Urol Res (1991) 19:375–380

Urological Research

Glycosaminoglycans, uric acid and calcium oxalate urolithiasis

F. Grases[1], A. Costa-Bauzá[1], J. G. March[1], and L. Masárová[2]

[1] Department of Chemistry, Faculty of Sciences, University of the Balearic Islands, Palma de Mallorca, Spain
[2] Department of Inorganic and Organic Chemistry, Faculty of Pharmacy, Comenius University, Bratislava, Czechoslovakia

Accepted: April 1, 1991

Summary. The interaction between calcium and glycosaminoglycans (GAGs) was studied using a calcium ion-selective electrode. The Ca-binding capacity of GAGs involved 16% of total calcium in the presence of chondroitin sulphate and 28% in the presence of pentosan polysulphate. The action of GAGs on the nucleation of uric acid and sodium urate was examined and inhibitory effects were observed. The action of uric acid as a heterogeneous nucleant of calcium oxalate was studied, and considerable promotion of the heterogeneous nucleation of calcium oxalate by uric acid was found, which could be inhibited by the action of GAGs. From these summarised in vitro results, we conclude that uric can constitute an important risk factor for calcium oxalate urolithiasis through heterogeneous nucleation and the GAGs can play an important role as preventive agents.

Key words: Calcium oxalate – Glycosaminoglycans – Uric acid

During the last decade, the role of urinary glycosaminoglycans (GAGs) in calcium oxalate urolithogenesis has been widely studied and discussed [16]. Thus, GAGs have been proposed as being inhibitors of calcium oxalate nucleation [6, 14, 17] and/or of calcium oxalate crystal growth [4, 8, 12, 13, 20]. On the other hand, the interaction between uric acid and GAGs has also been broadly discussed. Several authors have suggested that the binding of GAGs to uric acid or sodium urate crystals enables those substances to act as anti-inhibitors by reducing the free urinary GAGs and thereby blocking their inhibitory activity on calcium oxalate crystallization [1, 15, 18, 21]. Some investigators have postulated that the binding of GAGs to uric acid or sodium urate crystals is favoured in the presence of divalent cations such as calcium or magnesium [5, 10].

Nevertheless, the role of uric acid in calcium oxalate stone formation remains a matter of controversy. Apart from the anti-inhibitor action of uric acid that arises through binding of GAGs, several authors have proposed the direct induction of calcium oxalate precipitation by uric acid (either in crystalline or colloidal form). Crystallographic considerations have suggested that uric acid might trigger off calcium oxalate crystallisation by epitaxy [11], but in vitro experiments have shown that calcium oxalate growth can more favourably occur on sodium urate crystals [3], although the latter have not been detected in fresh urine and a rarely found in stones. For this reason, the promoting action of uric acid on calcium oxalate crystal formation seems more feasible. In the present study, we investigated the Ca-GAG interaction, the influence of uric acid on the heterogeneous nucleation of calcium oxalate, and the effects of GAGs on such heterogeneous nucleation.

Materials and methods

Reagents and apparatus

Solutions of calcium chloride (Panreac), sodium oxalate (Panreac), ammonium chloride (Panreac), ammonium acetate (Panreac), acetic acid (Panreac), sodium chloride (Probus), sodium citrate (Probus), tetrasodium pyrophosphate (Merck), D-glucurone-3,6-lactone (Aldrich), chondroitin sulphate B disodium salt (Serva) and pentosan polysulphate sodium salt (Sigma) were prepared in water. A solution of urate (0.012 M) was prepared from uric acid (Aldrich) by adding drops of 0.1 M sodium hydroxide (Probus) until total dissolution was achieves (final pH = 10).

Potentiometric measurements were performed by means of a Crison 2002 micro-potentiometer using a calcium ion-selective electrode (Ingold) coupled with a silver/silver chloride electrode, which was separated from the cell solution by an intermediate junction containing potassium nitrate. All experiments were repeated at least three times to ensure their reproducibility. Turbidimetric measurements were taken using a single-beam spectrophotometer (Bausch and Lomb, Spectronic-21). The usual cell compartment was substituted by a spiral copper tube that surrounded a cylindrical glass vial measuring 2.3 cm in diameter and 22 ml in volume. The vial was stirred magnetically, and the absorbance was measured at 500 nm.

