Urol Res (1982) 10:185–189

Urological Research

May Enzyme Activity in Urine Play a Role in Kidney Stone Formation?

R. Azoury[1], N. Garti[1], S. Perlberg[2], and S. Sarig[3]

[1] Casali Institute of Applied Chemistry
[2] Department of Urology, Hadassah Hospital, The Hebrew University of Jerusalem, Jerusalem, Israel
[3] Casali Institute, Hebrew University, Jerusalem, Israel

Accepted: April 16, 1982

Summary. It has been found that calcium oxalate stone formers have low UGOT and UGPT activity compared to healthy individuals. The urine of 23 stone formers and 19 controls has been tested for combined UGOT and UGPT activity. The effect of L-aspartic acid, alanine and L-glutamic acid on calcium oxalate precipitation has been tested. Only L-glutamic acid exerted a significant retardation effect at physiological concentrations. As GPT and GOT convert alanine and aspartic acid respectively into glutamic acid, a possible mechanism of retardation of kidney stone formation involving enzyme steps via glutamic acid creation in situ is suggested.

Key words: Calcium oxalate, GOT, GPT, L-glutamic acid, Enzyme, Kidney stone.

Introduction

Studies on calcium oxalate stone formation have shown quite clearly that it is a multifactorial disorder. Among the factors found to be of importance are age, sex, occupation, dietary and fluid intakes, geographical location and climate [1, 2]. In addition, certain metabolic disorders predispose to calcium stone formation. Some workers believe that a genetic factor may be involved [3]. However, stone formation seems to be of limited occurrence. There is evidence [4] that urine from healthy persons retards calcium oxalate crystal formation, whereas urine from stone formers has much less inhibitory power [5].

Natural inhibitors in urine have been extensively studied [6, 7]. It is known that magnesium, citrate, pyrosphosphate and other small ions [5, 7] are effective inhibitors in vitro [8], but they are able to affect calcium oxalate crystallisation only at concentrations which are much higher than those encountered in natural urine. Marked effects of substances such as nucleosides and amino acids, particularly alanine and glutamic acid, have also been observed in vitro test systems. McGeown's [9] experiments indicated that glutamic acid significantly reduced the incidence of calculi in rats supplied with this acid in their food. No difference was found in the pH of the urine of the animals given glutamic acid and in that from the controls. Chow reported the same observation with alanine [10].

Some of the proteins present in urine have been recognised as inhibitors or promoters. The presence of other proteins has been reported but they have not been correlated with kidney stone formation. For instance, about 30 enzymes have so far been detected in urine; these include oxidoreductases, hydrolyase and lyases [11]. Only a few of these enzymes have proven their usefulness in the diagnosis of kidney diseases and these are mainly LDH, AP, β-glucoronidase and amino acid arylamidase (AA) [11]. The enzymes so far studied in urine are sensitive indicators of pathological processes but, to the best of our knowledge, no correlation has been found between the level of enzyme activity and the inhibitory or promoting mechanisms of of kidney stone formation.

In the present study we present a possible retardation mechanism of calcium oxalate kidney stone formation, involving enzymes steps.

Materials and Methods

First urine samples were collected from patients in the Urology Department of the Hebrew University Hadassah Hospital in Jerusalem. The patients had all recently had kidney stones removed. The stones were analysed and the urine of the calcium oxalate stone formers only was used for the present study. The controls were random contributors, considered healthy. Fresh mimic urine was prepared, based on the procedure proposed by Gardner et al. [12] except for the calcium concentration. The urine (human or mimic) was equilibrated in a thermostatic bath at 37 °C and 20 ml sample taken for the test. The experimental details have been described elsewhere [13, 14].

0300-5623/82/0010/0185/$01.00

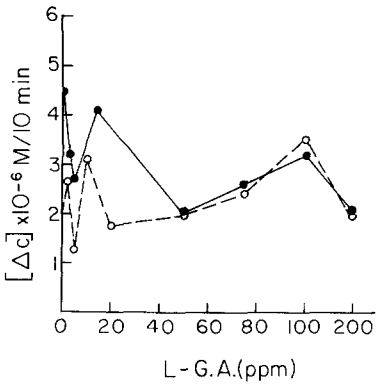

Fig. 1. The change of the sedimentation rate of calcium ion as a function of L-glutamic acid concentrations (change of concentration per 10 min)

