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Biochemical and quantitative analysis of Tamm Horsfall protein in rats

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Abstract The involvement of Tamm Horsfall protein (THP) in nephrolithiasis is currently under investigation in several laboratories. Although rat is a commonly used species as an in vivo model for such studies, there is only limited information available about the biochemical properties and excretion profile of THP in normal rats. In order to characterize rat THP, we purified and analyzed normal male rat THP, and compared it with normal human male urinary THP by gel electrophoresis. Both THPs migrated at approximately 90 KDa, and stained similarly for protein (Coomassie blue) as well as carbohydrates (periodic acid Schiff reagent). Compositional analysis revealed that rat THP was largely similar to human THP in amino acid and carbohydrate contents but showed differences in the individual sugar components from other mammals. There was considerable variation in the day-to-day urinary excretion of THP in normal rats, with values ranging from 552.96 μg to 2865.60 μg and a mean value of 1679.54 μg per 24 h. It was concluded from this study that rat THP did not contain any unusual biochemical components and was primarily similar to human THP in composition and mean urinary concentration.

Key words Sprague-Dawley rats · Tamm Horsfall protein · Gel electrophoresis · Amino acids · Carbohydrates

Introduction

Tamm Horsfall protein (THP), the most abundant protein in human urine [14], has received considerable attention for its normal function as well as its involvement in nephrolithiasis. Recently, a comprehensive study of 48 vertebrate species by Howie et al. [12] has demonstrated by immunohistochemistry that THP, which is absent in birds and reptiles, shows some antigenic differences between various species. Since its first identification in human urine, several reports have focused on biochemical characterization [6–8] and quantification [15, 12] of urinary THP from normal subjects as well as stone formers. Furthermore, numerous studies have been performed to examine the effect of THP on calcium oxalate crystallization in vitro [11, 23]. Thus, in addition to clinical data, a substantial amount of information on THP regarding its biochemical composition, as well as functional characterization in the context with stones, has been derived from studies on human THP.

Rat is commonly used in experimental studies of calcium oxalate nephrolithiasis [16]. In our laboratory, we use male Sprague-Dawley rats as the in vivo model to study experimentally induced nephrolithiasis, specifically to investigate the participation of THP, if any, in calcium oxalate (CaOx) stone formation. Consequently, a detailed knowledge of the biochemical properties of THP from this strain, as well as its urinary concentration from normal untreated rats is vital information for our studies. Furthermore, if one compares the normal urinary composition of rats and humans, it becomes evident that rats tolerate considerably higher (threefold higher than human) oxalate levels, and yet seldom form kidney stones [16]. Therefore, it was also of interest to know if biochemically, rat THP has any unusual components, which could at least partially contribute to the rarity of spontaneous stone formation in this species.

Purification of rat THP by precipitation with sodium chloride has been reported by Hoyer et al. [13], who used the antibody made against this preparation to localize THP in rat kidney. However, the only report on partial characterization of rat THP was by Kirchner and Bichler [17], who isolated "uromucoid" from rat urine by using an affinity column made with an antibody to human THP. Further characterization by polyacrylamide disc gel electrophoresis revealed that it showed

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migration similar to human THP. Thus, in contrast to human THP, data on characterization of rat THP are extremely limited.

The urinary THP excretion in humans ranges from 20 to 100 mg/day, with 50 mg/day as the generally accepted average value [14]. In contrast, in the literature, normal rat urinary THP excretion shows a rather wide range of normal values which appear to differ between various strains of rats [1, 27]. Therefore it is difficult to assume a "normal" mean value of THP excretion, which can be used as a standard for different strains of rats.

Based on the background discussed above, the primary objective of this study was to perform detailed biochemical characterization and quantification of urinary THP from male Sprague-Dawley rats and to generate a specific polyclonal antibody to rat THP for future use. For comparison, THP from a healthy male donor was purified and analyzed in a similar manner.

Materials and methods

Urine collection from rats

Male Sprague-Dawley rats weighing 125–150 g were housed in metabolic cages and given food and water ad libitum. Urine was collected at 4°C, with 0.02% sodium azide, either separately for each rat or as a pooled sample, and stored frozen at −20°C until further use.