The crystals were observed with a Kyowa Microlux-11 optical microscope or with a Hitachi S-530 scanning electron microscope. All observations were repeated ten times to ensure the statistical significance of the results.

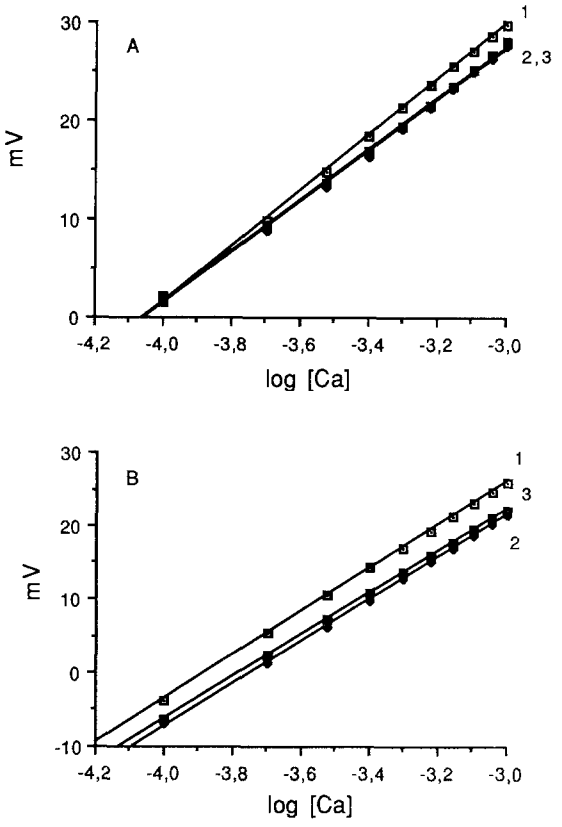

Fig. 1 A, B. Electrical signal (mV) versus log [Ca] in the presence or absence of glycosaminoglycans and uric acid (pH = 5.26, T = 25°C, $[NH_4Cl] = 0.1$ M). **A** *1*, Without chondroitin sulphate and uric acid; *2*, with 2.5 g/l chondroitin sulphate; *3*, with 2.5 g/l chondroitin sulphate and 9.5×10^{-3} M uric acid. **B** *1*, Without pentosan polysulphate or uric acid; *2*, with 2.5 g/l pentosan polysulphate; *3*, with 2.5 g/l pentosan polysulphate and 9.5×10^{-3} M uric acid

Calcium-binding capacity of glycosaminoglycans

Experiments were carried out by adding ten 0.1 ml aliquots of calcium chloride stock solution (0.2 M) to a thermostatted (25° ± 0.2°C) solution of ammonium chloride (0.1 M) in either the presence or the absence of GAGs (chondroitin sulphate or pentosan polysulphate) and uric acid. The analysis of free calcium was carried out by potentiometric measurements followed by interpolation on a calibration graph. The final volume of the working solution was 200 ml. When neccesary, the pH value was previously adjusted by the addition of drops of ammonium hydroxide or hydrochloric acid.

Precipitation of uric acid in the presence of GAGs

Turbidimetric measurements were carried out in a glass vial placed in the photometer (see Reagents and apparatus) that contained 1.2×10^{-3} M urate solution (final volume, 20 ml), to which 0,5 ml acetic acid-ammonium acetate buffer solution was added (total concentration 5 M). The systems were thermostatted by the circulation of water at a constant temperature of 25° ± 0.2°C. The final pH in all instances was 5. The turbidity of the solution was measured in the presence or absence of chondroitin sulphate, pentosan polysulphate, D-glucurone-3,6-lactone, sodium citrate and tetrasodium pyrophosphate.

