Characterization of the urinary albumin degradation pathway in the isolated perfused rat kidney

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This study examines the existence of the urinary albumin degradation pathway and the proposed role of receptor-mediated endocytosis in this process using the isolated perfused rat kidney (IPK) model. Albumin-derived peptides in IPK urine are analyzed in terms of their relative size distribution using radioactivity and absorbance at 214 nm, and their susceptibility to trypsin digestion. The effects of perfusing kidneys with concanamycin A and myristoyl trimethyl ammonium bromide (MTMAB), inhibitors of the receptor-mediated endocytosis regulators vacuolar-type H^+ ATPase (v-ATPase) and dynamin GTPase, respectively, are examined. Normal IPK urine contains mildly degraded (defined as ~10–40 kDa; 43.0 ± 8.3%) and heavily degraded (defined as <10 kDa; 22.6 ± 7.7%) albumin peptides as well as intact albumin (34.5 ± 4.1%). The relative size distribution of the peptides is similar by radioactivity and absorbance at 214 nm, and both profiles are reduced to very small peptides following trypsin digestion. Administration of concanamycin A or MTMAB causes a significant increase in the proportion of intact albumin (concanamycin A: 55.8 ± 11.6%; MTMAB: 50.0 ± 11.9%) excreted compared with normal IPK urine. This coincides with a reduction in the proportion of mildly (concanamycin A: 27.6 ± 9.8%; MTMAB: 39.9 ± 11.5%) and heavily degraded (concanamycin A: 16.6 ± 7.4%; MTMAB: 10.0 ± 2.5%) albumin present and is not associated with changes in glomerular permeability to albumin because no significant change is observed in the fractional clearance of Ficoll (radius range 20–60 Å) in the presence of concanamycin A. This study demonstrates the existence of albumin peptides in IPK urine and suggests that receptor-mediated endocytosis plays a role in urinary albumin degradation. (J Lab Clin Med 2006;147:36–44)

Abbreviations: BSA = bovine serum albumin; DMSO = dimethylsulfoxide; dpm = disintegrations per minute; GBM = glomerular basement membrane; GCW = glomerular capillary wall; GFR = glomerular filtration rate; HPLC = high-performance liquid chromatography; IgG = immunoglobulin G; IPK = isolated perfused rat kidney; K_{av} = fractional available volume; MTMAB = myristoyl trimethyl ammonium bromide; PBS = phosphate buffered saline; RSA = rat serum albumin; SD = standard deviation; UFR = urine flow rate; v-ATPase = vacuolar-type H^+ ATPase; V_e = elution volume; V_o = void volume; V_t = total volume

It is widely documented that albumin is filtered and excreted intact in urine. However, our recent research demonstrates that filtered albumin and other proteins are normally degraded during renal passage. More than 95% of excreted albumin undergoes extensive degradation via the "degradation pathway" to produce small peptides (<10 kDa). Urinary albumin peptides derived from the degradation pathway, identified primarily by radioactivity or nonspecific protein assays, have been observed in humans,[1,2] isolated perfused rat

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kidneys,[3,4] rat models,[5–12] and proximal tubular cells.[12] Despite this large body of evidence, however, the existence of the degradation pathway remains a controversial issue. Norden et al[13] recently examined protein and peptide excretion in human urine by amino acid and proteomic analyses following size-exclusion chromatography. This technique detected only very low excretion of protein peptides >750 Da, which suggests peptides are not present in normal human urine. Size exclusion chromatography and differential filtration through size selective membranes, however, has revealed that most proteinaceous material in normal human urine is in the molecular weight range of 200–10,000 Da. Detailed analysis of this peptide peak by Strong et al[14] identified a predominance of small peptides with molecular weights in the range of 300–500 Da.

