Renal Proximal Tubular Albumin Reabsorption: Daily Prevention of Albuminuria

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Although the glomerular filtration coefficient of albumin is small, the daily filtered load can be as much as 8 g. To prevent such massive losses of albumin, quantitative reabsorption along the proximal tubules is accomplished by “receptor”-mediated endocytosis. Albumin reaches the lysosomes where it is degraded to amino acids.

What is albumin? The term albumin derives from albus, the Latin word for white (12), and dates back to the recognition that the portion of an egg that appears white after coagulation, consists mainly of proteins. This part of the egg is also called albumen. In the early days of plasma protein separation, the water-soluble fraction was called albumin. Proteins of the less water-soluble fraction were denominated globulins. Nowadays, albumin is generally regarded to mean serum or plasma albumin, a single protein species.

In quantitative terms, albumin is the most important plasma protein. At a concentration of 45 g/l (652 × 10^-6 mol/l), albumin represents ~60% of all plasma proteins. The molecular mass

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Thus the only mechanism able to mediate albumin reabsorption is endocytosis.

Quantitative aspects of albumin filtration

Analysis of urine of healthy individuals shows that only very little albumin (30 mg/day) undergoes renal excretion (10). When one considers the plasma concentration of albumin and the high rate of renal blood flow, excretion of albumin with the final urine is very low. Nevertheless, this measurement shows that albumin reaches the tubular lumen. Furthermore, there are pathophysiological states in which the daily albumin excretion can be dramatically increased, resulting in the so-called albuminuria.

How can a molecule of the size and charge of albumin reach the tubular lumen? The composition of glomerular ultrafiltrate depends on the intrinsic permeability of the glomerular capillary wall (4). Molecules with effective radii >2 nm are retained with increasing efficiency as the radius rises. Furthermore, negatively charged macromolecules are restricted to a greater extent than neutral molecules of comparable size (4, 10). For albumin, the effective radius is in the range of 7.5 nm (4, 10), resulting in a low fractional filtration, which is even further impaired by the negative net charge. As a result, fractional filtration of albumin is in the range of 0.05–0.1% (10, 15). Multiplication of plasma albumin concentration (45 g/l), with the fractional filtration of 0.1%, results in an ultrafiltrate concentration for albumin of 45 mg/l. This value is in good agreement with albumin concentrations determined by micropuncture studies (10, 15). Assuming a daily glomerular filtration rate of 180 liters in a healthy adult, one can estimate the daily filtered load of albumin to be 8,100 mg. This corresponds to ~6.5% of total plasma albumin.

So far, the fate of albumin in the kidney can be summarized as follows. Per day, up to 8,100 mg of albumin reach the urinary space by glomerular filtration, but only ~30 mg are excreted with the final urine. Hence, more than 99% of filtered albumin (i.e., up to 8,070 mg/day) must be reabsorbed along the nephron. Extensive micropuncture studies show that reabsorption takes place almost exclusively along the proximal tubule (e.g., Refs. 2 and 15).

Albumin is reabsorbed by endocytosis

Albumin seems to be almost evenly reabsorbed in early and late proximal convoluted tubules and straight tubules (15). Because of its size, albumin cannot leave the tubular lumen on the paracellular route across the tight junctions. Furthermore, albumin is not cleaved in the tubular lumen and therefore does not cross the apical membrane of the proximal tubular cell in the form of free amino acids (10, 15). Thus the only mechanism able to mediate albumin reabsorption is endocytosis. Hereby, small endocytic invaginations are formed at the microvillar base, at so-called clathrin-coated pits, which contain a certain volume of the extracellular (in this case tubular) fluid and thereby also molecules dissolved in the fluid. The endocytic invaginations are untied to form endocytic vesicles that transport their content to the endosomal compartment. From the endosomal compartment, part of the material taken up reaches the lysosomal compartment via late endosomes, whereas another part is brought back to the plasma membrane via recycling endosomes. One can roughly distinguish two forms of endocytosis: 1) fluid-phase endocytosis and 2) adsorptive endocytosis (10).

