Clinica Chimica Acta 402 (2009) 94–101

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Molecular heterogeneity of urinary albumin in glomerulonephritis: Comparison of cardiovascular disease with albuminuria

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ARTICLE INFO

Article history:
Received 9 September 2008
Received in revised form 19 December 2008
Accepted 19 December 2008
Available online 31 December 2008

Keywords:
Urinary albumin
Glomerulonephritis
Cardiovascular disease
Nonreducing SDS PAGE
Albumin zymography
MALDI-TOF MS

ABSTRACT

Background: Despite the unstable structure of urinary albumin in kidney diseases, urinary albumin fragments have been identified by denaturing methods such as two-dimensional electrophoresis. This study examined the relationship between the structural heterogeneity of urinary albumin and protease effects.
Methods: Urine samples from patients with glomerulonephritis (GN), cardiovascular diseases (CVD), and healthy subjects were analyzed by non-reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE), Western blot, diagonal 2-dimensional non-reducing/reducing (d2D) SDS PAGE, and albumin zymography.
Results: The major band was monomer albumin in CVD and healthy subjects; however, 13 urinary albumin bands ranging from 55 to 172 kDa were identified by non-reducing SDS PAGE in GN. The results from d2D SDS PAGE showed urinary albumin polymerization between disulfide bridges, interactions with other proteins, and reduction induced degradation in GN patients. The results from albumin zymography showed that low-molecular mass forms of albumin did not necessarily correspond to high protease activity. Furthermore, concentrated healthy urine showed similar protease digestion as in GN without low-molecular mass of albumin.
Conclusions: The molecular alterations observed cannot be explained only by urinary proteases. The specific alteration of urinary albumin molecules in GN can be attributed to different mechanisms to CVD.

1. Introduction

Glomerular filtrated urinary albumin is physiologically fragmented (>90–95%) into smaller peptides of molecular masses of 1–15 kDa in both rat and human [1–3]. In patients with type 1 diabetes mellitus, intact (monomer) albumin excretion is increased and the fragmentation ratio (fragmented: intact) is reduced [2]. These fragments are not detected by standard immunochemical assays [4,5].

Recently, not only the immunochemically undetectable fragments, but antibody detectable albumin fragments with relatively high molecular masses were reported. Candiano et al. showed that about 40 albumin derived fragments in the range 20–64 kDa were identified using 2-dimensional electrophoresis (2DE), Western blot, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis in the urine of patients with minimal-change nephropathy, focal segmental glomerulosclerosis, and membranous glomerulonephritis [6]. Candiano et al. indicated that the fragmentation was caused by the up-regulation of urinary proteases specific for albumin.

These recent proteome analyses have led to a better understanding of the mechanisms of urinary albumin excretion and degradation. However, there was no consideration of the typical albumin structures of internally cleaved forms held together by disulfide bonds [7–9]. For example, Osicka et al. reported that urinary intact albumin was partly fragmented into smaller peptides when analyzed by reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) in diabetes mellitus patients [9]. These findings suggest that urinary albumin in patients with kidney diseases is unstable in reducing conditions, but previous reports on albumin fragments have been made using denaturing and reducing methods.

The present study tested the hypothesis that urinary albumin is more unstable in patients with glomerulonephritis (GN) compared with those with cardiovascular disease (CVD) with albuminuria and in healthy

Abbreviations: 2DE, 2-dimensional electrophoresis; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; SDS PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; GN, glomerulonephritis; CVD, cardiovascular diseases; d2D SDS PAGE, diagonal 2-dimensional nonreducing/reducing SDS PAGE; IgAN, immunoglobulin A nephropathy; ANCA, antineutrophil cytoplasmic antibodies; TIA, turbidimetric immunoassay; CBB, Coomassie brilliant blue R-250; PVDF, polyvinylidine fluoride; DTT, dithiothreitol; TFA, trifluoroacetic acid; CHCA, α-cyano-4-hydroxycinnamic acid; DHB, 2,5-dihydroxybenzoic acid.

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doi:10.1016/j.cca.2008.12.026

