Cloning and Sequencing of cDNA Encoding Rabbit Inter-α-Trypsin Inhibitor Heavy-Chain 1 Precursor

Atsuo Suzuki[1], Hiromi Kato[1] and Akira Iritani[1, 2]

Summary

Complementary DNA encoding a precursor of heavy chain 1 (HC 1) of the inter-α-trypsin inhibitor was amplified from rabbit liver total RNA by reverse-transcription polymerase chain reaction (RT-PCR) and rapid amplification of cDNA end (RACE), cloned and sequenced. The cDNA spanned a stretch of 2,899 nucleotides with open reading frame coding for 906 amino acid residues. The amino acid sequence of HC 1 precursor was 82, 79, 79, and 79% identical with those of the HC 1 precursors from man, mouse, pig and hamster, respectively.

Key words: cDNA sequencing of inter-α-trypsin inhibitor heavy chain 1, Rabbit

Introduction

The inter-α-trypsin inhibitor (ITI) is a family of structurally related plasma serine proteinase inhibitors (1-2). This family is composed of multiple proteins made up of a given combination of polypeptide chains after complex posttranslational maturation (3-4). Members of ITI family can be divided into two subclasses depending of their composition with or without bikunin (a Kunitz-type trypsin inhibitor). When bikunin is present, mature heavy chains named HC 1, HC 2, HC 3 are covalently linked to bikunin by an unusual cross-link named protein glycosaminoglycan protein (5-7).

PK120 (8) is a member of the bikunin-uncompelled subclass (also known as ITI heavy chain-related protein or HC 4). It is a single chain glycoprotein of 120 kDa, cleaved into 85- and 35-kDa fragments when plasma is incubated at 37℃. PK120 is highly sensitive to plasma kallikrein.

Although ITI was isolated more than 30 years ago, its physiological role is still unclear (9). Recent findings indicate that ITI is required for the maturation of oocytes (10) and may also play a role in inflammation (11-12). It is necessary to establish an appropriate animal model in order to get further insight into the physiological role of the ITI family. This paper describes the cloning and sequencing of HC 1 of rabbit ITI, and comparison of the sequences with those of known four species, man, mouse, pig and hamster.

Materials and Methods

Materials—QuickPrep Total RNA Extraction Kit, terminal deoxynucleotidyl transferase (TDT), 2'-deoxyadenosine 5'-triphosphate (dATP) and oligonucleotides were purchased from amersham pharmacia biotech. RNA LA PCR Kit (AMV) Ver. 1. 1 and pGEM-T Easy vector system were obtained from Takara Shuzo Co. and Promega respectively. Restriction enzymes were obtained from Takara Shuzo Co. and BioLabs. Plasmid purification kit was obtained from QIAGEN. DNA sequencing kit (BigDye Terminator Cycle Sequencing) was obtained from Applied Biosystems.

Isolation of Total RNA from Rabbit Liver and Sequencing Strategy for cDNA—Total RNA was prepared from rabbit liver by the method of Chirgwin, J. M *et al* (13) using QuickPrep Total RNA Extraction kit. The complete cDNA sequence encoding HC 1 was determined by the following three step strategy, (i) RT-PCR of the region specific for HC 1, (ii) 3'-RACE, and (iii) 5'-RACE. RT-PCR was carried out essentially by the method of Lynas *et al.* (14) using the RNA LA PCR kit, and the RACEs were performed by the method of

1. Institute of Biology-Oriented Science and Technology, Kinki University, Kainan, Wakayama, 642-0017, Japan
2. Department of Genetic Engineering, Kinki University, Wakayama 649-6493, Japan The nucleotide sequence data reported in this paper have been submitted to the DDBJ/EMBL/GenBank nucleotide sequence database with the accession number AB035707

Frohman *et al.* (15). The PCR products were ligated into pGEM-T Easy vector and sequenced by the dideoxynucleotide chain termination method (16) using the BigDye Terminator Cycle Sequencing kit on a Genetic Analyze System model 310 (Applied Biosystems). The protocols and primers are briefly described below and in Fig. 1.

