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Cancer-Related Urinary Proteinase Inhibitor, EDC1: A New Method for Its Isolation and Evidence for Multiple Forms

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Abstract During the past several years, numerous laboratories have reported isolation and purification of proteinase inhibitors from human urine. Many of these molecules were incompletely characterized and some of them may have been artifacts in part because of harsh procedures used for their isolation. Consequently, there is disagreement and confusion regarding the biochemical characteristics of these inhibitors. We previously reported the isolation of a proteinase inhibitor, EDC1, from the urine of a leukemic patient. This molecule, M_r 30 kDa, was antigenically related to plasma inter-α-trypsin inhibitor (IATI) and inhibited the growth of a virally transformed B cell line. Immunoreactive EDC1 was also the major component of low molecular weight proteinuria observed in cancer patients. We now report a new method for the isolation of EDC1 from urine of patients with adenocarcinomas of colon and lung and melanoma and compare its partial amino acid sequence with that of HI 30, a proteinase inhibitor previously isolated from pooled normal urine by Hochstrasser et al. [Hoppe-Seyler's Z Physiol Chem 357:153–162, 1976]. Our method involves i) a batchwise cation exchange, ii) gel filtration chromatography, iii) anion exchange chromatography on FPLC, and iv) reverse phase C18 chromatography on HPLC. This method is mild and results in an overall yield of 0.4 to 1.2 mg of EDC1/liter urine. On the basis of the partial N-terminal amino acid sequence of its N terminal (38 residues) and middle regions (29 residues), EDC1 appears to be identical with HI30. Surprisingly, during this isolation procedure, another proteinase inhibitor, M_r 22 kDa, which cross-reacted with antisera to EDC1 and IATI, was also isolated. The 22 kDa molecule was a major component of the IATI related urinary molecules and was identical with the 30 kDa EDC1 in which first the 15 N terminal residues were clipped. The lower M_r inhibitor was not an artifact formed during storage or isolation procedure because the Western blot analysis of fresh cancer and normal urine revealed the 30 and 22 kDa molecules. Thus, both the 30 kDa EDC1 (or HI30) and its clipped variant, the 22 kDa molecule, are physiologic components of IATI related urinary proteinase inhibitors and excretion of both forms may be increased in patients with advanced cancer.

Key words: plasma proteinase inhibitors, urinary trypsin inhibitor, inter-α-trypsin inhibitor, tumor markers

There have been several reports regarding the isolation of proteinase inhibitors from human urine. In 1909, Bauer and Reich were the first to identify the trypsin inhibitory activity in normal urine [1]. Subsequently others also reported isolation and partial characterization of urinary molecules capable of inhibiting proteolytic activity [2–17]; Table I summarizes data on representative urinary inhibitors reported in literature. Most of these molecules are heat and acid stable and are antigenically related to the plasma inter-α-trypsin inhibitor (IATI), M_r 180 kDa. Elevated amounts of these inhibitors were noted in pregnancy and in rheumatic fever, infectious diseases, and renal dysfunction [2,6,18–20]. Only a few of the urinary proteinase inhibitors were isolated in a homogeneous form and partly or fully characterized; the amino acid sequences of these molecules are summarized in Table II. The only completely characterized urinary proteinase inhibitor is HI30. It was isolated from pooled normal urine [21] and its complete amino acid sequence was determined [22 and refer-

Abbreviations used: IATI, Inter-α-trypsin inhibitor; HPLC, High performance liquid chromatography; SDS-PAGE, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PVDF, Polyvinylidene difluoride membrane.

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TABLE I. Representative Urinary Trypsin Inhibitors Reported in Literature*

