ULTRASTRUCTURAL LOCALIZATION OF γ-GLUTAMYL TRANSPEPTIDASE IN RAT KIDNEY AND JEJUNUM

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1. Introduction

γ-Glutamyl transpeptidase, a membrane-bound enzyme, catalyzes the initial step in the degradative metabolism of glutathione by transferring the γ-glutamyl moiety to a number of acceptors such as amino acids, peptides and water. High activities of this enzyme are found in epithelial cells of the renal proximal tubules, jejunal villi, choroid plexus, bile ducts, seminal vesicles, epididymis, and ciliary body, consistent with its proposed role in transport processes [1,2]. Histochemical studies at light microscopic level were suggestive of the enzyme's localization in the brush border of renal and jejunal epithelial cells [2]. This inference has been supported by subcellular fractionation studies which showed that the enzyme activity was enriched in brush border membranes [3–10]. However, these conclusions have been challenged by claims that the enzyme is neither localized in the renal brush border membranes nor in the basal–lateral membranes [11,12]. We report here the ultrastructural localization of γ-glutamyl transpeptidase in tissue slices that provides direct evidence that the enzyme is indeed primarily located in the brush border membranes of the rat kidney proximal tubules and of the tip cells of the jejunal villi. Activity is also seen in the membranes of the foot processes of glomerular podocytes. Furthermore, immunocytochemical studies using ferritin–antibody conjugates and results from papain treatment of intact membrane vesicles show that the enzyme is located on the outer (luminal) surface of the brush border membranes. Such localization is consistent with the enzyme's ability to metabolize both extracellular glutathione as well as intracellular glutathione translocated to membrane surface, a process reported to occur in various tissues [13,14].

2. Materials and methods

L-γ-Glutamyl-(4-methoxy)-2-naphthylamide, ferritin, and osmium tetroxide were obtained from Polysciences. 4-Aminophthalhydrazide was purchased from Calbiochem. Antibodies against the highly purified rat kidney γ-glutamyl transpeptidase [15] were prepared in rabbits as in [16] and the γ-globulin fraction was purified [17]. These antibodies have been shown to crossreact with the enzyme extracted from various rat tissues [18]. Conjugation of the IgG fraction with ferritin was carried out as in [19]. Rat kidney brush borders were prepared as in [8]. Papain was purchased from Sigma.

Ultrastructural localization of γ-glutamyl transpeptidase activity in rat tissues was carried out essentially as in [20]. Cortical segments of the kidney ~ 1 × 2 mm (from male Sprague-Dawley rats (300 g)) were fixed for 30 min at 4°C with 1% formaldehyde (prepared by depolymerization of paraformaldehyde). The fixed segments were washed overnight in 0.01 M phosphate buffer (pH 7.4) containing 0.15 M NaCl (phosphate–NaCl), then frozen in liquid nitrogen and sliced into ~ 0.2 mm sections. The sections were floated on the incubation medium [20] containing γ-glutamyl-(4-methoxy)-2-naphthylamide, Gly–Gly and freshly diazotized 4-aminophthalhydrazide for 60 min at 25°C. In controls, the transpeptidase activity was inhibited by the addition of L-serine and borate (5 mM each) [21,22] to the incubation medium. At the end of the incubation, the slices were

washed with several changes of the phosphate–NaCl buffer, fixed overnight at 25°C in 1% osmium tetroxide, dehydrated in alcohol and embedded in Araldite. Ultrathin sections were cut on a LKB ultramicrotome, stained for 10 s with 0.01% lead citrate, and observed in a JEM-100B electron microscope. Segments of the rat jejunum were similarly treated for localization of transpeptidase activity.

Rat kidney brush border membranes were treated with papain as follows: the membranes were suspended in phosphate–NaCl buffer containing 10 mM 2-mercaptoethanol (final conc. 1 mg protein/ml). Papain (2.5 mg/ml in phosphate–NaCl buffer containing 10 mM 2-mercaptoethanol) was added to an aliquot of the membrane suspension to final conc. 1 μg papain/10 μg total membrane proteins. After incubation for 2 h at 37°C, the membranes were separated by centrifugation at 29 000 × g for 30 min and the pellet resuspended in phosphate–NaCl buffer. The suspension and the supernatant after papain treatment were assayed for γ-glutamyl transpeptidase, alkaline phosphatase and dipeptidase activities [8]. Dipeptidase activity was determined with *S*-benzyl-L-cysteinyl-*p*-nitroanilide and with L-leucyl-*p*-nitroanilide [23].

