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ORIGINAL PAPER

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Alpha-1-microglobulin: inhibitory effect on calcium oxalate crystallization in vitro and decreased urinary concentration in calcium oxalate stone formers

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Abstract In the past few years, alpha-1-microglobulin (α1m) has been copurified from human urine with bikunin, a potent inhibitor of calcium oxalate (CaOx) crystallization in vitro. In this study, we have purified α1m without bikunin contamination and investigated its possible role in CaOx crystallization by in vitro and in vivo studies. Alpha-1m was purified with an anti-α1m antibodies CNBr-activated sepharose column. Two molecular species of α1m of respectively 30 and 60 kDa were purified. For each protein, two blots of 30 and 60 kDa cross-reacted with anti-α1m antibodies, suggesting that these two forms were derived one from the other. Both protein species inhibited CaOx crystallization in a dose-dependent manner in two in vitro tests. In the first test, the presence of α1m of 30 kDa (8 μg/ml) in a medium containing 0.76 mM $CaCl_2$ (with ^{45}Ca) and 0.76 mM $Ox(NH_4)_2$ inhibited CaOx crystallization by 38% as estimated by supernatant radioactivity after 1 h of agitation. In the second test, CaOx kinetics were examined for 3 to 10 min in a turbidimetric model at 620 nm. The presence of α1m of 30 kDa in a medium containing 4 mM $CaCl_2$ and 0.5 mM Na_2Ox inhibited CaOx crystallization by 41.5%, as estimated by the slope modification of turbidimetric curve. Alpha-1m can be considered as another inhibitor of urinary CaOx crystal formation, as shown by the present in vitro studies. Using an ELISA assay, we found that urinary α1m concentration was significantly lower in 31 CaOx stone formers than in 18 healthy subjects (2.95 ± 0.29 vs 5.34 ± 1.08 mg/l respectively, $P = 0.01$). The decreased concentration of α1m in CaOx stone formers could be responsible in these patients, at least in part, for an increased risk of CaOx crystalluria.

Key words Alpha-1-microglobulin · Calcium oxalate · Crystallization · ELISA · Human · Urine

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Introduction

Supersaturation of calcium oxalate (CaOx) in biological fluids is a fundamental condition required, but not always sufficient, for the occurrence of CaOx crystal formation. Stone formation does not occur in urine of normal healthy subjects despite frequent CaOx supersaturation because several urinary inhibitors are also present in urine, allowing an equilibrium between promoters and inhibitors of crystallization [11]. It has been suggested that in stone formers this equilibrium may be disturbed, either by an excess of one or several promoters, a deficit of one or several inhibitors, or both. To date, a number of macromolecular inhibitory substances have been identified using in vitro tests, including glycoproteins and glycosaminoglycans [29, 31]. The latter were long believed to be the only potent inhibitors. In recent years, attention has been focused on several proteins, in particular glycoproteins, responsible for the inhibitory activity in urine, including the Tamm-Horsfall protein (THP) [21], nephrocalcin [29], the crystal matrix protein [13], uropontin [33], a protein with osteopontin fragment [34], and uronic acid-rich protein (UAP) [4]. The last compound has been identified to date as the light chain of inter alpha trypsin inhibitor (ITI), i.e. bikunin [7].

Initially the preparation of UAP was frequently contaminated with another urinary protein, namely alpha-1-microglobulin (α1m), as described by Atmani et al. [5]. This contamination, as shown by a cross-reaction of the protein with anti-α1m antibodies, may influence the inhibitory activity attributed to bikunin alone. Although inhibitory activity in urine is not a

mass-dependent parameter and the urinary activity of two inhibitory proteins is not necessarily the sum of the activity of each, it appeared of interest to test the activity of α1m using in vitro models of CaOx crystallization in vitro, after its purification by immunoaffinity.

Therefore, we compared the urinary excretion of α1m in normal men presenting no crystals in their first morning urine specimen and in hypercalciuric male stone formers with CaOx dihydrate crystals in their urine specimen.

Materials and methods

Materials

Primary rabbit monospecific anti-human α1m antibodies and primary rabbit monospecific anti-human ITI antibodies were purchased from Dako, Denmark (reference A 256 and A 0301, respectively). Primary rabbit anti-human albumin (HSA) antibodies and anti-rabbit IgG peroxidase conjugate, developed in goat, were purchased from Sigma, USA (reference A 0433 and A 9169). Polystyrene microtitration plates were from Nunc Immuno, Denmark, ^{45}Ca (specific activity, 37 mBeq/ml) from Amersham, England, CNBr-activated sepharose 4B and Sephadex G25 from Pharmacia, Sweden, and peroxidase, glutaraldehyde, 4-chloro-1-naphtol, O-phenylenediamine dihydrochloride (OPD) and all usual chemical products from Sigma.