Protein	Source of urine	M_r	Characteristics	Reference
1. UTI	Normal	16 K	Anticoagulant; pI 2.8	J Biol Chem 213:655, 1955
2. UTI	Pregnancy	70 K	Antigenically related to IATI	J Lab Clin Med 79:491, 1972
3. HC protein	Tubular proteinuria	30 K		FEBS Lett 70:239, 1976
4. UTI	Normal	67 K	pI 2.8	Thromb Res. 11:747, 1977
5. α1-Microglobulin	Plasma cell leukemia	29 K	Antigenically related to IATI; pI 4.8	Biochem. 17:2815, 1978
6. UTI-A and UTI-B	Pregnancy urine	50 K, 33 K, and 24 K	Antigenically related to IATI;	Biochim Biophys Acta 580: 154, 1979
7. UTI-I and UTI-II	Normal			Invest Urol 17:465, 1980
8. UTI-I and UTI-II	Normal	67 K and 23 K	Antigenically related to IATI	Enzyme 26:122, 1981
9. UTI	Multiple myeloma	20 K	Antigenically related to IATI	Biochim Biophys Acta 667: 303, 1981
10. UTI 68	Normal	68 K	Cleaved to two proteins of M_r 49 K and 25 K by trypsinolysis	J Biochem 91:1391, 1982
11. UTI-A and UTI-B	Pregnancy	44 K and 22 K	Antigenically related to IATI. Inhibits human granulocyte elastase.	Hoppe-Seylers Z. Physiol Chem 363:1167, 1982
12. H-UTI	Normal			Biochem Biophys Res Commun 109:1247, 1982
13. UTI	Normal	44 K	Antigenically related to IATI	Biochem Med 27:56, 1982
14. TATI	Ovarian cancer	6.2 K	Not antigenically related to IATI; pI 5.8; Identical with human pancreatic trypsin inhibitor	J Biol Chem 257:13713, 1982
15. UTI	Premature neonates	33 K	Dimer of identical peptide chains	Biochem Med 27:168, 1982
16. UTI	Normal	26 K and 32 K	Antigenically related to IATI	Biol Chem Hoppe-Seyler's 68:47, 1987

*Does not include EDC1 (reference 26) or HI30 (reference 22).

ences cited therein; see Table 2]. Its cDNA sequence was found to contain an mRNA encoding not only for HI30 but also for α1-microglobulin [23,24]. HI 30 contained two Kunitz type inhibitory domains in tandem and was re-named Bikunin in 1989 [25].

Previously, we reported the isolation of a proteinase inhibitor, M_r 30 kDa, from the urine of a patient with acute myelogenous leukemia [26]. This heat and acid stable glycoprotein, labeled EDC1 after the name of the patient from whose urine it was first isolated, was antigenically related to plasma IATI and was a competitive inhibitor of trypsin, chymotrypsin, elastase, and plasmin [27,28]. Immunoreactive EDC1 was a major component of the low molecular weight proteinuria encountered in patients with different types of neoplasia and its excretion correlated with tumor burden [29,30]. Interestingly EDC1 stimulated growth of endothelial cells [31] and inhibited the growth of a virally transformed B cell line [32].

This paper reports a mild method for the isolation of a pure and homogeneous preparation of EDC1 in excellent yield from urine of patients with malignant melanoma and with adenocarcinomas of colon and lung. Primary structure of the cancer urine derived EDC1 and the normal urine derived HI30 is also compared. Interestingly, we find that while the M_r and amino acid sequence of the two molecules are identical, the major component of IATI-related

TABLE II. Amino Acid Sequence of Urinary Trypsin Inhibitors Reported in Literature

HI30 [references 22 and 24]	1 2 3 4 5 6 7 8 9 10 Ala. val. leu. pro. gln. glu. glu. glu. gly. ser.* 11 12 13 14 15 16 17 18 19 20 gly. gly. gly. gln. leu. val. thr. glu. val. thr 21 22 23 24 25 26 27 28 29 30 lys. lys. glu. asp. ser. cys. gln. leu. gly. tyr. 31 32 33 34 35 36 37 38 39 40 ser. ala. gly. pro. cys. met. gly. met. thr. ser. 41 42 43 44 45 46. 47. arg. tyr. phe. tyr. asn. gly*. thr. .....(entire sequence of 143 amino acids reported)
HC protein	Gly. pro. val. pro. X. pro. pro. asp. asp. ile.
Identical with number 3 and 5 of table 1. Also identical with α1-microglobulin	gln. glu. asp. X. phe. leu. ser. arg. ile. tyr. glu. arg. trp. (Only partial amino acid sequence reported as shown)
UTI_c (number 16 of Table I)	Ala. val. leu. pro. gln. (Only partial amino acid sequence reported as shown. Corresponds to residues 1 through 5 of EDC1/HI30)

The underlined residues correspond to the known sequence of 22 K molecule in Table IV.

proteinuria is not the 30 kDa molecule but its derivative with M_r 22 kDa.