Treatment of the renal brush border membranes and jejunal segments with the ferritin conjugate of anti-rat kidney γ-glutamyl transpeptidase IgG was carried out as in [19]. In controls, the tissues were exposed to the unconjugated anti-IgG prior to the treatment with ferritin–IgG conjugate. After washing the membranes and jejunal segments to remove the excess ferritin–IgG, tissues were processed for electron microscopy by conventional methods and embedded in Spurr embedding medium.

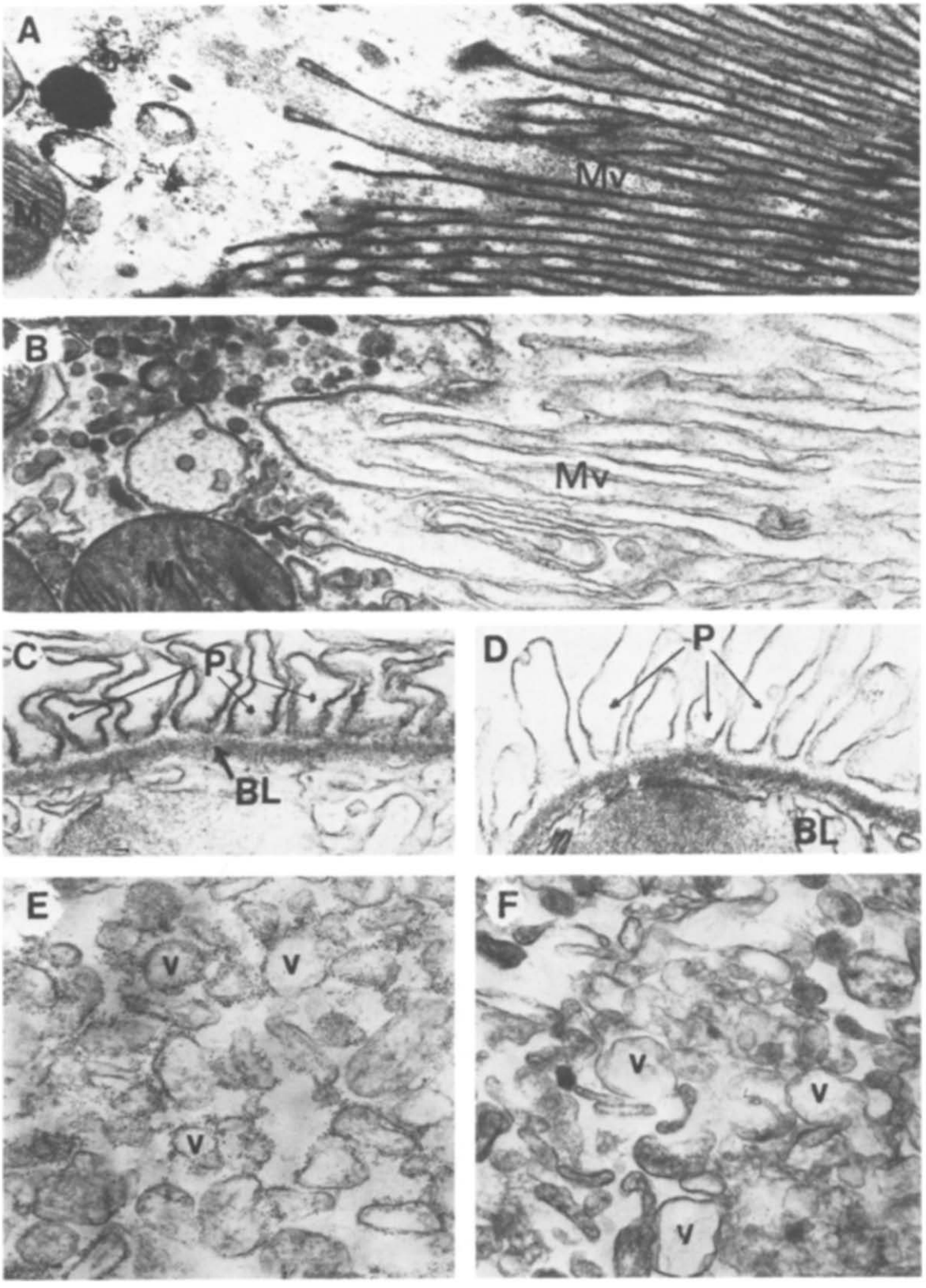
3. Results

Ultrastructural localization studies showed that γ-glutamyl transpeptidase activity in rat kidney is seen in the membranes of the proximal tubule microvilli (as indicated by thicker and darker appearance of these membranes (fig.1A) due to deposition of the lipophobic, osmiophilic reaction product at the site of enzyme activity, as compared to the appearance of these membranes in the corresponding control (fig. 1B)). Occasionally activity was also seen in membranes of, what appear to be, endocytotic vesicles (results not shown). Transpeptidase reaction product was not observed in sections obtained from segments of the inner medulla or calyx. An interesting finding was the demonstration of transpeptidase activity in membranes of the foot processes of podocytes (fig.1C). These glomerular epithelial cells, which are in close proximity to the capillaries, are continuous with, and developmentally related to, the cubiodal epithelium of the proximal convoluted tubule.