Urine collections

First morning urine specimens were obtained from healthy men of the laboratory for α1m purification and for the control group (n = 18) of healthy subjects in the in vivo study. The subjects were all asymptomatic, with no history of renal stone disease and receiving no medications.

Hypercalciuric patients regularly followed in the Nephrology division at Necker Hospital, who had CaOx dihydrate crystals in their first morning urine constituted the stone former group (n = 31).

The age of control and lithiasis subjects ranged from 18 to 65 years (mean ± SEM = 39 ± 3.9 vs 47 ± 2.8 years respectively, P = NS).

Methods

Alpha-1m purification procedure

Two litres of pooled first morning urine obtained from five healthy subjects were collected, dialyzed 24 h at 4°C against distilled water in the presence of sodium azide (0.1% w/v) and phenylmethylsulfonyl fluoride (0.1 μM). Then urinary volume was concentrated approximately 10-fold across a dialysis membrane of 8000 Da cut-off, in the presence of polyethylene glycol 17 500 Da. Some urinary proteins, especially the THP protein, as estimated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) were partially precipitated by adding ammonium persulfate (17% saturation). Then 6 mg of the supernatant proteins, after dialysis, concentration and equilibration in a 10 mM phosphate saline buffer (PBS) pH 7.4, were applied on a CNBr-activated sepharose column coupled with rabbit anti-human α1m, according to the recommendations of the manufacturer. Seven ml of gel were coupled with 2 mg of antibodies and packed in a 10-ml column (1 cm × 10 cm). After washing the column with 10 volumes of PBS, α1m was eluted with 30 ml of glycine 0.1 M, HCl pH 2.5. The collected fractions were immediately neutralized with concentrated Tris. In one experiment, α1m was eluted from the column with 1.0 M NaCl.

After dialysis and concentration, the protein purity was verified by SDS PAGE, and visualized by silver staining or immunoblotting using various antibodies, especially anti-α1m, anti-ITI and anti-HSA.

Electrophoresis and Western-blotting

SDS PAGE was carried out according to Laemmli [25] with a concentrating gel of 4% acrylamide and a separating gel of 12% acrylamide on a mini protean II electrophoresis cell (BioRad, USA). Electrophoresis of urine proteins were carried out in native conditions as well as after denaturation and reduction using 1% SDS and 0.5% dithiothreitol.

The proteins were stained with a silver technique (kit from Biorad). Immunoblotting was carried out on 0.2 μm nitrocellulose membranes. The primary antibodies (anti-α1m, anti-ITI and anti-HSA) were diluted 200-fold, and the secondary antibodies coupled with peroxidase were diluted 12-000-fold. Immunoblot revelations were made with 4-chloro-1-naphtol (0.05% in PBS 50 mM, pH 7.4, added to 30 μl/100 ml of 30% hydrogen peroxide solution).

ELISA procedure for α1m quantification in urine

The polystyrene plates were coated overnight at 4°C with 100 μl anti-α1m antibodies, 300-fold diluted in PBS buffer pH 7.4. After washing with PBS-Tween (Tween 0.2%, v/v), the wells were saturated with PBS containing 2% BSA (bovine serum albumin, w/v). Then, after washing with PBS-Tween, 100 μl of urine samples diluted in PBS-BSA (with a protein concentration ranging from 20 to 1000 ng/100 μl) were added and incubated at room temperature for 2 h. After washing with PBS-Tween, 100 μl of peroxidase anti-α1m conjugate (labeled with peroxidase according to Avrameas and Terninck (8) with a two-step procedure) were added to the wells and the plates were held overnight at 4°C. After washing with PBS-Tween, 200 μl of OPD solution (Sigma fast OPD kit) were added. Then, the reaction was stopped after coloration by addition of 50 μl of 3 N HCl and optical density was read at 490 nm.

The calibration curve of ELISA performed with the α1m of 30 kDa purified by affinity was linear up to a concentration of approximately 100 μg/l (10 ng/well). The minimum measurable concentration of α1m was approximately 5 μg/l (0.5 ng/well). Each urine sample was assayed in octuplicate (four different dilutions in duplicate).