MATERIALS AND METHODS:

Materials

All chemicals, enzymes, and reagents were of highest purity available from BioRad, Richmond, CA, Sigma Chemicals Co., St. Louis, MO, Fisher Scientific Co., Atlanta, GA, and Scientific Products, Atlanta, GA. HPLC solvents were from J. T. Baker, Phillipsburg, NJ. Antiserum to EDC1 was available in our laboratory [26b,27], and antiserum to IATI was obtained from Behring Biochemicals (LaJolla, CA). All studies related to human subjects were approved by the Emory University School of Medicine Human Investigations Committee and appropriate informed consent was obtained.

Methods

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyses were performed according to our previously published procedures [33]. Amino acid sequencing was performed using Edman degradation technique according to Hunkapiller et al. on a gas phase microsequencer with an on line HPLC system for identification and quantification of the amino acid derivative [34]. Also the amino acid sequences of certain proteins or their fragments were analyzed according to the procedure of Matsudaira [35]. The molecule was analyzed on an SDS-PAGE gel, eluted to a PVDF membrane, and stained. The band corresponding to the molecule was excised and sequenced as described above. Antitryptic activity and protein concentrations were assayed according to the published procedures [28,29].

Isolation and Purification of EDC1

Twenty-four hour urine was collected from patients in plastic jars containing 50 ml of acetic acid, stored at 4°C, and usually processed further within 24 h of collection. The isolation of EDC1 involved the following steps.

Concentration of urine and batchwise cation exchange. One 24 h urine collection was mixed with about 20 g of Biorex cation exchange resin and the suspension was adjusted to pH 3.0 with 1 M acetic acid. The slurry was shaken gently at 4°C for 16 h. The supernatant had no significant antitryptic activity and was, therefore, discarded. The resin was next suspended in sodium acetate buffer and its pH was adjusted to 8.0. The supernatant fluid was dialyzed at 4°C against 0.1 M acetic acid for 72 to 96 h, and then lyophilized.

Gel filtration. The lyophilized material was chromatographed at 4°C on a precalibrated AcA 44 column using 0.1 M acetic acid as an eluant and fractions with antitryptic activity were ana-

lyzed by SDS-PAGE. Their total protein concentration was also assayed.

Fast protein liquid chromatography with anion exchange column. The above material was chromatographed on a mono Q column (Pharmacia, Piscataway, NJ) using a linear gradient of 60 min formed by solvent A (0.25 M Bis-Tris buffer, pH 6.5) and solvent B (solvent A plus 1.0 M NaCl). Antitryptic activity was detected in fractions eluting at the NaCl concentrations of ~0.05 M NaCl and ~0.4 M NaCl. These fractions were collected and analyzed by SDS-PAGE. The first fraction had the M_r of 30 kDa while the second fraction was a doublet with M_r 22 kDa and 20 kDa.

High performance liquid chromatography using a reverse phase column. EDC1, eluting as 30 kDa material with 0.05 M NaCl in the previous step, was next chromatographed by HPLC on a C-18 reverse phase column using a linear gradient of 40 min formed by mixing solvent A (0.05% trifluoroacetic acid in water) with solvent B (0.05 percent trifluoroacetic acid in acetonitrile). The material collected at ~40 percent acetonitrile was rechromatographed and analyzed by SDS-PAGE and its antitryptic activity and protein concentration were assayed.

SDS-PAGE and Western Blot Analysis of Urine

Aliquots of fresh 8 h collection urine from normal and cancer subjects were dialyzed against 0.1 M acetic acid at 4°C for 6 h, lyophilized, and reconstituted in a small volume of water. Aliquots of reconstituted urine were electrophoresed by SDS-PAGE and electroeluted to a nitrocellulose membrane as described previously [33]. The membrane was washed with a gelatin solution, treated with rabbit antiserum to IATI, washed, treated with anti-rabbit gamma globulin linked to the horseradish peroxidase (second antibody), and then with 4-chloronaphthol [33].

