Glomerular handling of native albumin in the presence of circulating modified albumins by the normal rat kidney

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Londono, I., and M. Bendayan. Glomerular handling of native albumin in the presence of circulating modified albumins by the normal rat kidney. Am J Physiol Renal Physiol 289: F1201–F1209, 2005.—Persistent hyperglycemia, as occurring in diabetes, induces changes in circulating as well as in structural proteins. These changes involve substitution of lysine residues by glucose adducts resulting in early Amadori products that evolve into toxic and active substances, the advanced glycation end adducts. In previous studies, we demonstrated that early glycated (Amadori) albumin infused into the circulation of normal animals induces transitory alterations of glomerular filtration. Attempting to elucidate the mechanisms underlying these changes, various molecular modifications were introduced in vitro to serum albumin. Glycation, acetylation, carboxymethylation, methylation, and succinylation, involving either a few or a significant number of amino acid residues, produced heavier and more anionic albumin variants as previously described (41) or purchased from Sigma. These glycated forms have two to three substituted lysine residues. Acetylated albumin, succinylated albumin, and methylated albumin were injected intravenously into normal rats, followed, 30 min later, by hapten-tagged native BSA. Changes in glomerular filtration were evaluated by morphometrical analysis of gold immunolabelings. Compared with native albumin, all the modified forms of albumin induced a deeper penetration of the tracer through the glomerular basement membrane revealing alterations in glomerular permselectivity. This was more evident for severely modified albumin molecules which displayed high labelings in the urinary macromolecules according to their size, charge, and structural conformation (7, 22). In pathological conditions such as in diabetes, the glomerular permselectivity is markedly impaired leading to glomerular hyperfiltration and proteinuria (62). Modification of circulating as well as structural proteins by glycation (nonglycated glycosylation) has been described as a major contributor to the development of glomerular dysfunction and diabetic nephropathy (8, 12, 38, 51). In particular, it has been shown that glycated albumin, an abundant form of circulating albumin in early diabetes (14), is an important trigger in the onset of glomerular dysfunction and proteinuria (10, 14).

Previous studies demonstrated that injection of glycated albumin or glycated serum proteins into the circulation alters the glomerular permselectivity in normal animals (41). Interestingly, the presence of glycated albumin in circulation also affects, in these normal animals, the glomerular filtration properties for other circulating native proteins (40, 41).

Evidence is available demonstrating that chemical modifications of serum proteins do occur in vivo. Circulating antibodies against malonaldehyde adducts of albumin and lipoproteins have been reported in healthy subjects as well as in pathological situations predisposing to atherosclerosis (29, 61). The presence of circulating antibodies against oxidized LDL is a good indicator of oxidative stress in the progression of chronic vascular diseases (48, 52). In alcohol consumers, malonaldehyde and acetaldehyde adducts seem to be formed by oxidative reactions promoted by ethanol (59). During hyperglycemia, a variety of advanced glycation end products (AGE) and the highly reactive dicarbonyl compounds 3-deoxyglucosone and methylglyoxal have been characterized in both serum and tissues and their role in the development of diabetic vascular complications has been emphasized (4, 8, 34, 45).

In the present study, different modifications were introduced into albumin molecules and these were then injected in the circulation of normal rats. The glomerular filtration properties in those animals were evaluated. These proteins were chosen according to their level of substitution in lysine or arginine residues, as in glycated proteins, and to their more drastic modifications which involved other amino acid residues.

The use of electron dense markers in electron microscopy has served in multiple opportunities to reveal changes in glomerular permeability (17, 22, 27). The immunogold approach combined with morphometrical evaluations have been repeatedly used to demonstrate changes in glomerular filtration properties (25, 41, 50), particularly in early stages of diabetes and in long-term proteinuric animals (5, 18, 21). Based on these well-established approaches, changes in the glomerular permselectivity properties were evaluated in normal animals injected with the different modified albumin molecules.