Purification of rat THP

THP was purified from pooled urine from male Sprague-Dawley rats by a method described by Hoyer et al. [13] with slight modifications (J.R. Hoyer, personal communication). All solutions contained 0.02% sodium azide to prevent protein degradation by bacteria. In the first cycle of precipitation, urine volume was measured and sufficient sodium chloride was added to make it 0.58 molar. THP was allowed to precipitate at 4°C for 7–10 days. The urine was then centrifuged at 7000 rpm for 25 min and the supernatant was discarded. This pellet (P1) from the first precipitation was used for all further extraction cycles. In the second cycle of precipitation, the pellet was washed once with 0.58 M sodium chloride solution in distilled water. THP was extracted from the pellet by mixing it thoroughly with water (pH 9.0) and letting it stand overnight at 4°C. It was then precipitated from the water-soluble phase by adding one-fifth volume of 3.5 M sodium chloride (final concentration 0.58 M). For the pellet P1, this sequence of extraction/precipitation was repeated four to six times until no more precipitate was formed. All precipitates were pooled and stirred overnight to obtain a homogeneous solution. The final product was then dialyzed extensively against distilled water, re-precipitated, dialyzed again, freeze-dried and stored at −20°C until further use.

Purification of human urinary THP

Urine was collected at 4°C from a healthy male volunteer over a 24 h period. THP was isolated by the method of Hunt and McGiven [14] as follows. The precipitation step included addition of sufficient sodium chloride so as to attain a molarity of 0.58 M. After stirring for 30 min at room temperature, the urine was centrifuged and the supernatant was discarded. The pellet was washed with cold 0.58 M sodium chloride (in distilled water), re-centrifuged, and the supernatant, which also contained most of the pigments, was discarded. Next, the gel-like viscous THP was dissolved in alkaline distilled water (pH 9.0), centrifuged, and the non-soluble contaminants were discarded. THP was re-precipitated from the solution and stirred overnight at 4°C to obtain a homogeneous suspension. After extensive dialysis with distilled water over 48 h, it was lyophilized and stored frozen until further use.

SDS-PAGE analysis

Electrophoresis of THP (10 μg) was performed on a 10% denaturing polyacrylamide gel according to the method of Laemmli [18]. Purity of the preparations was determined by SDS-PAGE (of 1 μg protein per lane) followed by the sensitive silver staining method by using a silver staining kit obtained from BioRad (Melville, N.Y.). The gels were stained with 0.2% Coomassie brilliant blue R250 for 10 min to visualize the proteins. The presence of carbohydrates was detected by periodic acid schiff (PAS) staining of 10 μg of THP on a polyacrylamide gel as described by Zacharius et al. [28].

Amino acid composition analysis

For amino acid analysis, after electrophoresis, the gels were blotted on to an Immobilon membrane (Millipore, USA) overnight in 10 mM MES buffer with 20% methanol (pH 6.0). The membrane was stained with 0.2% Coomassie R250 for 5 min and de-stained with 50% methanol and 10% acetic acid for 15 min. The proteins were analyzed for amino acid composition by the Protein Core Laboratory at the University of Florida, by the acid hydrolysis method.

Carbohydrate composition analysis

THP was analyzed for monosaccharide composition as follows: the trimethylsilyl (TMS) derivatives of the methyl glycoside were obtained followed by gas chromatography (GC) and then combined GC and mass spectrometry (MS). Inositol was added as an internal standard before derivatization.

Western blotting

The protocol described below was used specifically to detect THP in rat urine. The gels were blotted overnight on nitrocellulose membrane at 4°C, in TRIS-glycine buffer with 20% methanol. The membrane was blocked with 2% bovine serum albumin (BSA) in TRIS-buffered saline with 0.1% Tween-20 (TBST). After incubation with the polyclonal antibody it was washed three times (10–15 min each) with TBST. Next it was incubated with the goat anti-rabbit alkaline phosphatase conjugate (Hyclone, Logan, Utah) at a dilution of 1:5000 for 30 min. After three washes with TBST, color was developed with NBT and BCIP (alkaline phosphatase kit, BioRad) for 5–15 min.