RESULTS

Isolation, Purification, and Characterization of EDC1

Table III summarizes the data on purification of EDC1 from cancer urine at various steps of the schema. Figure 1 shows the results of the SDS-PAGE analyses of antitryptic fractions at various stages of purification. Lane 2 shows the analysis of the material obtained after gel filtration and lane 3 is the 22 kDa/20 kDa doublet isolated after the anion exchange chromatography. Lane 4 shows the purified EDC1 which electrophoresed as a single band of M_r 30 kDa and eluted as a sharp peak on C18 reverse phase chromatography (Fig. 2). The 30 kDa EDC1 was isolated from urine of four different patients with yields ranging from 0.4 to 1.2 mg/liter. Surprisingly, a major component of the proteinase inhibitor was a doublet with an M_r of 22/20 kDa (Fig. 1; lanes 2 and 3; yield 2 to 6 mg/liter urine). This material, like the 30 kDa EDC1, was a proteinase inhibitor and cross reacted with antisera to EDC1 and IATI and could be separated from the 30 kDa EDC1 only by anion exchange chromatography on a mono Q column on FPLC. The quantification of EDC1 by antitryptic assays reported in steps 1 and 2 in Table III, therefore, included the 30 kDa material along with the 22 kDa/20 kDa doublet; the amount of EDC1 in steps 3 and 4 includes only the 30 kDa molecule. The homogeneity of EDC1 in each batch was established by the following criteria: i) The molecule moved as a single band on SDS-PAGE using silver stain (up to 12.0 μg as in lane 4 of Fig. 1) and Western blot analysis using antiserum to EDC1 and commercial antiserum to IATI as probes; ii) It eluted as a single sharp peak on gel filtration, ion exchange, and reverse phase chromatography; and iii) It released a single amino acid residue/cycle by Edman degradation on gas phase microsequencer for up to 38 cycles (see the next subsection). EDC1 was considered homogeneous and pure

TABLE III. Stepwise Yield of EDC1/Liter Urine During its Isolation

Step[a]	Amount of protein (mg)	Amount of EDC1 (mg)	Percent purity
1. Extraction from Biorex cation exchange adsorption	24	8	33
2. Gel filtration	10	4	40
3. Anion exchange Mono Q chromatography	1.6	1.4	90
4. Reverse phase	0.9	0.9	100

[a]The amount of EDC1 in steps 1 and 2 is estimated on the basis of the trypsin inhibitory capacity of the fractions [28], the amount of protein in all steps by the BioRad dye binding assay [29]. For steps 1 and 2, the amount of EDC1 includes both the 22 K and 30 K molecules. For steps 3 and 4, the amount of EDC1 reflects only the 30 K form. See text.

