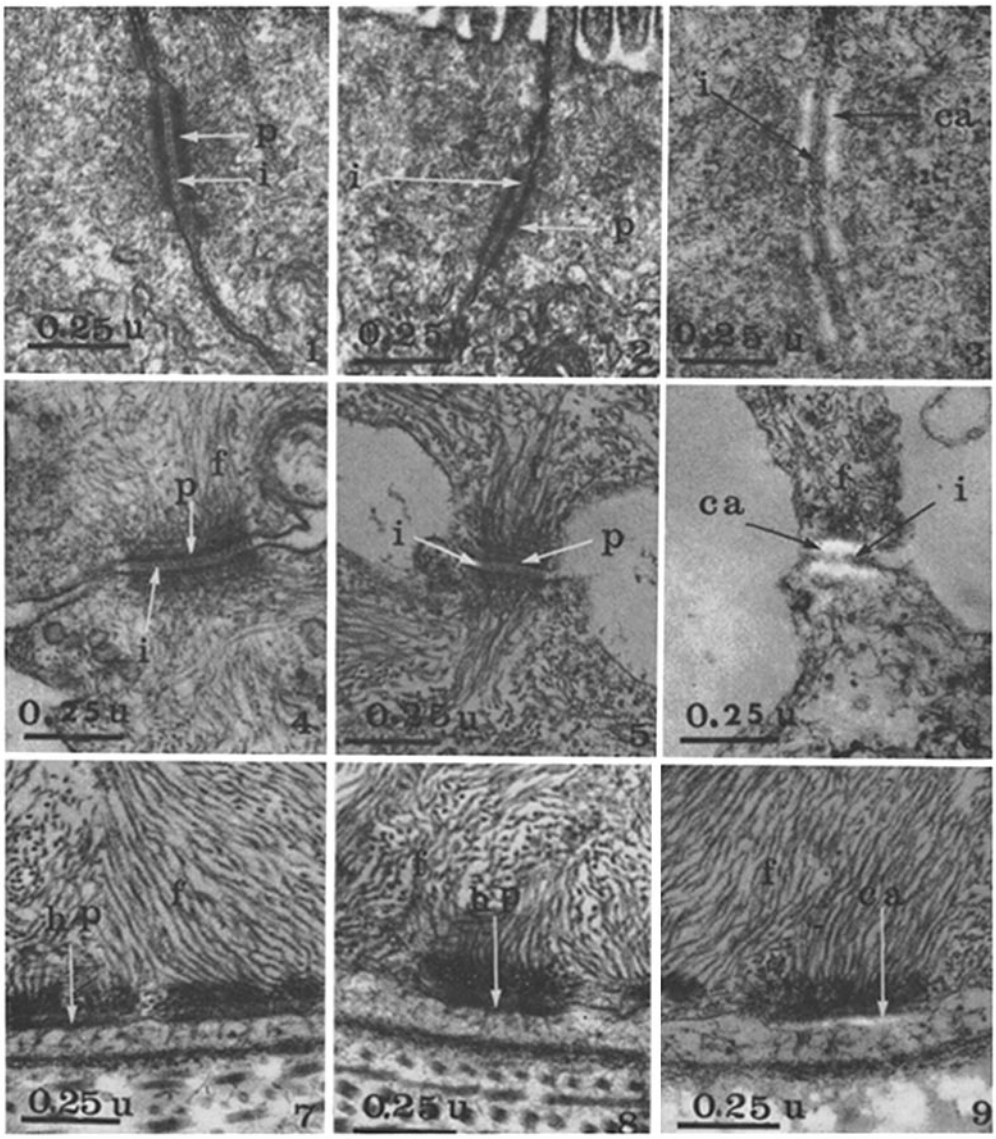
FIGURE 5 This thin section of amphibian epidermis was oxidized in 5% H_2O_2 for 10 min at 23°C, then incubated in distilled water pH 6.8 for 50 min at 37°C, and stained with uranyl acetate and lead citrate. The desmosomal plaques (*p*), intercellular contact substance (*i*) and tonofilaments (*f*) are not affected by the treatment. × 53,000.

FIGURE 6 This thin section of amphibian epidermis was oxidized in 5% H_2O_2 for 10 min at 23°C, incubated in 0.3% protease pH 6.8 for 50 min at 37°C, and stained with uranyl acetate and lead citrate. The plaques of the desmosome are now electron lucent (*ca*), but the intercellular contact material (*i*) and tonofilaments (*f*) are not affected. × 53,000.

FIGURE 7 This thin section of amphibian skin stained in uranyl acetate and lead citrate reveals the normal structure of the hemidesmosome including the plaque (*hp*) and tonofilaments (*f*). × 53,000.

FIGURE 8 After oxidation in 5% H_2O_2 for 10 min at 23°C, incubation in distilled water pH 6.8, and staining with uranyl acetate and lead citrate, the plaques of the hemidesmosomes (*hp*) and the tonofilaments (*f*) are unaffected. × 53,000.

FIGURE 9 After oxidation in 5% H_2O_2 for 10 min at 23°C, incubation in 0.3% protease pH 6.8 for 50 min at 37°C, and staining with uranyl acetate and lead citrate, the plaque of the hemidesmosome (*ca*) is no longer dense, but the tonofilaments (*f*) are unaffected. × 53,000.



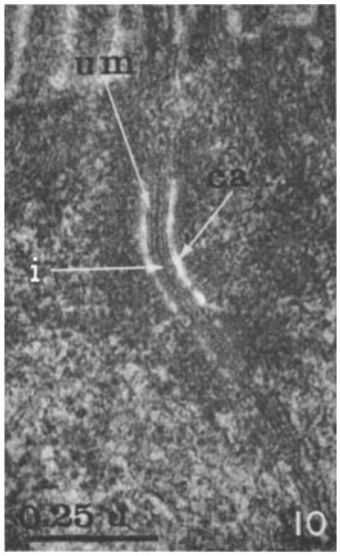


FIGURE 10 In this section from catfish small intestine oxidized in 5% H_2O_2 for 10 min at 23°C, incubated in 0.3% protease pH 6.8 for 50 min at 37°C, and stained with uranyl acetate and lead citrate, the plaques of the desmosome are no longer evident (*ca*), but the leaflets of the unit membranes (*um*) and the intercellular contact substance (*i*) are unaffected. × 71,000.

(4), and Rambourg and Leblond (5) have provided evidence that the intercellular component of desmosomes contains acid mucopolysaccharide, sialic acid, and polysaccharide. However, Kelly (3) noted an increase in electron opacity in the area between the plaque and the cell membrane in desmosomes treated with ruthenium red, which indicates the presence of an acid mucopolysaccharide binding between the plaque and the membrane. Our observations suggest that, if acid mucopolysaccharide is present in this zone, the amount of it there is less than in the intercellular space and it is probably admixed with protease-labile protein.

Recently Rash et al. (6) reported specific extractions of desmosomes when freshly excised chick muscle was treated with molar concentrations of urea. This observation, coupled with our findings, strongly suggests that the hemidesmosome and desmosome plaques consist of polypeptide or protein that differs markedly from that of tonofilaments and plasma membranes. Since pepsin and chymotrypsin act on peptide links of aromatic amino acids and pepsin on dicarboxylic amino acids (14), our results suggest that the plaques contain protein or polypeptide rich in aromatic amino acids.

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