The mechanism of endocytosis involved in the uptake of albumin by renal cells before its degradation, however, has not been confirmed. It is generally recognized that the renal degradation of filtered albumin in the kidney most probably occurs within lysosomes of tubular cells located distal to the GBM. This is supported by histochemical and electron microscopical studies that previously viewed the lysosomal degradation of albumin and horseradish peroxidase in proximal tubular cells after endocytosis.[15,16] Lysosomal inhibitors such as ammonium chloride and lysine have also been shown to modulate the size of albumin peptides excreted in the IPK system.[4] Both pinocytosis and receptor-mediated endocytosis are recognized as possible candidates; however, selectivity of protein reabsorption by tubular cells suggests that this is a receptor-mediated process.[17–22] The receptor-albumin complex is sequestered and internalized in clathrin-coated pits that give rise to clathrin-coated vesicles for albumin transport to lysosomes.[22]

Several reports have demonstrated that receptor-mediated endocytosis in the renal proximal tubules is regulated by the components of the acidification processes in the endosomal/lysosomal system including the v-ATPase.[23–25] Within the proximal tubule, the highest v-ATPase activity is observed in the apical endosomal membrane where it regulates endosomal acidification via the translocation of proteins from the cytoplasm to the endosomal lumen using the energy released from ATP hydrolysis.[26] This acidic environment is essential for maintaining intracellular vesicle trafficking and the recycling of receptors for the further uptake of albumin destined for lysosomal degradation.[24]

Another well-documented regulator of receptor-mediated endocytosis is the dynamin GTPase. This protein is localized to clathrin-coated pits in both neuronal and non-neuronal cells[27] and is essential for the formation of the clathrin-coated vesicles as well as their release from the plasma membrane.[28] Several working models have been proposed that describe dynamin's function in this process.[27] Despite their major differences, each model generally agrees that dynamin self-assembly stimulates GTP hydrolysis that in turn prompts the release of the clathrin-coated vesicles from the plasma membrane.

The aim of this study is to demonstrate the existence of the urinary degradation pathway and examine the role of receptor-mediated endocytosis in this process using the IPK model. The existence of albumin-derived peptides in normal (control) IPK urine is shown using radioactivity and absorbance at 214 nm (measures peptide bonds that predominate in IPK urine). Urinary albumin peptides are analyzed in terms of their relative size distribution and susceptibility to proteolytic digestion using trypsin. The involvement of receptor-mediated endocytosis in the degradation pathway is examined using the v-ATPase inhibitor concanamycin A and the dynamin GTPase inhibitor MTMAB. We also examine the size selectivity of the GCW using Ficoll (a spherical polysucrose that is not reabsorbed by the tubules) in the presence and absence of concanamycin A to provide evidence that the permeability properties of the filtration barrier are not altered by the inhibitors.

MATERIALS AND METHODS

Experimental animals. Male Sprague–Dawley rats (300–350 g) were obtained from the Australian Resource Centre (Perth, Australia). The experimental procedures used in this study were approved by the Biochemistry, Anatomy and Microbiology Animal Ethics Committee of Monash University.

Materials. [Carboxyl-^{14}C]inulin (2.3 mCi/g) and [^{3}H]water (0.25 mCi/g) were purchased from NEN Life Science Products (Boston, Mass). Sodium boro-[^{3}H]hydride (8.0 Ci/mmol) was purchased from Amersham Biosciences (Uppsala, Sweden). BSA (fraction V) was purchased from JRH Biosciences Inc. (Kan). RSA (approximately 99%, agarose electrophoresis), Ficoll 70, immunoglobulin G, transferrin, MTMAB (approximately 99%), trypsin (product number T4665; from bovine pancreas), and concanamycin A (from Streptomyces species) were purchased from Sigma Chemical Co. (St. Louis, Mo).

Solutions and buffers. PBS, pH 7.4, contained (mmol/L) 136.9 NaCl, 2.68 KCl, 8.1 Na_2HPO_4, and 1.5 KH_2PO_4. The Krebs–Henseleit buffer, pH 7.4, contained (mmol/L) 122 NaCl, 4.6 KCl, 0.115 $MgSO_4$, 24.9 $NaHCO_3$, and 0.1 $CaCl_2.H_2O$. Kidney perfusate solution, pH 7.4, was 5% BSA in Krebs–Henseleit buffer that also contained 5-mmol/L glucose, oxygen radical scavengers, and amino acids, as described previously.[4]

Tritium labelling. RSA, IgG, and transferrin were tritiated with sodium boro-[^{3}H]hydride using the reductive methylation technique described by Tack et al.[29] The activities achieved were [^{3}H]albumin (for IPK procedure), 6.87×10^{9}