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The Paradox of Inhibition and Enhancement of the Formation of Urinary Stones

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Summary. Nucleation (B^o) and linear crystal growth (G) rates, average particle size ($\bar{L}_{1,0}$) and total mass (M_T) of calcium oxalate dihydrate crystals were measured in artificial urine with and without polylysine, polyglutamic acid or heparin. The purpose of the study was to see if any of these polymers had effects on crystallisation similar to those created by addition of 5% natural urine to artificial urine, wherein B^o had increased but G, $\bar{L}_{1,0}$ and M_T decreased. Polylysine addition had insignificant effects. Heparin increased B^o and decreased G, $\bar{L}_{1,0}$ and M_T significantly, and polyglutamate had similar but more marked effects than did heparin. It is concluded that properly structured organic polymers can significantly inhibit calcium oxalate dihydrate crystallisation by paradoxical enhancement of nucleation. It is possible that such polymers may act as nucleation substrates.

Key words: Urolithiasis, Calcium oxalate dihydrate, Crystallisation, Inhibition, Polymers, Glycoseaminoglycans.

Introduction

The concentration of the inorganic constituents in human urine which can form urinary stones often exceeds the solubility of the stone forming salts such as calcium oxalate or calcium phosphates. As the majority of humans go through life without forming a stone, much research has been directed towards finding the inhibitory materials present in normal human urine. Many inorganic inhibitors of urinary stone formation have been found and their activities have been tested and recorded. However, on close scrutiny it becomes clear that even the most powerful inhibitor in this group, pyrophosphate, is not a likely candidate to be the major inhibitor since "only a fraction of total inhibition can be accounted by the amount of pyrophosphate present" [1]. This would suggest that the factors responsible for most inhibition are organic substances excreted in urine [2].

The inhibitory material extracted from urine contains strongly acidic peptides and mucopolysaccharides (glycoseaminoglycans) [2]. Leal and Finlayson [3] showed that mucopolysaccharides readily absorbed to calcium oxalate crystals according to the Langmuir isotherm. The"progressive coverage by the protein fraction in the fact of surface saturation by the carbohydrate fraction" suggested either heterogeneity in the protein-polysaccharide system or loose bonds between the protein and the polysaccharide backbone. The very special affinity between the acidic peptides and calcium ions is illustrated further by the calcium binding properties of γ-carboxyglutamic acid in blood clotting proteins [4]. Lian assumed therefore that proteins involved in functions where calcium ions take part should contain this specific amino acid. He looked for and found γ-carboxyglutamic acid in calcium containing urinary stones. Significantly this acid was absent from uric acid or cystine stones [4].

It would seem that the acidic peptides associated with mucopolysaccharides or contained in the matrix might be the inhibitory factor. However, comparison of mucopolysaccharide content in the urine of healthy individuals and stone-formers showed no significant differences [5].

We have attempted to show that this paradox of quantities is merely apparent. We offer the hypothesis that the phenomenon of inhibited precipitation may be viewed on the basis of an enhanced heterogeneous nucleation on structurally compatible microsubstrates [6] together with significant reduction in linear crystal growth rate which, taken together, still results in reduction of total mass precipitated.

Methods

The nucleation rate of calcium oxalate in synthetic urine with and without the presence of mucoproteins was measured. These determinations were carried out using MSMPR "population balance" analysis of steady state systems [7]. The analysis also yielded the linear growth rate of the individual particles (not to be confused with the process of agglomeration which contributes to stone formation)

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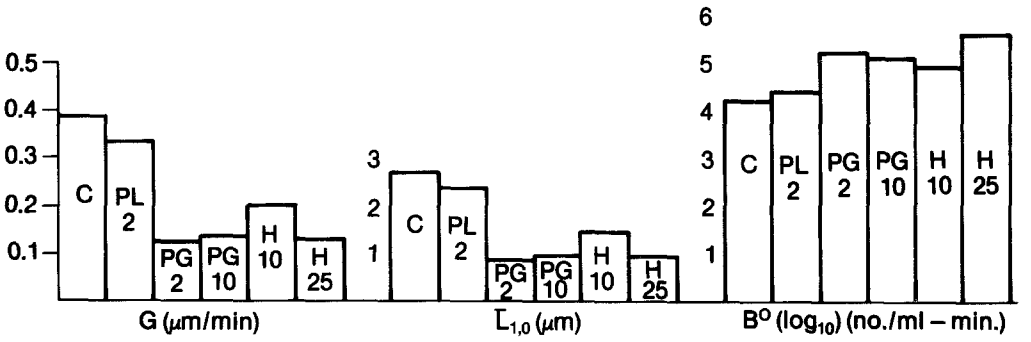