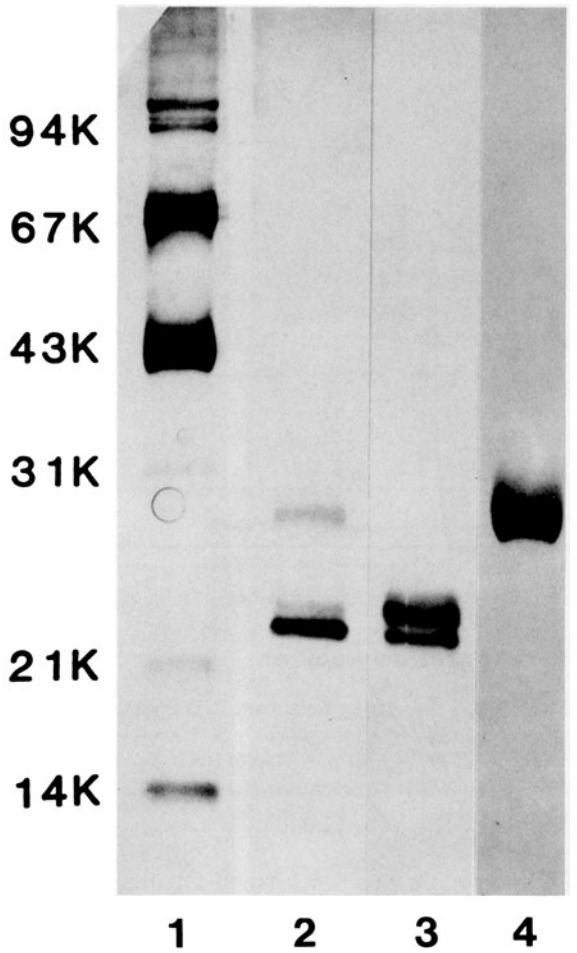

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of fractions obtained during purification of EDC1. A 16 cm × 15 cm × 0.75 mm gel was used (3% stacking and 9% resolving) under reducing conditions. **Lane 1** is the standard mixture of proteins. **Lane 2** is the material after gel filtration, **lane 3** is the 22 K/20 K doublet after Mono Q ion-exchange chromatography, and **lane 4** is the purified EDC1 (12 μg) after the final purification on HPLC. Samples were electrophoresed at 11 mA constant current in stacking gel and at 22 mA in the resolving gel at 15°C until the tracer dye was within 1 cm of the lower edge. The gel was treated with a mixture of methanol, acetic acid, and water and then silver stained according to manufacturer's instruction.

within these criteria. Additional chromatography of the 22/20 K doublet by HPLC or FPLC did not resolve the mixture into its components.

Amino acid sequence of the 30 kDa EDC1, the 22/20 kDa doublet, and a major fragment obtained by proteolytic fragmentation of the 30 kDa EDC1. The results of the N-terminal amino acid sequence of EDC1 (38 residues) are in Table IV. The PTH derivatives were identified by an on-line reverse phase HPLC. The molar yields of residues ranged from 88 to 17%, the lower yields noted toward the end of the analysis. This sequence was identical with the first 38 residues reported for HI30 [22]; Table II.

In another experiment, EDC1 was reduced with Cleland reagent, alkylated with 4-vinylpyridine (personal communication Guy Salvesen, University of Georgia, Athens, GA) and digested with Staph aureus V8 protease. The digest was analyzed on SDS-PAGE and then blotted on a PVDF membrane according to the procedure of Matsudaira [35]. A major band, corresponding to M_r of ~12 kDa, was excised and sequenced. Its partial amino acid sequence which corresponds to residues 70 to 98 of HI30 is shown in Table IV. The N-terminal amino acid sequences of the 22 kDa and 20 kDa bands were also determined according to the procedure of Matsudaira [35] and are shown in Table IV. These sequences correspond to that of the 30 kDa EDC1 from which the N terminal residues 1 through 15 (22 K band) and 1 through 18 (20 kDa band) have been clipped.

Western Blot Analysis of Urine

Figure 3 shows the results of this analysis of urine from cancer and normal subjects. Silver staining the acrylamide gel after its elution to the nitrocellulose membrane revealed that >90 percent of protein loaded on the gel had eluted to the nitrocellulose membrane. Lanes 1 and 2 show the analysis of normal urine and lanes 3 and 4 show the analysis of urine from cancer subjects. In all samples, the 22 kDa band was predominant. The 30 kDa band was detectable in the cancer urine but was faint in the normal urine even after loading a five fold excess of normal urine on the gel.

DISCUSSION

Most of the trypsin inhibitory activity in urine is attributed to the excretion of glycoproteins antigenically related to plasma IATI, although a small part of the activity is ascribed to urinary excretion of α-1 proteinase inhibitor—especially in patients with renal dysfunctions—and to pancreatic secretory trypsin inhibitor [15,36]. The IATI related antitryptic activity is acid and heat stable while that associated with other inhibitors is heat labile. Our interest in urinary proteinase inhibitors arose as a result of our observation of low molecular weight proteinuria in