In vitro inhibition tests of CaOx crystallization

Model using ^{45}Ca as an indicator of remaining soluble Ca The proteins were assayed in the CaOx crystallization system developed by Atmani et al. (4). In a test tube, 0.5 ml of a 2 mM ammonium oxalate solution in 50 mM Tris HCl buffer, pH 7.4, were added to 0.3 ml of 0.15 M NaCl or 0.3 ml of 2.5–30 μg of proteins dissolved in 0.15 M NaCl. The assay was initiated by adding 0.5 ml of 2 mM $CaCl_2$ in 50 mM Tris HCl buffer, pH 7.4, containing ^{45}Ca as tracer. After 1 h of stirring at room temperature, the tubes were centrifuged at 10 000 g for 5 min and 0.5 ml of the supernatant were counted for ^{45}Ca radioactivity in a liquid scintillation counter. Each point of protein concentration was measured in duplicate.

Model based on a turbidimetric method The proteins were assayed in the CaOx crystallization system developed by Hennequin et al. (19). In each test cuvette, the CaOx precipitation kinetics were followed for 3–10 min by reading the turbidimetry at 620 nm. The presence of proteins (2.5–25 μg/ml) in a medium containing 4 mM $CaCl_2$ and 0.5 mM Na_2Ox (Tris HCl pH 6.8, NaCl 0.15 M) inhibited CaOx crystallization as estimated by slope decrease of the turbidimetric curve. The time of induction (ti) corresponds to the time between the addition of oxalate and the moment at which the crystal growth is experimentally measurable, i.e. 2.5% of

the maximal OD value [20]. Each point of protein concentration was measured in duplicate.

Urinary biochemical determinations

Urinary samples were analyzed for Ca (ortho-cresolphthalein method, Boehringer kit), and creatinine (Jaffe method, Boehringer kit) on a multiparametric analyser Hitachi 717 (Boehringer, Mannheim). Oxalate concentration was determined by ionic chromatography and protein concentration by a modified Bradford method (Biorad protein microassay). Urinary crystals were analyzed by polarizing microscopy according to the recommendations previously published by Bader et al. (9).

Expression of results and statistical analysis

The results of the in vivo study have been expressed as means ± SEM. Analysis of variance (ANOVA) was used to compare the mean results of stone formers and healthy subjects.

Results

Urinary proteins cross-reacting against rabbit anti-human α1m antibodies

After non-denaturing PAGE of proteins from native urine and transfer on nitrocellulose membrane, two immunoblots were detected after incubation with anti-α1m antibodies (Fig. 1, trace A). However, when the urine samples were dialyzed against distilled water and lyophilized before electrophoresis, only one protein species reacted with the antibodies (Fig. 1, trace B).

In the absence of lyophilization procedure before SDS PAGE, the visualization using anti-α1m antibodies indicated two spots of 30 and 60 kDa apparent molecular mass. After lyophilization of the urinary sample and SDS PAGE, only the protein of 30 kDa was observed on Western blotting against anti-α1m antibodies. An increase of 1–5% in SDS concentration in the solubilizing buffer did not modify the distribution of the blots visualized above (not shown).

Urinary α1m purification using immunoaffinity column

The protein eluted with 0.1 M glycine HCl, pH 2.5 from the anti-α1m-CNBr-sepharose column, was composed of only one protein species of 30 kDa as shown on SDS PAGE after silver staining (Fig. 2, trace A). After Western blotting, the anti-α1m antibodies cross-reacted with two immunoreactive bands of proteins of 30 and 60 kDa (Fig. 2, trace B). This purified protein did not cross-react with anti-ITI and anti-albumin antibodies (Fig. 2, traces C and D).

On the other hand, two proteins of 60 and 67 kDa were eluted by replacing the acid elution buffer of the affinity column by a 1 M NaCl solution (Fig. 2, trace E, silver staining). Two bands of 30 and 60 kDa were detected, after Western blotting and anti-α1m visualization, as previously described (Fig. 2, trace F). At the same time, the Western blot of the purified protein against anti-human albumin antibodies showed a spot at 67 kDa (Fig. 2, trace G).