METHODS

BSA, FITC-BSA, and -dextran T70 (FITC-dextran) were obtained from Sigma (Oakville, ON, Canada). Glycated albumin was prepared as previously described (41) or purchased from Sigma. These glycated forms have two to three substituted lysine residues. Acetylated albumin and carboxymethylated BSA were also purchased from Sigma. Carboxymethylated BSA is described as containing around 30 homocysteine residues. On the other hand, BSA was modified by reductive alkylation according to described procedures (43). Briefly, methylated BSA was prepared on ice by adding 5 mg of sodium borohydride to 10 mg/ml of BSA in borate buffer at pH 9. For lightly methylated BSA, 15 μl of 37% formaldehyde were added every 20 min during...
Table 1. Levels of substitution in lysine residues of albumin molecules

<table>
<thead>
<tr>
<th>Lysine Residues Available, %</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Native</td>
<td>100</td>
</tr>
<tr>
<td>Glycated</td>
<td>96</td>
</tr>
<tr>
<td>Acetylated</td>
<td>19</td>
</tr>
<tr>
<td>Carboxymethylated</td>
<td>40</td>
</tr>
<tr>
<td>Lightly methylated</td>
<td>95</td>
</tr>
<tr>
<td>Moderately methylated</td>
<td>62</td>
</tr>
<tr>
<td>Lightly succinylated</td>
<td>53</td>
</tr>
<tr>
<td>Moderately succinylated</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

1 h, while for moderately methylated albumin, 100 μl were added every 10 min during 50 min. Reactions were stopped 30 min after the last addition, by extensive dialysis. Succinylated albumin was prepared according to Klapper and Klotz (36): to an aqueous solution of 20 mg/ml BSA at pH 7.0, 1 mg succinic anhydride was added every 15 min during 30 min for lightly succinylated BSA and every 5 min during 50 min for moderately succinylated albumin. Reactions were stopped 45 min after the last addition, by extensive dialysis. The degree of protein modification was determined by the fluorescamine method (60). According to this method, the methylated and succinylated forms contained up to 50 substituted lysine residues (Table 1).

Characterization of the Modified Albumins

**SDS-PAGE.** Changes in molecular weight for the modified albumin molecules were evaluated by electrophoresis on 10% acrylamide gels, using 20 μg of protein, according to standard procedures.

**Isoelectric focusing.** Charges of native and modified albumin molecules were assessed by isoelectric focusing according to described procedures (26). Briefly, albumin was solubilized in a buffer containing 4% CHAPS, 8 mol/l urea, and 2 mol/l thiourea. Samples were sonicated and vortexed intermittently for 30 min, while keeping them on ice. Immobilized linear pH gradient (IPG) 7-cm (pH 3–10) strips (Amersham Pharmacia Biotechnology, Bais d’Urfe ´, QC, Canada) were used. IPG strips were hydrated in the different albumin samples overnight. The isoelectric focusing was run on a Multiphor system (Pharmacia) according to manufacturer’s instructions. After the run, the strips were immersed in 10% TCA for 10 min and washed in 0.1% TCA three times for 5 min. At the end, the sticks were digitalized and compared.

**Fluorescence quenching by binding to bilirubin.** Bilirubin binds to epsilon-amino lysine residues in the albumin molecule through its COOH groups. The binding of bilirubin to albumin induces a quenching of the protein fluorescence which is dose dependent and varies according to the number of lysine residues available. Native BSA as well as modified forms were diluted in 100 mM phosphate buffer to 5 μM and measured spectrophotometrically at A_{280}. According to described procedures (33, 39, 47), fresh bilirubin stock solution was prepared by dissolving 4 mg of bilirubin in 1 ml of 10 mM NaOH containing 1 mM EDTA and then brought to 2 mM with distilled water. A dilution of 1/20 was prepared and then added to the protein solution to a final concentration of 1 or 5 μM. In some experiments, increasing concentrations from 0.05 to 5 μM were prepared. All experiments were performed in dim light to prevent bilirubin degradation. Fluorescence measurements were obtained in a Turner 430 spectrofluorometer using 295 nm for excitation and 330 to 360 nm for emission. The fractional quenching values (Q) were obtained according to the formula Q = (F0 – F)/F0, where F0 is the fluorescence at bilirubin:albumin ratio zero, F is the fluorescence at different bilirubin:albumin ratios (0.2, 0.4, . . . , 1), and F1 is the fluorescence at ratio 1. The maximal fluorescence values are obtained in the absence of bilirubin and the maximal quenching is given by F0-F1 (39).

