Characterization and Localization of Human Renal Kininogen*

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Kininogen was isolated from human urine by batch adsorption with immobilized antibody to the immunologically identical heavy (H) chains of both high molecular weight (HMW) and low molecular weight (LMW) human plasma kininogens. All releasable kinin in the guanidinium chloride eluate was associated with kininogen antigen in gel filtration fractions. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the eluate gave major stained and antigenic bands corresponding to the major form of plasma LMW kininogen. Also, the staining patterns and antigenic profiles obtained upon alkaline disc gel electrophoresis of the urinary and plasma LMW kininogens were strikingly similar. When antibody to H chain was used in an indirect immunofluorescence technique, cytoplasmic staining was observed in cells of distal tubules and cortical and medullary collecting ducts of human kidneys. No fluorescence was observed using antibody to the unique light (L) chain of plasma HMW kininogen and no intact HMW kininogen was found in urine by radioimmunoassay. We conclude that the kidney is a source of urinary kininogen, while the L chain antigen in urine probably represents filtered degradation products of plasma HMW kininogen.

Several years ago we detected kininogen antigen in concentrated human urine by Ouchterlony immunodiffusion using antibody to plasma LMW® kininogen, but it was not known whether the urinary antigen represented kinin-containing protein or filtered degradation products of plasma kininogens. Subsequently, however, we showed that kinins could be generated in urine by uropepsin under acid conditions (1) and by trypsin or human urinary kallikrein under neutral conditions (2). These results clearly indicated the presence of a kinin-containing protein, which was named urokininogen (2).

Human plasma is known to contain both HMW and LMW kininogens (3) which appear to be very similar to the more extensively studied bovine plasma kininogens. Both HMW and LMW kininogens consist of heavy (H) and light (L) chains. In the case of the bovine molecules, the L chains derived from HMW and LMW kininogens show clear differences in molecular weights and chemical properties, while the H chains have similar molecular weights and amino acid compositions and are immunologically identical (4). Recent studies on human plasma kininogens show similar results: immunologically distinct L chains but immunologically identical H chains (5, 6).

It is generally believed that the kallikrein-kinin system plays an important role in renal function because kinins are potent vasoactive peptides which are both rhesus- and duretic. To support this concept, it must be assumed that kinins are generated intrarenally, an event which requires the presence of both kallikrein and kininogen. However, while the occurrence of renal kallikrein is well documented, the origin of its substrate has not been determined. Information on the nature and origin of urokininogen is, therefore, of prime importance to our understanding of the role of the kallikrein-kinin system in the kidney. We now report on the characterization of urokininogen isolated by immunoadsorption using immobilized antibody to the H chains of human plasma kininogens. This antibody was also used to localize kininogen in cells of distal nephrons of human kidneys by immunofluorescence. These data provide the first rational basis for the intrarenal generation of kinins.

MATERIALS AND METHODS

The following materials were obtained commercially: hog pancreatic kallikrein (Canada Packers Ltd., Toronto, Canada), TPCK-trypsin (Worthington), SDS-PAGE molecular weight standards and Sephadex G-150 (Pharmacia), Nα°(Amersham), and pepstatin (Peninsula Laboratories Inc., Belmont, CA). All other chemicals were of reagent or ultrapure grade.

24-h Urine Collections—Samples of urine were collected from normal individuals. Each voiding was divided into two equal portions. One portion was collected in a plastic bottle containing 10 ml of 6 × HCl and 100 μg of pepstatin, while the other portion was collected in a bottle to which no additions had been made. Between voidings the bottles were kept refrigerated. At the end of the 24-h period, total volumes were recorded. Aliquots were then taken, made 0.025% in NaCl, and frozen until assayed. The samples collected in acid/pepstatin were used to measure free and total kinin, while kininogen antigen assays were performed on the acid-free samples.