Inhibitory effect of the purified α1m(s) on calcium oxalate crystallization in vitro

The protein eluted from the immunoaffinity column by acid pH buffer inhibited the calcium oxalate crystallization, in a dose-dependent manner, in the test of inhibition estimated by supernatant radioactivity, as shown in Fig. 3. In the inhibition test, using the kinetic turbidimetric model, this protein increased the induction time and furthermore, it decreased the turbidimetric slope, and consequently increased the percent of inhibition proportionally to the protein concentration, up to 24 μg/ml (Table 1).

The proteins eluted from the immunoaffinity column with 1 M NaCl also inhibited the calcium oxalate crystallization in the inhibition model based on ^{45}Ca radioactivity, similarly to the protein eluted with the acid pH buffer (Fig. 3). An inhibitory activity was also observed in the turbidimetric test (Table 1).

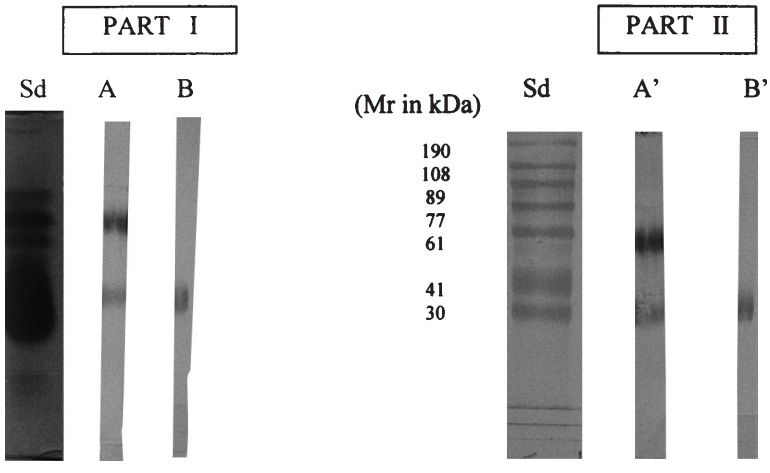

Fig. 1 Western blotting analysis of urinary proteins with anti-α1m antibodies. Part I: native conditions, polyacrylamide gel electrophoresis (PAGE) (before A and after B lyophilization of the samples). Part II: denaturing and reducing conditions, sodium dodecyl sulfate (SDS) PAGE (before A' and after B' lyophilization of the samples). *Sd* molecular weight of standard proteins

Human serum albumin, assayed in the test estimated by supernatant radioactivity, at similar concentrations up to 24 μg/ml, did not modify the crystallization process (not shown).

Human study: comparison between urinary excretion of α1m in healthy subjects and in renal stone formers. Relations with urinary oxalate and calcium excretion

A marked hypercalciuria was observed in the first morning urines of the CaOx stone formers selected, as compared with those of the healthy subjects: 6.20 ± 0.55 versus 2.90 ± 0.34 mMol/l respectively, $P = 0.0001$ (Table 2). The mean urinary oxalate concentration was not significantly different between the two groups, as was urinary pH (not shown). The CaOx product was significantly higher in urine of renal stone formers than in urine of healthy subjects ($P = 0.022$), as well as the calcium/oxalate ratio ($P = 0.017$), in agreement with the presence of CaOx dihydrate crystals in the first morning urine of stone former patients (Mean ± SEM = 12.43 ± 4.64 /mm^3).

The urinary α1m concentration was significantly lower in lithiasis subjects than in healthy subjects, whether it was expressed in concentration (2.95 ± 0.29 versus 5.34 ± 1.08 mg/l respectively, $P = 0.01$) or in grams per mole of creatinine (0.31 ± 0.04 versus 0.47 ± 0.1 respectively, $P = 0.047$) (Table 2). No difference between the two groups was observed in total protein concentration. On the other hand, it was possible to correlate positively urinary α1m concentration and total protein concentration ($r^2 = 0.24$, $P = 0.02$, not shown).