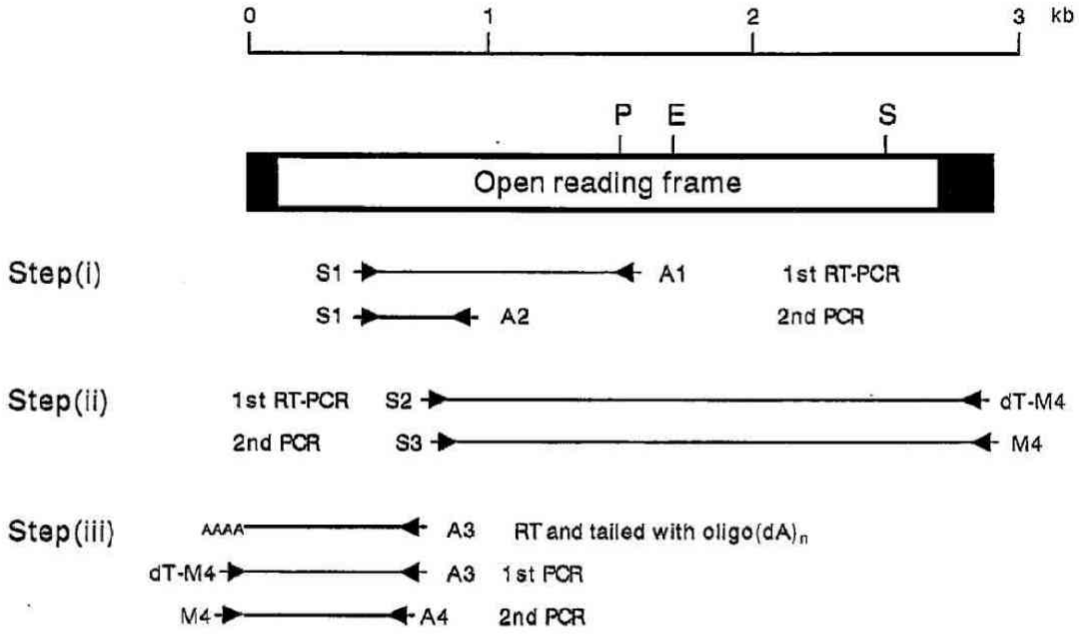

Figure 1. Schematic representation of primer positions and the PCR strategy for cloning of the cDNA encoding the HC1 precursor of rabbit ITI. The restriction endonuclease sites are only those used in the sequence determination. P, Pst I ; E, EcoRI ; S, Sph I.

Sequencing of cDNA Encoding the HC1 Precursor—Step (i) : A portion (1 μg) of total RNA was incubated with avian myeloblastosis virus reverse transcriptase in the presence of an antisense primer A1 (21bp : TGC TAC CAC GAT CTC GGA GCC), which is complementary to nucleotides 1501-1521 (Fig. 2), for 30 min at 50℃, and then heated for 5 min at 99℃ prior to cooling at 5℃ for 5 min. Thereafter, the reaction mixture was subjected to PCR amplification using Takara La Taq polymerase in the presence of a sense primer S1 (20bp : AAA CGG CAG GCC GTG GAT AC), corresponding to positions 335-354 (Fig. 2). PCR was performed at 94℃ for 30 sec, 55℃ for 30 sec, and 72℃ for 1.0 min for a total 30 cycles. The final extension step was 7 min at 72℃. The resulting first round PCR product was further amplified using S1 and an antisense primer A2 (21bp: CAT TGA GCC GCT GAT GTC AAT), positions 874-893 (Fig. 2). The sequence of these primers was specific for HC 1, as judged from the corresponding of the human, mouse, and hamster HC1s. The PCR product was analyzed by electrophoresis on a 1% agar gel and staining with ethidium bromide. The single band material (about 550bp) separated on the gel was eluted and ligated into the pGEM-T Easy vector, and then sequenced with the BigDye Terminator Cycle Sequencing kit according to the manufacture's instructions.