Preparation of anti-rat THP

A polyclonal antibody to the THP isolated from male rats was made in rabbits, in collaboration with Kel Farms, Alachua, Florida. Animals were pre-bled from the ear vein (15 ml) for pre-immune sera at least 14 days prior to injection of the antigen. They were injected as follows: 300 μg of the purified THP in saline was emulsified with 0.5 ml of complete Freund's adjuvant by passing through a syringe (total volume of the mixture 1.0 ml). This mixture was injected at two sites intramuscularly and at three sites subcutaneously (0.2 ml each). Next, at 30 days, a second dose of 200 μg of the protein was injected in a similar manner. After 44 days at 2–3 week intervals, 100 μg protein was injected. Rabbits were bled periodically and the antibody titer was checked with western blotting. Finally, anti-THP antiserum was collected and stored frozen at −20°C until further use.

Characterization of antibody

Initially, a polyclonal antibody to rat THP was a kind gift from Dr John Hoyer (Children's Hospital, Philadelphia, Pa.). The newly generated antibody (described above) was compared with Hoyer's antibody by titration in ELISA for its reactivity as follows: 96-well plates were coated overnight with 1 μg/ml of purified rat THP. After blocking the non-specific binding sites with 2% BSA-PBS, either Hoyer's or the new antibody was added as twofold serial dilutions in the range of 1:5000 to 1:640 000. After appropriate incubation and washes, a second antibody (alkaline phosphatase conjugated goat-anti-rabbit-immunoglobulin G) was added and finally color was developed with the NBT-BCIP as the substrate. The plates were read at 405 nm in a microplate reader.

Antigen capture assay for quantitation of rat urinary THP

Urine was collected from rats individually in separate tubes over a 10-day period and stored frozen at −20°C until further use. Samples at 1, 4, 6, 8, and 10 days were used for the antigen capture assay. Immunoglobulin G (IgG) was purified from the anti-rat polyclonal THP antibody (described previously) by passing through a sepharose B column conjugated to protein A (Sigma, St Louis, Mo.). It was further biotinylated by the method described by Goding [10], for use as a second antibody in this assay.

A standard curve was run with each individual experiment. A stock was made from the purified protein (1 μg/ml) followed by six twofold serial dilutions (up to 0.0156 μg/ml). All washes were performed with 200 μ l PBS containing 0.2% Tween 20 and 0.02% sodium azide. Based on previous pilot experiments, three dilutions (1:320, 1:640 and 1:1280) of each urine sample were run in duplicates. Between each step, plates were washed three or four times with TBST.

Plates were coated with the purified IgG overnight at a concentration of 5 μg/ml (50 μl/well) in PBS. After washes, the nonspecific binding sites were blocked with 1% BSA (in PBS containing 0.01% azide) overnight at 4°C. Urine samples (or dilutions of purified rat THP) were added to the wells and incubated at 37°C for 30 min. After washes, biotinylated anti-rat THP was added to each well at a dilution of 1:1000 and incubated for 30 min at 37°C. Following washes, plates were incubated with a 1:1000 dilution of streptavidin conjugated to alkaline phosphatase (Fisher Scientific, USA) at 37°C for 30 min. Excess antibody was removed by washes, and the plates were incubated with the alkaline phosphatase substrate (Sigma, Calif) at 37°C for 30 min and plates were read at 405 nm in a microplate reader.

Calculations were performed with the statistical software Sigma Plot (Jandel Scientific Calif). Calculations for average daily THP excretion was based on values from 20 separate samples over 9 days (samples taken at day 1, 4, 6, 8, and 10).