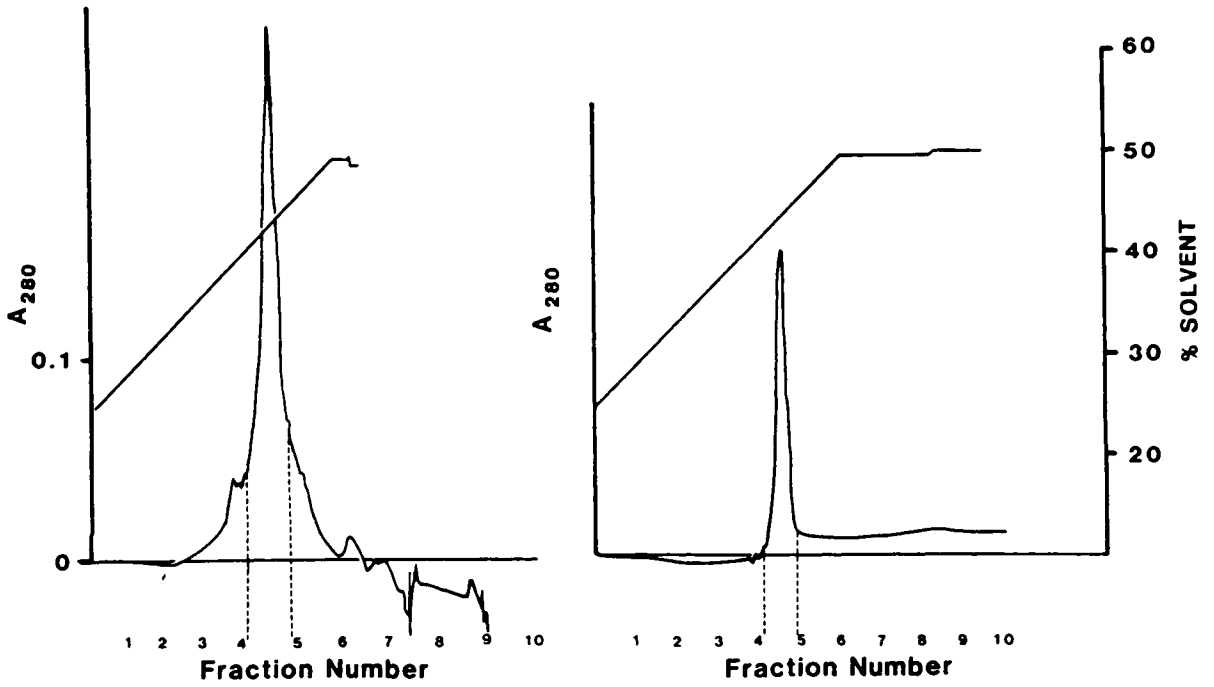

Fig. 2. HPLC (C18 reverse phase) of the 30 K EDC1 obtained after FPLC ion-exchange treatment. The sample was desalted and chromatographed using the solvent gradient described in the text. Four minute fractions (3.2 ml) were collected and tested for their trypsin inhibitory capacity and immuno reactivity against antiserum to EDC1. Fraction obtained in the left panel was rechromatographed as shown in the right panel. The vertical axis shows A_{280} at AUFS of 0.2 units; the solvent gradient is also shown. EDC1 elutes at ~ 40% solvent B.

cancer subjects [26b]. Subsequently, on the basis of its immunoactivity, we reported EDC1, M_r 30 kDa, to be a major component of this proteinuria. This glycoprotein was originally isolated from urine of a leukemic patient [26b] by a procedure involving dialysis, repeated gel filtration (repeated three to four times) and ion-exchange chromatographic analyses (two to three times). This procedure, although mild, was laborious and resulted in poor yields: < 0.1 mg/liter urine. The new method reported here is less arduous and unlike procedures reported elsewhere [2,20] does not involve treatment with any harsh chemicals or trypsin affinity columns. Furthermore, this procedure was used to isolate the urinary inhibitor from four different cancer patients and the M_r and N terminal amino acid sequence of all molecules were identical indicating no patient to patient variations in the nature of urinary molecules.

Surprisingly, the major component of IATI-related proteinuria in health or disease is not the 30 kDa EDC1 but a molecule of M_r 22 kDa which is a truncated form of EDC1 with the first 15 residues clipped (see Tables II and IV). The 22 kDa molecule is a proteinase inhibitor and cross reacts with antisera to IATI and EDC1. This molecule was not formed during collection and/or isolation procedure since we stored urine during collection at 4°C in acid and usually processed it within 24 h of its collection using relatively mild conditions. Also, the 22 kDa molecule was not formed as an artifact during the storage of 30 kDa EDC1. When the 30 kDa material was stored at 4°C in 1 M acetic acid for up to 2 weeks and analyzed at various intervals by SDS-PAGE, its M_r did not change. That the 22 kDa molecule is a physiologic entity was further confirmed by its detection in an aliquot of fresh 8 h collection of urine (first void in the morning) from normal and cancer subjects by SDS-PAGE/Western blot analysis performed promptly using antiserum to IATI as a probe (Fig. 3). Therefore, the major component of IATI-related proteinuria in both normal and cancer subjects is the 22 kDa molecule. The 20 kDa molecule was not detected in the Western blot of fresh urine and may be an artifact formed during isolation of EDC1.