Step (ii): The 3'half of the HC1 precursor transcript was amplified by 3'-RACE using a sense primer S2 (20bp : ACC TCC TGG TGG CCA ACA AC), positions 791-810 (Fig. 2) and an antisense primer oligo- (dT) 20-M4 (Takara Shuzo Co.) ; the resulting first round RACE product was further amplified using a sense primer S3 (20bp : CCT GAA GAA CAT GAG CAA GA), positions 840-859 (Fig. 2) and an antisense primer M4 (Takara Shuzo Co.). These sense primers had been determined through the above RT-PCR. Experimental conditions are identical to those described in Step (i). The single band material (about 2 kbp) separated on the gel was eluted, ligated and sequenced described above. The pGEM-T Easy vector was

digested with restriction enzyme Pst I or Sph I or EcoRI to shorten the inserted cDNA, and then sequenced. These restriction sites were in the multi-cloning-site of pGEM-T Easy vector and inserted cDNA.

Step (iii) : The 5 'half of the HC 1 precursor transcript was amplified by 5 '-RACE. Briefly, liver mRNA was reverse transcribed using an antisense primer A 3 (20bp : CAC GTC GAT CTC AAA GTG CT), positions 548-567 (Fig. 2) and then tailed with oligo (dA) n ; the product was subsequently amplified by PCR using oligo (dT) 20-M 4 and A 3 , and then further amplified using an antisense primer A 4 (20bp : TTC ACT CTG ATG ACG ATG TT), positions 511-530 (Fig. 2) and M4. These antisense primers also had been determined in Step (i). Experimental conditions are the same described above.