Fig. 1. Effects of some nucleating agents on G, $\bar{L}_{1,0}$, B°. Linear growth rate (G), average particle size ($\bar{L}_{1,0}$) and nucleation rate (B°) for control (C) polylysine (PL), polyglutamate (PG) and heparin (H) experiments. Numbers below additive refer to milligrams of additive per litre of synthetic urine

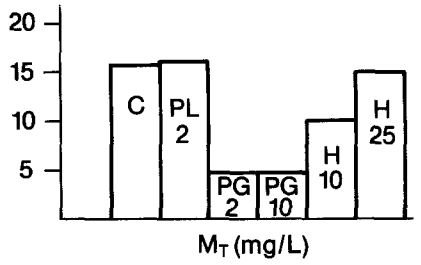

Fig. 2. Effects of some nucleating agents on M_T. Total mass of crystal produced with various additives. Designations as in Fig. 1

and the total mass of the precipitate. The experimental system set up to measure these parameters and described in detail in previous studies [8–10], does not necessarily simulate the process of calcium oxalate precipitation in urine. It does allow the examination of calcium oxalate crystallisation in urine-like solutions. The solutions and mode of operation were adjusted to give reproducible measurements of the relevant parameters [8]. When the potential inhibitory materials below were added to the standardised urine-like solutions in turn, it was possible to evaluate their separate effects on the main parameters.

Effects of two commercially produced peptides, polyglutamic acid (M.W. ~14,000 D) and polylysine (M.W. ~14,000 D), and one natural peptide polymer, heparin (M.W. ~ 25,000 D), on calcium oxalate precipitation were studied, using the MSMPR technique and the experimental system used in the previously published studies [8–10]. The aim was to establish if these polymers had effects similar to those exerted by natural urine and to show possible differences between peptides with acidic and basic functional groups.

Results

The values of crystallisation characteristics of calcium oxalate precipitate from synthetic urine in the presence of added peptides are summarised in Figs. 1 and 2. Experiments without additive served as reference controls. Comparison between control experiments confirmed statisfactory reproducibility of the measurement of all the relevant parameters.

The addition of 2 ppm polylysine changes slightly the values of B^o, G and $\bar{L}_{1,0}$. However the change induced by the presence of 2 ppm polyglutamic acid is spectacular: growth rate and average particle size were reduced by 70%, while the nucleation rate increased by a factor of 9. The amount of total precipitate was reduced by a factor of 3.6. Such a steep decrease in material deposition is usually classified as inhibition of precipitation. However, in sharp contrast to inhibitory effects which involve suppression of nucleation [9], the present inhibitory effect is accompanied by enhanced nucleation rates with decreased growth rates.

In experiments with 10 ppm of polyglutamic acid this inhibition was excessive; whereas turbidity in the crystalliser appeared in control experiments after four retention times (about 28 min), no turbidity was visible until after nine retention times (about 63 min). The "induction period" prolongation was observed in previous studies and has been used as an index of "crystallisation inhibition" [11, 12]. The appearance of turbidity in this experiment was rapidly followed by deposition of crystal agglomerates on the bottom of the crystalliser and on the walls, rendering the measurements of growth and nucleation less exact than in the former runs. However, experiments with polyglutamate and heparin show that inhibition can be detected either visually or by the decreased weight of the deposited material and can be accompanied by remarkable enhancement of nucleation.

The effect of 10 ppm heparin is slightly lower but consistent with the effect of 2 ppm polyglutamic acid thus grading the activity of the peptides polyglutamic acid > heparin ≫ polylysine, in apparent direct correlation with the content of carboxylic groups.

Discussion

The results indicate that these acidic polypeptides at very low concentration (in the range of 10^{-8} M to 10^{-7} M) exert a marked enhancing effect upon the nucleation rate of calcium oxalate coupled with significant decrease in the rate of linear crystal growth. The course of crystallisation is thus altered, the resulting phenomenon being inhibition of precipitation characterised by decreased growth rate, small crystal size and low yield. We are aware that oxalate assay of supernatant would have assisted in defining which process was most affected, but precise oxalate assay was not available when these studies were done.

