Urinary Calculi Matrices and Urine Polyelectrolytes[1] (37710)

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(Introduced by M. L. Hopwood)

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In most calculi, there are organic matrices. These matrices, mainly proteins and mucoproteins (1, 2), are composed of amino acids, neutral sugars, and aminosugars (3–5). There have been many studies and discussions concerning the origin of matrices and the role of matrices in calculi formation (2, 6), but conclusive evidence about either topic is not available. In earlier publications (7–9), we proposed that the randomly aggregated urine proteineous materials (urine polyelectrolytes), which include the high-molecular-weight proteins and mucoproteins as well as the low-molecular-weight peptides, could be involved in the formation of calculi matrices. The objective of this study was to investigate whether calculi matrices were formed from preformed proteineous macromolecules or from the randomly aggregated polyelectrolytes in urine.

Materials and Methods. Sheep and rat calculi were obtained from animals fed a phosphate calculi-producing semipurified ration which had been successfully used in our laboratory (10). Bovine and canine calculi were obtained from the Glover Veterinary Hospital of the College of Veterinary Medicine and Biomedical Sciences, and human calculi from Dr. L. D. Dickey, a urologist in Fort Collins.

The urine polyelectrolytes (UPE) were isolated from the urine of sheep fed either a semipurified or hay ration. The latter is a noncalculi-producing ration (7). The two fractions of UPE, the high-molecular-weight fraction F1 (proteins and mucoproteins) and the low-molecular-weight fraction F2 (peptides), were prepared by Bio-Gel P-30 filtration as previously described (9).

For amino acid analysis, the pulverized calculi and UPE were hydrolyzed in 6 *N* HCl at 100° for 20 hr following the procedures of Zumwalt *et al.* (11). The *N*-trifluoroacetyl *n*-butyl esters of amino acids with internal standard, 4(aminomethyl)cyclohexanecarboxylic acid (Aldrich Chemical Co.), were prepared and analyzed with a Varian Aerograph Model 1700 gas chromatograph with a flame ionization detector according to the technique of Roach and Gehrke (12).

For the preparation of anticalculi rabbit serum, 18 mg of the pulverized phosphate calculi from sheep was suspended in 1 ml of complete Freund's adjuvant (Difco). Two milliliters of this suspension was injected subcutaneously into young albino New Zealand rabbits at weekly intervals for 6 weeks. Two weeks after the last injection, blood was collected intracardially, and anticalculi serum was separated and stored at −20°. Rabbit antiserum to sheep serum was purchased (Hyland Division Travenol Laboratory, Inc., Los Angeles, CA).

The immunoelectrophoresis was performed in 1% agar medium on a 1 × 3 in. slide in barbital acetate buffer, pH 8.6, ionic strength 0.05, for 4 hr at 100 V. The unreacted proteins were washed off with 1% NaCl solution. The precipitin bands were viewed either unstained or stained with 0.1% thiazine red R in 2% acetic acid.

Results. The amino acid composition of calculi matrices of various types from different species are tabulated in Table I. Tryptophan is not recorded because it is destroyed

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TABLE I. Amino Acid Composition of Calculi Matrices, in Molar Ratio Based on Serine.