Absorption spectrum of bilirubin bound to BSA. Spectrophotometric data of bilirubin-BSA complexes reveal changes in protein conformation. The absorption spectrum of bilirubin bound to each form of modified albumin molecules was obtained as follows (47): to 1 ml of 5 μM solution of BSA in 100 mM phosphate buffer pH 8, bilirubin was added to a final concentration of 5 μM. After 15-min incubation at a 37°C, the spectrum was recorded from 330 to 550 nm (optimal absorbance for bilirubin is 440 nm). Proteins do not absorb in this range of wavelength.

**Experimental Protocol**

One-hundred- to one-hundred-twenty-gram Sprague-Dawley rats were used in this study. The animals were handled following the guidelines of the Canadian Council on Animal Care (CCAC). At least three animals were used for each experimental condition. Animals were anesthetized with an intraperitoneal injection of urethane and the abdominal cavity was opened. Fifty milligrams of native or modified BSA were injected in the cava vein. After 30 min, 50 mg of native BSA tagged to FITC were injected. In another set of experiments, native or glycated BSA were introduced into the circulation and after 30 min, 150 mg of de dextran T70 tagged to FITC were injected. Five minutes later, the fixative, 1% glutaraldehyde in 0.1 mol/l phosphate buffer pH 7.2, was floated into the abdominal cavity to begin the fixation of the kidneys in situ. Then, slices of renal cortex were sampled, cut in small pieces, and immersed in the same fixative for 2 h at room temperature. Tissues were rinsed three times with phosphate buffer and postfixed with 1% osmium tetroxide for 90 min. They were then dehydrated in graded ethanol and embedded in Epon.

The 30 min was chosen as we found that protein tracers injected in the circulation reach saturation in the subendothelial spaces at this time point (24, 40). Fixation was started in situ within the abdominal cavity and further by immersion to avoid washing out serum proteins from the blood vessels and to retain our tracer molecules within the glomerular wall.

**Immunocytochemical labeling.** Renal tissue sections were mounted on grids and treated with NaNO₃ for 20 min, washed with distilled water, and quenched with 150 mM glycine in PBS and 1% ovalbumin. Grids were then incubated on a drop of the anti-FITC (1/200, Dakopatts, Cederlane, Canada) for 2 h at room temperature and washed with PBS. Finally, they were incubated on a drop of protein A-gold (10-nm diameter, A_{550} 0.5) for 30 min, at room temperature.

**Morphometrical determinations.** The distribution of the gold particles through the glomerular basement membrane (GBM) was evaluated by morphometry using an image analysis system (Clemex Vision, Longueuil, QC, Canada). Pictures were recorded at ×38,000 and gold-labeling distributions of FITC-BSA were obtained as follows: the distance between each gold particle in the GBM and the abluminal endothelial cell membrane (a) was recorded, as well as the GBM thickness (b) at the very same point. The ratios R (R = a/b) were calculated and presented as histograms. R varied between 0 (gold particle at the base of the endothelial cell) and 1 (gold particle located at the base of the epithelial cell). An average of 500 gold particles was evaluated per animal, which corresponds approximately to the study of three glomeruli per animal and per condition. Labeling densities over the capillary lumen and the urinary space were also evaluated to assess the presence of albumin in the urinary space under the different conditions. Densities were obtained by direct planimetry and gold-particle counting using the Videoplan 2 image processing system. For comparative evaluations, ratios of labeling densities between these two compartments were calculated.

Statistical evaluations were performed using the Mann-Whitney U-test.

