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The Amino Acid Factor in Stone Formers' and Normal Urines

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Summary. The composition of amino-acids in kidney stone matrices and inhibitory materials from normal urines, reported in different independent studies has been reviewed. No obvious difference was found between the composition of amino acids from healthy and pathological sources. Studies carried out in this laboratory showed a specific marked effect of glutamic acid on the crystallization of calcium oxalate while aspartic acid and alanine affected the process very slightly. It is known that aspartic acid and alanine are transformed into glutamic acid by enzyme activity. A short survey performed in this laboratory showed that the relevant enzyme activity was relatively high in healthy urine and low in stone formers' urine. The AA factor (F_{AA}) proposed is $F_{AA} = [\text{Glutamic acid}]/\{[\text{Aspartic acid}] + [\text{Alanine}]\}$ its value in fractions of non-potent inhibitory material and in kidney stone matrices is below 0.6. In potent portions of the inhibitory material separated from healthy people and young animals urine the value is 0.8 and above.

Key words: Amino Acids, Urine, Kidney stones.

Introduction

This report reviews and compares several studies concerning the role of acidic peptides in the inhibition of calcification and especially of their effect on kidney stone formation, both as preventive agents and matrix stone binders. The studies were reported during the period 1965–1980. The aim of this report was to systematize the various results by the introduction of a factor to distinguish between a stone forming and a normal urine. The factor was formulated on the basis of a series of recent studies in this laboratory.

All the authors included in the present survey have recognized the effect of acidic peptides on sparingly soluble calcium salts crystallization. Another common denominator of these studies was the conclusion that the presence, absence or inactivation of these substances may be a decisive factor in calcification and stone formation. In each one of the studies a detailed analysis of the acidic peptides composition (whether extracted by a multi-step procedure from urines or isolated from kidney stone matrix) was presented.

Comparing the lengthy lists of the amino acid compositions from different studies there was no obvious difference between the composition of amino-acids in a stone matrix and in the super active residue of the healthy urine. Even when a single urine sample was split into an "inactive fraction" and "active" portion, the amino acid composition data in both fractions were similar [1]. The authors did not attribute the "activity – inactivity" to any specific component or to any configuration of them.

In the present survey an exploratory "AA factor" has been proposed to distinguish between the amino acids composition in the stone matrix, pathological urine, "inactive fractions" on one hand and between their composition in healthy urine and "active fractions" on the other hand. The formulation of the AA factor was an attempt to explain the puzzle which emerges from the survey. The AA factor was the relationship between three ordinary acids which were investigated in a series of studies in this laboratory and which were specifically dealt with in the reviewed literature.

The Scope of Active/Inactive Amino Acid Compositons

In Table 1 two sets of analyses of amino acids in urine are presented. Each set is a pair, the members of which could be reasonably expected to have recognizably different compositions. The first set represents the average composition of four acidic polypeptides isolated from normal human urine [2] versus the composition of organic matrices from kidney stones [3]. Both compositions are expressed in weight percent.

It is interesting to note that glutamic and aspartic acids, which are the most abundant components in the mixture

Table 1. Amino acids compositions in urine and kidney stone matrices

Amino Acid	Set I: Independent studies (weight percent)		Set II: Gel electrophoresis of one sample (molar percent)	
	Active inhibitors in healthy urine	Organic matrix of CaOx stone	Active peak	Inactive fractions
Reference	2	3	1	4
Aspartic acid	12.9	34.0	17.6	12.5
Threonine	8.2	4.7	5.8	7.4
Serine	7.7	9.0	7.2	10.6
Glutamic acid	16.9	21.5	19.2	11.6
Proline	2.1	3.4	11.0	11.5
Glycine	13.6	5.3	9.5	12.9
Alanine	6.3	3.7	6.7	7.4
Cysteine		1.0		
Valine	5.9	2.7	4.6	5.6
Methionine			1.1	1.3
Isoleucine	6.1	1.1	4.4	5.3
Tyrosine	2.8	1.1		
Phenylalanine	8.2	1.6	2.3	3.0
Hydroxylysine				
Lysine	3.1	2.9	3.3	3.1
Histidine	1.7	2.3	2.8	1.5
Arginine	2.8	2.1	2.4	2.2

of the active inhibitors [2], appeared in higher relative proportion in the stone matrices of the kidney stone [3]. It may be inferred that these acids were either preferentially adsorbed on calcium oxalate crystals or start nucleation as templating agents [4], suggesting that they may not be the sought after inhibitory agents. Which are the most probable candidates for the inhibitory function is still an open question.