Antiserum to H Chain—A sheep was immunized with 1.84 mg of human plasma LMW kininogen B2a (7) on day 0 and boosted with 0.89 mg of B2a on day 29 by the same method used to prepare antiserum to human plasma HMW kininogen B4 (8). A large bleed on day 37 gave 900 ml of serum. Ouchterlony double diffusion in 1.0% agarose gel (9) of this serum, diluted 1:3.3, showed a single precipitin line with the immunogen (0.2 mg/ml), but two equally strong lines with normal human plasma. One of the lines showed identity with B2a, while the other line was identical with plasminogen (0.1–0.2 mg/ml). Kininogen B2a at a concentration of 5 mg/ml gave a moderately strong precipitin line with commercial rabbit antiserum to human plasminogen (Behringwerke AG), as well as with 1:3.3 diluted sheep

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1 The abbreviations used are: LMW, low molecular weight; RIA, radioimmunoassay; HMW, high molecular weight; H, heavy; L, light; GdmCl, guanidinium chloride; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TPCK, 1-p-tosylamido-2-phenyl-ethyl chloromethyl ketone; PMSF, phenylmethylsulfonyl fluoride; NaP, sodium phosphate buffer; PBS, phosphate-buffered saline.
anti-Bα2 antisera from which the antikininogen antibodies had been removed by human low molecular weight kininogen immobilized on agarose (6). To remove the antiplasminogen antibodies, 130 ml of the sheep antisera was passed slowly at 5°C and pH 7.0 through a column (3.4 x 2.7 cm) of human plasminogen covalently bound to cyanogen bromide-activated Bio-Gel A-50m agarose (Bio-Gel A-50m agarose from Bio-Rad). The plasminogen was purified from a plasma fraction by affinity chromatography using lysine-agarose (10). The filtrate gave only one precipitin line with human plasma by Ouchterlony analysis and was thus judged to be monospecific. This preparation was prepared in sheep by immunization with highly purified Bα2a-LMW kininogen (7). Radioimmunoassays—The RIA for the H chains of all human kininogens uses a procedure identical with that previously described for the L chain assay (8). The only differences are that the antibody was prepared in sheep by immunization with highly purified Bα2a-LMW kininogen, as described above, and that the immunogen was used as the standard. All values for H chain antigen are expressed as microgram equivalents of this standard.

For the L chain assay, plasma HMW kininogen (7) was used as a standard, and all values for L chain antigen are given as microgram equivalents of this standard.

Measurement of Free and Total Urinary Kinins—Free urinary kinins were measured by RIA as previously described (11). Total kinin was measured after treatment with trypsin or hog pancreatic kallikrein and generated kinins were determined by difference. To generate kinins with hog pancreatic kallikrein, urine was adjusted to pH 8.0 and centrifuged for 5 min at 1500 x g. A 2-ml sample of supernatant was mixed with 1.9 ml of 0.02 M Tris-HCl, 4 mM EDTA, pH 8.0, and 0.1 ml of a 0.1 mg/ml solution of kallikrein. The mixture was incubated at 37°C for 30 min and aliquots were assayed by RIA. Free kinins were assayed under identical conditions but with kallikrein replaced by buffer. When trypsin was used to generate kinin, urine was boiled for 6 min at pH 2.0, cooled in ice, adjusted to pH 8.0, and centrifuged as above. A 2-ml sample was incubated for 30 min at 37°C with 1.8 ml of 0.02 M Tris-HCl, pH 8.0, and 0.2 ml of a 2 mg/ml TPCK-trypsin solution in 0.1 M Tris-HCl and 0.025 M CaCl2, pH 8.0. The samples were then boiled for 6 min, cooled to room temperature, adjusted to pH 6.4, and centrifuged. Aliquots of the supernatant were assayed. Free kinin was measured in samples in which the TPCK-trypsin was replaced with buffer. Since denatured trypsin interfered considerably with antigen-antibody binding, a trypsin blank consisting of 1.8 ml of 0.02 M Tris-HCl, pH 8.0, and 0.2 ml of the TPCK-trypsin solution was also put through the incubation procedure and used to correct all samples including standards, contained an identical concentration of denatured trypsin.

Immunofinity Adsorption—Six liters of male urine were collected over a period of 3 h in a stainless steel container immersed in ice. A total of 440 ml of 0.1 M NaCl, 0.01 M NaPO4, 1 mM EDTA, 1 mM ethylene glycol bis(β-aminoethyl)ether)-N,N,N',N'-tetraacetic acid, 0.01% NaN3, and 0.4 mM PMSF, pH 8.5 (Buffer A) and 50 ml of deionized water, a procedure which took approximately 3 h. The adsorbed urokinogen was eluted at 5 to 20°C with 20 ml of 8.0 M Gdm-Cl, and 0.01 M NaPO4, pH 4.5 (Buffer B), followed by water. The Gdm-Cl eluate (35 ml) was concentrated to ~0.5 ml by pressure dialysis. In order to ensure complete removal of Gdm-Cl, the sample was then applied to a column (0.69 x 27.8 cm) of Bio-Gel P-2 and eluted with ~10 ml of the equilibration buffer, 0.25 M NaCl and 12.5 mM Na2B4O7, pH 7.2. The Gdm-Cl-free protein fraction was called H chain eluate.