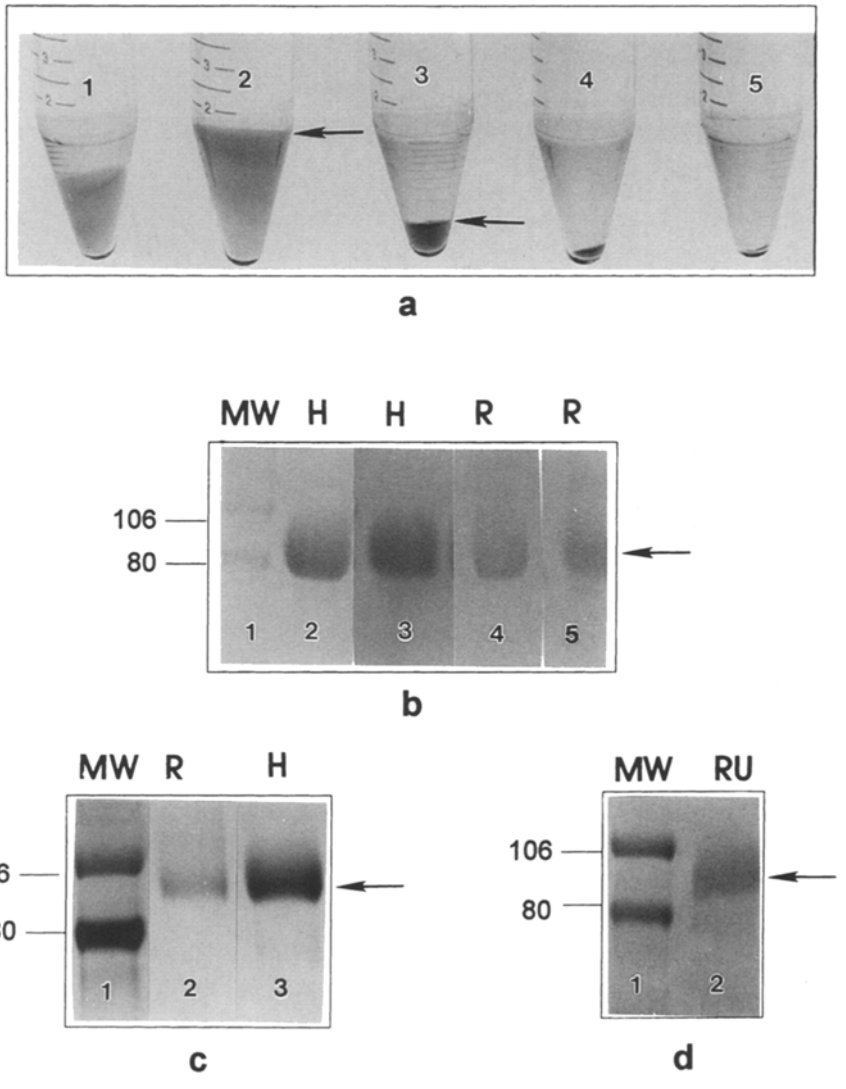

Fig. 1a–d Purification and analysis of normal rat Tamm-Horsfall Protein (THP). **a** Typical precipitation cycles (1 through 5) showing viscous gel-like precipitates (*arrows*). **b** Human and rat THP stained for protein and carbohydrates. *Lane 1* molecular weight markers (MW). *Lanes 2 and 3* Coomassie blue (protein) and PAS (carbohydrate) staining for human THP (*H*) respectively. *Lanes 4 and 5* Coomassie blue (protein) and PAS (carbohydrate) staining for rat THP (*R*) respectively. Note that rat and human proteins show similar staining. **c** A silver-stained gel showing purified rat and human THP. *Lane 1* molecular weight markers (*MW*). *Lane 2* rat (*R*) and *Lane 3* human (*H*), **d** Western blot showing the specificity of the newly made polyclonal antibody to rat THP. *Lane 1* molecular weight markers (*MW*), *Lane 2* rat urine (*RU*, 10 μl) probed with anti-rat THP antibody, showing a single band of THP (*arrow*).

Results

Purification of rat THP

The purification and characterization of rat THP is shown in Fig. 1. Typically, at least four or five precipitation cycles (Fig. 1a) were necessary to precipitate all of the THP present in the urine sample. Typically, the yield from 500 ml of pooled normal rat urine was 10–11 mg of THP.

Purification of human THP

A single precipitation with sodium chloride appeared to be sufficient to precipitate most of the THP from the human urine. Although there was probably some loss of sample during the processing, a substantial amount of THP could be isolated from a 24 h urine collection (e.g., 25 mg in one sample). On a 10% polyacrylamide gel, all THP samples showed a band at approximately 90 KDa. On western blots, sheep anti-human THP antibody (The Binding Site, Calif.), confirmed the identity of these preparations (data not shown).

Gel electrophoresis and staining for protein and carbohydrate

When stained with Coomassie blue for protein and the PAS method for carbohydrates, both human and rat THP samples showed staining of similar intensity. At this level of analysis, this indicated that the protein (blue) and carbohydrate (purple) contents of these samples were very similar (Fig. 1b).