Any speculation will be refuted by the comparison within the second pair (see (Table 1, set II). In this study gel electrophoresis eluted inhibitory material enriched with peptides that were "strongly acidic and high in proline" [1]. However, no distribution of proline between the active and inactive fractions was effected, and thus the significance of "high proline" remains unclear. The composition shows the dominace of aspartic and glutamic acids in both the active and inactive fractions. Even if one could find a distinguishing factor, by any arbitrary configuration of acids, between the active and inactive fractions in the particular study [1], what is the confidence that this factor will be valid in general? The random differences in the composition of amino acids in studies [1, 2] indicated that the diversity stems from natural variation yet in the "active fraction" composition [1] possibly rested an important part of the inhibitory potential of healthy urine.

The key may have been a ratio between some of the components. We present a series of studies performed in this laboratory and reported by others on the effect of amino acids on calcium oxalate crystallization. The results produced a basis for for the formulation of a distinguishing factor.

Experimental Evidence of Acidic Amino Polymers and Monomers Effect on Calcium Oxalate Crystallization

Much experimental evidence that acidic amino acids both as polymers and monomers affect the crystallization of calcium oxalate in vitro and in vivo can be found in the literature.

Polyglutamic acid efficiently retards calcium oxalate precipitation from highly supersaturated solutions; polyaspartic acid exerts a similar effect [4]. Amino polymers which have no free acidic functional groups, such as polylysine have a comparatively insignificant effect [5]. When the acidic functional groups in polyaspartic acid were blocked as in polybenzyl aspartate, no retarding effect was detected [6].

McGeown, citing 40 year old observations and her own results [7] maintained that amino acids increase the solubility of calcium and magnesium phosphates. Moreover, administration of glutamic acid monomers prevented formation of calculi in rats fed on a vitamin A deficient diet [8].

The lack of effect of glutamic acid monomer on calcium oxalate crystallization [6] was retested and disproved; glutamic acid has a complex oscillating pattern of influence, either retarding or accelerating calcium oxalate precipitation [9]. Incidentally at 100 ppm level this effect was hardly detectable [6]. Aspartic acid monomers had a comparatively slight effect [9].

Considering the similarity of glutamic and aspartic acids which differ only by one CH_2 group, the specificity of the effect on calcium oxalate crystallization is noteworthy. An interesting analogue was recently reported in a study of the effect exerted by organic acid on the crystallization of calcium sulfate [10]. The effect, specific to di-carboxylic acids of certain chain length, was attributed to structural compatibility between the organic molecule and the cationic spacing in the crystal. Several earlier studies explored the idea of specific active interaction based on structural compatibility between crystals and polyacids [5, 11, 12].

Structural compatibility could explain the dual effects of glutamine and aspartic polymers as both growth retarding and nucleation enhancement agents. The distance between the two carboxylic groups in glutamic acid is about equal to the distance between the cationic sites in calcium oxalate dihydrate. This would explain the preferential activitiy of glutamic acid monomer as well as the effect of the free flexing polymers of both glutamic and aspartic acids. In agreement with the finding that monocarboxylic acids have no effect on calcium sulphate crystallization, it was ascertained that alanine has very little effect on calcium oxalate.

However, a basic study on oxalate urolithiasis revealed that alanine and pyruvate at appropriate levels may be beneficial in preventing oxalate urolith formation in male

Table 2. The AA factor for amino acid compositions in urines and kidney stone matrices

Data concerning urine/stone	Reference	AA Factor
Inactive fraction (in gel electrophoresis)	1	0.58
Organic matrix in CaOx stone	3	0.57
Calcium oxalate stone matrix	18	0.56
Purified inhibitors (*no* inhibitory potency)	19	0.53
Active peak (in gel electrophoresis of urine)	1	0.79
Active inhibitors in healthy urine	2	0.88
Urine polyelectrolytes (urine of lambs)	20	0.91, 0.86 1.05, 1.05, 1.06
Kidney disease (tabular protein uria, no stone)	21	0.82, 0.85
At maximal reported urinary concentration	22	1.91

rats [13, 14]. The lack of effect of alanine on in vitro crystallization and its obvious preventive effect on in vivo stone formation led to a hypothesis formed in this laboratory that the administered alanine may be transformed into glutamic acid in situ via enzymatic activity in urine.