Adsorption of GAGs on uric acid

A selected amount of GAGs were dissolved in 87.7 ml sodium urate solution (1.2×10^{-2} M, pH = 10). When the experiment was performed in the presence of calcium, 1 ml 0.5 M calcium chloride was added. Uric acid was precipitated by adding 5 ml acetic acid-ammonium acetate buffer solution (total concentration, 5 M; pH = 5). Sodium urate was precipitated following adjustment of the pH to 7 by adding drops of hydrochloric acid and 11.3 ml 4 M sodium chloride. In all instances, the final volume was 100 ml. The systems were thermostated at 25° ± 0.2°C. The suspension was stirred for 1 h and then filtered through a 0.45 µm Millipore filter. The analysis of GAGs in solution was carried out using the photometric method proposed by Blumenkrantz and Asboe-Hansen [2]. Uric acid or sodium urate crystals were characterised by optical and scanning electron microscopy.

Heterogeneous nucleation measurements

Nucleation experiments were performed in a Pyrex glass vessel with a capacity of approximately 250 ml and were followed potentiometrically. The solutions were maintained at 28° ± 0.1°C by the circultion of thermostatted water; working solutions were stirred magnetically. Uric acid crystals used as a heterogeneous nucleant were formed just before their use by the addition of 2.5 ml acetic acid-ammonium acetate buffer solution (total concentration 5 M; pH = 5.10) to 50 ml 1.8×10^{-2} M sodium urate followed by 1 min stirring. Heterogeneous nucleation processes on uric acid were initiated by th addition of 0.2 M sodium oxalate to a 200-ml final volume containing calcium chloride (different concentrations), 0.1 M ammonium chloride and 50 ml of the uric acid suspension prepared as described above.

The electrodes (except on the membrane) were covered by a plastic film, which was renewed after each experiment to prevent calcium oxalate deposits that might induce crystalline growth. In general, maximal care was taken to avoid calcium oxalate deposits in all material. Induction periods were calculated from the experimental electric potential-time curves (Fig. 5). All experiments were repeated at least three times to ensure their reproducibility. Crystals formed by heterogeneous nucleation were characterised by means of scanning elelctron microscopy.

Results

The interaction between Ca^{2+} ions and GAGs (chondroitin sulphate and pentosan polysulphate) was studied using a calcium ion-selective electrode. The results are shown in Fig. 1, in which the electric signals (mV) corresponding to several total calcium concentrations, total calcium concentration in the presence of the GAGs tested and total calcium concentration in the presence of GAGs and uric acid crystals are plotted. Calcium concentration values were represented as log [Ca] so as to obtain straight lines. The diminution induced by the presence of GAGs in the electric signal reflecting the total calcium concentration corresponds to complexation of the polymer and calcium. From these results, the percentage of bound calcium was calculated and expressed as:

$$\frac{\text{Total calcium} - \text{free calcium in the presence of polymer}}{\text{Total calcium}} \times 100.$$

In Fig. 2, the percentage of calcium binding versus total calcium for several pH values and GAG concentrations