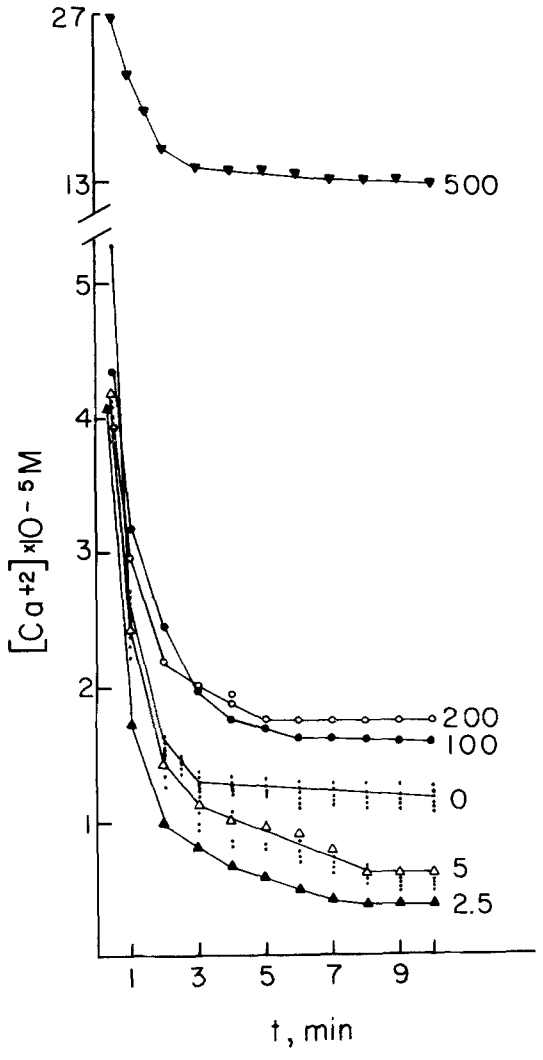

Fig. 2. The decrease in calcium ion concentration with time in mimic urine without and with variable concentration of L-glutamic acid monomer (ppm)

Measurement of the Enzyme Activity

The urine Glutamic-Oxalacetic-Transaminase (UGOT) activity and the urine Glutamic-Pyruvic-Transaminase (UGPT) activity were measured using the Technion SMAC System analysis at the Hadassah Hospital.

SEM Photomicrographs

At the termination of the tests the calcium oxalate precipitates were filtered and dried. The samples were gold coated and viewed under SEM (Jeol JPm-35).

Results and Discussion

The method of Sarig et al. [5] discriminates between the stone formers' urine and normal urine. The urine of healthy persons retards the precipitation of calcium oxalate (low Discriminating Index), while the urine of stone formers has little effect (high Discriminating Index). Mimic urine has also very little effect on calcium oxalate precipitation.

Precipitation of calcium oxalate in mimic urine in the presence of L-alanine, L-aspartic acid and L-glutamic acid has been studied. L-sapartic acid and L-alanine had no influence on the precipitation process of calcium oxalate at physiological concentrations (2.5–200 ppm). On the other hand, L-glutamic acid had a dramatic influence on this process in the concentration range of 2.5–500 ppm.

Figure 1 shows an effect of retardation (100–500 ppm) and an unusual effect of acceleration of precipitation at 2.5–5 ppm [15]. The relation of a physical property to concentration follows, as a rule, a certain trend; be it either a gradual decrease or increase with or without leveling out effect. The reverse of a trend needs an explanation.

In addition to the unusual concentration/retardation effect pattern of L-glutamic acid as compared to the orderly pattern of polyglutamic acid [16] there is also a unique effect on the nature of sedimentation of calcium oxalate. It has been stressed that the major part of calcium oxalate in the tests for the Discriminating Index (DI) determination precipitates in the first 30 s [4]. This precipitate forms a visible sediment in the reaction vessel, in the normal and pathological urines as well as in mimic urine, regardless of the presence of any added inhibitory agent which, when present, slows down the rate of calcium ion concentration decrease. However, in the presence of L-glutamic acid the solution turns turbid, but no visible sedimentation can be detected, even with the very small addition of 2.5 ppm of glutamic acid.