Results and discussion

The nucleotide sequence of the rabbit ITI HC 1 precursor cDNA and the deduced amino acid sequence are shown in Fig. 2 . The sequence spans a stretch of 2899 nucleotides with one open reading frame coding for 906 amino acid residues. When the deduced amino acid sequence of this polypeptide was aligned with amino acid sequences of human (17) and mouse HC 1 precursor (18), a putative signal peptide of 24 amino acids residues was identified. A consensus hexapeptide (Asp-Pro-His-Phe-Ile-Ile : positions 667-672, Fig. 2) sequence which is cleaved by one or more processing enzymes at the Asp-Pro junction and added protein-glycosaminoglycan-protein/bikunin to the Asp residue, was also conserved in rabbit HC 1 precursor. Four potential N-glycosylation sites, Asn-X-Ser/Thr, were present in the deduced sequence at amino acid positions Asn-283, Asn-583, Asn-745, and Asn-749. The only third site is well conserved in human, mouse, pig and hamster.

```
      tggtctgcgcgaagggacagggcagcgggagcctggggactcctccgtacagtgcc   -1
   ATGGGGCCTCGGGGACTGCTGTGCGTGTGCCTGGTGTCCCTCCTCATCTTGCAGGCCAGG   60
 1 M  G  P  R  G  L  L  C  V  C  L  V  S  L  L  I  L  Q  A  R
   CCTGCTCCGAGCTCAGCCACAGGCAGGTCCAAGGGCAGTCAGAAGCGACAGGCTGTGGAC  120
21 P  A  P  S  S  A  T  G  R  S  K  G  S  Q  K  R  Q  A  V  D
   AGAACAGTGGATGGCGTGCTCATCCGGAGTTTGAAAGTCAACTGCAAAGTCACCTCTCGC  180
41 R  T  V  D  G  V  L  I  R  S  L  K  V  N  C  K  V  T  S  R
   TTCGCCCACTACGTCATCACCAGCCAGGTGGTCAACAGCGCCAACGAACCCAGGGAAGTG  240
61 F  A  H  Y  V  I  T  S  Q  V  V  N  S  A  N  E  P  R  E  V
   GCCTTCGATGTGGAAATTCCCAAGACAGCCTTCATCAGCGACTTCGCCATCACGGCCAAT  300
81 A  F  D  V  E  I  P  K  T  A  F  I  S  D  F  A  I  T  A  N
   GACAATGCCTATGTTGGGAACATAAAGGACAAAGCAGCCGCGTGGAAGCAGTACCGGAAG  360
101 D  N  A  Y  V  G  N  I  K  D  K  A  A  A  W  K  Q  Y  R  K
   GCAGCCATCGCAGGGGAGAATGCCGGCTTAGTCAGGGCCTCGGGGAGGACGATGGAGCAG  420
121 A  A  I  A  G  E  N  A  G  L  V  R  A  S  G  R  T  M  E  Q
   TTCACCATCCACATCACCGCCAGTCCCCGCAGCAAGGTCACCTTCCAGCTGACCTACGAG  480
141 F  T  I  H  I  T  A  S  P  R  S  K  V  T  F  Q  L  T  Y  E
   GAGGTGCTGAAGCGAAGACTGGGGCAGTACAACATCGTCATCAGAGTGAAGCCCAAGCAG  540
161 E  V  L  K  R  R  L  G  Q  Y  N  I  V  I  R  V  K  P  K  Q
   CTGGTGCAGCACTTTGAGATCGACGTGGACATATTTGAGCCACAAGGGATCAGCAAGCTG  600
181 L  V  Q  H  F  E  I  D  V  D  I  F  E  P  Q  G  I  S  K  L
   AACGCCCAAGCCCCCTTCCTCCCCAAGGAACTGGCGGCTCGAACTATCAAGAAGTCCTTC  660