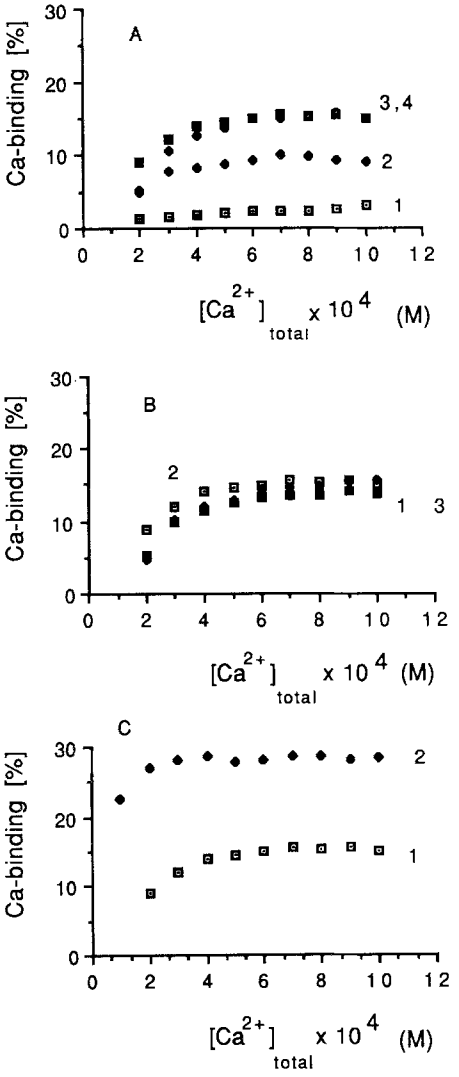

Fig. 2A–C. The percentage of Ca-binding capacity of glycosaminoglycans versus total calcium concentration (T = 25°C, $[NH_4Cl]$ = 0.1 M). **A** Different chondroitin sulphate concentrations and pH = 5.26: *1*, 1 g/l; *2*, 2 g/l; *3*, 2.5 g/l; *4*, 3.5 g/l. **B** Different pH values and 2.5 g/l chondroitin sulphate: *1*, pH = 5.26; *2* pH = 6.09; *3* pH = 6.59. **C** pH = 5.26 and 2.5 g/l glycosaminoglycans: *1*, chondroitin sulphate; *2*, pentosan polysulphate

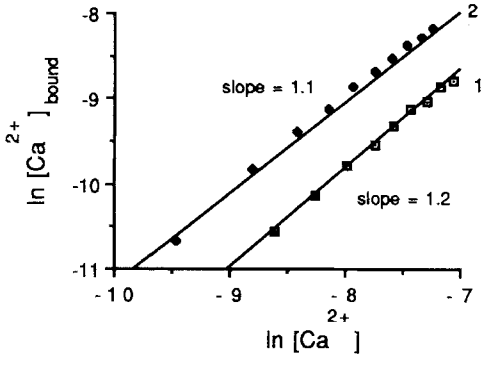

Fig. 3. In $[Ca^{2+}]_{bound}$ ($[Ca^{2+}]_{total} - [Ca^{2+}]$) versus in $[Ca^{2+}]$ in the presence of glycosaminoglycans (pH = 5.26, T = 25°C, $[NH_4Cl]$ = 0.1 M) *1*, in the presence of chondroitin sulphate (2.5 g/l); *2*, in the presence of pentosan polysulphate (2.5 g/l)

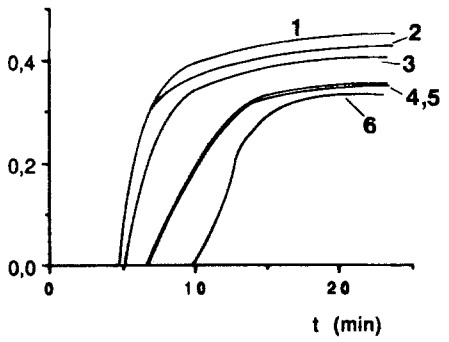

Fig. 4. Kinetics of uric acid precipitation: in the absence (*1*) and presence of D-glucurone-3,6-lactone (0.5 g/l, *2*), sodium citrate (0.5 g/l, *3*), tetrasodium pyrophosphate (0.5 g/l, *4*), chondroitin sulphate (0.5 g/l, *5*), and pentosan polysulphate (0.5 g/l, *6*). (pH = 5, T = 25°C, $[CH_3COO^- + CH_3COOH] = 1.3 \times 10^{-1}$ M, [uric acid] $= 1.2 \times 10^{-3}$ M

are presented. The maximal percentages obtained were 16% of total calcium in the presence of chondroitin sulphate and 28% in the presence of pentosan polysulphate. Under all conditions studied, a typical saturation curve corresponding to a stoichiometric complexation reaction was obtained. These results enable us to assume that each polymer (P) has a defined number of active bindings points (n); consequently, the reaction can be expressed as follows:

$$n\,Ca^{2+} + P \rightleftharpoons Ca_nP. \tag{1}$$

The apparent dissociation constant yields

$$K_D = \frac{[Ca^{2+}]^n \times [P]}{[Ca_nP]}. \tag{2}$$

A calcium mass balance indicates

$$[Ca_nP] = \frac{[Ca^{2+}]_{total} - [Ca^{2+}]}{n}. \tag{3}$$

Combining Eqs. 2 and 3, we obtain

$$\ln([Ca^{2+}]_{total} - [Ca^{2+}]) = \frac{\ln[P] \times n}{K_D} + n \times \ln[Ca^{2+}], \tag{4}$$

where $[Ca^{2+}]$ is deduced from the potential measure.