The "down and up" effect, i.e. increase and decrease of precipitation rate repeats itself. Though the overall effect may be summarised as decrease of precipitation rate (expressed as change of Ca ion concentration during 10 min) with increase of L-glutamic acid concentration (Fig. 2), the non-uniform trend needs a special explanation. To provide a basis for it the precipitates have been examined.

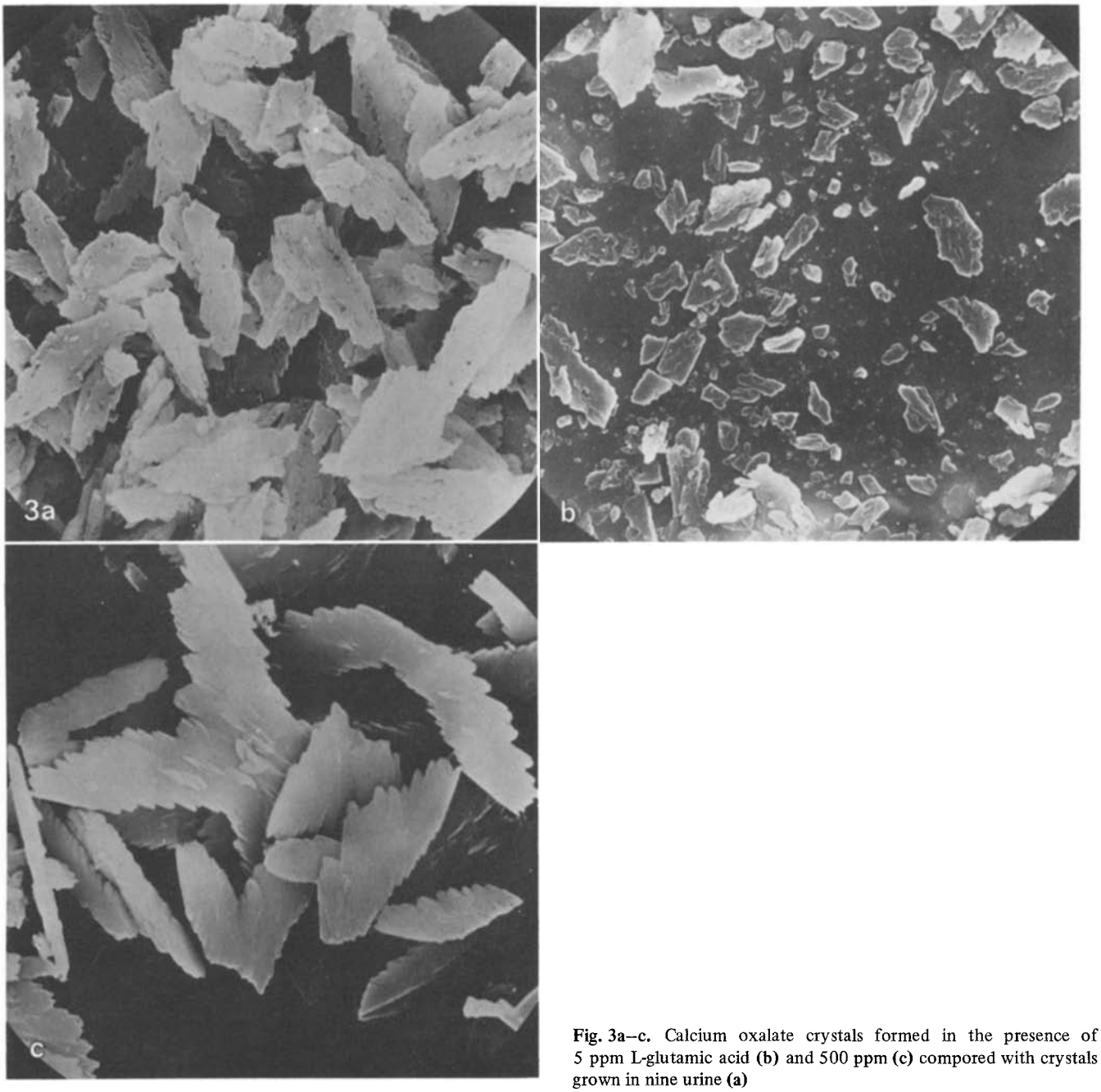

Fig. 3a–c. Calcium oxalate crystals formed in the presence of 5 ppm L-glutamic acid **(b)** and 500 ppm **(c)** compored with crystals grown in nine urine **(a)**

The turbid solutions were filtered through 0,2 μm millipore filters and viewed under SEM. The photomicrographs (Fig. 3) show calcium oxalate crystals formed in the presence of various L-glutamic acid concentrations to crystals formed in the same conditions in mimic urine without admixture, which can serve as controls (Fig. 3a). The crystals formed with 5 ppm of L-glutamic acid are significantly smaller than the controls (Fig. 3b). Similar effects of an impurity on crystal size have been reported in literature [17]. In the present study the interesting feature is that the crystals formed in the presence of higher L-glutamic acid concentration (500 ppm) are larger than those grown with 5 ppm – compare Figs. 3b and 3c.

