

## Molecular cloning and sequence analysis of the cDNA encoding the human acrosin-trypsin inhibitor (HUSI-II)

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A complete cDNA clone encoding the human acrosin-trypsin inhibitor HUSI-II has been isolated from a cDNA library of human testis and completely sequenced. The cDNA of 594 bp contained an open reading frame of 252 base pairs. The deduced amino acid sequence comprised the complete amino acid sequence of rHUSI-II [1] and a putative signal peptide. Northern blotting analysis revealed that HUSI-II is synthesized in testis, epididymis and seminal vesicle, but not in the prostate gland.

Kazal-type inhibitor; Acrosin-trypsin inhibitor (human seminal plasma); Pancreatic secretory trypsin inhibitor (human)

### 1. INTRODUCTION

The acrosin-trypsin inhibitor HUSI-II of human seminal plasma [1] belongs to the family of Kazal-type serine proteinase inhibitors [2,3]. The physiological function of HUSI-II is believed to be the blocking of deleterious degradation of proteins by acrosin released from spermatozoa. The amino acid sequence of HUSI-II was elucidated recently [1], but nothing was known on the precursor form of HUSI-II, on the organisation of its gene and on the regulation of its production. In the course of our studies on these aspects of HUSI-II and other Kazal-type inhibitors we isolated and characterized the cDNA of HUSI-II.

### 2. MATERIALS AND METHODS

Restriction and modification enzymes were from Gibco/BRL or Boehringer. <sup>32</sup>P- or <sup>35</sup>S-labeled dATP, dCTP and ATP were from Amersham. Nylon filters were purchased from Pall, nitrocellulose filters from Schleicher and Schuell. Oligonucleotides used for screening and sequencing were synthesized on a Pharmacia Gene Assembler. All methods employed were according to [4] or to the instructions of the kit manufacturers.

#### 2.1. Screening of cDNA libraries

A cDNA library of human seminal vesicle mRNA in Lambda gt11 [5] was screened with a 5'-labeled 68-mer oligonucleotide deduced from the amino acid sequence of HUSI-II [1]. One positive clone could be isolated, its insert was subcloned into the EcoRI site of pTZ19R (Pharmacia, Freiburg). The randomly primed (kit from Boehringer, Mannheim) <sup>32</sup>P-labeled cDNA-insert of the seminal vesicle clone was used to screen a testis cDNA library (Genofit, Heidelberg), 11 positive clones were isolated and subcloned as above.

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#### 2.2. Sequence analysis

Both strands of cDNA subclones in pTZ19R were completely sequenced by the dideoxy chain termination method using double-stranded DNA and a T7 polymerase sequencing kit (Pharmacia). Sequence data were analyzed using the MicroGenie sequence analysis program, version 5.0 (Beckman).

#### 2.3. Northern blot analysis

Randomly primed <sup>32</sup>P-labeled full-length or partial cDNA was used to probe Northern blots on nylon membrane of RNA from human prostate, seminal vesicle, epididymis and testis. For size estimation of the transcript, RNA markers from Gibco/BRL were used.

#### 2.4. Genomic Southern blot analysis

Genomic DNA from human placenta (20 µg) was restricted with EcoRI or HindIII, electrophoresed through 0.8% Agarose/TAE gels and blotted on nylon membranes. The blots were hybridized with the <sup>32</sup>P-labeled insert of the full-length cDNA clone.

### 3. RESULTS AND DISCUSSION

A 68-mer oligonucleotide of the antisense sequence corresponding to amino acids +22 to +46 of the HUSI-II ([1], Fig. 1) was designed following the suggestions of Lathe [6] (5' ATTTTCATGCACAGGGTGC- ACTCGTTGGCATAGGTGGACATGTCAGAGCC- ACACACAGGATTGAAGTG3'). When a human seminal vesicle cDNA library in Lambda gt11 was screened twice using this probe, only a single, incomplete cDNA clone could be isolated which contained an insert (SV1) corresponding to nucleotides 225 to 594 in Fig. 1.

