Immunocytochemical localization of \(\gamma\)-glutamyl-transferase on isolated renal cortical tubular fragments

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Summary. The localization of \(\gamma\)-Glutamyltransferase (\(\gamma\)-GT, E.C.2.3.2.2) was studied on isolated tubular fragments from rat kidney cortex immunocytochemically. Monospecific antibodies raised in the goat against rat kidney \(\gamma\)-GT were used. Antigoat immunoglobulin from the rabbit conjugated with ferritin was used for visualisation of the antibody binding sites. The enzyme was found to be localized at the brush border membrane of proximal tubules, the luminal membrane of distal tubules and collecting duct segments. The role of this basolateral \(\gamma\)-GT localization in context with the kidney’s ability to extract over 83% of the renal arterial glutathione (GSH) input during a single passage is discussed.

Introduction

\(\gamma\)-GT (E.C.2.3.2.2) plays a crucial role in the regulation of glutathione (GSH), an ubiquitous tripeptide that is essential for protecting cells against toxic substances and antioxidative processes (Wendel 1980). The enzyme is a transmembrane glycoprotein (Tsao and Curthoys 1982) that catalyses the initial step in the degradative metabolism of glutathione and in mercapturic acid formation from glutathione-S-conjugates (Tate 1980).

The renal proximal tubular brush border membrane has been established as the major site of the former process (George and Kenny 1973; Glossmann and Neville 1972; Silbernagl et al. 1978), which occurs as part of the interorgan turnover of GSH (Silbernagl et al. 1978; Hahn et al. 1978; McIntyre and Curthoys 1980).

The effectiveness of extracellular GSH degradation and the impermeability of some cells (Hahn et al. 1978) to the tripeptide results in a very low extracellular steady state concentration. The GSH contained within the kidney also turns over rapidly (Sekura and Meister 1974). It is translocated from the tubular cell to the tubular lumen (Griffith and Meister 1979a and b) and immediately degraded. The resulting amino acids then enter the cell at the luminal pole and are, thus, made available for protein or GSH synthesis (Silbernagl et al. 1978, 1982). The amount of plasma GSH removed by the kidney is in excess of that filtered by the glomeruli (McIntyre and Curthoys 1980). Therefore, it is likely that the renal tubular epithelium possesses a transport system which enables the uptake of GSH from the antiluminal side.

A few years ago, we initially identified \(\gamma\)-GT on the luminal membrane by electron microscopic analysis of tissue prepared following in vivo et situ administration of a specific antibody and subsequent labelling with a ferritin conjugated immunoglobulin (Silbernagl et al. 1978). This technique, however, could not be used to investigate the possible association of \(\gamma\)-GT with antiluminal membranes since the junctional complexes of nephron epithelia are impermeable to the antibody and the ferritin label. In order to avoid the need for fixation of the organ before exposing the peritubular surface to antibody, we have used a suspension of tubular segments isolated from rat kidney.

Material and methods

Enzyme and antibody. \(\gamma\)-GT was purified according to the procedure described by Hughey and Curthoys (1976). Specific antibodies were prepared by immunizing a goat with monthly injections of 1 mg of the pure enzyme protein in Freund's adjuvant.

The globulin fraction of the goat serum was precipitated with 50% (NH₄)₂SO₄ and dialyzed against 50 mM potassium phosphate/150 mM NaCl (pH 7.2). This antibody has been used to specifically immunoprecipitate \(\gamma\)-GT from a Triton X-100 solubilized crude homogenate of rat kidney. The specific antibody was purified by affinity chromatography using a protein-A coated sepharose CL-4B (Sigma) column.

Preparation of renal tubular fragments. Tubular fragments were prepared from kidneys of Spraque Dawley rats fasted over night and anesthetised with Inactin (Byk Gulden). After perfusion of the organ with cold Krebs-Henseleit buffer (Guder et al. 1971) kidney cortex was dissected, washed in cold buffer and forced through a plastic tea sieve, and the obtained tissue brei was then weighed.

Thereafter, cortex chips were washed, separated from broken material and treated with collagenase CLS II (Worthington, Freehold N.J. USA) as described by Guder (1979).

Isolated tubular fragments were incubated with the specific antibody for 30 min at room temperature and washed 5 times with Krebs-Henseleit buffer. The tubular suspension was then incubated with ferritin labelled rabbit anti goat serum (Miles-Yeda Corp.) for 30 min and again washed in Krebs-Henseleit buffer. Thereafter, the tubular suspension was fixed in 1% glutaraldehyde for 1 h at 4°C, postfixed with 1% OsO₄, dehydrated in acetone and embedded in Durcopan ACM (Fluka, Switzerland). Sections were stained with bismut subnitrate to improve visualization of the ferri-
Fig. 1. Brush border of proximal convoluted tubule (S₂-segment). Arrows indicate specific binding of the antibody-immunoglobulin-ferritin complex to the cell's luminal membrane

Fig. 2. Brush border of proximal straight tubule (S₃-segment) showing a higher binding density of the antibody than the S₂-segment

Fig. 3. Specific antibody binding to the luminal membrane of the distal convoluted tubule

Fig. 4. Typical binding pattern of anti γ-GT antibody at the luminal membrane of cortical collecting duct cells

Fig. 5. Distribution of anti γ-GT antibody binding sites on the basolateral membrane domain of proximal convoluted tubules. Arrows indicate sites of specific binding

Fig. 6. Antibody binding to the basolateral membrane of distal convoluted tubules

Fig. 7. Typical appearance of immunoglobulin-ferritin complexes at the brush border region of proximal tubules as revealed under control conditions (Control-experiment I and II)

Fig. 8. Appearance of the immunoglobulin ferritin label in the basolateral intercellular spaces after control experiments I and II