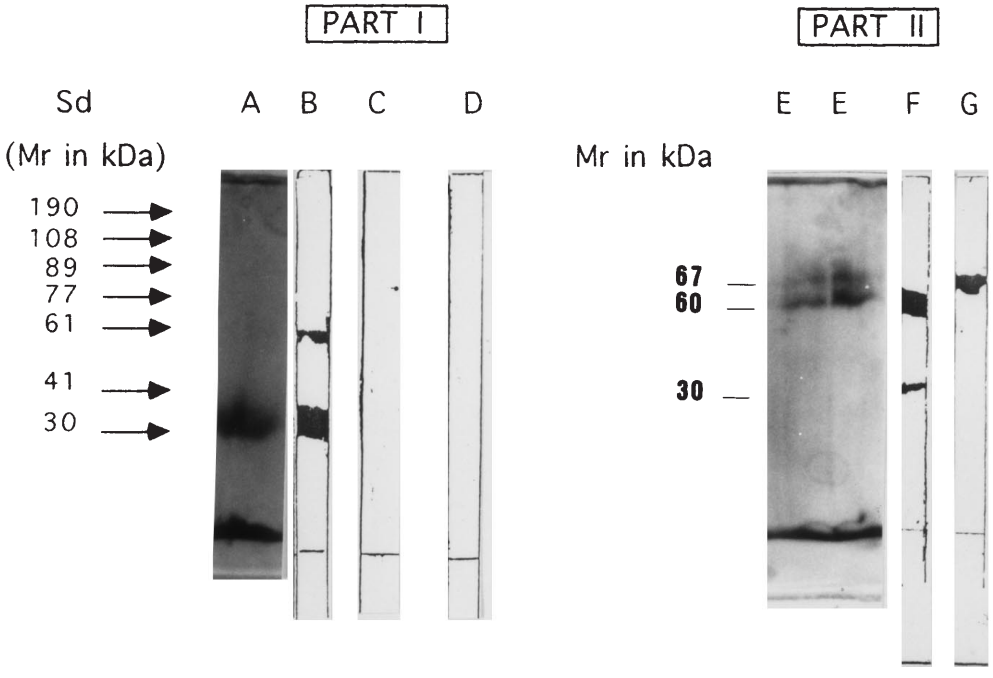

Fig. 2 SDS PAGE analysis of the purified proteins isolated by immunoaffinity from urine. Part I: elution with 0.1 M glycine, HCl pH 2.5. *Line A* protein staining, *lines B*, *C* and *D* Western blotting with anti-α1m, anti-ITI and anti-albumin antibodies, respectively. Part II: elution with 1 M NaCl. *Line E* protein staining, *lines F* and *G* Western blotting with anti-α1m and anti-albumin antibodies, respectively. *Sd* molecular weight of standard proteins

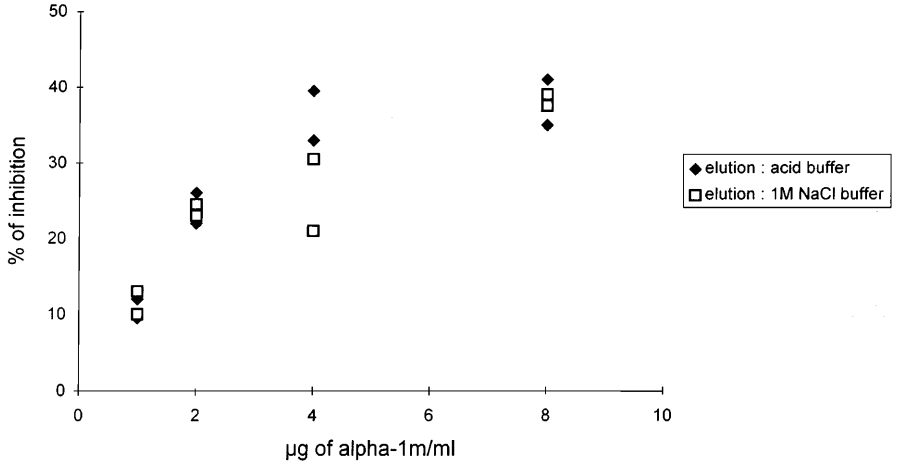

Fig. 3 Inhibitory activity of the purified proteins eluted from anti-α1m CNBr-sepharose column either with 0.1 M glycine, HCl pH 2.5 or with 1 M NaCl. The inhibitory activity was determined in presence or absence of the protein in a test tube containing 0.76 mM $CaCl_2$ (with ^{45}Ca) and 0.76 mM Ox $(NH_4)_2$. After 1 h of agitation, the radioactivity of the supernatant corresponding to the Ca remaining in solution was counted

Table 1 Inhibitory activity of the purified proteins eluted from anti-alpha-1-microglobulin (α1m) CNBr-sepharose column either with 0.1 M glycine, HCl pH 2.5 or 1 M NaCl. The inhibitory activity was determined in presence or absence of protein, by slope modification of turbidimetric curve read at 620 nm in a cuvette containing 4 mM $CaCl_2$ and 0.5 mM Na_2Ox