In Fig. 3, $\ln([Ca^{2+}]_{total} - [Ca^{2+}])$ is plotted as a function of $\ln[Ca^{2+}]$. For each polymer studied, straight lines exhibiting a slope of close to 1 were obtained, suggesting that under the present experimental conditions, [P] can be considered to be constant and the presence of one (n = 1) binding point can be assumed. If numerous binding points were involved, the curve in Fig. 3 could not be a straight line. On the other hand, the presence of uric acid or ammonium urate (depending on the pH) either in solution or in colloidal form did not affect the Ca-GAGs binding capacity (Fig. 1).

The action of GAGs on the nucleation of uric acid and sodium urate was turbidimetrically studied. Inhibitory effects on such nucleation were observed (Fig. 4). The uric

Table 1. Precipitation of uric acid or sodium urate in the presence of GAGs at 25°C and a uric acid concentration of 1×10^{-2} M

GAG	Added (g/l)	Found[a] (g/l)	Other experimental conditions
Chondroitin sulphate	0.50	0.50	pH = 5[b], $[CH_3COO^- + CH_3COOH] = 0.25$ M
	0.50	0.47	pH = 7[c], [NaCl] = 0.45 M
	0.50	0.49	pH = 5[b], $[CH_3COO^- + CH_3COOH] = 0.25$ M, $[CaCl_2] = 5 \times 10^{-3}$ M
	0.50	0.50	pH = 7[c], [NaCl] = 0.45 M, $[CaCl_2] = 5 \times 10^{-3}$ M
Pentosan polysulphate	4.00	3.70	pH = 5[b], $[CH_3COO^- + CH_3COOH] = 0.25$ M

[a] Average of 4 determinations
[b] Uric acid precipitate
[c] Sodium urate precipitate

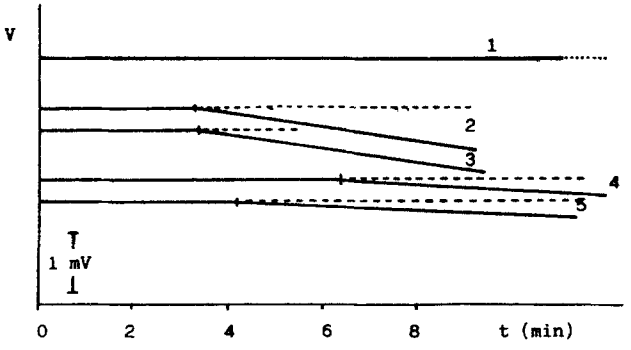

Fig. 5. Nucleation runs used to evaluate the induction period obtained potentiometrically under several conditions T = 28°C, pH = 5.10, 0.1 M ionic strength, $[Ca^{2+}]_{total} = 6 \times 10^{-4}$ M, $[C_2O_4^{2-}]_{total} = 6 \times 10^{-4}$ M). *1*, Reference without uric acid and without additives; *2*, [uric acid] = 4.8×10^{-3} M; *3*, [uric acid] = 4.8×10^{-3} M; [Chondroitin sulphate] = 0.2 g/l; *4*, [uric acid] = 4.8×10^{-3} M; [Chondroitin sulphate] = 2 g/l; *5*, [uric acid] = 4.8×10^{-3} M; [Pentosan polysulphate] = 0.15 g/l

Table 2. Kinetic data for calcium oxalate nucleation in the absence and in the presence of uric acid seed crystals and GAGs