What is the relationship between EDC1 and its clipped form, the 22 kDa molecule, and other IATI-related urinary proteinase inhibitors reported in literature (Table I)? On the basis of its M_r and partial amino acid sequence, EDC1 seems

TABLE IV. Partial Amino Acid Sequence of EDC1, its Clipped Forms and Proteolytic Fragments

EDC1. First 38 N terminal residues	1. 2. 3. 4. 5. 6. 7. 8. 9. 10. *Ala. val. leu. pro. gln. glu. glu. glu. gly. ser.* 11. 12. 13. 14. 15. 16. 17. 18. 19. 20 *gly. gly. gly. gln. leu. val* X. *glu. val.* X. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. *lys. lys. glu. asp. ser.* X. *gln. leu. gly. tyr.* 31. 32. 33. 34. 35. 36. 37. 38. *ser. ala. gly. pro. cys. met. gly. met.* (Sequence of the first 38 N terminal amino acids; homologous to the corresponding region of HI30).
Peptide obtained by fragmentation of EDC1 by Staph aureus V 8 protease	1. 2. 3. 4. 5. 6. 7. 8. 9. 10. Lys. glu. cys. leu. gln. thr. cys. arg. thr. val. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. ala. ala. cys. asn. leu. pro. val. ile. arg. gly. 21. 22. 23. 24. 25. 26. 27. 28. cys. arg. ala. phe. ile. gln. leu. trp. (Corresponds to residues 70 to 98 of HI30).
22 K Fragment	1. 2. 3. 4. 5. 6. 7. 8. 9. 10. Val. thr. glu. val. thr. lys. lys. glu. asp. ser. 11. 12. 13. 14. 15. 16. 17. 18. 19. gly. gln. leu. gly. thr. ser. ala. gly. pro. (Homologous to residues 16 through 34 of EDC1 and HI30 and to sequence 1 through 9 of the inhibitor from multiple myeloma; see Table II).
20 K Fragment	1. 2. 3. 4. 5. 6. 7. 8. 9. Val. thr. lys. lys. glu. asp. ser. X. gln. (N terminal sequence corresponds to residues 19 through 27 of EDC1 and HI30).

Italicized residues of the N terminal region correspond to the corresponding region of HI30. Underlined residues correspond to N terminal sequences noted for the 22 K and 20 K molecules.

to be identical to HI30 [21] and molecule number 16 in Table I. However, its relationship with other molecules with M_r of ~30 K (Table I) cannot be established categorically because the amino acid sequence of the other molecules are not known. Nonetheless, probably peptide chains of other inhibitors with M_r ~30 K, e.g., numbers 6 and 15, are identical to EDC1/HI30. Slight differences in their reported Mr may be due to laboratory to laboratory variations or to the extent of the glycosylation of the molecules. Similarly, it may be speculated that molecules with M_r ~60 K or higher, e.g. numbers 2, 8, 10, 11, and 13, and antigenically related to IATI have the same peptide chain as EDC1/HI30 and may have more extensive glycosylation. The partial N terminal amino acid sequence of the clipped 22 kDa molecule is identical to that reported for the inhibitor isolated from urine of patients with multiple myeloma (molecule number 9 in Table I). Other low M_r inhibitors with M_r ~ 22 K are probably identical with the truncated form of EDC1. It is also of interest to point out that molecules 5 and 6 of table 1 are identical with α-1 microglobulin [24], a protein which is encoded by the same mRNA that encodes the 30 kDa molecule urinary inhibitor.

Previously, we reported large quantities of immunoreactive EDC1 in urine of patients with advanced neoplastic diseases which were generally correlated with tumor burden [29,30]. These levels declined when the patients went into remission and increased during relapse. The current studies point out that the immunoassay of EDC1 included both the 30 K EDC1 and the 22 kDa molecule and presumably the excretion of both of these molecules increased in cancer. Interestingly, our observations on elevated IATI-related proteinuria in cancer and inflammatory and infectious diseases [30] have been confirmed by recent reports by other groups in patients with different types of neoplasia, renal dysfunction, inflammatory diseases, and myocardial infarction [38–43].