Elution buffer	Protein concentration (μg/ml)	Induction time (min)	Inhibition of CaOx crystallization (%)
0.1 M glycine buffer pH 2.5	0	0.8	0
	8	1.8	9.8
	16	1.9	34.1
	24	1.5	41.5
1 M NaCl	0	0.8	0
	4	1.0	15.5
	8	1.2	21.7

CaOx Calcium oxalate

Discussion

In this study, we have shown that in the urine of healthy subjects the anti-α1m antibodies revealed one or two molecular species (after SDS PAGE and Western blotting), according to the urine preparation procedure. On immunoaffinity column, a protein of 30 kDa was purified, which corresponds to α1m [15]. Another form of 60 kDa has been partially purified. These two proteins inhibited in vitro calcium oxalate crystallization in two different experimental models.

More than 20 years ago α1m (also called protein HC) was described and isolated from the urine of patients with chronic cadmium poisoning, associated with tubular proteinuria [15]. Alpha-1m is synthesized in liver cells as α1m-bikunin precursor protein and, after cleavage in Golgi apparatus, it is secreted in free form into the bloodstream [10]. In human serum, α1m is complexed with IgA [18] and in rat serum with the alpha 1 inhibitor 3 [16] and fibronectin [17]. In vitro, α1m has been described as possessing an immunosuppressive function, mitogenic and migration-interfering properties [26, 27, 3], and it has been suggested that some of its functions may be exerted via its carbohydrate moieties [1]. In contrast, to date nobody has attributed a calcium oxalate inhibitory activity to α1m.

To carry out this study, we have developed an original immunoaffinity technique. After electrophoresis in SDS PAGE and silver staining α1m, when purified on anti-α1m antibody column, was constituted of only one band of 30 kDa, as classically reported in the literature [2, 15]. However, on Western blots of this protein, anti-α1m antibodies detected two spots, localized at 30 and 60 kDa (Fig. 2). This discrepancy may be explained by the difference in the relative sensitivity of the two visualizing types. Furthermore, these two forms were also observed in native urine (Fig.1).

Alpha-1m forms spontaneously no covalently linked dimers that would be easily dissociable in reducing media [15, 36]. Ekström and Berggard [15] have shown that anti-α1m antibodies, produced in their laboratory, reacted in urine against further proteins, and may be polymers of α1m or complexes of α1m with other proteins. Imaï et al. [22] have shown that anti-α1m antibodies (obtained from Dako) revealed three proteins in urine and two molecular species of purified α1m. We have produced rabbit anti-human-α1m antibodies, using α1m purified by non-specific methods (no immunoaffinity, but gel filtration, ion exchange chromatography and specific electro-elution of the 30-kDa protein from electrophoresis gels). This antibody, as the commercial antibody used in this work, also detected the same bands of 30 and 60 kDa in native urine. Considering the molecular mass of the two immunoreactive bands in urine and their transformation into a single band after lyophilization, it is probable that the band revealed at 60 kDa is a dimer of α1m, which is difficult to dissociate, rather than a heteroprotein having a common epitope with α1m. This small fraction may not have been detectable by silver staining but was identified by Western blotting because of the greater sensitivity of the immunological technique.

In the literature, urinary α1m concentrations were relatively low in humans (3–10 mg/l). For this reason, and especially in the first steps of purification procedures, it is often useful to lyophilize the samples, a procedure which may alter some proteic structures, as well as lyophilization used as final means of preservation, which may lead to modifications of the purified proteins.

Alpha-1m eluted from the affinity column with acid pH, which was predominantly constituted of the 30 kDa

Table 2 Comparison of calciuria, oxaluria, urinary proteins and α1m concentrations in healthy subjects and in CaOx stone formers

	Healthy (n = 18)	CaOx stone formers (n = 31)	Significance
Ca (mmol/l)	2.90 ± 0.34	6.20 ± 0.55	P = 0.0001
Oxalate (mmol/l)	0.46 ± 0.07	0.40 ± 0.04	NS
CaOx (mmol/l)^2	1.45 ± 0.33	2.62 ± 0.32	P = 0.022
Ca/Ox	10.94 ± 2.89	20.09 ± 2.27	P = 0.017
Total proteins (mg/l)	39.22 ± 3.97	47.03 ± 2.81	NS
Creatinine (mmol/l)	13.74 ± 1.65	11.93 ± 1.11	NS
α1m (mg/l)	5.34 ± 1.08	2.95 ± 0.29	P = 0.01
α1m g/mol of creatinine	0.47 ± 0.10	0.31 ± 0.04	P = 0.047

