Protein-Bound Molecules: A Large Family With a Bad Character

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Summary: Many small solutes excreted by the kidney are bound to plasma proteins, chiefly albumin, in the circulation. The combination of protein binding and tubular secretion allows the kidney to reduce the free, unbound concentrations of such solutes to lower levels than could be obtained by tubular secretion alone. Protein-bound solutes accumulate in the plasma when the kidneys fail, and the free, unbound levels of these solutes increase more than their total plasma levels owing to competition for binding sites on plasma proteins. Given the efficiency by which the kidney can clear protein-bound solutes, it is tempting to speculate that some compounds in this class are important uremic toxins. Studies to date have focused largely on two specific protein-bound solutes: indoxyl sulfate and p-cresyl sulfate. The largest body of evidence suggests that both of these compounds contribute to cardiovascular disease, and that indoxyl sulfate contributes to the progression of chronic kidney disease. Other protein-bound solutes have been investigated to a much lesser extent, and could in the future prove to be even more important uremic toxins.
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Many small solutes excreted by the kidney are reversibly bound to plasma proteins. At first glance, protein binding would seem to pose a barrier to solute removal by the kidney because it reduces glomerular filtration of a solute. But the effects of toxic solutes on body tissues presumably are related to their free, unbound rather than their total concentration in the plasma.[1] This being the case, the combination of protein binding with tubular secretion can provide more effective renal solute removal than could be accomplished by glomerular filtration and tubular secretion alone, as summarized in Table 1. The potential capacity of the kidney to reduce the free concentrations of protein-bound solutes to very low levels was described by Marshall,[2] who also provided an unequivocal demonstration of solute secretion by the renal tubules. It is analogous to the clearance of unconjugated bilirubin by the liver, and likewise depends on the rapid reversibility of solute-protein binding and on the presence of a fenestrated capillary endothelium, which allows the close approach of plasma to the cells responsible for solute uptake. The role of the tubules in uremic solute excretion is described in more detail by Masereeuw et al in this issue.[3]

A further advantage of protein binding may be to provide a buffer for toxic solutes. With protein binding, the increase in the effective, free concentration after a rapid solute load is less than the increase in concentration that would result if the same solute load were distributed throughout extracellular or total body water. Clearance by the kidney, which varies remarkably little through the day, then gradually can eliminate the solute.

A list of protein-bound uremic solutes derived from prior reports is provided in Table 2.[4–7] Protein binding for these solutes is presumed to be rapidly reversible similar to that of bilirubin, fatty acids, and many protein-bound drugs.[8–11] Dissociation rates for the uremic solutes, however, have not been measured. Solutes that are protein bound in different ways, including modified amino acids that accumulate both in the free form and incorporated into circulating proteins, are not listed.[12] It should be noted that the current list undoubtedly is incomplete, and that recent metabolomic studies suggest that a large number of protein-bound solutes are normally efficiently cleared by the kidneys and accumulate in uremia.[7] Moreover, protein binding has in some cases not been quantified by direct measurement but only assumed based on the solute's clearance rate or chemical structure. The solutes listed in Table 2 are thought to bind largely

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Table 1. Potential Reduction of the Free Plasma Solute Concentration by Plasma Protein Binding and Tubular Secretion

Solute	Free Fraction	Clearance Mechanism	Clearance Total, mL/min	Clearance Free, mL/min	Concentration Total, mg/dL	Concentration Free, mg/dL
A	1.0	Filtration	120	120	1.0	1.0
B	1.0	Filtration and secretion	600	600	0.2	0.2
C	0.1	Filtration and secretion	240	2400	0.5	0.05

