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Review

Albumin heterogeneity in low-abundance fluids. The case of urine and cerebro-spinal fluid ☆

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ABSTRACT

Background: Serum albumin is a micro-heterogeneous protein composed of at least 40 isoforms. Its heterogeneity is even more pronounced in biological fluids other than serum, the major being urine and cerebrospinal fluid. Modification 'in situ' and/or selectivity of biological barriers, such as in the kidney, determines the final composition of albumin and may help in definition of inflammatory states.
Scope of review: This review focuses on various aspects of albumin heterogeneity in low 'abundance fluids' and highlights the potential source of information in diseases.
Major conclusions: The electrical charge of the protein in urine and CSF is modified but with an opposite change and depending on clinical conditions.
In normal urine, the bulk of albumin is more anionic than in serum for the presence of ten times more fatty acids that introduce equivalent anionic charges and modify hydrophobicity of the protein. At the same time, urinary albumin is more glycosylated compared to the serum homolog. Finally, albumin fragments can be detected in urine in patients with proteinuria.
For albumin in CSF, we lack information relative to normal conditions since ethical problems do not allow normal CSF to be studied. In multiple sclerosis, the albumin charge in CSF is more cationic than in serum, this change possibly involving structural anomalies or small molecules bindings.
General significance: Massively fatty albumin could be toxic for tubular cells and be eliminated on this basis. Renal handling of glycosylated albumin can alter the normal equilibrium of filtration/reabsorption and trigger mechanisms leading to glomerulosclerosis and tubulo-interstitial fibrosis. This article is part of a Special Issue entitled Serum Albumin.

1. Introduction

Serum albumin is the major protein synthesized in the liver [1,2]. After synthesis, it is directly flooded into hepatic blood and, hence, in circulation from where it disappears within 30–40 days (half-life 20 days). Degradation follows mechanisms not entirely defined: in plasma it is degraded into peptides; in the kidney it is filtered at the glomerular level, reabsorbed at the proximal tubule and degraded to peptides.

Distribution of plasma albumin takes place over hours/days and involves 30–40% of the albumin pool. Overall, lymph and skin represent the main sites of extra-vascular albumin: in the former case the ratio with plasma varies from 0.4 to 0.9; in skin it contained the 30–40% of the extra-vascular pool [3–5]. Low amounts of albumin are present in urine, cerebrospinal fluid, tears, bile, sweat, gastric juice, eye and other tissues [6]. Distribution of albumin in low abundance fluids is a part of the overall metabolism that is of minor importance in most cases, but not for urine since it represents the site of excretion and/or of recycling. Persistent albeit modest increment of albumin loss in urine, as those occurring in diabetes mellitus [7–9] and or hypertension, may be associated with degenerative changes of the renal tissue. Moreover, in other compartment such as the cerebrospinal fluid, albumin levels increase in inflammatory states and may be a part of the mechanism of inflammation. Characterization of the physicochemical characteristics of albumin in normal urine may furnish information about degradation pathways. Studies addressing albumin features during pathological states associated with mid albumin increment are part of the discovery of mechanisms of diseases.

2. Albumin in urine: from normality to disease

2.1. Renal handling of albumin and normal albumin excretion

Urine contains proteins deriving from plasma and filtered according to a selective process based on their dimension, charge and configuration

Abbreviations: CSF, cerebrospinal fluid; pI, ioelectric point

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[10,11]. Studies originally performed in early eighties showed that proteins with a molecular mass corresponding to albumin (67 kDa) are readily filtered at the glomerular level but in the case of albumin, it is the charge (pI 4.6) that limits this mechanism since anionic molecules are repulsed by anionic charges of the glomerular basement membrane. Moreover, re-absorption at the proximal tubular level reduces the excretion to 10% of the filtered amount. At this site, albumin binds megalin and cubulin that are the two specific receptors localized at the surface of proximal epithelia [12,13]; after binding, albumin is internalized and digested to produce aminoacids that are re-utilized for protein synthesis.

The final result is that, in humans, normal urine contains about 10 mg albumin per litre that means a minimal amount compared to plasma (clearance of albumin 2.5×10^{-4} ml min). Overall, albumin excretion into urine represents a minimal part of the metabolism. Considering that for a normal man of 50 kg, the pool of albumin is about 400 g (200 intravascular and 200 interstitial/lymph and tissues) and in view of the life of albumin of 40 days, 10 g of protein is recycled every day; loss of 10 mg seems of no value. It becomes instead important to consider minimal variation since the current dominant idea is that modest increments in albuminuria may play deleterious effect on the kidney provided that it persists over years. For reasons that we do not entirely know minimal amount of albumin in urine (also known as microalbuminuria) in selected clinical conditions such as diabetes mellitus [7,8] and hypertension [9] potentially determines chronic tissue degeneration such as glomerulosclerosis, tubulo-interstitial fibrosis and renal failure. In this view, it seems important to understand the mechanisms for microalbuminuria and to do this we should first consider structural features of the protein in normality and during mid alterations.