Mean ± SEM, n number of subjects

molecular species, inhibited calcium oxalate crystallization in vitro. An inhibition of about 30% was observed with 5 mg/l of α1m and reached 40% with 8 mg/l in the test estimating non-precipitated calcium as residual radioactivity which takes into account the different steps of crystallization, i.e. nucleation phase, crystal growth and aggregation phases. In the turbidimetric test we have also observed an inhibition of approximately 40% with 24 μg/ml of α1m. This test allows us to distinguish between the nucleation step (with the measure of the induction time) and the crystal growth step, even though the two steps may not be strictly individualized [20]. By comparison, incompletely purified UAP and pure bikunin had an inhibitory activity of about 60–70% and of 82%, respectively, at the same concentration of 8 mg/l [5, 28]. These results show firstly a lower specific inhibitory activity of α1m compared with bikunin, and secondly that urinary inhibitory activities are not additive.

On the other hand, we have shown that the protein eluted with 1 M NaCl solution, mainly constituted by the 60-kDa form of α1m, had an inhibitory activity as well, although it was contaminated by albumin. Human albumin had no inhibitory effect itself on CaOx crystallization. Thus the specific inhibition resulting from the 60-kDa form alone may be superior to the one obtained with the 30-kDa form.

Urinary α1m excretion increases together with the proteinuria observed in renal tubular dysfunction. Its normal urinary concentration in healthy humans ranged from 0.05 to 14 mg/l, with average values of 2.93 ± 0.77 mg/l, 3.53 ± 0.77 mg/l, or 0.62 ± 0.4 g/mol of creatinine, according to the type of assay, including turbidimetry, automated latex immunoassay and radial immunodiffusion, as well as the standard protein used as reference (23, 35). These concentrations are comparable to those measured in the present work (5.34 ± 1.08 mg/l in the urine of healthy subjects) and for which we have observed for the first time an inhibitory activity on CaOx crystallization in vitro, using two different experimental models.

The present results are in keeping with a recent report, demonstrating a selective incorporation of α1m, among other proteins, into the protein matrix of kidney stones, even though all urinary proteins and particularly the most abundant one, i.e. THP, were not detected (12, 14). Furthermore, the selective incorporation into the matrix probably occurred by binding to albumin rather than by direct binding to stone crystals.

The results of our in vivo study showed that the urinary α1m concentrations were significantly decreased in CaOx stone formers suggesting that this protein could influence the risk of crystallization in vivo. However, α1m cannot be considered as a specific marker for hypercalciuria in stone patients with calcium oxalate dihydrate (COD) crystals since no correlation was found between either the number or the size of COD crystals and urinary α1m concentration or between hypercalciuria and urinary α1m concentration. The concentration of bikunin has been determined elsewhere [28] in urine from the same cohort of healthy subjects and of CaOx stone formers studied in the present work. It was also significantly decreased in CaOx stone formers and bikunin was demonstrated to be a potent inhibitor of CaOx crystallization in vitro. A positive correlation between α1m and bikunin concentration has been found in normal urine but not in that of stone patients [28]. As to the fate of these two proteins in the organism, some steps are common to both proteins, such as cosynthesis in the liver and similar degradation and utilization by renal tubule cells. It is possible that in stone formers these steps are disturbed, leading to a concomitant decrease in urinary excretion. On the other hand, the lack of correlation between α1m and bikunin excretion in stone formers may result from metabolic steps where the two proteins have distinct pathways, such as their plasma binding to different proteins leading to a different proportion of ultrafilterable free form of proteins in stone formers. Furthermore, a difference in the renal handling of these two proteins in stone formers in comparison with normal subjects cannot be excluded.

Among the glycoproteins inhibiting calcium oxalate crystallization, THP probably plays an important part not only in vitro, but also in vivo since it has been shown recently that its excretion is decreased in some lithiasis subjects [32]. On the other hand, glycoproteins such as UAP, nephrocalcin and THP may have molecular structures which differ between nephrolithiasis patients and healthy subjects [6, 24, 30]. Further studies using a molecular approach in the investigation of α1m structure deserve to be developed.

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