NOTE. The table summarizes clearance rates and equilibrium plasma concentrations for three hypothetical small-molecule solutes that are each produced at a rate of 1.2 mg/min and cleared exclusively by the kidneys. Solute A is not protein bound and is cleared exclusively by glomerular filtration. The clearance is equal to a GFR of 120 mL/min and is the same whether expressed in terms of the total plasma solute concentration or the free plasma solute concentration, which are both 1.0 mg/dL. Solute B is not protein bound and is cleared both by glomerular filtration and tubular secretion. In this example, secretion is so efficient that the solute is removed almost completely from the plasma flowing through the kidney, and the clearance approaches the renal plasma flow rate, which is approximately five times the GFR. As a result of five-fold higher clearance, the same rate of solute excretion is achieved with a five-fold lower plasma solute level of 0.2 mg/dL. Solute C is 90% bound to plasma proteins so that the free plasma concentration is only one tenth of the total plasma concentration. Solute clearance by the glomerulus is reduced but the solute still can be cleared efficiently by active tubular transport.[7] The clearance expressed in terms of the total plasma concentration is below the theoretical limit of the renal plasma flow rate but the clearance expressed in terms of the free plasma concentration can be much higher. The total plasma concentration is therefore higher than for a solute that is unbound and cleared efficiency by secretion, but the free plasma concentration to which body tissues are exposed can be much lower.

to albumin. Pharmaceuticals are known to bind to other proteins as well, albeit to a lesser extent than albumin, but the participation of these other proteins in the binding of uremic solutes remains to be explored.

Several of the protein-bound uremic solutes listed in Table 2 are indoles and many of the others are phenyl compounds containing a benzene ring. The indoles are derived from the amino acid tryptophan and the phenyl compounds from food chemicals and from the amino acids tyrosine and phenylalanine. Many of the bound solutes are formed by conjugation of precursor compounds with sulfate, glucuronide, glycine, or glutamine. The conjugation reactions are the same as those responsible for the metabolism of pharmaceuticals, and generally are thought to render solutes more soluble and susceptible to the action of transport proteins. Both the sulfate and glucuronide are formed from several precursor compounds.[13] Where both conjugates have been detected in human beings, the sulfate predominates and is bound more tightly, but conjugation reactions and protein binding may be different in other species. In some cases the unconjugated precursor compound also has been detected in uremic plasma, but this may reflect in part disruption of the conjugate during sample processing. It is further notable that many precursor compounds are produced partially or entirely by colon microbes, including indole and p-cresol, which give rise to the most extensively studied of the protein-bound uremic solutes, indoxyl sulfate and p-cresyl sulfate. The role of the colon in uremic toxin generation is described in more detail in the articles by Jankowski et al and Meijers et al of this issue.[14,15]

Given the efficiency with which the kidney can clear protein-bound solutes, it is tempting to speculate that other compounds in this class also might be important uremic toxins. As summarized later, investigations to date have focused largely on indoxyl sulfate and p-cresyl sulfate, which are produced in relatively large quantities and were among the first protein-bound uremic solutes to be identified.

PROGRESSIVE RENAL INJURY

There is substantial evidence that accumulation of bound solutes may contribute to progressive renal injury in chronic kidney disease (CKD). The evidence is strongest for indoxyl sulfate.[16–19] Administration of indoxyl sulfate to 5/6-nephrectomized rats promoted the progression of CKD accompanied by enhanced gene expression of transforming growth factor-β1 (TGF-β1), tissue inhibitor of metalloproteinase-1, and proα1(I)collagen in the kidney.[18,19] The protein metabolite hypothesis was proposed by Niwa et al[20–23] to emphasize that endogenous protein metabolites such as indoxyl sulfate play a significant role in the progression of CKD.

Indoxyl sulfate normally is excreted into urine mainly via active secretion by the proximal tubular cells. Organic anion transporters (OAT1 and OAT3) play an important role in the transcellular transport of indoxyl sulfate in the tubular cells and in the induction of its nephrotoxicity.[24,25] Indoxyl sulfate in the blood is taken up by OAT1 and OAT3 at the basolateral membrane of tubular cells, and is accumulated in the tubular cells at high concentration in CKD patients.[26] The accumulation of indoxyl sulfate generates reactive oxygen species (ROS), reduces superoxide scavenging activity, and consequently causes tubular cell injury by impairing the kidney's anti-oxidative system.[27] The damaged tubular cells produce TGF-β1 as well as