In the jejunal epithelium, activity was confined primarily to the brush border membranes of the villus tip cells (fig.2A). The poorly developed microvilli of the crypt cells showed no activity (fig.2C). These results are consistent with the findings of a gradient of transpeptidase activity in serial sections obtained from villus tip to crypt, the tip cells exhibiting 4–5-fold higher activity than the cells from crypt region [9,24].

Treatment of renal brush border membranes and jejunal segments with anti-transpeptidase ferritin–IgG conjugate showed that the conjugate binds to the outer surface of the membrane vesicles as well as to the luminal surface of jejunal microvilli (fig.1E and 2D, respectively), indicating that the antigenic determinants of the enzyme are located on the external surface of these membranes. Previous studies had shown that renal brush border vesicles used here have right-side-out configuration [8]. The more extensive labeling of the apical tips of the jejunal microvilli (fig.2D) compared to the sparse labeling along the sides, may in part be due to poor penetration of the relatively large molecule such as the ferritin–IgG conjugate through the mucoprotein coat surrounding the microvilli. Attempts to remove the mucoprotein coat by treating the jejunal segments with various agents (e.g., hyaluronidase, salts, low guanidine–HCl concentrations) resulted in extensive morphological damage in this tissue and hence were not pursued. The fact that enzyme activity (fig.2A) appears to be evenly distributed along the microvilli membranes indicates that the enzyme is present in all regions of these membranes. However, the cytochemical technique as used here is not quantitative and further workneeds to be done to exclude the possibility that there is indeed a non-uniform distribution of the enzyme in the microvilli.

Papain treatment of the intact rat kidney brush border vesicles resulted in 89% and 94% release of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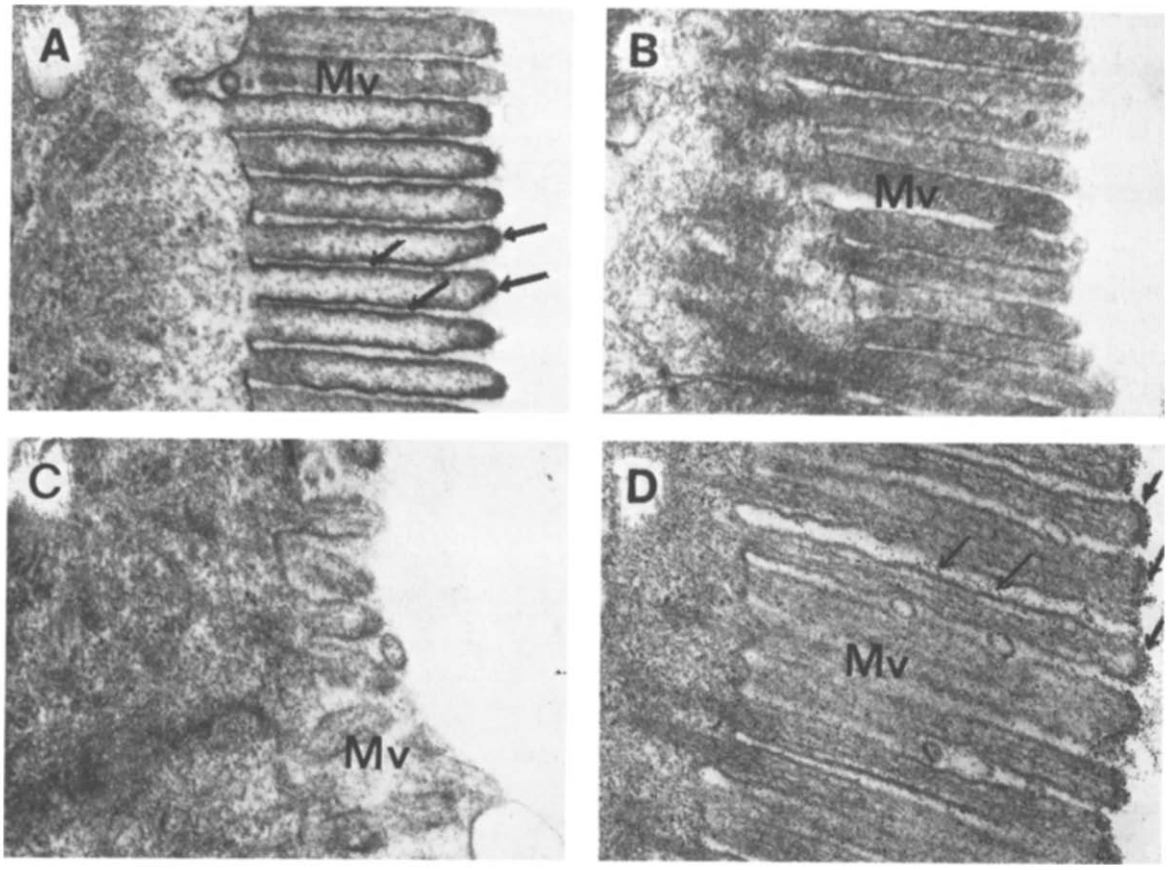