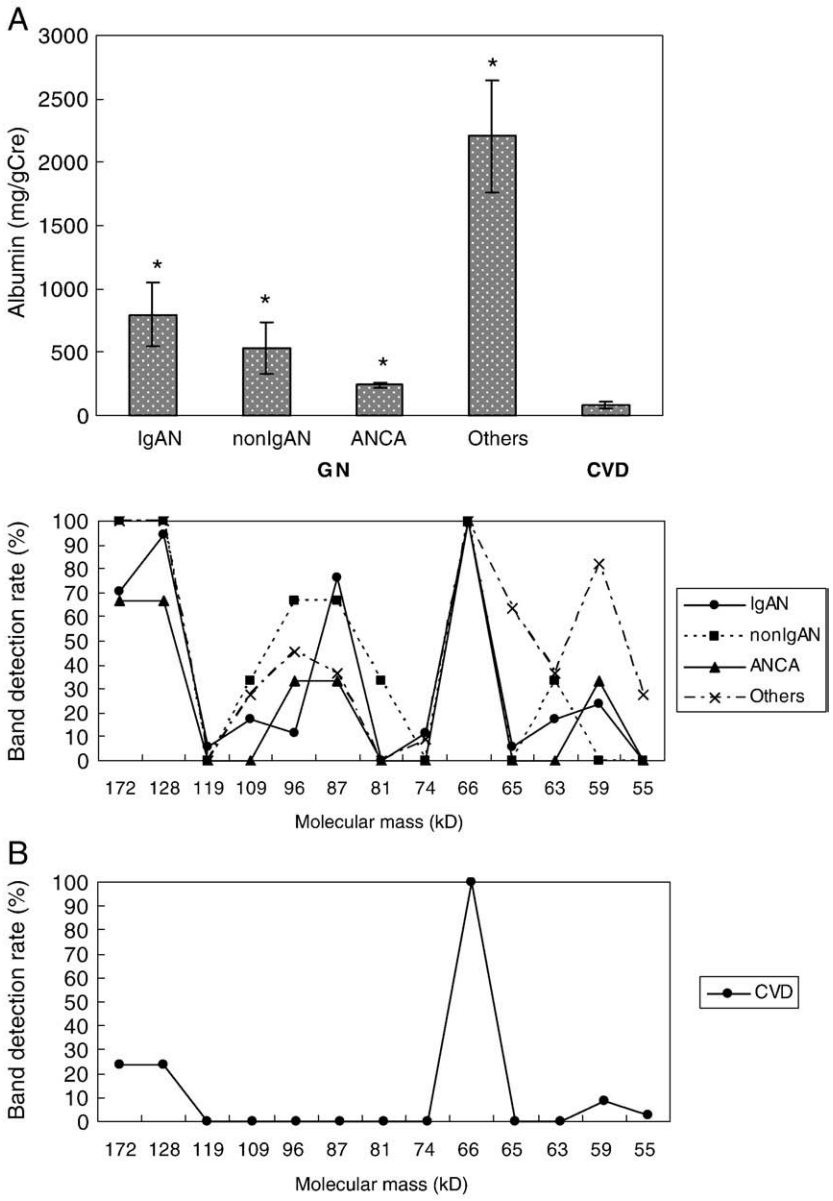

Fig. 1. Comparison of urinary albumin mg/gCre values and detection ratios of each albumin band. A) Albumin mg/gCre values in patients with CVD or one of four forms of GN. The albumin mg/gCre values were highest in patients in the Other nephropathies group, because these included diseases with relatively severe albuminuria. *Statistically significant difference at $p<0.05$ compared with CVD subjects; B) Detection ratios of albumin bands in GN and CVD from Western blot results of 34 subjects. Western blotting shows that a variety of urinary albumin bands were recognized in the GN group compared with CVD patients with a similar degree of albuminuria.

subjects using both non-reducing SDS PAGE and diagonal 2-dimensional non-reducing/reducing SDS PAGE (d2D SDS PAGE). The heterogeneity of urinary albumin in GN was clarified and a better understanding of the molecular structure of urinary albumin was obtained.

2. Methods

2.1. Subjects

The Ethics Committee of Niigata University Graduate School of Medical and Dental Sciences approved the protocol for this study. Informed consent was obtained from all study subjects. Spot urine samples were collected from 34 patients (15 men and 19 women; mean age ± SD = 48.4 ± 16.0) admitted to Niigata University Hospital with various forms of GN. Renal biopsies were performed in all patients as described previously [10] and the patients were classified into 4 groups: 17 patients with IgA nephropathy (IgAN), 3 with non IgA nephropathy (nonIgAN), 3 with ANCA associated nephropathy (ANCA), and 11 with other nephropathies (Other) including membranous nephropathy, lupus nephritis, thin glomerular basement membrane disease, diabetic glomerulosclerosis, renal amyloidosis, and crescentic glomerulonephritis.

Spot urine samples were also collected from 34 patients (18 men and 16 women; mean age ± SD = 68.5 ± 8.14 y) with various type of CVD who consulted Tokyo Medical and Dental University Hospital Faculty of Medicine. Patients with underlying hypertension included 28 out of 34 (82.4%) subjects. In addition, 19 out of 34 (55.9%) subjects exhibited hypertension in combination with hyperlipidemia, hyperuricemia, arrhythmia, ischemic heart disease, and myocardial infarction. Patients without hypertension comprised 6 out of 34 (17.6%) subjects, and they were affected by ischemic heart disease or myocardial infarction. Patients with complications of diabetic mellitus or any other kidney disease were excluded from this study.

Urine samples were also collected from healthy subjects comprising laboratory staff. All fresh urine samples were centrifuged (1500 ×g, 5 min, 4 °C) and the supernatant was stored at −80 °C until analysis in small aliquots to avoid deterioration due to freezing–thawing.

2.2. Laboratory evaluations

Urinary total protein was measured using the pyrogallol red method (Wako Pure Chemical Industries, Ltd., Osaka, Japan), urinary albumin was measured using a turbidimetric immunoassay (TIA; Nitto Boseki Co., Ltd., Tokyo, Japan) and urinary creatinine was measured using an enzymatic method (Nitto Boseki). These