The varying effect of L-glutamic acid concentration on the size of calcium oxalate crystals correlates well with the retardation/acceleration of the various concentrations on the rate of calcium oxalate precipitation and substantiates the existence of this curious phenomenon. The speculative explanation may be the ability of the L-glutamic acid monomer to act both as nucleation modifier and growth retardant by adsorption on crystal faces. It may be that at low concentrations most of the molecules are used at the nucleation stage giving a large number of small crystals [17]. Above a certain concentration there is also a sufficient amount left to adsorb on the growing crystals, slowing down the growth rate and thus causing both retardation

Table 1. Urine glutamic-oxalic-transaminase (UGOT) and glutamic-pyruvic-transaminase (UGPT) activity (units/litre) in normal controls' urine and in stone-formers' urine

Stone formers	5, 9, 9, 20, 9, 21, 9, 11, 22, 8, 15, 9, 15, 16, 6, 7, 15, 8, 19, 12, 10, 10, 15
Normal controls	22, 20, 26, 32, 54, 55, 24, 29, 20, 51, 60, 21, 55, 54, 21, 55, 39, 46, 26
Mean of (UGOT + UGPT) ± S.E.	
Stone formers	12.1 ± 4.85
Normal controls	37.4 ± 14.8

of precipitation and the growth of relatively large crystals (Fig. 3c). The dual effect of the L-glutamic acid monomer may give rise to a more complex concentration dependent pattern than that exerted by L-polyglutamic acid which evidently acts principally as a nucleation modifier [16].

More detailed proof of the proposed hypothesis will have to be worked out either by a special experimental design or by comparison with similar systems. However, the present study shows that L-glutamic acid has a definite effect on calcium oxalate precipitation. It is worth noting that both L-aspartic acid and alanine do not have a comparable effect under the same conditions. In addition, the apparent lack of effect of L-glutamic acid monomer reported previously [4] was due to accidental use of 100 ppm concentration. The effect is more pronounced either at significantly lower concentrations (2.5–5 ppm) or at much higher concentrations (200–500 ppm).

It is known that L-glutamic acid, L-alanine and L-aspartic acid are present in the urine. Patients with idiopathic renal calculi have lower than normal urinary amino acids content, as reported by McGeown [18]. It is interesting that the matrices of renal calculi which contain 50% aspartic acid and glutamic acid have more aspartic acid than glutamic acid. GOT and GPT convert aspartic acid and alanine respectively to glutamic acid in the presence of α-keto-glutaric acid and cofactors. There is some evidence concerning GOT and GPT activities in natural urine [11]. In the literature there are many reports on the difference between the urine of stone formers and that of healthy controls [19]. For the major constituents the differences do not follow a recognisable pattern, therefore it is very interesting to find whether the difference for minor constituents may be significant. For this purpose we wanted to establish whether the normal controls and the stone formers differ in the urine GOT and GPT activity.

In each natural urine sample the GOT and GPT activities were tested. The results (Table 1) indicate a difference between enzyme activity of the normals and of stone formers. The GOT and GPT activities are higher in the normals (37.4 u/l). We have observed a significant positive correlation between UGOT and UGPT activity and the ability of retardation of kidney calcium oxalate stone formation in the population sample examined. A negative correlation between UGOT and UGPT activity and the stone former group has been also established.

Two separate phenomena have been presented in this study. First, it has been shown that there is a difference in the enzyme activity between stone formers and healthy people in a statistically valid sample. Second, the retarding effect of L-glutamic acid on the crystallisation of calcium oxalate in vitro has been established, both by the present study and in the literature [9]. L-glutamic acid is the end product of GOT and GPT activity.