201 N  A  Q  A  P  F  L  P  K  E  L  A  A  R  T  I  K  K  S  F
   TCAGGGAAAAAGGGTCACGTGCATTTCCGGCCCACCGTGGCCCAGCAGCAGTCCTGCCCC  720
221 S  G  K  K  G  H  V  H  F  R  P  T  V  A  Q  Q  Q  S  C  P
   ACGTGCTCCACATCCCTGCTGAATGGGGACTTCAGGGTGACCTACGACGTCAATCGGGAC  780
241 T  C  S  T  S  L  L  N  G  D  F  R  V  T  Y  D  V  N  R  D
   AAGCTCTGTGACCTCCTGGTGGCCAACAACCACTTTGCCCATTTTTTCGCCCCCCAAAAC  840
261 K  L  C  D  L  L  V  A  N  N  H  F  A  H  F  F  A  P  Q  N
   CTGAAGAACATGAGCAAGAGCCTGGTTTTTGTGATTGACATCAGCGGCTCAATGGAAGGC  900
281 L  K  N  M  S  K  S  L  V  F  V  I  D  I  S  G  S  M  E  G
   CAGAAAGTGAAGCAGACCAAAGAGGCACTGCTTAAGATCCTGGGCGACATCCGGCCAGAG  960
301 Q  K  V  K  Q  T  K  E  A  L  L  K  I  L  G  D  I  R  P  E
   GACTACTTCGACCTGGTCCTCTTCGGCTCTCGAGTGCAGTCGTGGAGGGGTTCACTGGTG 1020
321 D  Y  F  D  L  V  L  F  G  S  R  V  Q  S  W  R  G  S  L  V
   CCAGCCAGCGAGGCCAACCTGCAAGCAGCCCGCGACTTCGTGCAGCGCTTCTCCCTGGCT 1080
341 P  A  S  E  A  N  L  Q  A  A  R  D  F  V  Q  R  F  S  L  A
   GGGGCCACAAACCTGAACGGCGGTTTGCTCCGGGGAATTGAGATCTTGAACAATGCCCAA 1140
361 G  A  T  N  L  N  G  G  L  L  R  G  I  E  I  L  N  N  A  Q
   GGGAACCTGCCCGCGGTCAGCAAGCACGCTGCGATTCTCATCATGCTGACCGACGGCGAG 1200
381 G  N  L  P  A  V  S  K  H  A  A  I  L  I  M  L  T  D  G  E
```

```
     CCCACCGAGGGGGTGACAGACCGCCCCCAAATCCTTAAGAACATCCGGAGTGCCATCGGG  1260
401  P  T  E  G  V  T  D  R  P  Q  I  L  K  N  I  R  S  A  I  G
     GGCAGGTTCCCGCTCTACAGCCTGGGCTTTGGCCACGACCTGGACTTCAACTTCCTGAAG  1320
421  G  R  F  P  L  Y  S  L  G  F  G  H  D  L  D  F  N  F  L  K
     AGCCTGTCCATGGAGAACAACGGGTGGGCCCAGAGGATCTACGAGGACCACGACGCCGCC  1380
441  S  L  S  M  E  N  N  G  W  A  Q  R  I  Y  E  D  H  D  A  A
     CAGCAGCTGCAGGGCTTCTACAACCAGGTGGCCAACCCCCTGCTGGTGGATGTGGAGCTG  1440
461  Q  Q  L  Q  G  F  Y  N  Q  V  A  N  P  L  L  V  D  V  E  L
     CTGTACCCGCAGGACGCTGTGGTGGCCCTCACCCAGCACCGCCATAAGCAGTACTACGAC  1500
481  L  Y  P  Q  D  A  V  V  A  L  T  Q  H  R  H  K  Q  Y  Y  D
     GGCTCAGAGATCGTGGTGGCCGGGCGCATTGCTGACCACAAGCTGGGCAGCTTCAAGGCT  1560
501  G  S  E  I  V  V  A  G  R  I  A  D  H  K  L  G  S  F  K  A
     GACGTGCGGGCCCGTGGGGAGGGACAAGAATTCCAGACCACCTGCCTGGTGGATGAGGAA  1620
521  D  V  R  A  R  G  E  G  Q  E  F  Q  T  T  C  L  V  D  E  E
     GAGATGAAGAAGCTGCTCCGGGAGCGCGGCCACATGCTGGAGAACCACGTGGAGCGCCTC  1680
541  E  M  K  K  L  L  R  E  R  G  H  M  L  E  N  H  V  E  R  L
     TGGGCCTACCTCACCATCCAGGAGCTGCTGGCCAGGCATGTGAAGGCGAAGGGGGAGGAG  1740
561  W  A  Y  L  T  I  Q  E  L  L  A  R  H  V  K  A  K  G  E  E