Uric acid ($M \times 10^3$)	GAG (g/l)	t_i[a] (min)
–	–	> 20
4.8	–	3.35
4.8	0.02 (CS)	3.35
4.8	0.2 (CS)	3.40
4.8	2.0 (CS)	6.28
4.8	0.15 (PP)	4.30

CS, Chondroitin sulphate; PP, pentosan polysulphate
[a] Average of 5 determinations
Experiments were carried out under the following conditions: $T = 28$°C; pH = 5.1; ionic strength, 0.1 M; $[CA^{2+}]_{total} = [C_2O_4^{2-}]_{total} = 6 \times 10^{-4}$ M

acid-GAG binding capacity was also assayed by the precipitation of uric acid or sodium urate in the presence of GAGs. The results (summarised in Table 1) show that no important binding occurred.

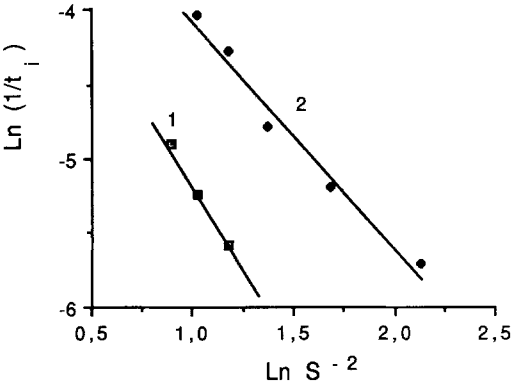

Fig. 6. $\ln(t^{-1})$ vs $[\ln(S)]^{-2}$ curves (T = 28°C, pH = 5.10, 0.1 M ionic strength). *1*, Absence of uric acid; *2*, [uric acid] = 5.9×10^{-3} M

The effects of uric acid as a heterogeneous nucleant of calcium oxalate trihydrate (COT) were studied in depth using potentiometry. Considerable promotion of the heterogeneous nucleation of calcium oxalate by colloidal uric acid was evident (Fig. 5, Table 2).

The interfacial energy values for the formed nuclei were calculated according to the nucleation theory [19]:

$$J = A \exp\{-B/[\ln(s)]^2\}, \quad (5)$$

being

$$B = \beta \times V_m^2 \times \sigma^3 \times N_A/(v^2 \times R^3 \times T^3), \quad (6)$$

where J represents the nucleation rate; A indicates the preexponential factor; S represents supersaturation, defined as the ratio of the ionic activity product of the precipitating phase (in this case, COT) to the thermodynamic solubility product $[a_{Ca} \times a_{Ox}/P_{s(COT)}]^{1/2}$; β is a dimensionless geometric factor equal to 32; V_m is the molar volume (M/d) equal to 9.82×10^{-5} m^3/mol; M_{COT} is 182.14 g/mol; d_{COT} is 1.855×10^6 g/m^3; σ represents the interfacial energy of the nucleus (J/m^2); N_A represents the Avogadro number; v is the number of ions in a molecular unit (= 2); $R = 8.314$ J/mol × K; and T represents the absolute temperature (K).

It is conventional to let $J = K \times t^{-1}$, where t as the time required for detect precipitation. A and B are evaluated by measuring $\ln(t^{-1})$ as a function of $[\ln(S)]^{-2}$. The interfacial energy value was calculated from the slope of $\ln(t^{-1}$ vs $[\ln(S)]^{-2}$ (Fig. 6).

The inhibitory action of GAGs on the heterogeneous nucleation of calcium oxalate by uric acid was also assayed. As can be seen in Fig. 5 noticeable inhibitory effects of GAGs on such heterogeneous nucleation were detected.