The Possible Role of Enzymes in Calcium Oxalate Stone Formation

It is well known that Glutamic-Pyruvic Transaminase (GPT) causes the transformation:

$$\text{Alanine} + \alpha\text{-ketoglutaric acid} \xrightarrow{\text{GPT}} \text{Pyruvic Acid} + \textit{Glutamic Acid.}$$

Glutamic Oxalacetic Transaminase (GOT) also yields glutamic acid from aspartic acid.

$$\text{Aspartic Acid} + \alpha\text{-Ketoglutaric Acid} \xrightarrow{\text{GOT}} \text{Oxalatic Acid} + \textit{Glutamic Acid}$$

A small scale survey was carried out to check whether stone formers' urines may have lower GOT and GPT enzyme activity than healthy individuals. The levels of enzyme activity in 23 first urine samples of normals and 19 stone formers was measured using Technicon SMAC System [9]. The results were clear: The mean of Urine Glutamic-Oxalilic Transaminase (UGOT) and Urine Glutamic-Pyruvic Transaminase (UGPT) activities in the stone formers group was 12.1 ± 4.85 while in the control group it was 37.4 ± 14.8 [9]. These results suggested that glutamic acid was one of the main modifiers of calcium oxalate crystallization in urine thus controlling the retarding or stone forming processes.

Clinical observations revealed that in Fanconi's syndrome, when there was an excess of urinary calcium and amino acids, urinary calculi were absent [15], suggesting that the presence of amino acids had an influence on the prevention of stone formation.

In order to strengthen the credibility of the hypothesis, an additional set of experiments was devised. Pathological urine taken from patients who had undergone surgery for calcium oxalate stone removal was examined and ascertained not to have any retarding potential toward standardized in vitro calcium oxalate precipitation. The results of the test are expressed on a Discriminating Index (DI) scale; values of DI between 0.6–2.9 indicate stone forming disposition while values of −0.5–0.7 are characteristic for normals [16]. The pathological urine in question had a DI value of 1.8 which classifies it unequivocally as stone former's urine.

This urine (100 cc) was incubated with 80 units of GOT. After 20 min the DI was 0.51, i.e., the pathological urine reacted with the precipitation of calcium oxalate as if it had originated from a healthy individual. It proved that GOT corrected the faulty retarding potential of urine. It is most probably that the restored retarding potential was due to creation of glutamic acid in situ.

The "AA Factor" for Retarding Potential in Urine

Glutamic acid is the most active modifier among the simple amino acids for calcium oxalate crystallization. Alanine and aspartic acid are comparatively inactive as shown by in vitro experiments [9]. Alanine was found to decrease stone formation incidence in extreme in vivo conditions [14]. One may infer that either alanine or its issue were active in the retarding effect of stone formation. The activity level of the enzymes which transfer alanine and aspartic acid is relatively high in healthy urine [9]. It follows that in healthy urine glutamic acid might arise from alanine and aspartic acid in situ. Therefore the ratio of glutamic acid to alanine and aspartic acid, which were used in the transformation, should be high in healthy urine and especially so in the active fractions. It could be relatively low in inactive fractions, stone formers' urine and in organic matrices of kidney stones.

Therefore the AA factor proposed as a first approximation is

$$F_{AA} = \frac{[\text{glutamic acid}]}{[\text{alanine}] + [\text{aspartic acid}]}$$

After the formulation of the "AA factor" and finding different values for pathological/healthy or active/inactive amino acid compositions, additional studies were reviewed and the simple ratio was computed for them. The results are summarized in Table 2.

The values of the AA factor for acid composition in the organic matrix of CaOx stone was below 0.6 [3, 18]. In

the fractionated amino acids with no inhibitory potency the AA factor was also below 0.6 [1, 19]. The very narrow distribution of AA factor values in these last four studies, undertaken for very diverse reasons and purposes was important.

On the other hand the lowest value of the AA factor in the active fractions was 0.79 [1]. In active inhibitors of healthy human urine a value of 0.88 was reported [2]. A study in which the urine of lambs was used reports values 0.86–1.06 for animals fed on different diets [20]. As a rule young animals are not predisposed to kidney stone formation, i.e. they have perfectly functioning control mechanisms. Urine from patients with impaired tubular function contains low molecular weight proteins. Urine from a patient with multiple myeloma (but no kidney stone) was studied with regard to trypsin inhibition. The AA factor of the amino acid content was 0.82 [21]. Finally, at the maximal reported urinary concentration [22], which was actually a synthetic picture of "super-healthy" urine the AA factor reached a value of 1.91.

All the investigators, who reported the amino acid compositions of stone matrix extract on one hand and the active inhibitory material on the other hand, did it for their own purposes. The lack of easily distinguishable features between the results was reflected in the apparent "Paradox of inhibition and enhancement in the formation of urinary stones" [5].

The formulation of the AA factor which discriminates between amino acid composition in healthy urine (0.8 and above) and in cases when the protective mechanism does not work effectively (below 0.6) resulted from information and ideas gained and developed in a recent series of studies [5, 6, 9, 11, 12, 16, 17]. Being based on the results of a significant number of sound and independent studies, it may be a piece in the highly incomplete puzzle of kidney stone formation.

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