Silver staining

Both rat and human normal male THP showed a molecular weight of approximately 90 KDa. The purity of the THP preparations was confirmed when single bands were seen in gels stained with the sensitive silver staining technique as shown in Fig. 1c.

Western blotting

The anti-rat THP antibody specifically detected THP in 10 µl of normal rat urine, when used at the dilution of 1:10,000 (Fig. 1d).

Amino acid analysis

A comparison of the amino acid analysis of human and rat THP obtained in this study and data on other mammals found in the literature is shown in Table 1. As shown in Table 1, amino acid composition of human and rat samples was found to be largely similar with minor differences. Moreover, the values generally agreed with the amino acid composition of other mammals shown. It is evident from Table 1 that acidic amino acids (aspartic and glutamic) were present in higher amounts than the basic amino acids (arginine and lysine), which are known to contribute to the low pI of THP.

Table 1 Comparison of amino acid composition of urinary Tamm-Horsfall protein (THP) (residues/molecule) from different sources: rabbit [19], human [6], and calf [26]. Values are taken from the literature for comparison with data obtained in this study

Amino acid	Human	Rabbit	Calf	Human (male)	Rat (Male)
Asp	10.90	10.17	10.32	11.24	11.43
Thr	7.63	8.96	8.83	7.34	8.06
Scr	7.86	8.13	7.65	7.44	8.54
Glu	8.44	9.79	9.80	10.71	12.82
Pro	4.26	3.99	2.52	5.93	5.46
Gly	8.41	8.56	9.05	10.50	9.39
Ala	6.79	4.89	6.76	7.08	4.82
Val	6.40	7.03	7.03	4.08	5.40
Met	2.05	1.54	1.34	1.85	1.75
Ile	2.46	3.05	3.03	2.73	3.39
Leu	7.58	7.96	9.24	8.78	8.47
Tyr	3.83	3.39	4.08	4.92	5.59
Phe	3.14	3.49	3.69	6.63	4.94
Lys	2.65	2.06	3.46	2.89	3.20
His	2.67	2.75	2.58	2.58	1.71
Arg	4.49	3.78	4.64	5.18	4.91

Carbohydrate analysis

Detailed analysis of the carbohydrate composition of rat and human THP obtained in this study and values for other mammals as found in the literature are given in Table 2. As shown in Table 2, human THP showed 18.1% of total carbohydrate complex type *N*-linked, highly branched oligosaccharides (based on the ratio of galactose to mannose) and highly sialated (based on the equal amounts of galactose and sialic acid). Normal rat THP showed a total of 19.3% carbohydrates, also with highly branched oligosaccharides. Although most of the components were similar, galactoseamine appeared to be considerably lower in the rat sample. The significance of this observation is not clear at this time.

Characterization of anti-THP polyclonal antibody

As shown in Fig. 2a, the new polyclonal antibody generated (designated UF, which was also used for the western blots shown in Fig. 1d) showed high reactivity and compared well with another anti-THP antibody.

Quantitation of rat urinary THP by antigen capture assay

The three dilutions of urine (1:320, 1:640 and 1:1280) gave comparable results (Fig. 2b). Therefore, for all

Table 2 Comparison of carbohydrate composition of normal human and rat THP obtained in this study, with other published values: human [6], rabbit [19] and calf [26]. *NR* not reported. All values are expressed as percentages. *Total* % is based on mass determination and represents total percentage of carbohydrates in a given THP sample. The percent values of the individual constituent sugars indicate their quantity in a total of 100% carbohydrate moiety. *GlcNAc* glucosamine, *GalNAc* galactosamine, *SA* sialic acid

	Total%	Glucose	Fucose	Mannose	Galactose	GlcNAc	GalNAc	SA
Human	28.0	NR	0.8	NR	NR	9.8	1.6	4.4
Rabbit	31.0	NR	<0.5	NR	NR	13.3	0.2	4.6
Calf	22.0	NR	0.9	4.63	4.82	7.36	1.38	2.93
Human	18.1	1.5	2.3	15.7	16.5	34.1	5.9	24.1
Rat	19.3	2.0	3.1	12.6	22.5	33.0	1.2	25.5