Discussion

The present study on the interaction between Ca and GAGs demonstrated that some, albeit rather slight, binding occurs. Such linkage seems to be a function of the number of sulphate groups in the GAG moleculae; thus, whereas chondroitin sulphate exhibited 16% binding under the conditions tested pentosan polysulphate (a semi-synthetic GAG), which displays a higher content of sulphate groups per dimer, showed 28% bindings. These results were in good agreement with those obtained through a very different methodology by Hesse et al. [10]

We felt that it would be interesting to compare these findings with results of previous investigations in which the inhibitory action of GAGs on the crystalline growth of calcium oxalate was studied [8]. We found that GAGs caused slight inhibition of calcium oxalate crystal growth, the extent of which was much lower than that produced by other substances that inhibit calcium oxalate crystal growth, such as citrate [7]. Pentosan polysulphate exhibited an inhibitory effect on crystal growth that was greater than that slown by chondroitin sulphate. These results seems to indicate that GAGs do not play a decisive role in preventing calcium oxalate crystalline growth in urolithiasis.

On the other hand, as can be deduced from Fig. 1, under the experimental conditions tested, the presence of colloidal uric acid hardly altered the GAG-Ca binding capacity. The uric acid-GAG binding capacity was also studied by the precipitation of colloidal uric acid or sodium urate in the presence of a known amount of GAG. As shown in Table 1, after the separation of uric acid or sodium urate by filtration, the GAGs remaining in solution were on the same order as the initial concentration, suggesting that no important binding between GAGs and uric acid or urate was established. Nevertheless, GAGs exhibited a considerable inhibitory effect on the nucleation of uric acid and urate, which can be explained in terms of an important dynamic interaction between uric acid and GAG. Besides, uric acid caused a noticable promotion of calcium oxalate crystallisation through its heterogeneous nucleation on the uric acid surface (Fig. 5). The surface energy values, σ, corresponding to the nucleation experiments carried out in the presence and absence of uric acid were 7.99 and 9.4 mJ/m^2 respectively (Fig. 6). In the presence of uric acid, a clear decrease in this energy occurs, demonstrating that heterogeneous nucleation is favoured by uric acid.

The heterogeneous nucleant capacity of uric acid and urate depends considerably on the size and morphology of the colloid formed [9]; thus, if GAGs are thought to affect uric acid or urate nucleation, they could therby modify the morphology of the colloid obtained as well as affecting its heterogeneous nucleant capacity. From these results it can be concluded that at urinary pH values, uric acid can constitute an important risk factor for calcium oxalate urolithiasis through heterogeneous nucleation processes. On the other hand, it seems clear that neither colloidal uric acid nor urate in urine acts as an anti-inhibitor by binding urinary GAGs and thereby blocking their inhibitory activity on calcium oxalate crystallisation, as has previously been suggested by some authors [1, 15, 18, 21].

The effects of GAGs on the heterogeneous nucleation of calcium oxalate by uric acid were also examined. As can be deduced from Fig. 5 and Table 2, the presence of GAGs caused considerable inhibition of the heterogeneous nucleation of calcium oxalate by uric acid. From these in vitro results, an important role for GAGs as an inhibitor of heterogeneous nucleation in the prevention of calcium oxalate urolithiasis at low urinary pH values can be assumed. Finally, it must be borne in mind that the action of GAGs on the heterogeneous nucleation of calcium oxalate could result in a change in the crystalline morphology of the calcium oxalate crystals formed; as a consequence, a number of crystalline characteristics such as aggregation capacity could also be modified. This explain the observations recorded in previous experiments in which agglomeration of calcium oxalate crystals was clearly favoured by the presence of large quantities of GAGs [8].

Acknowledgements. This work was supported by the Direccion General de Investigacion Cientifica Tecnica (grant PB 89-0423). One of the authors (L. M.) whishes to thank the University of the Balearic Islands for a fellowship awarded during the course of this work and would like to express the gratitude to Prof. F. Devinsky for his help and for the use of his facilities.