SDS-PAGE—Unreduced samples were electrophoresed in 10%/v/v cylindrical polyacrylamide gels according to the method of Laemmli (12). Gels were stained overnight with 0.1% Coomassie brilliant blue R-250 in 10:50:40% acetic acid/methanol/water and destained in 7.5:587.5% acetic acid/methanol/water. Antigenic profiles for gels were approximately 5°C through Pyrex glass wool over a 1-h period. The clear filtrate (pooled urine in Table II was further cooled at 2°C and the pH was adjusted from 6.62 to 6.48 with 5 M HCl. The filtrate was then stirred vigorously for 3 h at ~3°C with a 46-ml bed volume of an immunosorbent of highly purified LMW kininogen antibodies covalently bound to Sepharose 4B (Ab α2a-agarose (7)). The adsorbent was recovered by rapid filtration through a coarse fritted glass funnel and the filtrate was retained for assay. The adsorbent was packed into a column (4.2 x 3.0 cm) and washed at 5°C with 235 ml of 0.15 M NaCl, 0.01 M NaPO4, 1 mM EDTA, 1 mM ethylene glycol bis(β-aminoethyl)ether)-N,N,N',N'-tetraacetic acid, 0.01% NaN3, and 0.4 mM PMSF, pH 8.5 (Buffer A) and 50 ml of deionized water, a procedure which took approximately 3 h. The adsorbed urokinogen was eluted at 5 to 20°C with 20 ml of 8.0 M Gdm-Cl, and 0.01 M NaPO4, pH 4.5 (Buffer B), followed by water. The Gdm-Cl eluate (35 ml) was concentrated to ~0.5 ml by pressure dialysis. In order to ensure complete removal of Gdm-Cl, the sample was then applied to a column (0.69 x 27.8 cm) of Bio-Gel P-2 and eluted with ~10 ml of the equilibration buffer, 0.25 M NaCl and 12.5 mM Na2B4O7, pH 7.2. The Gdm-Cl-free protein fraction was called H chain eluate.

\[ \text{TABLE I} \]

| Urokinogen in 24 h urines | Sample | \( \mu g/24 \text{h} \) |
|-------------------------|--------|-----------------|
|                         | H chain antigen | L chain antigen | Free kinin | Total kinin |
| Male                    |        |                |            |            |
| 1                       | 1500   | 160            | 5.6        | 9.8        |
| 2                       | 2500   | 210            | 19.2       | 11.0       |
| 3                       | 1300   | 240            | 7.0        | 8.0        |
| 4                       | 1000   | 440            | 7.0        | 8.4        |
| 5                       | 1400   | 380            | 9.8        | 11.8       |
| Mean ± S. E.            | 1560 ± 270 | 306 ± 52      | 7.9 ± 0.9  | 9.8 ± 0.7  |
| Female                  |        |                |            |            |
| 1                       | 2500   | 310            | 7.0        | 10.8       |
| 2                       | 1700   | 280            | 11.4       | 14.6       |
| 3                       | 1200   | 340            | 8.0        | 10.2       |
| 4                       | 900    | 180            | 5.2        | 6.4        |
| 5                       | 220    | 9.4            |            | 10.6       |
| Mean ± S. E.            | 1575 ± 390 | 270 ± 28      | 8.2 ± 1.0  | 10.5 ± 1.3 |

*Expressed as microgram equivalents of LMW kininogen standard.