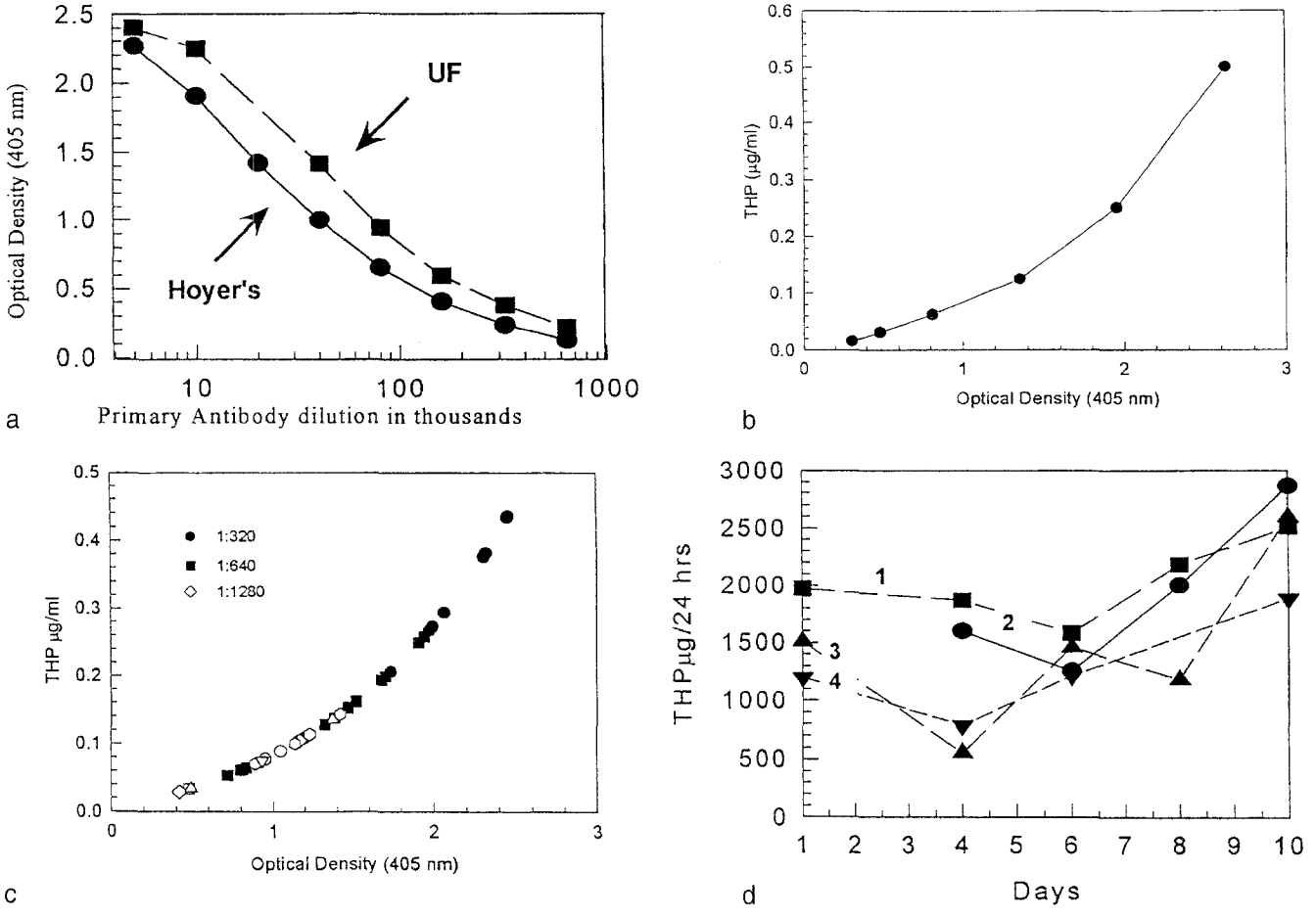

Fig. 2a–d Characterization of the anti-THP antibody and quantitative determination of rat urinary THP. **a** Determination of reactivity of the polyclonal antibody to rat THP (*UF*) by ELISA. Note that the antibody shows high reactivity and compares well with *Hoyer's* antibody. **b** Determination of optimum dilution for rat urine by antigen capture assay. All three dilutions (1:320, 1:640 and 1:1280) gave values that followed the same curve, but covered different ranges. Based on these results, to eliminate interference by other urinary factors, the highest dilution (1:1280) was selected for all further experiments. **c** A typical standard curve for antigen capture assay showing detection of purified THP in the range of 1 µg/ml to 15 ng/ml, by the polyclonal antibody (dilution of 1:10 000). **d** A graph showing daily THP excretion of four rats over a 10-day period. Note that all rats show day-to-day variation in THP excretion

calculations, the highest dilution of 1:1280 was selected as a precaution to avoid interference by other factors in the assay.