Our present studies do not establish the mechanism by which the 30 kDa EDC1 and the 22

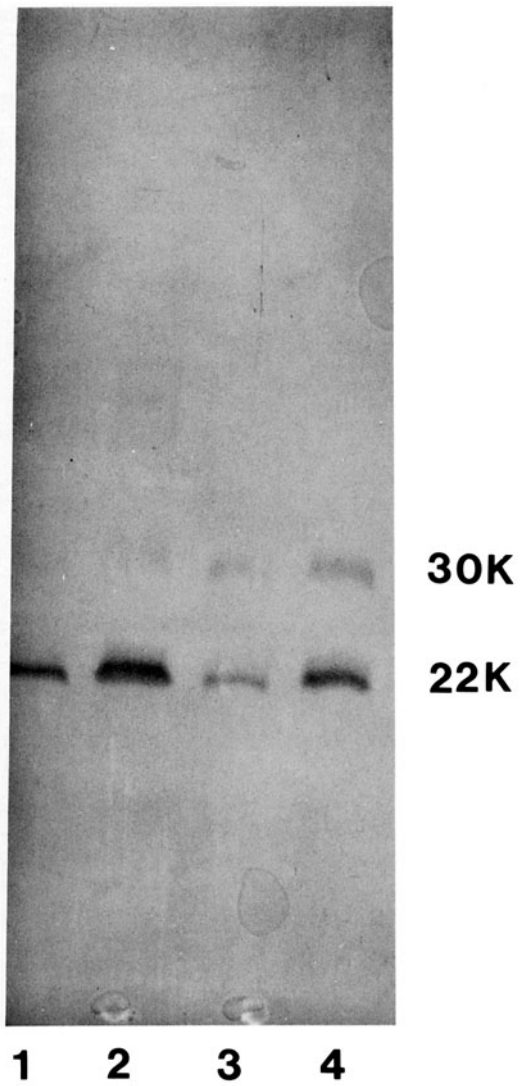

Fig. 3. SDS-PAGE/Western blot analysis of normal (**lanes 1 and 2**) and cancer (**lanes 3 and 4**) urine. Aliquots of fresh urine, 35 ml of normal and 3 ml of cancer urine, were dialyzed against 0.1 M acetic acid, lyophilized, and reconstituted in a small volume of water. Aliquots of urine corresponding to 6 ml of normal and 2 ml of cancer urine were electrophoresed and electroeluted to a nitrocellulose membrane, and visualized according to our procedure [reference 32]. Both cancer and normal urine show the 30 K and 22 K bands; the latter is the predominant band in both normal and cancer urines.

kDa molecule are formed. The urinary 30 kDa molecule, according to recent studies [24], corresponds to the light chain of the IATI molecule and is responsible for its antiproteolytic activity. IATI has been shown to be composed of one light chain and two heavy chains, transcribed from three different chromosomes and joined together by a glycosaminoglycan [25]. The 30 kDa EDC1 may arise by an enzymatic dissociation of IATI into heavy and light chains [44,45] or its gene may be under separate control. The origin of the 22 kDa moiety is not clear but it may arise by proteolysis of the 30 kDa molecule either by a post translational modification or in the kidney. Other mechanisms, such as a separate gene for its synthesis, cannot be totally eliminated at this stage.

The physiologic functions or the physiologic target enzymes of EDC1 are not known. Recent studies here [46] rule out granulocytic elastase or cathepsin G as its target physiologic enzymes. Of interest in this regard are studies by us [32] and by McKeehan et al. [31] regarding the capacity of EDC1 to inhibit the growth of a virally transformed B cell line and to stimulate the growth of endothelial cells. Interestingly, the 22 kDa molecule, unlike the 30 kDa glycoprotein, had no effect on the growth regulation of B cells [32 and unpublished data from our laboratories].

In conclusion, the present study has identified a mild method for the isolation of urinary EDC1 in good yield from urine of patients with different types of cancer and also demonstrated that i) the amino acid sequence of EDC1 is identical with that of HI30; ii) more significantly, the major form of the urinary proteinase inhibitor in health and disease is the 22 kDa molecule; and iii) other urinary proteinase inhibitors described in literature are either EDC1/HI30 or their derivatives. Perhaps, the biologic role of this molecule is to function as a regulator of cellular growth.

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