One can deduce that one of the possible retardation mechanisms of calcium oxalate stone formation may involve L-glutamic acid which forms in vivo due to UGOT and UGPT activity. Healthy people have a considerable activity of UGOT and some of UGPT. This activity may retard the calcium oxalate crystal growth rate via L-glutamic acid produced in situ and thus prevent stone formation. Stone formers do not have this advantage.

Sucessful stone treatment using DL-alanine [10], which by itself does not retard calcium oxalate precipitation, can be explained by transformation of alanine into glutamic acid by UGPT and the subsequent activity of the acid. Further study in this area is in progress.

Acknowledgments. Thanks are due to: Miss Karpuj Idit, for the assistance in performing the experiments; Mrs. Kupfer Gila, for the assistance in the enzymes assays; Mrs. Mila Folhan, for the SEM microphotographs.

References

1. Andersen DA (1969) Historical and geographical differences in the pattern of incidence of urinary stones considered in relation to possible aetiological fac'ors. In: Nordin BEL, Hodgkinson A (eds) Proceedings of the Renal Stone Research Symposium. Churchill, London, p 7
2. Andersen DA (1973) Environmental factors in the aetiology urolithiasis. In: Cifuentes De Latte L, Rapado A, Hodgkinson, A, Urinary Calculi. Karger, Basel, p 130
3. Resnick M, Pridgen DM, Goodman HO (1968) Genetic predisposition to formation of calcium oxalate renal calculi. N Engl J Med 278:1313–1318
4. Robertson WG, Peacock M (1972) Calcium oxalate crystalluria and inhibitors of crystallization in recurrent renal stone formation. Clin Sci 43:499

5. Sarig S, Garti N, Azoury R, Wax Y, Perlberg S (in press) A method for discrimination between calcium-oxalate kidney stone formers and normal. Invest Urol
6. Meyer JL, Smith LH (1975) Growth of calcium oxalate crystals II, Inhibition by natural urinary crystal growth inhibitors. Invest Urol 13:36
7. Smith LH, Meyer JL. McCall JT (1973) Chemical nature of crystal inhibitors isolated from human urine, Urinary Calculi, In: International Symposium on Renal Stone Research. Karger, Basel, p 318
8. Welshman SG, McGeown MG (1972) A quantitative investigation of the effects on the growth of calcium oxalate crystals on potential inhibitors. Br J Urol 44:677
9. McGeown MG (1957) The urinary amino acids in relation to calculus disease. Urol 78:318
10. Chow Fu-Ho-C, Myrtle ID, Durayne WH, Udal RH (1975) Control of oxalate urolithiasis by DL-alamine. Invest Urol 13:113
11. Mattenheimer H, Bergmeyer HU (ed) Enzyme in alanine In: Methods of Enzymatic Analysis. Verlag Chemie Weinheim, Academic Press Inc, New York London, p 62
12. Gardner GL, Doremus RH (1978) Crystal growth inhibitors in human urine and effect on calcium oxalate kinetics. Invest Urol 15:478
13. Garti N, Sarig S, Tibika F (1980) Retardation of calcium oxalate formation by polyacidic peptides. Invest Urol 18:149
14. Garti N, Tibika F, Sarig S, Perlberg S (1980) The inhibitory effect of polymeric carboxylic amino-acids and urine on calcium oxalate crystallization. Biochem Biophys Res Commun 1154
15. Sarig S, Kahana F (1976) On the association between sparingly soluble carbonates and polyelectrolytes. J Cryst Growth 35:145
16. Garti N, Tibika F, Sarig S, Perlberg S (1981) The inhibitory effect of polymeric carboxylic amino acid and urine on calcium oxalate crystallization. In: Smith LH, Robertson WG, Finlayson B (eds) Urolithiasis, Clinical and Basic Research. Plenum Press, New York London, p 657
17. Sarig S, Glinio O (1976) A mechanism for retarded precipitation based on time evolution of particle size and relative number density. J Phys Chem 80:256
18. McGeown MG (1959) The urinary excretion of amino acids in calculus patients. Clin Sci 18:185
19. Tiselius HG, Larsson L (1981) Inhibition of calcium oxalate crystal growth in patients with urolithiasis. In: Smith LH, Robertson WG, Finlayson B (eds) Urolithiasis: clinical and basic research. Plenum Press, New York London, p 623

Dr. Sara Sarig
Casali Institute
Hebrew University
Jerusalem
Israel 02-584522