Species:	Sheep	Rat	Canine	Bovine	Bovine	Human	Human
Type of calculi:	Phosphate	Phosphate	Phosphate	Silica	Phosphate	Urate and uric acid	Oxalate
No. of samples analyzed:	10	14	4	3	2	4	3
	Mean ± SD[a]	Mean ± SD	Mean ± SD	Mean ± SD	Mean	Mean ± SD	Mean ± SD
Glycine	1.35 ± 0.51	0.90 ± 0.24	1.65 ± 0.65	1.20 ± 0.28	1.04	6.26 ± 6.15	2.18 ± 2.45
Proline	0.78 ± 0.14	0.54 ± 0.11	0.52 ± 0.07	0.76 ± 0.14	0.62	0.63 ± 0.13	0.57 ± 0.12
Hydroxyproline	0.10 ± 0.13	0.05 ± 0.05	Trace	0.05 ± 0.02	Trace	Trace	Trace
Alanine	1.05 ± 0.23	0.86 ± 0.31	1.90 ± 0.53	1.05 ± 0.12	0.86	0.78 ± 0.16	0.73 ± 0.29
Valine	0.76 ± 0.17	0.51 ± 0.12	0.70 ± 0.12	0.67 ± 0.13	0.64	0.76 ± 0.08	0.75 ± 0.03
Isoleucine	0.37 ± 0.12	0.27 ± 0.07	0.39 ± 0.13	0.30 ± 0.08	0.31	0.31 ± 0.45	0.70 ± 0.36
Leucine	1.18 ± 0.21	0.94 ± 0.20	1.03 ± 0.14	1.27 ± 0.10	0.94	1.08 ± 0.05	1.18 ± 0.22
Threonine	0.75 ± 0.17	0.44 ± 0.08	0.55 ± 0.14	0.60 ± 0.05	0.47	0.64 ± 0.19	0.88 ± 0.18
Phenylalanine	0.44 ± 0.08	0.41 ± 0.06	0.45 ± 0.08	0.39 ± 0.04	0.37	0.56 ± 0.07	0.71 ± 0.31
Tyrosine	0.24 ± 0.05	0.09 ± 0.08	0.08 ± 0.12	0.30 ± 0.09	0.16	0.47 ± 0.14	0.40 ± 0.09
Aspartic acid	1.51 ± 0.27	1.22 ± 0.20	1.31 ± 0.21	1.21 ± 0.15	1.03	1.99 ± 0.55	1.95 ± 0.26
Glutamic acid	1.81 ± 0.45	1.49 ± 0.29	1.99 ± 0.37	1.52 ± 0.21	1.25	1.73 ± 0.30	1.97 ± 0.53
Lysine	1.04 ± 0.33	1.02 ± 0.35	1.58 ± 0.13	1.12 ± 0.13	1.18	1.66 ± 0.50	1.23 ± 0.01
Arginine	Trace	Trace	Trace	Trace	Trace	0.40 ± 0.13	0.60 ± 0.27
Serine	1.00	1.00	1.00	1.00	1.00	1.00	1.00
% Total amino acids / Calculi	2.33 ± 1.20	0.55 ± 0.37	0.46 ± 0.06	11.71 ± 4.56	0.24	3.82 ± 0.82	2.49 ± 2.04

[a] SD = standard deviation.

TABLE II. Amino Acid Composition of Urine Polyelectrolytes, in Molar Ratio Based on Serine.

Ration:	Semipurified		Hay	
Sample:	UPE F1	UPE F2	UPE F1	UPE F2
No. of samples analyzed:	6	6	15	15
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Glycine	2.30 ± 0.70	9.98 ± 4.20	0.96 ± 0.24	6.27 ± 1.93
Proline	1.01 ± 0.47	1.82 ± 0.14	0.89 ± 0.02	1.86 ± 0.33
Hydroxyproline	0.44 ± 0.14	2.01 ± 0.38	0.13 ± 0.01	2.06 ± 0.30
Alanine	1.24 ± 0.20	1.29 ± 0.43	1.07 ± 0.10	1.52 ± 0.22
Valine	0.73 ± 0.17	0.50 ± 0.14	0.88 ± 0.14	0.46 ± 0.10
Isoleucine	0.25 ± 0.10	0.47 ± 0.48	0.29 ± 0.10	0.18 ± 0.14
Leucine	1.01 ± 0.14	0.64 ± 0.10	1.26 ± 0.26	0.62 ± 0.01
Threonine	0.82 ± 0.02	0.63 ± 0.14	0.84 ± 0.10	0.87 ± 0.36
Phenylalanine	0.42 ± 0.01	0.45 ± 0.17	0.48 ± 0.01	0.21 ± 0.01
Tyrosine	0.24 ± 0.01	0.25 ± 0.17	0.31 ± 0.10	0.13 ± 0.01
Aspartic acid	1.70 ± 0.28	1.83 ± 0.47	1.45 ± 0.14	2.11 ± 0.20
Glutamic acid	2.07 ± 0.44	2.81 ± 0.34	1.78 ± 0.17	2.59 ± 0.33
Lysine	0.85 ± 0.14	0.84 ± 0.14	1.38 ± 0.24	1.15 ± 0.36
Serine	1.00	1.00	1.00	1.00
% Total amino acid / UPE	35.83 ± 7.9	13.6 ± 10.7	63.3 ± 9.3	30.0 ± 8.8