During fluid-phase endocytosis, the concentration of any given substrate in the endocytic invagination is the same as in the extracellular space, indicating that these substrates are not enriched at the plasma membrane, and thus uptake increases proportionally to the extracellular concentration of the substrate. Classical substrates for this kind of endocytic uptake are dextran or inulin, markers for determination of glomerular filtration rate. Obviously, uptake in the form of fluid-phase endocytosis is a very slow process, as can be easily derived from the fact that virtually all inulin filtered in the glomeruli reaches the final urine.

During adsorptive endocytosis, substances are bound to the cell membrane, and the concentration in the endocytic invagination exceeds the concentration in the extracellular space several-fold, e.g., in the case of albumin, up to 40-fold. The enrichment in the endocytic invaginations
makes adsorptive endocytosis far more effective than fluid-phase endocytosis and renders quantitative uptake possible.

As pointed out above, albumin is reabsorbed quantitatively along the proximal tubule. Hence, reabsorption of albumin must be accomplished by adsorptive endocytosis. Comparison of the uptake rates of fluid-phase markers like dextran or inulin (Fig. 1A) with the uptake rate of albumin in cultivated proximal tubule-derived cells (7, 14) or in isolated perfused proximal tubules (10) supports this notion. As shown in Fig. 1A, the rate of uptake of albumin 1) exceeds the rate of uptake of dextran by >20-fold and 2) is a saturable process, whereas the uptake of dextran increases linearly with the extracellular concentration. These data show that there is an enrichment of albumin with a limited capacity at the plasma membrane.

What attracts albumin to the plasma membrane?

Enrichment of proteins at the plasma membrane could be either due to interactions with the negative surface charges of adjacent microvilli ("selective constraint model," Ref. 10) or due to a specific binding site. However, charge interactions cannot explain the quantitative reabsorption of native albumin, which has a negative net charge (see above). Furthermore, uptake of albumin is specific in the sense that endocytosis of labeled albumin can be prevented by an excess of unlabeled albumin (7), indicating the presence of binding sites for this protein. Functional characterization of albumin binding to the apical membrane of proximal tubule-derived opossum kidney (OK) cells reveals one specific binding site with an apparent dissociation constant ($K_d$) of $290 \times 10^{-9}$ mol/l, corresponding to 20 mg/l (Fig. 2B; Ref. 9). For comparison, binding affinity of glycoproteins to surface asialoglycoprotein receptors is in the range of $10^{-8}$ mol/l. Binding of labeled albumin can be almost completely prevented by a 1,000-fold excess of unlabeled albumin but not by transferrin or lactalbumin. Unlabeled albumin not only inhibits binding of labeled albumin but also stimulates its dissociation from the apical membrane, with half-maxi-
mal dissociation occurring at \(260 \times 10^{-9}\) mol/l unlabeled albumin (9). The value of the binding affinity \((K_d)\) is almost the same as the value of apparent affinity [Michael-Menten constant \((K_m) = 370 \times 10^{-9}\) mol/l] for albumin endocytosis in OK cells and in isolated proximal tubules (7, 10, 14). These data show that there is an albumin binding site in the apical membrane of proximal tubular cells with a \(K_m\) in the range of physiological albumin concentrations in the tubular lumen. Furthermore, the data indicate that this binding site mediates adsorptive albumin endocytosis along the proximal tubule (Fig. 1C).