By subsequent Northern blot analysis of RNA from human prostate, testis, seminal vesicle and epididymis using SV1 as a probe (Fig. 2) the strongest hybridization signal was obtained with poly A<sup>+</sup>-RNA of testis indicating that HUSI-II mRNA is most abundant in this organ. Therefore, a human testis cDNA library was

HUSI-II	GGGGAGCAGCTCCCTGCGAGTGGCGAGGTAACAGACGGAGGGGGAGAGACAGGGCGGGAGGG	68
HPSTI	CAACACAGCTCTAACTTCTGCTGACTTTTCAACTCAACCA	41
HUSI-II	ATG GCG CTG TCG GTG CTG CGC TTG GCG CTG CTG CTC CTG GCA GTC ACC TTC	119
	M A L S V L R L A L L L A V T P	
	-20	-10
HPSTI	TGGACGGCAGAACTTCAGGG ATG AAG CTA ACA CGC ATC TTT CTT CTC ACT GCG TTG	96
	M K V T G I F L L S A L	
	-20	
HUSI-II	GCA GCC TCT CTG ATC CCT CAA TTT GGT CTG TTT TCA AAA TAT AGA ACG CCA	170
	A A S L I P Q F G L P S K Y R T P	
	-1 +1	+10
HPSTI	GCC CTG TTG AGT CTA TCT GGT AAC ACT CGA CCT GAC TCC CTC GCA AGA GAG	147
	A L E S L S G M T G A D S L G R E	
	-10	+1
HUSI-II	AAC TGG TCT CGG TAT AGA TTA CCA CGA TGT CGG AGA CAC TTT AAC CCT CTG	221
	N C S Q Y R H P G Q P R H F N H Y	
	+20	
HPSTI	GCC AAA TGT TAC AAT CGA CTT AAT CGA TGG ACC AAG ATA TAT GAC CCT GTC	198
	A K C Y H E H H Q G Q T K I Y D H Y	
	+10	+20
HUSI-II	TGT GGC AGT GAC ATG TCC ACT TAT GCC ATT CGA TGT ACT CTG TGC ATG AAA	272
	Q Q S D M S H X A N R Q T H Q M K	
	+30	+40
HPSTI	TGT GGG ACT CAT CGA AAT ACT TAT CGG ATT CGA TCC GTG TTA TGT TTT GAA	249
	Q Q T D G H H X P N R G V H Q F E	
	+30	+40
HUSI-II	ATC AGG GAA GGT GGT CAT ATT ATT AAA ATC ATT CGA ATT CGA CCC TGC TGA	323
	I R E G H N H K I R N Q H Q	
	+50	+60
HPSTI	ATT CGG AAA CGG CAG ACT TCT ATC CTC ATT CGA AAA TCT CGG CCT TGC TGA	300
	N R K R Q T S H L H Q K S Q H G	
	+50	
HUSI-II	TGGAGCAGTTACAGAAAAAGAAGATGGAGAGACCCCTCACTGGCAGACTAGataaaATTGCATTCC	391
HPSTI	GAACCAAGGTTTGAAATCCATCAGGTACCGCGAGGCTGACTGGCCTTATTGTTGCAATAATGTA	368
HUSI-II	CCTTTCTCTTTCCCTGTGTTCTTACATGAGATTGTTAACACACATTTCTGAGAGCAGGTATG	459
HPSTI	TCTGAATAATCA	379
HUSI-II	GAAGACAGCCATGTGTAGTGATGGATAATTAAAGAAAAAGAATCCTGTTCTGGCTTTGCTC	527
HUSI-II	CTGGAGTTAAGCTTACTGCCAGGTGACTGTGCATTGCTTATTAGTTGAAATAATCAGCATT	594

Fig. 1. Alignment of the cDNA and amino acid sequences of HUSI-II with those of the human pancreatic secretory trypsin inhibitor (HPSTI, [11,14]). Polyadenylation signals are underlined, a modified, non-functional polyadenylation signal in HUSI-II is printed in lowercase letters; amino acids which are identical in mature HUSI-II and mature HPSTI are shaded.

screened with insert SV1 as a probe. Eleven positive clones could be isolated. Two of these had identical cDNA inserts with an overall length of 594 bp. This length is in good agreement with the mRNA size of about 0.6 kb estimated by the Northern hybridization (Fig. 2), indicating that the cDNA clone is of full length.