by acid hydrolysis. Methionine, histidine, cysteine, and cystine are not included in the Table because they occurred in only insignificant quantities. Results in Table I show that the percentage of matrices (represented by total amount of amino acids) varied with different calculi. There were also variations in the amino acid composition of individual calculi. However, every matrix analyzed had more acidic than basic amino acids, and thus, were negatively charged.

Table II contains the amino acid composition of the two fractions, F1 and F2, of the urine polyelectrolytes of sheep fed the semipurified and hay rations. Similar to calculi matrices, UPE had more acidic than basic amino acids. There was a distinct difference in quantities of glycine, proline, and hydroxyproline among the UPE. These three amino acids were in greater amount in the UPE of semipurified ration than that of the hay ration. They were also greater in F2 than in F1.

Immunoelectrophoresis revealed that UPE F1 consisted of components of serum proteins (Fig. 1A) and also that calculi matrices and UPE F1 had common entities (Fig. 1B). According to the mobilities of the precipitin bands, the common constituents in matrix and UPE F1 could have originated from serum proteins.

Discussion. The amino acid composition of calculi matrices varied with type and species (Table I). This variation was also observed with the same type of calculi from the same species. Furthermore, the amount of matrix, expressed by the total amino acid content, demonstrated a similar variation between the individual calculus and among the calculi from different types and species. These variations indicate that the calculus matrix is not composed of preformed compound but consists of randomly aggregated proteineous debris excreted in urine. Some of the proteineous compounds in matrices can be UPE F1, as indicated by immunoelectrophoresis (Fig. 1). However, the role of the UPE F2 fraction in the matrix can be only speculated, because its small molecular size renders it nonantigenic.

According to the high proportion of glycine and appreciable amount of hydroxyproline (Table II), part of the F2 fraction can be derived from connective tissues or collagen. The F2 fraction also contains large amounts