**RESULTS**

Modified albums were evaluated for changes in size, molecular weight, and in bilirubin binding capacity. Results
from the SDS-PAGE (Fig. 1) show minor changes in molecular weight for the glycated, lightly and moderately methylated, and lightly succinylated forms (lanes 2, 5, 6, and 7, respectively) compared with native BSA (lane 1). In contrast, the acetylated, carboxymethylated, and moderately succinylated forms (lanes 3, 4, and 8, respectively) display significant increases in molecular weight compared with the native form. This is probably due to significant substitutions in these BSA molecules. Concerning the isoelectric focusing (Fig. 2), two to three protein bands were observed between pI 5.9 and 6.2 for native and glycated albumin (lanes 1 and 2), while four to seven bands between pI 5.7 and 6.2 were found for the methylated forms and the lightly succinylated forms (lanes 5 to 7) suggesting the presence of isoforms with various degrees of substitution. Acetylated and carboxymethylated albumin molecules display bands around pI 3.9 and pI 4.2 (lanes 3 and 4), respectively, whereas moderately succinylated albumin displays bands at pI 4.7 (lane 8), notably more anionic than native BSA.

Fluorescence quenching and visible spectroscopic measurements of bilirubin binding to albumin molecules allow for the evaluation of changes in the binding capacity and three-dimensional conformation of albumin molecules. Fluorescence quenching curves depending on the bilirubin to albumin ratios are presented in Fig. 3. Only small differences in fluorescence quenching were detected for lightly and moderately methylated albumin molecules. Glycated albumin was significantly less quenched by bilirubin compared with the methylated and native forms. The acetylated albumin was the least quenched by bilirubin suggesting the lowest binding capacity probably due to high levels of modification. During acetylation, amino acid residues such as tyrosine were also affected. Surprisingly, moderately succinylated albumin yield higher quenching values than native albumin. Because intrinsic fluorescence of this succinylated form was higher than the one of native albumin (results not shown), it is probable that succinylation induced conformational changes in the molecule that enhanced its binding capacity (35, 44). Due to problems of solubility for the carboxymethylated form, we could not include it in this assay.

However, values of intrinsic fluorescence recorded for this form were 50% lower compared with native albumin.

The visible absorption spectrum of bilirubin displayed a peak at 440 nm (Fig. 4). Binding of bilirubin to native BSA induces a shift to the right, with a maximum at ~465 nm. Similar changes were observed on binding to glycated, methylated, and lightly succinylated forms and in lesser extent, to the moderately succinylated form. In contrast, binding to acetylated and carboxymethylated albumins did not induce any significant shift in the absorption peak, suggesting absence of binding.

Gold immunocytochemistry revealed the presence of FITC-BSA in different renal locations with patterns that differ depending on the form of albumin molecule injected. When
native BSA is injected into the circulation of normal animals, FITC-BSA was mostly located to the subendothelial side of the glomerular wall, at the lamina rara interna (Fig. 5A) reflecting the restriction of this molecule and intact glomerular permselectivity properties. In animals injected with lightly modified albumin molecules, higher labelings were observed in the lamina rara interna spreading to the lamina densa and the lamina externa with few gold particles reaching the urinary space of the Bowman’s capsule (Fig. 5B). This distribution pattern reflects alteration of the permselectivity with loss of albumin into the urinary space. In animals injected with moderately modified proteins such as acetylated and carboxymethylated albumins, labelings were more randomly distributed through the GBM and were more significant in the urinary space (Fig. 5C and D). Furthermore, in the presence of these modified forms, labelings for FITC-BSA were intense in the proximal convoluted tubules, associated with the microvilli and within the endosomal compartments of the epithelial cells (Fig. 6). These results indicate major changes in glomerular permselectivity with large amounts of albumin in the urinary space and significant reabsorption activity at the level of proximal tubules.

When FITC-dextran was used as a tracer in combination with native albumin, labelings were restricted to the capillary lumen and the GBM (Fig. 7A). In the presence of glycated albumin, on the other hand, labeling for dextran was abundant throughout the GBM and in the urinary space (Fig. 7B), again indicating alteration of the glomerular properties.

