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Fibrillar ultrastructure of the glomerular basement membrane of the rat kidney as revealed by digestive treatment.

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Abstract

Rat kidney glomerular basement membrane (GBM) was isolated and digested with alpha-amylase and elastase. Electron microscopy revealed a meshwork structure composed of fibrils 3 nm in width. They appeared to be type IV collagen fibrils. We succeeded in clarifying a significant ultrastructural aspect of the GBM which had been unclear until now. The findings are consistent with our previously proposed GBM molecular sieve theory.

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— Brief Note —

Fibrillar Ultrastructure of the Glomerular Basement Membrane of the Rat Kidney as Revealed by Digestive Treatment

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Rat kidney glomerular basement membrane (GBM) was isolated and digested with α-amylase and elastase. Electron microscopy revealed a meshwork structure composed of fibrils 3 nm in width. They appeared to be type IV collagen fibrils. We succeeded in clarifying a significant ultrastructural aspect of the GBM which had been unclear until now. The findings are consistent with our previously proposed GBM molecular sieve theory.

Key words : type IV collagen, glomerular basement membrane, enzymatic digestion, fibrillar ultrastructure

Glomerular permeability studies have indicated that the glomerular basement membrane (GBM) is the main filtration barrier (1), but the morphological structure of the GBM is not well understood. By transmission electron microscopy, the GBM appears as fine osmiophilic granules embedded in an amorphous matrix, and infrequently a large fibril about 10 nm wide is found on the endothelial side (1). The GBM does not appear to have pores, presumed to exist by physiologists (2), when conventional methods of fixation and embedding are used. We previously demonstrated that the GBM was made up of a three dimensional molecular sieve composed of pores and strands by negative staining and electron microscopy (3-5).

To date, there have been few reports concerning the ultrastructure of the GBM after enzymatic digestion is revealed by transmission electron microscopy (TEM). In 1983, Goto *et al.* observed collagen fibrils in a muscle after digestion with α-amylase and elastase (6). These enzymes removed materials other than collagen and revealed the collagen fibrils. In this study, the same enzymes were used to observe the ultrastructure of the GBM, and collagen fibrils were successfully visualized.

GBM was isolated from the kidneys of Wistar rats by Sipro's method (7). The basement membrane was digested in 0.05 M phosphate buffer solution (PBS), pH 6.6, containing 0.5% α-amylase and elastase at 37°C for 5 h. For the control experiment, a part of the isolated GBM was treated under the same conditions in PBS without the enzymes. The specimens were washed three times by suspending in PBS and centrifuging at 4°C, and then fixed with 2.5% glutaraldehyde for 2 h. They were sieved through a no. 250 metal mesh (Ikemoto Co., Ltd.) to remove debris. The samples were postfixed in 1% osmium tetroxide for 1 h at 4°C, dehydrated in a graded ethanol series

Fig. 1

Fig. 2

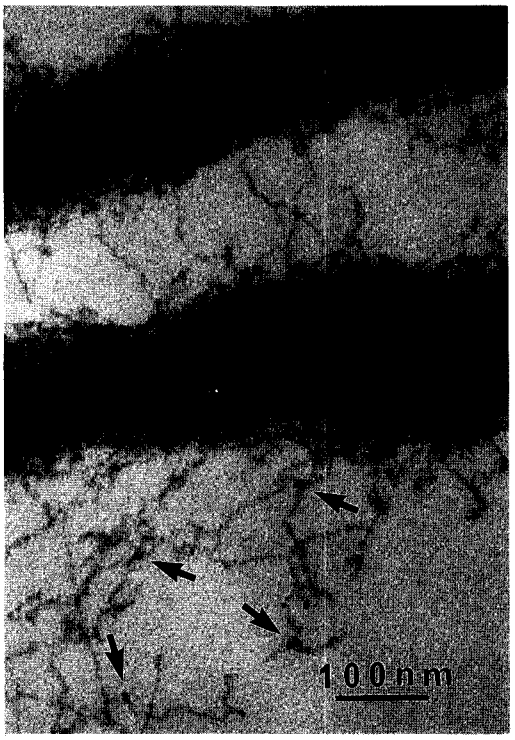

Fig. 1 Transmission electron micrograph of isolated rat glomerular basement membrane (GBM) treated with α-amylase and elastase. Fibrillar structure is seen on both sides of the disentangled GBM. ×40,000

Fig. 2 Partial enlargement of Fig. 1. Crisp string-shaped fibrils, approximately 3 nm in width, and globules (arrows) are seen. ×120,000

and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed under a Hitachi H300 electron microscope at 75 kv.

The specimens were confirmed to be GBM by light microscopy and low magnification electron microscopy. At higher magnifications, the GBM appeared smooth and amorphous, and no fibrils were seen in undigested control specimens. After digestion, the surface of the GBM seemed rather rough, and the inner part of it was electron dense and had a spongy appearance (Fig. 1). At a much higher magnification, many fibrils were seen both inside and outside the GBM. Most of the fibrils outside were continuous with those on the surface of the GBM as the GBM became disentangled. The fibrils were 3 nm in width, crisp, string-shaped, and linked to one another. Globules were observed on the fibrils (Fig. 2).

The presence of filtration pores was demonstrated by Ota *et al.* through the study of selective permeability of the GBM using negative staining (3, 4). With this method, the GBM had a spongy appearance and was composed of a network of fibrils about 3 nm in width, forming polygonal pores about 3 nm in diameter. These pores were connected to one another and formed channels in the GBM.

Biochemical and immunohistochemical studies have shown that the GBM is composed of type IV collagen and some non-collagenous proteins (8, 9). The main GBM structure is probably constituted of collagen

like that of the basement membrane of other organs. Timpl *et al.* reported that monomeric type IV collagen forms curly string-like fibrils 400 nm in length and 2 to 3 nm in width with a globular domain at one end, and that polymeric type IV collagen has a branching structure with globules on the fibrils (10).

The fibrils we observed in the present study are probably type IV collagen because of the close morphological resemblance to the type IV collagen described by Timpl *et al.* Furthermore, the size of the fibrils coincides with our previous study employing negative staining (3-5). The globules observed in our experiment are almost identical morphologically to those described by Timpl *et al.* (10). The globules probably represent a non-collagenous domain. To the best of our knowledge, this is the first transmission electron microscopic study demonstrating that the GBM is made up of fine fibrils.

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