Three-dimensional ultrastructure of the brush border glycocalyx in the mouse small intestine: a high resolution scanning electron microscopic study

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Summary. The three-dimensional ultrastructure of the filamentous glycocalyx of the brush border in the mouse small intestine was successfully demonstrated by high resolution scanning electron microscopy (SEM). The specimens were fixed with 2% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.4), and rinsed with buffered solutions with differently adjusted pH values (pH 3.0, 7.0 or 11.0). They were then osmicated, dried, spatter-coated with gold (1.0-1.5 nm), and observed under a high resolution SEM. The glycocalyx on the luminal surface of the intestinal villi covered the top of the microvilli of the epithelial cells and were well preserved in the specimens treated with an alkaline buffer (pH 11.0). The glycocalyx was observed as filamentous structures, 7 to 15 nm thick in diameter. These filaments repeatedly branched and anastomosed with neighboring ones to form an actual network or plexus as a whole, in contrast with superimposed images in transmission electron microscopy (TEM) which suggested that such anastomoses were pseudo-networks. The filaments thickened globularly at the sites of the filament bifurcation or branching. On the other hand, specimens rinsed with an acid or neutral buffer showed no glycocalyx on their microvilli, whose naked top had knob-like structures.

Thus, the pH values of the washing buffer solutions were considered to affect the preservation of the surface coat due to molecular characteristics.

Introduction

An early transmission electron microscopic (TEM) study of the mouse gall bladder epithelium showed that minute branching filaments project from the plasma membrane of the microvilli; this phenomenon was coined the “antennulae microvillares” (Yamada, 1955). Later, it was confirmed that polysaccharide-rich components were, in fact, ubiquitous on all cell surfaces, resulting in the proposal of the general term of “glycocalyx” for all such cell coats (Bennet, 1963). The enteric glycocalyx or surface coat on the intestinal epithelial cells has been considered to constitute an important diffusion barrier for nutrients seeking digestive and transport sites on the outer intestinal membrane (Smithson et al., 1981).

The ultrastructural feature of the enteric glycocalyx has been shown by TEM of conventional specimen preparations (Ito, 1965; Fawcett, 1965) and freeze-etched samples (Swift and Mukherjee, 1976). In these transmission images, the glycocalyx were well preserved on the epithelial surface but not three-dimensional. Some scanning electron microscopic (SEM) studies (Jones and Murphy, 1994; Jongbloed et al., 1999) gave us three-dimensional image of the coat, but it was not always sufficiently preserved. Thus, the present study aims to show the three-dimensional ultrastructure of the well-preserved enteric glycocalyx using high resolution SEM, and shows the fine meshwork structure of the glycocalyx.

Received November 26, 2004

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Materials and Methods

Under deep anesthesia with diethyl ether, the abdominal cavities of Balb/c mice (10–12 weeks old) were opened. The small intestine was excised and its lumen was tenderly washed with 0.01 M phosphate buffered saline (pH 7.4).

Scanning electron microscopy (SEM)

The small intestine was fixed with 2.0% glutaraldehyde in a 0.1 M phosphate buffer, pH 7.4, for 6–12 h at room temperature and then rinsed for 6 h with 0.1 M NaH₂PO₄ or 0.1 M Na₂HPO₄, whose pH value was adjusted to 3.0, 7.0 or 11.0 with 1 N HCl or 1 N NaOH. After rinsing, the specimens were immersed in a 1% OsO₄ aqueous solution for 1–2 h at room temperature, washed with distilled water, dehydrated through ethanol, and dried with n-butanol using a freeze drier (ES-2030; Hitachi). After ion sputter coating with platinum-palladium of 1.0–1.5 nm, the specimens were observed in a high resolution SEM (S-900; Hitachi; Equipped in the Central Research Laboratory, Okayama University Medical School) at an accelerating voltage of 15 kV.

Transmission electron microscopy (TEM)

Some of the dried specimens for SEM were immersed again in absolute ethanol and embedded in epoxy resin as usual. They were cut into ultra-thin sections and observed in a TEM (H-700H; Hitachi; Equipped in the Central Research Laboratory, Okayama University Medical School).

Results

The luminal surface of the mouse intestinal villi, which was rinsed with the acid or neutral buffer (pH 3.0 or 7.0), was well cleaned, and the naked tips of the microvilli on the absorbing epithelial cells were rendered clearly visible, SEM showing their closely packed standing arrangement (Fig. 1a, b). Numerous knob-like hemoglobin protrusions of 10–20 nm in diameter were observed on the surface of each microvillus (Fig. 1b). In specimens rinsed with a phosphate buffer at a pH value of 11.0, a certain obscure membrane or film-like structure veiled the luminal surface of the intestinal villi. When the samples were observed at a higher magnification, such a membranous veil was revealed as a fine meshwork of filamentous glycocalyx on the microvilli (Fig. 1c). On the surface of the goblet cell, however, no such filamentous meshwork of the glycocalyx could be observed.