References

1. Baggio B, Gambaro G, Cicerello E, Marchini F, Borsatti A (1985) Further studies on the possible lithogenetic role of uric acid in calcium oxalate stone disease. In: Schwille PO, Smith LH, Robertson WG, Vahlensieck W (eds) Urolithiasis and related clinical research. Plenum Press, New York, p 785
2. Blumenkrantz N, Asboe-Hansen G (1973) New method for quantitative determination of uronic acids. Anal Biochem 54:484
3. Coe FL, Lawton RL, Golstein RB, Tembe V (1975) Sodium urate accelerates precipitation of calcium oxalate in vitro. Proc Soc Exp Biol Med 149:926
4. Fellström B, Danielson BG, Ljunghall S, Wikström B (1985) The inhibition of calcium oxalate crystal growth by chondroitin sulphates, heparin, pentosan polysulphate and tammhorsfall glycoprotein. In: Schwille PO, Smith LH, Robertson WG, Vahlensieck W (eds) Urolithiasis and related clinical research. Plenum Press, New York, p 887
5. Fellström B, Lindsjö M, Danielson BG, Ljunghall S, Wikström B (1986) Binding of glycosaminoglycans to sodium urate and uric acid crystals. Clin Sci 71:61

6. Grasses F, Costa-Bauzá A (1990) Potentiometric study of the nucleation of calcium oxalate in presence of several additives. Clin Chem Enzym Commun 3:319
7. Grases F, Millan A, Garcia-Raso A (1988) Polyhydroxycarboxylic acids as inhibitors of calcium oxalate crystal growth: relation between inhibitory capacity and chemical structure. J Crystal Growth 89:496
8. Grases F, Gil JJ, Conte A (1989) Glycosaminoglycans: inhibition of calcium oxalate crystalline growth and promotion of crystal aggregation. Colloids Surfaces 36:29
9. Grases F, Gil JJ, Conte A (1989) Urolithiasis inhibitors and calculus nucleation. Urol Res 17:163
10. Hesse A, Wurzel H, Krampitz G, Vahlensieck W (1987) Experimental determination of the kinetics of calcium-binding with chondroitin sulphate and the effects of uric acid on this process. Urol Res 15:93
11. Mandel NS, Mandel GS (1981) Epitaxis between stone-forming crystals at the atomic level. In: Smith LH, Robertson WG, Finlayson B (eds) Urolithiasis: clinical and basic research. Plenum Press, New York, p 469
12. Martin X, Werness PG, Bergert JH, Smith LH (1984) Pentosan polysulphate as an inhibitor of calcium oxalate crystal growth. J Urol 132:786
13. Norman RW, Scurr DS, Robertson WG, Peacock M (1985) Inhibition of calcium oxalate crystallization by pentosan polysulphate in control subjects and stone formers. Br J Urol 56:594
14. Osswald H, Weinheimer G, Schütt ID, Ernst W (1988) Effective prevention of calcium oxalate crystal formation in vitro and in vivo by pentosan polysulphate. Urol Res 16:230
15. Pak CYC, Holt K, Zerwekh JE (1979) Attenuation by monosodium urate of the inhibitory effects of glycosaminoglycans on calcium oxalate nucleation. Invest Urol 17:138
16. Robertson WG, Peacock M (1985) Pathogenesis of urolithiasis. In: Schneider HJ (ed) Urolithiasis: etiology diagnosis. Springer, New York Berlin Heidelberg p 185
17. Robertson WG, Scurr DS (1986) Modifiers of calcium oxalate crystallization found in urine: I. Studies with a continuous crystallizer using an artificial urine. J Urol 135:1322
18. Ryall RL, Hibberd CM, Marshall VR (1986) The effect of crystalline monosodium urate on the crystallisation of calcium oxalate in whole human urine. Urol Res 14:63
19. Söhnel O, Mullin JW (1988) Interpretation of crystallization induction periods. J Colloid Interface Sci 123:43
20. Tiselius HG (1985) The effect of sodium sulphopenstosan on the crystallization of calcium oxalate. In: Schwille PO, Smith LH, Robertson WG, Vahlensieck W (eds) Urolithiasis and related research. Plenum Press, New York, p 895
21. Zerwekh JE, Holt K, Pak CYC (1983) Natural urinary macromolecular inhibitors: Attenuation of inhibitory activity by urate salts. Kidney Int 23:838

Prof. Dr. F. Grases, PhD
Departamento de Quimica
Facultad de Ciencias
Universidad de las Islas Baleares
E-07071 Palma de Mallorca
Spain