Analysis of data from 20 separate urine sample is presented as daily total excretion, excretion per ml per 24 h and per 100 gm body weight (Table 3). Over a 9-day period THP values ranged from a minimum of 552 µg/day to 2865 µg/day with a mean of 1679.54 µg/day. When converted to mg/l, based on individual urine volumes of 16.2 and 15.9 ml respectively, the urinary excretion of rat THP translated into a range of 34.5 ± 38.6 to 180 ± 38.6 mg/l. The mean THP excretion for all samples over 9 days was 101.79 mg/l, based on the average output of urine of 16.5 ml per day.

Table 3 Quantitative measurements of urinary THP excretion. As described in the text, values in the three columns (left to right) indicate daily total excretion, daily concentration per milliliter and excretion per 100 g body weight

	THP excretion (μg)/24 h	Urinary THP concentration (μg)/ml per 24 h	THP concentration (μg)/100 g body weight per ml
Mean	1679.54	101.79	46.60
Standard deviation	617.85	48.01	16.27
Minimum	552.96	36.86	14.74
Maximum	2865.60	229.24	75.16
Sample size	20	20	20

Although there was a minor variation in readings from plate to plate, standard curves from all plates were similar and consistent. An example of a typical standard curve is shown in Fig. 2c. A graphical representation of THP values of four rats are shown in Fig. 2d. As can be seen from the graph, there was considerable variation in the daily excretion of THP in a single rat over 10 days. For example, rat 3 showed a value of 1500 μg on day 1, followed by 500, 1500, 1200 and 2400 μg at days 4, 6, 8, and 10 respectively.

Discussion

Purification of THP by precipitation with sodium chloride is a commonly used technique which is based on the method originally described by Tamm and Horsfall [25]. Other methods of purification include use of antibody affinity columns [13], and chromatography [2, 24]. In the salt precipitation method, during precipitation of a protein by a salt, neutralization of surface charges by the salt leading to changes in structural conformation is the major element which results in the precipitation of a protein [4]. Therefore, the effectiveness of this method depends on the surface charges of the particular protein, as well as other factors in the milieu. During the present study, initial attempts to purify THP by using a single precipitation step with sodium chloride protocol were unsuccessful. Although the reason for this is not clear at this time, it is possible that there was an intrinsic physicochemical difference between human and rat THP, which could explain the observation that the single precipitation method was not effective for this molecule. Later in the study, based on the suggestions by Hoyer [13] (J.R. Hoyer personal communication), the protocol of several cycles of precipitation with sodium chloride and extraction with water was optimized and adopted. We found that even though an identical precipitation procedure was used for all samples, the profile of THP precipitation differed with each sample. In some samples, most of the THP precipitated in the first cycle, and in others, maximum THP precipitated in the later cycles. To precipitate all of the THP from each sample, it was necessary to repeat the cycles at least four or five times, as shown in Fig. 1a.

Homology in the coding sequences of rat and human THP has been shown by Fukuoka et al. [9]. A comparison of THP from man [6, 7] and other mammals such as rabbit [5, 19], hamster [2], and calf [26] has shown that the composition, especially of the protein component of THP, is largely comparable in these species. Similarly, we found that rat THP has an amino acid composition very similar to that of human THP, with only minor differences. Both rat and human THP contained the acidic amino acids (aspartic and glutamic) in slightly higher amounts (10%–12%) than the other constituents. However, in contrast to other urinary proteins involved in nephrolithiasis such as osteopontin, which contains 22% acidic amino acids [3], this value is not sufficiently high to classify THP as a typical acidic protein.