*Expressed as microgram equivalents of HMW kininogen standard.

\[ \text{FIG. 1. Sephadex G-150 gel filtration of pooled human urine.} \]

\[ \text{Column, 1.6 x 91 cm; equilibration buffer, 0.01 M Tris-HCl, 0.15 M NaCl, and 1 mM EDTA, pH 7.4; sample volume, 2 ml; flow rate, 7.4 ml/h; fraction volume, 1.85 ml. Column was calibrated with ferritin, catalase, aldolase, hemoglobin, chymotrypsinogen, and ribonuclease as standard proteins.} \]

\[ \text{FIG. 2. Sephadex G-150 gel filtration of H chain eluate. Conditions were as described for Fig. 1. Sample, 0.35 ml (0.94 A_280 unit) of H chain eluate diluted to 2 ml.} \]
obtained using duplicate, unstained gels which were frozen on dry ice and sliced into 2-mm sections with a Bio-Rad slicer system. Each slice was homogenized in 0.1% Tris- HCl, 0.025% NaCl, and 0.1% bovine serum albumin, pH 7.4, and left overnight at 4°C. The supernatants were then assayed for kininogen antigen. A different duplicate gel was used to assay for each antigen.

Alkaline-PAGE—Discontinuous alkaline-PAGE was performed in 7% (w/v) polyacrylamide gels according to the method of Davis (13). Gels were stained or sliced and assayed for antigen as described above.

Immunofluorescence—Three fresh human kidneys, removed surgically for treatment in each case of a single localized tumor, were fixed by renal arterial perfusion with buffered ethylene glycol tetraacetate solution (0.01 M NaPi, 0.15 M NaCl, and 36.6 mM ethylene glycol tetraacetate, pH 7.4) followed by 95% ethanol at 22°C (14). Small sections of kidney were processed for paraffin embedding according to the method of Saint-Marie (15). Sections, 4 μm in thickness, were deparaffinized, placed in phosphate-buffered saline (PBS), treated with PBS, pH 7.4. For indirect immunofluorescence studies, sections were treated with either the sheep antisera to the H chains of both HMW and LMW human plasma kininogen described above or purified antibody to the unique L chain of HMW plasma kininogen (8), each diluted 1:10, followed by rabbit anti-sheep y-globulin conjugated with fluorescein isothiocyanate (Melyo Laboratories, Springfield, VA). The slides were examined with a Leitz fluorescence microscope equipped with a UV epi-illuminator system, cube A (BP 340–380 exciting filter, LP 430 suppression filter), and 200-watt mercury lamp. Normal sheep serum, diluted 1:10, and PBS alone were used as controls.

RESULTS

Validation of Radioimmunoassays for Urine—The assay for H chain is capable of detecting a minimum dose of 170 pg of antigen/tube using a bound per cent (B/Bo) of 96 ± 1 (mean ± S. D.) as the limit of detectability. All human plasma kininogens (7) were immunologically identical with the standard and were equipotent on a molar basis. Bradykinin did not cross-react. Human urine contained H chain antigen and cross-reacted in a manner parallel to the standard curve. In six experiments, recovery of standard B2a-LMW kininogen added to urine was 102 ± 4% (mean ± S. E.). The intra-assay coefficient of variation (S.E./mean) was 2.5% (n = 6), while the interassay coefficient of variation (n = 6) was 5%.

Urine also contained antigenic L chain which cross-reacted in a manner parallel to the standard curve for the L chain RIA over the range of dilutions tested. In eight experiments, recovery of standard HMW kininogen added to urine was 100 ± 4% (mean ± S. E.). Since urine cross-reacted in both assays in a manner parallel to the standard curves, the RIAs could be used to quantify urinary kininogen antigens.

Studies on 24-h Urines—The data obtained when 24-h urines were analyzed for their content of H and L chain antigens, as well as for free and total kinin, are shown in Table I. No appreciable sex difference was seen for any antigen. The daily excretion of H chain antigen is about 1600-μg equivalents, while about 300-μg equivalents of L chain antigen are excreted in 24 h. The mean of daily free kinin excretion was about 8 μg and treatment of urine with trypsin or kallikrein released about 2 μg of additional kinin.

Gel Filtration and Affinity Adsorption Studies—The antigenic profiles obtained when a sample of pooled urine was gel filtered on Sephadex G-150 are shown in Fig. 1. The H and L chain profiles parallel each other fairly well with each showing a major peak with apparent molecular weight of 130,000. In addition, a peak of L chain antigen was eluted very close to the total volume of the column and was too small for molecular weight determination. Unfortunately, the fractions contained levels of kinin which were too low to be measured accurately. A concentration procedure was obviously needed. To achieve this concentration and to obtain highly purified urokininogen, an immunoadsorption procedure was used. When the H chain eluate was gel filtered (Fig. 2), the profile for releasable kinin paralleled that for kininogen antigen, appearing as two peaks with apparent molecular weights of 290,000 and 135,000.