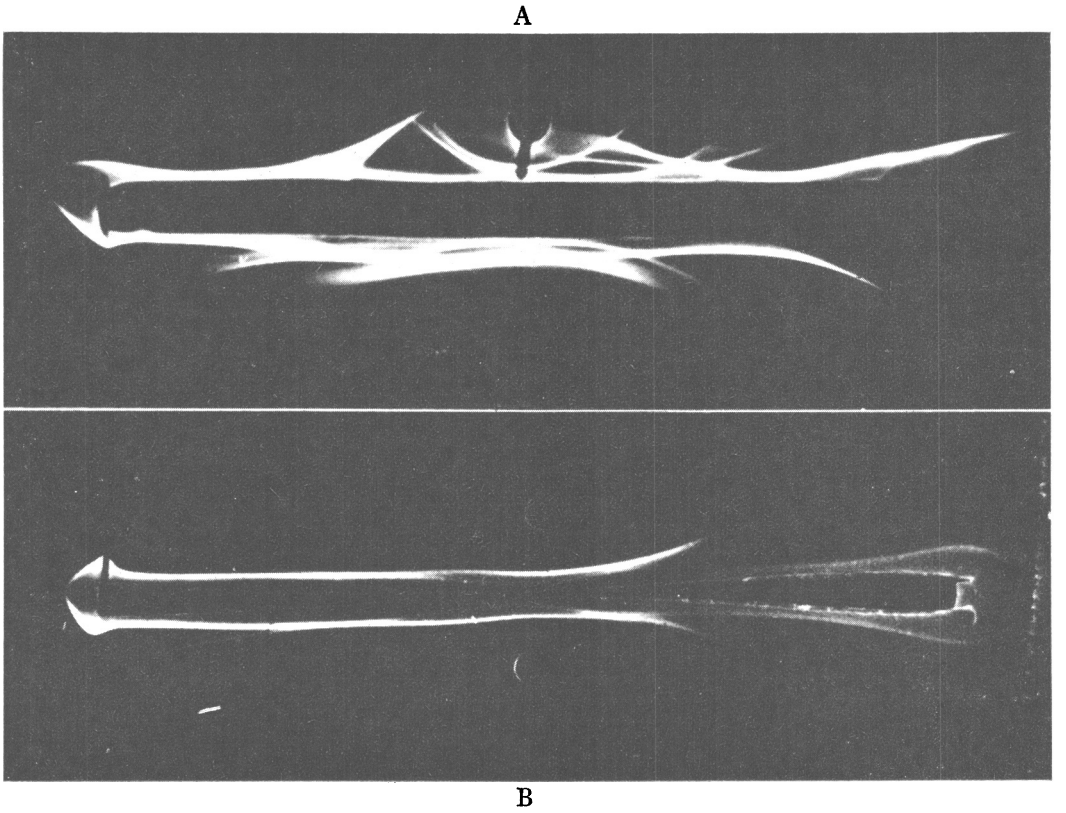

FIG. 1. Immunoelectrophoresis: (A) center trough, antisheep serum; top, sheep serum; bottom, urine polyelectrolytes (UPE F1) of semipurified ration. (B) Center trough, anticalculi serum; top and bottom, UPE F1 of semipurified ration.

of aspartic acid and glutamic acid. This suggests that F2 is composed of different kinds of molecules. One kind are segments of collagen, while another kind are molecules containing large amounts of aspartic acid and glutamic acid.

Since calculi matrices do not always contain a high quantity of glycine and hydroxyproline, the part of F2 derived from collagen presumably is not directly associated with calculus matrix. The observation that collagen does not readily seed crystal formation appears to support this interpretation (13). However, the presence of large amounts of aspartic acid and glutamic acid containing molecules of F2 can encourage aggregation of polyelectrolytes in urine (8). Because of their small molecular size, these molecules have their active groups exposed. Thus, the carboxyl groups of aspartic acid and glutamic acid can be readily available for cation binding. In previous studies, we have found that UPE can associate among/between the high-molecular-weight molecules (F1) and the low-molecular-weight molecules (F2) in the presence of divalent cations (8). We have also found that UPE can provide the primary binding sites to the divalent cations and can result in the formation of UPE–calcium/magnesium–phosphate complex (9). Therefore, the participation of the aggregated urine polyelectrolytes F1 and F2 in the formation of calculus matrix is feasible.

Summary. The amino acid composition of the calculus matrix of various types from different species and the two fractions of urine polyelectrolytes were analyzed by gas chromatography. All matrices and the urine polyelectrolytes had more acidic amino acids than basic amino acids, and, thus, are

negatively charged. Nevertheless, there were variations of amino acid compositions between individual calculi and among calculi from different types and species. Apparently, calculus matrix is not composed of preformed compounds.

Immunoelectrophoretic tests revealed that part of the calculus matrix was common to the high-molecular-weight urine polyelectrolytes. Because of the readily available carboxyl groups of aspartic acid and glutamic acid in the small-molecular-weight urine polyelectrolytes for cation binding, they could initiate the aggregation of the charged molecules. Therefore, the participation of the aggregated urine polyelectrolytes, including the high- and the low-molecular-weight fractions, in the formation of calculus matrix is feasible.

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