A similar effect has been found on addition of human urine to precipitation runs of calcium oxalate precipitation from synthetic urine [9]. The effect of these pure synthetic and natural peptides is relatively stronger than that of urine. The high activity of both polyglutamic acid and heparin indicates that acidic polypeptides and maybe other compounds with appropriate structures and surface charges can act as nucleation enhancers as well as causing delay in precipitation and ultimate production of smaller crystallites.

The study of the effects exerted by e.g. polyglutamic acid and heparin on calcium oxalate precipitation seems an efficient way to ascertain which of the likely inhibitory candidates in urine has a major effect on the process. Isolation of a certain group of substances from the extremely complex biochemical system of urine, which is subject to extensive variations, is difficult and tedious. The technique employed, adapted from the field of engineering, allows clear quantitative definition of the basic phenomena in the crystallisation process stages at the time of the solid phase formation.

Polyglutamic and polyaspartic acids have been shown to retard calcium oxalate precipitation in a separate assay system [13] and to comprise a major portion of the amino acids in "matrix" [14]. The most effective of the compounds used in this study, polyglutamic acid, was found also to have a strong effect on the crystal habit of $CaSo_4 \cdot 2H_2O$ [15]. This effect was explained as specific nucleating ability of polyglutamic acid with respect to $CaSO_4 \cdot 2H_2O$. The effect probably results from good structural fit between the polymer and the crystal. The average distances between the carboxylic groups on the peptide chain are about 8 Å and there are Ca^{2+} sites which are 8.2 Å apart in the $CaSO_4 \cdot 2H_2O$ crystal lattice. Significantly polyglutamic acid does not affect the crystal habit of $CaSO_4 \cdot 1/2H_2O$ in which the calcium ions are at distances shorter than 8 Å. To the best of our knowledge the crystal structure of $CaC_2O_4 \cdot 2H_2O$ has not been worked out in sufficient detail to enable the calculation between Ca^{2+} sites in its lattice. As $C_2O_4^{2-}$ is of a size comparable to that of SO_4^{2-} it stands to reason that irrespective of symmetry groups and exact cell dimensions, the distances between Ca^{2+} sites in the $CaC_2O_4 \cdot 2H_2O$ will be closer to those in $CaSO_4 \cdot 2H_2O$ than $CaSO_4 \cdot 1/2H_2O$, i.e. that there are Ca^{2+} site distances in the 8 Å range in the $CaC_2O4 \cdot 2H_2O$ crystal lattice.

One Possible Role of Mucoproteins in Urinary Stone Formation

The concept of nucleation on suitable substrates, or epitactic growth is neither strange nor new in urinary stone research. Boyce [16] recognized it as early as 1956 from his detailed microscopic studies where the association between the matrix and the inorganic deposit is evident. But though his study was very through and very detailed the technique was not suitable to obtain uniquivocally convincing proof. One hypothesis to resolve this problem was given by Lian: "... it is tempting to speculate the the γ-carboxyglutamic acid may serve as nucleation substrate, although an equally pausible argument can be advanced that they function just the opposite way" [4].

Chow et al. [17] speculated that urine polyelectrolytes may form the calculus matrix and thus promote calculus formation. Lian and Finlayson [3] rejected this proposal not only because it had not been proved but also on the grounds that it was "sterically implausible". They themselves proved beyond any reasonable doubt the mucopolysaccharides and mucoproteins adsorb readily on the surfaces of calcium oxalate crystals according to strict physical laws. Such tight adsorption probably brings about the observed decrease in linear growth rate.

Nucleation on microsubstrates might resolve one of the dilemmas encountered in Leal and Finlayson's study: the average matrix content in urinary stone is 2.5% [3]. Only 16% (not 40% as stated by mistake) of this amount can be accounted for by adsorption on the separate particles which constitute the stone. What part does the remainder play? Leal and Finlayson [3] report a study by Maki and Suzuki who aggregated calcium carbonate powder with a natural polymer. The mechanism involves adsorption, inter-particle bridging and compaction by tumbling. The resulting structure resembles gallstone. The extrapolation of the concept to urinary stones seems to be straight forward [3]. If the particles are covered by mucoprotein and mucopolysaccharide monolayer [3] their growth rate is retarded and it stands to reason that the smaller the particles, the better their chance to stay glued to each other after having formed interparticle bridges. In fact agglomeration processes proceed as the square of particle number density [18] and thus "bursts" of small nuclei might well enhance the tendency to form agglomerates.