At higher magnification, thin filaments, about 7–15 nm thick, repeatedly branched and anastomosed with each other to form a plexus or meshwork (Fig. 2). These filaments often swelled globularly, in particular at the branching points, while they contacted the tips of the microvilli and extended to the lumen. The glycocalyx filaments that associated with one microvillus also anastomosed to those associated with its neighboring microvilli.

TEM of tissues which had been prepared for SEM showed that the cytoplasmic membrane and other cellular components of villous epithelial cells were well preserved after rinsing with an alkaline buffer for 6 h. In these specimens, the glycocalyx was clearly observed on microvilli of the epithelial cells, though it was removed from the tips in some parts (Fig. 3). The filamentous appearance and branching pattern of the glycocalyx well corresponded to SEM images in the present study.

Discussion

The present study has employed SEM to show clearly the three-dimensional ultrastructure of the glycocalyx covering the surface of the microvilli of epithelial cells in the mouse small intestinal villi. The glycocalyx or surface coat was well preserved and remained in the alkaline-washed specimens. In previous SEM studies, the intestinal villous surface was cleanly washed to reveal naked microvilli (Kendall et al., 1991). As shown in the present study, the glycocalyx is easily removed from the microvillous tips during washing with a neutral or acid buffer solution. The reason for this phenomenon may be as follows. The

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Fig. 1. Scanning electron micrographs of the villous surface of the mouse small intestine. a: The surface of the epithelial cells in acid buffer-rinsed specimens have their microvillous tips markedly exposed, losing the enteric surface coat or glycocalyx. b: A highly magnified image of neutral buffer-washed specimens shows aggregations of hemoglobin knob-like structures (arrowheads) on the microvillous tips. c: Samples treated with the alkaline buffer show that the surface coat on microvilli is sufficiently preserved to cover the absorbing epithelial cells. Note the surface of the goblet cell (G) without microvilli which is not covered with the glycocalyx. a: ×30,000, b: ×210,000, c: ×30,000
Fig. 1. Legend on the opposite page.
Fig. 2. Highly magnified SEM image of the sample treated with the alkaline buffer. Filaments of the glycocalyx repeatedly branch and anastomose to form a layer of the enteric surface coat on the brush border microvilli (M). × 200,000
enteric glyocalyx consists of mucinous glycoconjugates or acid glycosaminoglycans, which contain richly ionized, negatively charged groups. In the acid condition, most of the acid groups are converted into non-ionized states; thus, the macromolecules lose their tertial and quaternary structure. In the alkaline condition, almost all of the acid groups including very weak ones may be ionized to maintain the macromolecular structure of mucin.

In our preliminary experiments, tannic acid was used for the preservation of polysaccharides in the glyocalyx (Chew, 1980), but this caused a deposition of a thick dirty complex on the microvilli (data not shown). Lanthanum staining enhanced with alcian blue or cetylpyridinium chloride fixation has been also used for glyocalyx or surface coat of the cells (Shea, 1971; van den Berg et al., 2003). However, these chemicals apparently formed a large artificial complex with polysaccharide molecules. Thus, for high resolution SEM studies of the glyocalyx, we avoided the use of tannic acid and other sugar binding reagents. The results show that postfixation simply with 1% OsO₄ is suitable for observation of the glyocalyx in tissues treated with the alkaline buffer.

The ultrastructure of the glyocalyx has been mostly studied by TEM of sectioned samples (Ito, 1965; Fawcett, 1965; Trier, 1969). In these studies, the well developed glyocalyx was observed as a meshwork of branched and anastomosed filaments although such an appearance was explained by the superimposition of filaments rather than actual anastomoses (Fawcett, 1981). In contrast, quick-freeze deep-etched images of the frog bladder epithelium showed an extensive network of glyocalyx filaments on its apical surface (Kachar et al., 1999). Our SEM studies have clearly shown that the enteric surface coat consists of actually anastomosed plexus of filamentous structures. It has been established that glycoprotein materials synthesized in the Golgi apparatus are conveyed to cell surfaces (Bennet...
and Leblond, 1970; Massey-Harroche, 2000). Furthermore, the filamentous glycoconvalyx on each enterocyte shows heterogeneity in molecular phenotypes (Maury et al., 1995). The meshwork structure of the glycocalyx suggests filamentous macromolecules may bind with neighboring ones in the extracellular space.

The filamentous glycocalyx in the brush border contains abundant negatively charged glycoconjugates to form the enteric surface coat with water, and maintains unstirred layers on the microvilli of the absorbing epithelial cells (Smithson et al., 1981). It has been suggested that these unstirred layers facilitate the passing of oligomers rather than monomers, thus playing an important functional role in the absorption of saccharides and oligopeptides and in the protection from a loss of hexoses and amino acids generated in the brush border (Pappenheimer, 2001). It is noteworthy that the filamentous glycocalyx originating from each microvillus anastomoses with neighboring ones to form firm meshworks without holes to then constitute stable, unstirred layers of the enteric surface coat.

Acknowledgements

The authors thank Mr. H. Urata (Central Research Laboratory, Okayama University School of Medicine), for his assistance in SEM, and, also thank Mr. H. Mizoguchi, Mr. M. Narasaki, Mr. H. Osugi, and Mr. T. Komiyama (Department of Human Morphology) for their technical help.

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