Besides these 2 full length clones 7 additional cDNA clones were characterized. The inserts of 4 clones were incomplete and 3 clones contained inserts with additional (intron) sequences, obviously representing incompletely spliced species of HUSI-II transcripts. The same phenomenon has been reported for cDNA species of the bovine pancreatic trypsin inhibitor [7].

The deduced amino acid sequence is presented in Fig. 1. The sequence +1 to +61 is identical with the HUSI-II amino acid sequence published recently [1]. In addition, the deduced sequence reveals a putative signal peptide, -1 to -23 in Fig. 1. According to the statistical evaluation by Heijne [8] of known signal peptidase cleavage sites the cleavage between -1 and +1 (Fig. 1) has a relatively low probability. However, for

two reasons we propose this cleavage site: (i) when HUSI-II is isolated from seminal plasma, multiple, partly proteolysed forms are obtained and the longest form ever characterized starts at glutamine +1 [1]; (ii) in all known genomic structures of Kazal-type proteinase inhibitors the first intron is localized within the signal peptide 14 nucleotides upstream of the codon of the N-terminal amino acid of the mature protein [9-12]. According to our preliminary analysis of the HUSI-II gene (unpublished data) the first intron is between nucleotides 123 and 124 which is 14 bases upstream of the suggested signal peptidase cleavage site.

The cDNA sequence also contained a 68 bp 5' non-coding sequence and a 274 bp 3' untranslated region with a canonical polyadenylation signal (AATAAA; [13]) 15 nucleotides upstream of the 3'-end of the clone. A sequence GATAAA (lowercase letters in Fig. 1) similar to the polyadenylation signal is found 53 bases downstream of the stop codon. In the human pancreatic secretory trypsin inhibitor gene, the functional polyadenylation signal is localized in nearly that same distance from the stop codon (Fig. 1, [11]).

1 2 3 4

kb E H M kb

8.8 9.1  
6.8 4.2

Fig. 2. Northern blot hybridization of RNA from human prostate gland (20  $\mu$ g total RNA, track 1), testis (10  $\mu$ g poly A<sup>+</sup>-RNA, track 2), epididymis (4  $\mu$ g poly A<sup>+</sup>-RNA, track 3) and seminal vesicles (4  $\mu$ g poly A<sup>+</sup>-RNA, track 4). The partial cDNA SV1 isolated from a seminal vesicle cDNA library was used as a probe.

Fig. 3. Southern blot analysis of restriction enzyme-cleaved human placenta DNA. The enzymes used were *Eco*RI (track E) and *Hind*III (track H), size markers were run in track M. Full-length cDNA was used as a probe.

The results of genomic Southern blot analysis using the full-length cDNA clone as a probe, are shown in Fig. 3. *Eco*RI cleaved human genomic DNA gave rise to two bands of 8.8 and 6.8 kb, whereas *Hind*III cleavage of the DNA yielded bands of 9.1 and 4.2 kb. The same hybridization experiment done with the partial cDNA clone SV1 results in hybridization of the smaller fragments only, indicating that the 8.8 and 9.1 fragments contain those parts of the gene upstream of the sequence of SV1. The results suggest that HUSI-II is encoded by a single gene.

The amino acid sequence of HUSI-II deduced from the cDNA sequence revealed the presence of a typical signal sequence thus clearly identifying the inhibitor as a secretory protein. The Northern blot analysis demonstrated that the polypeptide is synthesized in testis, epididymis and seminal vesicles, but not in prostate gland. This result is fully consistent with data obtained by radioimmunoassay studies on the presence of immunoreactive HUSI-II in various tissues (unpublished). More extended studies on expression and localization of HUSI-II in various tissues are part of our cur-

rent investigations on the biological function of HUSI-II.

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