Results for the immunoadsorption procedure are given in terms of antigen per liter of pooled urine (Table II). Batch adsorption with AbH-chain-agarose gave an eluate containing about 70% of the H chain antigen from urine and about 30% of the L chain antigen. In absolute terms, the H to L chain ratio was about 16. Recovery in eluate plus filtrate was greater

![Table II](image-url)

| Fraction | Volume | Protein | H chain antigen | L chain antigen |
|----------|--------|---------|----------------|----------------|
| Pooled urine | 1000 | 775 (100) | 102 (100) |
| H chain eluate | 0.43 | 1.03 | 535 | 69 |
| Filtrate | 1000 | 132 | 17 |

![Fig. 3. SDS-PAGE of H chain eluate](image-url)

![Fig. 4. SDS-PAGE of H chain eluate: antigenic profile](image-url)
than 80% for both antigens. Recovery data for releasable kinin are difficult to give accurately since levels in the filtrate were too low to be reliably measured. The H chain eluate, however, was a major source of releasable kinin, containing 30–35% of the levels in pooled urine.

**Electrophoretic Studies**—When SDS-PAGE was performed on the H chain eluate (Fig. 3), three strongly stained bands were seen corresponding to molecular weights of 88,000, 67,000, and 61,000. In addition, there were several weaker bands in the range 20,000–30,000 and one very narrow band immediately behind the dye front which was too small for molecular weight determination. When the stained band pattern was compared to the antigenic profiles obtained with duplicate gels (Fig. 4), all visible bands were associated with some antigenic activity. The 67,000/61,000 doublet was the major antigen-containing region and represented about 35% of the total H chain antigen in the slices.

Alkaline gel electrophoresis of the H chain eluate produced a stained band pattern which was strikingly similar to that seen for plasma B2α-LMW kininogen (Fig. 5). The antigenic profile for B2α-LMW kininogen (Fig. 6A) showed that virtually all of the H chain antigen was associated with the three major bands and, as expected, negligible L chain antigen was observed. The antigenic profile for the H chain eluate (Fig. 6B) was again very similar to that for LMW kininogen, with the H chain antigen associated with the three main bands. The major difference was that L chain antigen was also detected and closely paralleled the H chain profile, but the levels of L chain in the slices were much lower than H chain levels.

**Immunofluorescence**—Indirect immunofluorescence studies with antibody to H chain revealed diffuse staining of distal nephrons including the distal tubules (Fig. 7, A and B) and the cortical (Fig. 7, A and C) and medullary (Fig. 7D) segments of the collecting ducts. Kininogen generally was localized uniformly throughout the cell cytoplasm and nuclei were free of staining and appeared as clear circular profiles (Fig. 7D). Occasionally, kininogen was localized in glomerular basement membranes (Fig. 7B) but proximal tubules and loops of Henle revealed no fluorescence. Adsorption of the antiserum with H chain prior to incubation with tissue slices blocked the fluorescence. Indirect immunofluorescence studies using antibody to L chain revealed no fluorescence in glomeruli or any tubular segment of the nephron.

**DISCUSSION**

Although considerable interest has been focused on the kallikrein-kinin system in the kidney, no clear physiological role has yet been determined. Human urine contains kallikrein as well as its inactive zymogen, prokallikrein (16), and urinary kallikrein is derived predominantly from the kidney (17). Both stopped-flow studies in the dog (18) and immunohistochemical studies in rat (19, 20) have localized kallikrein in the distal nephron. It is difficult, however, to suggest any role for the kallikrein-kinin system in the kidney unless it can be shown that the enzyme has access to its natural substrate, kininogen. An important step in this direction was made when it was shown that kinin could be generated from human urine, indicating the presence of a kinin-containing protein which was named urokininogen (2).

In the present study, measurements on 24-h urines confirmed the existence of both releasable kinin and H chain antigen. In addition, urine was also shown, for the first time, to contain L chain antigen and the levels of all of these antigens were determined using radioimmunoassays.