Because binding of albumin to the apical membrane of proximal tubular cells has been quantitated and specificity as well as saturability and reversibility have been shown, it is now of obvious importance to determine the nature of the binding site. Knowledge about albumin binding proteins in renal epithelial cells is very limited in contrast to data available on albumin-binding proteins in endothelial cells (e.g., Ref. 13). Four endothelial albumin-binding proteins have been described with different preferences for native or modified albumin. One of these albumin-binding proteins, the so-called gp60 or albondin, seems to be responsible for binding and transcytosis of native serum albumin in endothelial cells (13). Apparent affinity of albumin binding to albondin is in the same range as binding to proximal tubular cells \((150 \times 10^{-9}\) mol/l) and is not inhibited by transferrin (13). Only recently, Cessac-Guillermet et al. (3) attempted to isolate albumin-binding proteins from renal brush-border membranes by affinity chromatography. They described two binding proteins with apparent molecular masses of 55 and 31 kDa. Immunohistochemistry shows these binding proteins to be present on microvilli, in endocytic vacuoles, and in lysosomes of proximal tubular cells, but no labeling is detected in the inner part of the outer medullary zone. Thus the data are in good agreement with micropuncture studies showing that reabsorption takes place in the proximal tubule only. These two proteins are good candidates for the binding site of albumin. However, the group of Christensen et al. (5) claimed that megalin, a 517-kDa monomeric protein in the proximal tubular brush border, is responsible for albumin endocytosis, according to their functional data. Obviously, there is a great discrepancy regarding the molecular mass of the supposed binding proteins. Possibly more than one protein of proximal tubular apical membrane has the basic ability to bind albumin, but not all are responsible for physiological reabsorption. Thus further detailed studies are required to unveil the true nature of the renal albumin-binding protein. Nevertheless, there is a more or less specific albumin-binding site in the apical membrane of proximal tubular cells that enables the accumulation of albumin in endocytic invaginations, despite its negative net charge, and thereby its preferential uptake as compared with dextran for example (Fig. 1C).

The importance of endosomal pH

Once albumin has been taken up by receptor-mediated endocytosis, it is directed to the endosomal compartment and its final destination, the lysosomes, where it will be cleaved to amino acids that exit the cell across the basolateral membrane (Fig. 2B). Hence, albumin must dissociate from the binding site before reaching the lysosomes, which could happen in the early endosomal compartment. The binding site returns to the apical membrane for further endo-

FIGURE 2. A: affinity constant \((K_m)\) of albumin binding is pH dependent. \(K_m\) increases with decreasing pH. Sharp decrease of affinity is observed at pH values similar to those in endosomes and lysosomes. B: after detachment from apical membrane, endocytic vesicles deliver albumin-binding protein complex to the endosomal compartment (1). Albumin dissociates from the binding protein (2) due to low endosomal pH. Binding protein recycles to the apical plasma membrane (3). Albumin itself reaches the lysosomal compartment and is cleaved to single amino acids (4). Amino acids exit the lysosomes and leave the cell via the basolateral membrane (5). This mechanism prevents loss of valuable amino acids but does not deliver intact albumin to peritubular space.

“...more than one protein of proximal tubular apical membrane has the ability to bind albumin...”
cytic cycles, whereas albumin undergoes lysosomal degradation. A common mechanism triggering receptor-ligand dissociation is the drop in pH in the different endocytic compartments (11). Because binding affinity for albumin is pH dependent (Fig. 2A; Ref. 9), its dissociation from the binding site is most probably triggered by the acidic pH (pH ~6.2). As long as pH ranges from 7.4 to 6.7, corresponding to the pH values at the beginning and at the end of proximal tubular lumens, binding affinity remains constant.

The functional importance of pH-dependent dissociation for effective albumin reabsorption has also been demonstrated experimentally (7). Endosomal alkalinization by either bafilomycin A₁ or NH₄Cl leads to a dramatic decrease of the apparent uptake affinity as well as of the maximal albumin uptake rate, whereas fluid-phase endocytosis is unaffected. At the same time, binding site recycling and albumin degradation are impaired. Reduction of the maximal uptake rate and uptake affinity is most probably due to reduced receptor recycling and enhanced reexocytosis (11). Enhanced renal albumin excretion in states of increased NH₄⁺ blood levels may be, at least in part, explained by endosomal alkalinization with the described changes of uptake affinity and rate (10).

As shown in Fig. 2B, the intracellular route of albumin can be summarized as follows. After detachment from the apical membrane, endocytic vesicles deliver the albumin-binding protein complex to the endosomal compartment. Albumin dissociates from the binding protein because of the low pH. The binding protein recycles to the apical plasma membrane, and albumin itself reaches the lysosomal compartment and is cleaved to single amino acids or small peptides. The amino acids exit the lysosomes and leave the cell via the basolateral membrane along an electrochemical gradient or in exchange for Na⁺. By this mechanism, >99% of filtered albumin is reabsorbed along the proximal tubules and <1% escapes into the final urine (Fig. 3A).