It would follow that the control mechanism which inhibits calcium oxalate deposition on kidney walls could be the same in normal and stone-forming urine: retardation of individual crystal growth rates (linear dimension) with provision of suitable nucleation sites for the formation of smaller crystallites which can then be safely washed away. The failure of the control system, i.e. the disease, becomes an example of over-regulation. The resulting crystallites are of enormous number and are very small – they are rapidly covered by the excess of mucomacromolecules and aggregate, thus beginning the agglomeration of stone formation. Due to the reduced linear growth rate, particles can only increase significantly in size by the process of agglomeration/aggregation. The stone embryo causes more irritation thus starting a vicious circle of increased mucoprotein. These compounds will further enhance nucleation, a step which entails additional decrease of particle size which inturn favors further agglomeration/aggregation. Ismail and Tawashi have reported that the crystallites of urinary calculi are indeed composed of very large numbers of very small particles, although the greatest volume of the stone is composed of very few large crystals [19].

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References

1. Ito H, Coe FL (1977) Acidic peptide and polyribonucleotide crystal growth inhibitors in human urine. Am J Physiol 233F-455-63
2. Rose M (1975) Renal stone formation. The inhibitory effect of urine on calcium oxalate precipitation. Invest Urol 12(6): 428–433
3. Leal JJ, Finlayson B (1977) Absorption of naturally occuring polymers onto calcium oxalate surfaces. Invest. Urol 14:278–283
4. Lian JB, Prien EL, Jr Glimcher MJ, Gallop PM (1977) The presence of protein bound gamma – carboxyglutamic acid in calcium containing renal calculi. J Clin Invest 59:1151–1157
5. Bichler KH, Ideler V, Hargmann R (1979) Uromucoid excretion in normal individuals and stone formers. Curr Probl Clin Biochem 9:309–324
6. Sarig S, Ginio O (1976) Mechanisms for retarded precipitation based on time evolution of particle size and relative number density. J Phys Chem 80:256–259
7. Randolph AD, Larsen MA (1971) Theory of Particulate Processes. Academic Press, New York
8. Miller JD, Randolph AD, Drach GW (1977) Observations upon calcium oxalate crystallization kinetics in simulated urine. J Urol 117:342–345
9. Drach GW, Randolph AD, Miller JD (1978) Inhibition of calcium oxalate dihydrate crystallization by chemical modifiers I. Pyrophosphate and methylene blue. J Urol 119:99–103
10. Drach GW, Thorson S, Randolph A (1980) Effects of urinary organic macromolecules on crystallization of calcium oxalate: enhancement of nucleation. J Urol 123:519–523
11. Sutor DJ (1976) A simple method for studying the formation of calcium oxalate. Br J Urol 48:177–182
12. Gardner GL, Doremus RH (1978) Crystal growth inhibitors in human urine. Invest Urol 15:478–485
13. Garti N, Sarig S, Tibika F (1980) Retardation of calcium oxalate formation by polyacidic peptides. Invest. Urol 18:149–150
14. Bommer J, Ritz E, Tschope W, Waldherr R, Gebhardt M (1979) Urinary matrix calculi consisting of microfibrillar protein in patients on maintenance hemodialysis. Kidney Int 16:722–728
15. Sarig S, Kahana F, Leshem R (1976) Selection of threshold agents for calcium sulfate scale control on the basis of chemical structure. Desalination 17:215–229
16. Boyce WH, Garvey FK (1956) The amount and nature of the organic matrix in urinary calculi: a review. J Urol 76:213–227
17. Chow FHC, Hamar DW, Udall RH (1973) Urinary calculi matrices and urine polyelectrolytes. Proc Soc Exp Biol Med 144: 912–916
18. Levich VG (1962) *Physico-chemical Hydrodynamics.* Prentice-Hall, Inc., Englewood Cliffs, N.J.
19. Ismail SI, Tawashi R (1980) Size distribution characteristics of mineral phase in renal stones. J Pharm Sci 69:829–831

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