To characterize urokininogen further, pooled urine was gel filtered. H and L chain antigenic profiles paralleled each other, with the exception of a peak of L chain antigen which was eluted close to the total volume of the column and, presumably, represents degradation products. About 70% of the antigenic activity in urine was recovered by immunoaffinity adsorption. When this eluate was gel filtered, releasable kinin tracked kininogen antigen. The observation of peaks of antigen which were eluted earlier than anything seen for pooled urine probably reflects aggregate formation caused by the Gdm-Cl procedure used to elute antigens from the immunoabsorbent. Gdm-Cl dissociates kininogen molecules, and its subsequent removal may allow dimerization of molecules or the formation of aggregates between H and L chains.
seems particularly likely since L chain has an affinity for negative surfaces (21) and can aggregate with other proteins (6, 22).

In view of the tendency for aggregate formation, it was decided to determine molecular weights under dissociating conditions using SDS-PAGE. Reducing conditions were not used since the antigenicity of H chain is lost in the presence of β-mercaptoethanol. When the H chain eluate was electrophoresed, a wide range of molecular weights was observed. All visible bands appeared to be associated with some antigenic activity, the lower molecular weight bands probably representing degradation products. It was particularly interesting that the 67,000/61,000 doublet should be the major antigenic region since intact LMW kininogen and/or its H chain shows similar molecular weights. The band at 88,000 contains the majority of L chain antigen, while the levels of H chain associated with this band are close to detection limits. The band is unlikely to represent an L chain aggregate in the presence of SDS and is too small to represent intact HMW kininogen, although it may represent some filtered degradation product of plasma HMW kininogen.

A comparison of the eluate with B2α-LMW plasma kininogen using alkaline disc electrophoresis revealed a striking similarity between both the stained band patterns and the antigenic profiles. The main difference between the antigenic profiles is that L chain antigen is also detected in gel slices from the H chain eluate. Although the H and L chain profiles do not track each other exactly, this could be because different duplicate gels were used to measure each antigen. Nevertheless, the large discrepancy between the levels of H and L chain antigen in the slices (approximately 13:1) excludes any possibility that they represent a stoichiometric ratio such as would be found in intact HMW kininogen (5, 21). In addition, the fact that H and L chain profiles do not track when the eluate is subjected to SDS-PAGE also shows that they are not part of the same molecule. Hence, while some of the L chain may be covalently linked to H chain, as in a degraded form of HMW kininogen, it seems likely that most of the L chain is loosely associated with H chain and that dissociating conditions such as those used in SDS-PAGE separate the two antigens. These data suggest that the H chain eluate is composed primarily of intact LMW kininogen and its degradation products, while the L chain antigen represents fragments of HMW kininogen which are covalently or noncovalently associated with the H chain and are thus adsorbed during the immunoaffinity step.

When the antibodies were used in an indirect immunofluorescence technique, H chain antigen was localized diffusely throughout the distal tubules as well as the cortical and medullary collecting ducts of human kidneys. L chain antigen, however, was not detected in any segment of the nephron. Using similar indirect immunofluorescence techniques with
sheep antiserum to rat urinary kallikrein, we have confirmed previous findings (19, 20) that kallikrein is localized in distal tubules and cortical and medullary collecting ducts. This indicates that the distal portion of the nephron has the substrate and enzyme to generate kinins locally within the kidney.

The occurrence of kininogen and kallikrein in tubular cells of distal nephron segments cannot be due to glomerular filtration of these substances followed by tubular reabsorption since numerous studies, including direct microperfusion experiments on isolated tubular segments (23), reveal that only the luminal aspect of the proximal tubule has the capacity to absorb protein molecules by endocytosis, a mechanism essentially absent in tubular segments distal to the proximal tubule. Moreover, following endocytic uptake by cells of the proximal tubules, proteins are transferred to lysosomes and degraded.

The present study indicates that urokininogen appears to represent intact LMW kininogen and that the kidney is a source of urokininogen. Although some of the urinary H chain antigen may be derived from filtered kininogen, the levels of H chain in urine are approximately 5-fold those which would be expected if filtration were to occur at a rate similar to that of albumin, again suggesting that the kidney is a major source of urokininogen. The L chain antigen in urine, however, was not detected in the kidney by immunofluorescence and probably consists of filtered degradation products of plasma HMW kininogen. This being the case, it would represent the first detectable protein molecule by endocytosis, a mechanism essentially absent in tubular segments distal to the proximal tubule.

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