**Regulation of albumin endocytosis**

It is well known that endocytosis in general can be regulated by several mechanisms, such as the concentration of cytoplasmic Ca²⁺, protein kinases, and G proteins. However, in the case of albumin reabsorption, little was known until recently about a possible regulation of this process. Two recent studies did shed some more light on the role of heterotrimeric G proteins, protein kinase A, and Ca²⁺ in albumin endocytosis (1, 8).

Overexpression of a G₁ protein subunit in proximal tubule-derived OK cells results in an increase of albumin uptake to ~200% of control (1). This stimulation of endocytosis is inhibited by pertussis toxin, an inactivator of G₁ and G₅ proteins. Furthermore, endocytosis in control cells is reduced by pertussis toxin to ~50% of control, whereas binding of albumin is not affected by pertussis toxin. Activation of G₅ proteins by cholera toxin has no effect on albumin endocytosis. The data presented in the study of Brunskill et al. (1) support a critical role for certain types of heterotrimeric G proteins in the regulation of albumin endocytosis, as was also shown for other endocytic processes. However, it is not known at present how and at which stage G proteins interact with albumin endocytosis. Accord-
impairment of endosomal acidification can affect endocytic albumin reabsorption.

Pathophysiological considerations

In healthy subjects, filtration and reabsorption of albumin are in an equilibrium in which <1% of filtered albumin reaches the final urine (Fig. 3A). Pathophysiological states with increased renal albumin excretion, the so-called albuminurias, can result, in principle, from two malfunctions (Fig. 3B). The first malfunction is an increase of the filtered load, which results in glomerular albuminuria. Because the enrichment of albumin at the brush-border membrane has a limited capacity (e.g., maximum transport rate of high-affinity reabsorption of albumin in isolated proximal tubules is in the range of 0.05 ng · min⁻¹ · mm tubular length⁻¹ (10)), an increase in the filtered load leads to a growing disequilibrium between filtration and reabsorption. The second malfunction is renal excretion of albumin increasing without any change of the tubular load, as in the case of a reduced tubular reabsorption capacity. If the reabsorption capacity decreases at a constant filtered load, a growing disequilibrium again originates and causes a tubular albuminuria. Thus, for enhanced albumin excretion, one should always consider a malfunction of proximal tubular reabsorption.

As already described, impairment of endosomal acidification can affect endocytic albumin reabsorption. Simulation of affinity changes by a simple mathematical model (7, 10) shows the high susceptibility of the system toward small changes of its kinetic parameters. Increasing the $K_m$ from $370 \times 10^{-9}$ to $1,300 \times 10^{-9}$ mol/l results in an increase in the percentage of albumin passing the proximal tubule, from values of ~1% to ~60% (corresponding to up to ~4,900 mg/day). However, changes in tubular uptake kinetics may also occur independently of vesicular pH homeostasis, as shown for the food contaminant ochratoxin A (6). Without affecting endosomal pH, ochratoxin A reduces the number of albumin-binding sites in the apical membrane by 30% and increases the rate of reexocytosis by 20%, thereby causing tubular proteinuria. The underlying molecular mechanism in this case has not yet been unveiled. Further points of attack for the generation of tubular albuminuria are the different mechanisms involved in the regulation of albumin reabsorption. Certainly, future studies will render more detailed insights into the regulatory processes and thereby broaden our pathophysiological knowledge.

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I apologize to those whose work I was not able to cite due to space restrictions.

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M. tubules are involved. Two subtypes of electrogenic import systems have systems in the basolateral and luminal plasma membranes of proximal renal 
transportation of organic cations. Electrogenic import systems and electroneutral export 
compounds like choline or monamine neurotransmitters may also be reabsorbed into 
the blood. In the kidney, the drugs can be excreted into the bile or reabsorbed into 
hepatocytes. From the hepatocytes, the drugs enter the liver where they are transported into 