     AAGGCCAATGTGTCGTCCGAGGCCCTGAAAATGTCCCTGGCCTACCAGTTTGTGACGCCG  1800
581  K  A  N  V  S  S  E  A  L  K  M  S  L  A  Y  Q  F  V  T  P
     CTGACCTCCATGACCATCAGAGGCATGGCGGACGAGGATGGCCTGGAGCCCACCATCGAC  1860
601  L  T  S  M  T  I  R  G  M  A  D  E  D  G  L  E  P  T  I  D
     AAGCCTCCCGAGGATTCGCAGCCCTTGGAGATGGTGGGACCCAGAAGGACGTTCGTGCTG  1920
621  K  P  P  E  D  S  Q  P  L  E  M  V  G  P  R  R  T  F  V  L
     CCAGCCGGGCAGCCCCCGCCTACCAGCTCCAGCTTGAACGTGCAGCAGCTGCCGGACCGA  1980
641  P  A  G  Q  P  P  P  T  S  S  S  L  N  V  Q  Q  L  P  D  R
     GTGACCGGCGTGGACACTGACCCCCACTTCATCATCCACGTGCCCCAGAAGGAGGACGCC  2040
661  V  T  G  V  D  T  D  P  H  F  I  I  H  V  P  Q  K  E  D  A
     CTGTGTTCCAACATCAACGAGCAGCCTGGGGTGATCCTGAGCTTGGTGCAGGACCCCAAC  2100
681  L  C  S  N  I  N  E  Q  P  G  V  I  L  S  L  V  Q  D  P  N
     ACAGGCTTTTCAGTGAATGGCCAGCTCATCGGGGACAAGGCCCGGAGCCCCGGGCGGCAC  2160
701  T  G  F  S  V  N  G  Q  L  I  G  D  K  A  R  S  P  G  R  H
     GGGGGCACGTACTTCGGGCGGCTGGGCATCGCAAACCCTGCCACAGGCTTTCAGCTGGAA  2220
721  G  G  T  Y  F  G  R  L  G  I  A  N  P  A  T  G  F  Q  L  E
     GTAACTCCTCAGAACATCACGCTGAACCCCAGCTCGGGTGGGCCCGTGTTCTCCTGGAGG  2280
741  V  T  P  Q  N  I  T  L  N  P  S  S  G  G  P  V  F  S  W  R
     GACCAGGCTGTGCTGCGGCGGGAGGGGGTGGTGGTGACCATCAACAGGAAGAAGAACCTG  2340
761  D  Q  A  V  L  R  R  E  G  V  V  V  T  I  N  R  K  K  N  L
     CTGGTGTCCGTGGACGGCAGGGCCACATTTGAGGTGGCCCTGCACCGAGTGTGGAAGGGG  2400
781  L  V  S  V  D  G  R  A  T  F  E  V  A  L  H  R  V  W  K  G
     AGCACAGCCCCCCAGGACTTCCTGGGCTTCTACGTGCTGGACAGTCACGGCATGCCGGCC  2460
801  S  T  A  P  Q  D  F  L  G  F  Y  V  L  D  S  H  G  M  P  A
     CGGACACACGGGCTGCTGGGACAATTCTTCCACCCCTTCCACTTTGAAGTGTCCGACCTC  2520
821  R  T  H  G  L  L  G  Q  F  F  H  P  F  H  F  E  V  S  D  L
     CGCCCAGGCTCCGACCCCACCAAGCCGGACGCCACCATGACGGTGAAGAACCGCCGGCTG  2580
841  R  P  G  S  D  P  T  K  P  D  A  T  M  T  V  K  N  R  R  L
     ACGGTCACCAGGGGCTTACAGAAGGATTACAGCAAGGACCCCCGGCACGGGGTGGCAGTG  2640
861  T  V  T  R  G  L  Q  K  D  Y  S  K  D  P  R  H  G  V  A  V
     TCCTGCTGGTTCGTGCATGACAACGGAGCTGGCCTGATCGACGGCGTGCACACTGACTAC  2700
881  S  C  W  F  V  H  D  N  G  A  G  L  I  D  G  V  H  T  D  Y
     ATCGTCCCCGACATCTTCTGAgctggcgccaagtacctgttccccaccaggcccattgcg  2816
901  I  V  P  D  I  F  *
     gtggcggcgaacagcaccccgacctgtggccaggccacgcctccagtgtcaaagcaccct  2876
     gagacttctattaaagagtgctg                                       2899
```