Morphometric evaluation of the tracer labelings through the GBM is an indicator of the integrity of the glomerular properties. Besides, it confirmed the subjective observations. For native BSA, a peak of labeling near the subendothelial side of the GBM, with an average R value of 0.38, was observed (Fig. 8). This represents a baseline and reflects normal function. The distribution patterns for FITC-BSA on infusion of the modified albumin molecules demonstrated a right-handed shift (toward the subepithelial side of the GBM), with average R values between 0.44 and 0.47 indicating changes in glomerular properties. Highest average R values were obtained in the presence of glycated, acetylated, carboxymethylated, and succinylated albumin forms. In the case of the lightly methylated form, although the histogram distribution remind the one of native BSA, the average R value was significantly higher (0.44). The moderately methylated albumin (MM) induced also a shift toward the epithelial side of the GBM but labeling was more widely and uniformly distributed through the GBM and the peak almost vanished (Fig. 8).

Fig. 4. Visible absorption spectra of bilirubin and bilirubin-protein complexes. The absorption spectra of bilirubin-native BSA (N) is shown in all panels as dotted lines. Binding of glycated BSA (G), lightly (M and S), and moderately (MM and SS) methylated and succinylated albumin to bilirubin induced a shift to the right. Bilirubin binding to G, M, and S yield spectra that were similar to the one displayed on binding to native BSA (N). Binding to MM and SS yield spectra that were lightly different from N. Conversely, binding to acetylated- and carboxymethylated-albumin (AC and CMT, respectively) yield spectra with similar characteristics to the bilirubin alone (BIL).
Table 2 displays the average $R$ values of the labeling distributions through the GBM for FITC-BSA in the presence of circulating native and modified albumins. It also displays the mean $R$ values obtained from labeling distributions of FITC-dextran and endogenous transferrin in the presence of native and glycated BSA. The mean $R$ values of FITC-BSA labeling distributions in the presence of circulating modified albumin molecules were significantly higher than that observed with native BSA, indicating a shift toward the epithelial side and alterations of the filtration properties of the GBM. Mean $R$ values for FITC-dextran and transferrin were also higher in the presence of circulating glycated BSA compared with native BSA, which again indicate changes in the glomerular properties likely induced by the presence of this modified protein.

Ratios between labeling densities for FITC-albumin in the urinary space vs. the capillary lumen appear in Table 3. Animals injected with native albumin display the lowest value, indicating that only 7% of the labeling recorded in the capillary lumen is found in the urinary space. Slightly higher although not statistically significant values were obtained from animals injected with glycated and lightly methylated albumin. In contrast, animals injected with other modified albumin molecules yield higher labeling density ratios indicating significant passage of albumin toward the urinary space. These alterations add to the changes in albumin distribution across the GBM reported above. However, for glycated and slightly methylated albumin molecules, the alteration appears not to be as significant. Care should be taken concerning these evaluations due to limitations in the retention of material in large openings such as the urinary space.

Thus once introduced in the circulation of normal animals, glycated albumin and all the other modified albumins tested (methylated, acetylated, carboxymethylated, or succinylated), whether lightly or moderately modified, induced changes in the glomerular handling of albumin as well as of other circulating molecules normally restricted by the glomerular wall.

**DISCUSSION**

In the present study, we made use of a hapten-tagged albumin as a probe to evaluate the glomerular handling of
native albumin in the presence of various types of circulating modified albumins. Hapten-tagged molecules constitute interesting tools for the morphological study of dynamic processes as strengthen by studies of Ghitescu and Bendayan (23, 24). Hapten-tagged molecules such as FITC-BSA and FITC dextran have been frequently used in studies of microvascular permeability (42, 53, 58, 64). At the electron microscope level, this FITC hapten can be detected under conditions of optimal ultrastructural preservation. The FITC-BSA probe can be unambiguously distinguished from the endogenous serum protein and provides a similar glomerular labeling distribution, showing that it is handled by the glomerular wall like endogenous albumin (41). An hapten-tagged albumin that is modified by glycation yields a glomerular labeling pattern different to the one observed for endogenous albumin or for FITC-BSA indicating alteration of the glomerular permselectivity (41). Furthermore, when glycated albumin is introduced in the circulation of normal rats as performed in this study, it alters glomerular handling of several circulating nonmodified molecules with an increased passage toward the urinary space. As glycated albumin disappears from circulation, glomerular permselectivity properties get restored (40).