2.2. Structural features of albumin in normal urine

Considering the selectivity properties of the renal filter and that proteins of the same molecular mass of albumin (67 kDa) are filtered, it is clear that the charge represents a factor of crucial importance for limiting urine loss. It is well known that the electrical charge of normal serum albumin is micro-heterogeneous in a range of pIs between 4.6 and 5.6; in normal human urine, albumin is more anionic with several isoforms with a pI between 4.0 and 4.6 (Fig. 1a). This seems a contradiction of the general role on the ultra-filtration of proteins based on their charge. Studies performed in mid eighties showed that variation in charge is correlated with variation in fatty acid content in which case the more anionic isoforms transport more fatty acids [14,15]. The same phenomenon can be observed in rat urine, in which case urinary albumin is more anionic and more fatted compared to the serum homolog [16] (Fig. 1c). This relation is explained by the fact that the interaction of fatty acids with albumin takes place through non-polar reaction leaving the carboxyl groups free to introduce negative charges (Fig. 2). The strict correlation between fatty acids, charge and micro-heterogeneity of albumin is interesting in consideration that albumin is the major transporter of fatty acids in serum (50% of serum fatty acids are in fact bound to this protein) and that the content in fatty acids of urinary albumin is higher by a factor of ten compared to serum albumin (20 vs 3 nmol/nmol). In parallel with a modification of the charge, increasing the fatty acid content modifies other important features of the protein that have a role in its renal selectivity. One potential modification is conformation another is hydrophobicity. Once again, studies performed in mid eighties addressed these aspects [17]. In particular, it was shown that considering the fluorescence quantum yield of Trp and Tyr as synonymous of conformational variation of albumin (this protein has a unique Trp in the second domain of the protein) no modification was seen in normal urine. Binding of fatty acids also influences affinity of albumin for other ligands and may finally modify its transport and anti-oxidant functions [18,19]. Overall, structural comparison of albumin in serum and in normal urine indicated that it is the fatty acid content to modulate excretion that may be interpreted as a mechanism based on hydrophobicity of the protein. We do not know how the tubular re-absorption of albumin modifies this aspect that is important since a potential mechanism causing renal fibrosis is linked to the quota of fatty acids reabsorbed at the tubular level [20,21]. It is now accepted that it is the amount of fatty acids bound to albumin that causes tubulo-interstitial damage [21] and that the definition of fatty acid-binding protein in urine reflects the clinical outcome and progression to renal failure in patients with non-diabetic renal disease [22].

Another interesting feature of normal urinary albumin is its carbohydrate content. Actually what we intend as carbohydrate content is a function of non-enzymatic glycosylation of this protein that in serum occurs spontaneously mainly with glucose [23]. This reaction takes place between the εamino residues of lysines 525 and 199 [24,25] and the C2 aldehydic groups of carbohydrates via the formation of a Schiff base followed by an Amadori rearrangement [23] that is a stable final product. It is intuitive that non-enzymatic glycosylation of albumin is important in diabetes mellitus in which case, and depending to the metabolic control, serum glycosyl albumin may double normal serum levels. Studies performed on glycosyl albumin in human beings in mid eighties [26–28] and in rats [29] have shown that glycosyl albumin is higher in urine compared to serum, once again, suggesting a privileged excretion of the modified counterpart [30]. Studies in humans documented ten times higher urinary levels of glycosyl albumin than in serum. In rats, clearance of endogenous glycosyl albumin as well as of ^{125}I-labeled glycosyl albumin was found twofold faster than the non-glycated counterpart confirming what is observed in humans [29]. Altogether, studies addressing selectivity in normal conditions constituted the basis for exploring diabetic nephropathy.

2.3. Micro and mid albuminuria

Mechanisms related to micro and mid-albuminuria may be involved in complications of diabetes mellitus [7,8]. In the first phase, the increased renal handling of glycosyl albumin may directly cause microalbuminuria [30]. When the phenomenon persists over time, the chronic passage of glycosyl albumin may produce deposition of albumin in basement membrane of glomerular capillaries as it occurs in rats [31,32]. In consideration of the potential link between micro-albuminuria and the metabolic milieu of diabetes mellitus, glycosyl albumin (that is, in turn, correlated to glucose levels) explains the crucial role of maintaining a strict control on albuminuria [14,33–35]. The same studies that addressed the characterization of urinary albumin in normal conditions considered the crucial point whether and how glycosylation of albumin could enhance the renal excretion of albumin and be implicated in diabetic albuminuria. Results turn out to be inconclusive leaving the question on diabetic microalbuminuria still poorly defined. The starting point was that serum levels of glycosyl albumin are much increased in diabetic patients compared to normal people with more evident increments in patients with mid and overt proteinuria [14,35]. However, this increment is not associated with an increase of urinary levels, since a progressive decrease of the renal selectivity and, hence, of urinary glycosyl albumin was observed in patients with mid and overt albuminuria (>300 mg/day). In the case of overt proteinuria, the percent of glycosyl albumin is the same in urine as in serum suggesting that the kidney is not selective any more. Owing to this change in selectivity the overall amount of urinary glycosyl albumin in diabetics with normal or mid-albuminuria is the same as in those patients with overt albuminuria. Overall, glycosylation may influence renal homeostasis by several mechanisms. First, it modifies secondary and tertiary structure of albumin and quenches its antioxidant properties [36,37]. Second, glycosyl albumin induces superoxide generation