Fig.2. Ultrastructural localization of γ-glutamyl transpeptidase in rat jejunal epithelium. (A) The microvilli membranes (Mv) of the villus tip cells exhibit intense staining for transpeptidase activity (compare with the corresponding control (B)). (C) An area from the villus crypt showing absence of enzyme activity in the microvilli (× 50 000). (D) A segment of the jejunum was treated with the ferritin–IgG conjugate. Heavy binding of the conjugate at the tips of the microvilli (section through a villus tip cell) is evident with lesser binding along the sides.

γ-glutamyl transpeptidase and dipeptidase, respectively, in the 29 000 × *g* supernatant. Alkaline phosphatase, another brush border marker enzyme, was not solubilized by this treatment (< 2% release). Papain treated membrane vesicles were not labeled by the ferritin–IgG conjugate (fig.1F) and thus serve as important control regarding the specificity of the antibody.

Fig.1. Ultrastructural localization of γ-glutamyl transpeptidase in rat kidney cortex. (A) The microvilli membranes (Mv) are stained intensely. Note the thicker and darker appearance of membranes in this micrograph of thin sections of kidney cortex, indicative of transpeptidase activity compared to the appearance of membranes in the control (B) in which the activity was inhibited by L-serine plus borate: × 45 000. (C) The membranes of the foot processes of glomerular podocytes (P) also exhibit transpeptidase activity. (D) Control as in B; × 50 000. BL, basal lamina. (E) Kidney brush border membrane vesicles (V), purified as in [8] and treated with the ferritin conjugate of anti-transpeptidase IgG. Note that almost all vesicles are decorated on the external surface with ferritin indicative of localization of the antigenic sites of the enzyme on the outside of these vesicles. (F) Kidney brush border vesicles pretreated with papain to solubilize ~ 90% of the transpeptidase, washed and then treated with the ferritin–IgG conjugate. No binding of the conjugate occurred. × 50 000.

4. Discussion

Ultrastructural localization studies provide direct evidence that the primary locations of γ-glutamyl transpeptidase in rat kidney and jejunum are the brush border membranes of renal proximal tubule and of the villus tip cells of jejunum. The poorly developed microvilli of the mitotically active villus crypt cells do not exhibit transpeptidase activity. An interesting finding is the presence of γ-glutamyl transpeptidase in the membranes of podocyte foot processes. These cells which are developmentally related to the proximal tubule epithelial cells, are believed to be involved in the synthesis and secretion of the basement membrane proteins and aid in glomerular filtration process. The presence of transpeptidase in foot processes of the podocytes raises an interesting possibility that the enzyme may play a role in the onset of the autoimmune disease, autologous immune complex nephritis [25,26]. A similar defect is caused in rats by deposition on the foot processes of auto-antibodies produced in response to antigens present in renal tubule epithelial cell membranes [27,28], a site of high transpeptidase activity. Further studies are required to determine whether the enzyme may serve as an antigen in the etiology of this defect.

Immunocytochemical studies using ferritin–IgG conjugates, together with results of papain treatment of intact brush border vesicles, demonstrate that transpeptidase is located on the external (luminal) surface of brush border membranes. The dipeptidase, which together with γ-glutamyl transpeptidase, could conceivably be involved in the extracellular metabolism of glutathione, also appears to be localized on the outer surface of these membranes. Thus the enzyme's location is consistent with its role in the metabolism of glutathione present in extracellular fluids as well as glutathione which is translocated to the surface of cell membranes [13,14].

Acknowledgements

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