Circulating glycated albumin has been recognized as a putative initiator of diabetic nephropathy (12). Glycated albumin stimulates the production of basement membrane components by glomerular endothelial (9) and mesangial cells (15) via activation of protein kinase C, transforming growth factor-β (TGF-β) and its receptor, TGF-β RII. In addition, glycated albumin increases the oxidative stress and activates NF-κB production in vitro (13). Correspondingly, advanced glycation end adducts (AGE) of circulating and structural proteins formed under chronic hyperglycemia must contribute to the development of diabetic complications by altering vas-

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**Table 2. Mean values of R**

<table>
<thead>
<tr>
<th>Tested Molecule</th>
<th>Tracer</th>
<th>Mean Values of R</th>
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</thead>
<tbody>
<tr>
<td><strong>Experiments with albumin (rat)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Native BSA</td>
<td>BSA-FITC</td>
<td>0.38±0.01</td>
</tr>
<tr>
<td>Glycated BSA</td>
<td>BSA-FITC</td>
<td>0.47±0.01*</td>
</tr>
<tr>
<td>Acetylated BSA</td>
<td>BSA-FITC</td>
<td>0.46±0.01*</td>
</tr>
<tr>
<td>Carboxymethylated BSA</td>
<td>BSA-FITC</td>
<td>0.46±0.01*</td>
</tr>
<tr>
<td>Lightly methylated BSA</td>
<td>BSA-FITC</td>
<td>0.44±0.02*</td>
</tr>
<tr>
<td>Moderately methylated BSA</td>
<td>BSA-FITC</td>
<td>0.47±0.01*</td>
</tr>
<tr>
<td>Lightly succinylated BSA</td>
<td>BSA-FITC</td>
<td>0.45±0.01*</td>
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<tr>
<td>Moderately succinylated BSA</td>
<td>BSA-FITC</td>
<td>0.47±0.01*</td>
</tr>
</tbody>
</table>

| **Experiments with dextran (rat)** | | |
| Native BSA      | Dextran-FITC   | 0.44±0.01        |
| Glycated BSA    | Dextran-FITC   | 0.52±0.01*       |

| **Experiments with transferrin (mouse)** | | |
| Native BSA      | Endogenous transferrin | 0.43 |
| Glycated BSA    | Endogenous transferrin | 0.47* |

Values are means ± SE, n = 3–4 animals. The values reported for transferrin come from Ref. 40. *P < 0.05 compared with native BSA.
circular permeability and inducing the release of growth-promoting cytokines (32).

Aiming at better understanding the characteristics of the glomerular alteration induced by glycated albumin, we introduced other types of modified albumin molecules into the circulation of normal rats. The results obtained demonstrate alterations of albumin distribution throughout the entire thickness of the GBM and higher amounts of albumin in the urinary space in conditions of exposure to modified albumin molecules. The interesting additional information revealed by such experiments is the fact that alteration of the perme selectivity is not the same for all molecules and while slightly modified albumin molecules induce little changes, the more severely modified ones do induce significant changes. Similarly, in long-term diabetic rats (5), in proteinuric patients (50), and after infusion of cationic probes (25), endogenous albumin, as revealed by immunogold, presented similar altered patterns of labeling distribution.

The mechanisms behind these changes are unknown. The chemical modifications introduced to BSA in the present study generated heavier and more anionic molecules compared with native albumin. These molecules are supposed to be restrained by the glomerular wall according to the classical concepts of glomerular size and charge selectivity (7, 22, 55). However, the presence of these proteins in circulation induced changes in glomerular perme selectivity permitting the passage of circulating proteins through the GBM and the glomerular wall. Furthermore, even dextran T70, a neutral molecule of similar size to albumin, displayed an increased passage toward the urinary space in the presence of circulating glycated albumin, which is suggestive of an alteration of the size-dependent perme selectivity of the GBM. It is possible that changes in shape or structural conformation of the modified albumin molecules influence their handling by the GBM (49) and affect the passage of other circulating proteins. Despite the fact that no major changes in conformation were observed for lightly modified albums, they did induce the same glomerular alterations as the highly modified ones (acetylated, carboxymethylated, and succinylated BSA molecules). In addition, the foremost point from these results is the enhanced passage of accompanying circulating nonmodified molecules solely by the presence of either lightly or moderately modified molecules. This has a major impact on the induction of proteinuria.