Analysis of the carbohydrate component of THP has revealed significant variations from species to species. This variation is not as much in the total percent of carbohydrates present, but is more pronounced in some of the individual components. For example, although total carbohydrate content of THP from the different mammals discussed above falls within a short range of 22%–31%, rabbit THP contains O-acetylated sialic acids [20] and hamster THP lacks sialic acids completely [2].

A comparison of our data on rat and human THP revealed that the overall carbohydrate content was similar, except that galactosamine was significantly lower in rats than human (1.2% vs 5.9%) THP. We found two major differences in the carbohydrate composition in the results of this study and previously published data. First, in our hands, the total carbohydrate content in the THP from the human as well as the rat samples was 18%–19%, a value significantly lower than reported for human THP earlier. Secondly, in both rat and human THP, the total sialic acid content was 24%–25%, a value significantly higher than found in the literature. Although individual components such as sialic acids have been observed to influence the biological activity of molecules like THP [2], the exact implication of such differences is not clear at this time. Nevertheless, this study confirms that the protein content of THP is remarkably similar between different species.

In a number of studies, several variations of ELISA have been used for quantitative determination of urinary THP in mammals [1, 19]. For humans, the average daily THP excretion is 20–100 mg, based on an average daily urine volume of 1–1.5 l. The values of THP excretion in mammals, specifically in various strains of rats, can be found expressed as different units. In the study by

Kirchner and Bichler [17] unidimensional agar Laurell technique revealed that the average daily THP excretion in rats (unspecified strain) was 197 ± 56 units. Wirdnam and Milner [27] used the radioimmunoassay technique and quantitated THP in serum (concentration in plasma: 43–240 ng/ml), urine and various tissues of normal Wistar rats. They used urine diluted to 1:500 in distilled water and concluded that normal excretion of THP in rats was 473 ± 34 mg/day in males and 383 ± 23 mg/day in females. In addition, there was a correlation in the amount of THP excretion and body weight in females but not in males. In a previous study, Wirdnam and Milner [27] reported that release of THP by rat kidney cortex slices into media was increased from 22.6 ± 0.6 to 30.9 ng/mg of tissue, when the sections were incubated with frusemide, a diuretic. In another study, Bachmann et al. [1] used radiolabeled antibody to determine excretion of THP in Brattleboro rats, a strain with hereditary hypothalamic diabetes insipidus. In control rats, the urinary THP excretion was 367 ± 41 μg/day per 100 g body weight. The authors concluded that rate of THP synthesis was linked to neither the process of urine concentration nor the ion transport activity in the TAL. By using a similar method, urinary THP in cats was found to be 49.2 ± 35.5 μg/ml [22].

We used a sandwich ELISA technique for accurate quantitation of rat urinary THP. In order to increase the sensitivity of ELISA, purified anti-THP rabbit IgG was used to bind the antigen as the first step, and a biotinylated form of the same polyclonal antibody to capture the bound antigen. After several experiments were performed to standardize the assay, it was determined that using highly diluted (1:1280) urine samples would eliminate interference of other urinary proteins in the assay. This approach made the assay extremely sensitive and consistent, as shown by the uniformity of standard curves obtained from all assays.

Our results showed that in urine samples taken from 20 normal rats, the daily THP excretion differed significantly. Moreover, the THP excretion for different days for one single rat was also remarkably different. A comparison of THP excretion values in other mammals with the values for rat seen in this study indicates that in general, rats excrete higher amounts of THP. This observation could be attributed to a combination of factors such as the particular strain of rats, type of assay and extreme sensitivity of the assay. We found that the amount of THP excreted by the rats was in the range of 552–2865 μg per 24 h, with a mean value of 1679.54 μg. If these values are converted to mg/l based on relevant urine volumes for the particular animal, the range of THP excretion in rats is similar to human THP excretion values (20–100 mg/1.5 l per day).

In conclusion, our results indicate that the biochemical composition as well as quantity of THP excretion in male Sprague-Dawley rats is largely comparable to that in humans. Moreover, as shown with THP from other mammals, especially the protein component of this glycoprotein is well conserved. In addition, rat THP does not contain any unusual components and is probably not the only element which contributes to the lack of spontaneous stone formation in these rats. Consequently, it can be speculated that various additional factors including other urinary proteins modulate the complex process of urinary stone formation.

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