Proteinuria is usually associated with renal dysfunction and results from an increase in glomerular permeability for normally nonfiltered plasma macromolecules such as albumin. In diabetic nephropathy, a transient increase in glomerular filtration rate is observed at the early stage of the kidney disease with the onset of hyperglycemia. This glomerular hyperfiltration occurs before any structural glomerular changes such as GBM thickening (21) and before albuminuria (16, 31). The hemodynamic factors are reported to play an important role in the induction of hyperfiltration (31). Different situations can induce transient proteinuria (protein overload, short-term hyperglycemia, exercise, hypertension, acute febrile illnesses) probably due to increases in glomerular capillary pressure (31, 55). Glomerular hyperfiltration may be caused by afferent arterial dilatation mediated by vasoactive hormones and cytokines such as nitric oxide, insulin-like growth factor, prosta glandins, and glucagon (11, 16). Glycated albumin, the predominant form of circulating albumin in early diabetes, as well as AGE adducts have been shown to modulate nitric oxide synthase and thromboxane production in murine endothelial cells (2). Whether the loss in glomerular perme selectivity induced by the presence of circulating modified albumin molecules is direct or induced by other factors triggered by their presence in circulation is still unknown. In patients suffering from focal segmental glomerular sclerosis (FSGS), the presence of a circulating factor (FSGS permeability factor or FSPF) that induces an increase in albumin permeability in isolated rat glomeruli has been described (57). The effects of FSPF on glomerular permeability are rapid and dose dependent and involve signaling through altered phosphorylation of cellular proteins (56). That the modified albumin molecules used in the present study could trigger the secretion of such active factors affecting glomerular permeability remains to be investigated.

Substantial evidence derived from morphological studies indicates that the primary filtration barrier in the glomerular wall is located at the subendothelial side of the GBM (5, 22, 50). In proteinuric states, loss of the glomerular perme selectivity is demonstrated by a further penetration of proteins in the GBM (5, 18, 21, 50). Interestingly, recent findings indicate a crucial role for the slit-diaphragm podocyte proteins nephrin, NEPH1, podocin, and CD2AP in the integrity of the glomerular filtration barrier. Mutations in the genes coding for these proteins result in heavy proteinuria (reviewed in Ref. 3). In these cases, the integrity of the slit diaphragm is lost by either a retention of these proteins in the rough endoplasmic reticulum (46) or a redistribution to apical surfaces (3). On the other hand, glomerular nephrin expression has been shown to decrease during diabetes (1). Furthermore, in human cultures podocytes incubated with glycated albumin, nephrin expression is also decreased (20). How circulating molecules would regulate the expression of podocyte slit-diaphragm proteins and thus affect glomerular permeability remains to be elucidated.

On its passage through the glomerular wall, FITC-BSA was absorbed by the proximal tubule epithelial cells. The intense labeling of the endosomal compartments of these cells confirms their important contribution in the catabolism of filtered proteins. Modified circulating molecules are rapidly cleared in the liver by either specific or scavenger receptors (28, 54, 65). AGE adducts formed during chronic hyperglycemia can be eliminated from circulation through specific receptors on endothelial and liver cells (30, 63) and are reabsorbed by podocytes and proximal convoluted tubules as demonstrated in diabetic animals (4). Circulating native albumin was internalized by proximal tubules in the presence of modified albums.
This selective reabsorption of native albumin by proximal tubules and the preferential excretion of modified albumins such as glycated albumin during diabetes and ageing have already been described (6, 37) and referred to as a physiological event (19).

In conclusion, the presence of either lightly or moderately modified albumin molecules in circulation rapidly induces significant changes in glomerular perme selectivity, the presence of the filtered protein in the urinary space, and the reabsorption at the level of proximal tubules. The changes induced may be related to the extent of the molecule modification but it is clear that modified proteins in circulation have a major impact in the induction of proteinuria in pathological situations including diabetes.

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GRANTS

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REFERENCES


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