Figure 2. Nucleotide and deduced amino acid sequences of the cDNA encoding the HCl precursor of rabbit ITI. The nucleotide and predicted amino acid residues are numbered on the right and left, respectively. Nucleotides preceding the start codon are presented as lower case letters, and numbered negatively. An asterisk shows the stop codon limiting the open reading frame and the ensuing untranslated nucleotides are shown as lower case letters.

The deduced amino acid sequence of rabbit HC 1 precursor shows 82% identity to that of human and 79% to that of mouse, pig (19) and hamster (20). The cysteine residue positions (65, 239, 242, 263, 535, 682, and 882) are conserved in the HC 1 precursors of all animals. In human, the segments containing Cys-682 and Cys-882 are cleaved off when the HC precursors are linked to bikunin and the mature HC 1 contains one free thiol group and two disulfide bridges of which one links two largely spaced cysteins residues (Cys-263 and Cys-535)(21).

All ITI heavy chains possess a von Willebrand type-A domain (18). Adhesion molecules such as integrins, collagen, proteoglycans and heparin are targets for proteins with von Willebrand domains. ITI stabilizes the cumulus extracellular matrix and supports the process of ovulation, possibly due to a hyaluronan-binding capacity (22). HC1 and HC 3 harbour a multicopper oxidase domain within their C-terminal segment that is trimmed off during chain assembly (18). But, whether the multicopper oxidase domain actually binds copper is currently unknown. Rabbit HC 1 precursor also possessed a von Willebrand type-A domain (amino acid positions 285-445) and a multicopper domain (positions 809-829).

For further functional studies of ITI, cloning of HC 2, HC 3, HC 4, and bikunin will be helpful. Experiment along such lines is currently in progress in this laboratory.

References

1. Hochstrasser, K., Bretzel, G., Feuth, H., Hilla, W., and Lempart, K. (1976) The inter-alpha-trypsin inhibitor as precursor of the acid-stable proteinase inhibitors in human serum and urine. Hoppe-Seyler's Z. Physiol. Chem. 357, 153-162
2. Potempa, J., Kwon, K., Chawla, R., and Travis, J. (1989) Inter-alpha-trypsin inhibitor. Inhibition spectrum of native and derived forms. J. Biol. Chem. 264, 15109-15114
3. Bourguignon, J., Sesboue, R., Diarra-Mehrpour, M., Daveau, M., and Martin, J. P. (1989) Human inter-alpha-trypsin inhibitor. Synthesis and maturation in hepatoma HepG 2 cells. Biochem. J. 261, 305-308
4. Th, I. B., and Enghild, J. J. (1995) Biosynthesis of bikunin proteins in the human carcinoma cell line HepG 2 and in primary human hepatocytes: polypeptide assembly by glycosaminoglycan. J. Biol. Chem. 270, 18700-18709
5. Jessen, T. E., Faarvang, K. L., and Ploug, M. (1988) Carbohydrates as covalent crosslink in human inter-alpha-trypsin inhibitor : a novel plasma protein structure. FEBS Lett. 230, 195-200
6. Enghild, J. J., Salvesen, G., Hefta, S. A., Thogersen, I. B., Rutherfurd, S., and Pizzo, S. V. (1991) Chondroitin 4-sulfate covalently cross-links the chains of the human blood protein pre-alpha-inhibitor. J. Biol. Chem. 266, 747-751
7. Morelle, W., Capon, C., Balduyck, M., Sautiere, P., Kouach, M., Michalski, C., Fournet, B., and Mizon, J. (1994) Chondroitin sulphate covalently cross-links the three polypeptide chains of inter-alpha-trypsin inhibitor. Eur. J. Biochem. 221, 881-888
8. Nishimura, H., Kakizaki, I., Muta, T., Sasaki, N., Pu, P. X., Yamashita, T., and Nagasawa, S. (1995) cDNA and deduced amino acid sequence of human PK-120 plasma kallikrein-sensitive glycoprotein. FEBS Lett. 357, 207-211
9. Salier, J.-P., Rouet, P., Raguenez, G., and Daveau, M. (1996) The inter-α-inhibitor family: from structure to regulation. Biochem. J. 315, 1-9
10. Chen, L., Mao, S. J., and Larsen, W. J. (1992) Identification of a factor in fetal bovine serum that stabilizes the cumulus extracellular matrix. A role for a member of the inter-alpha trypsin inhibitor family. J. Biol. Chem. 267, 12380-12386
11. Daveau, M., Rouet, P., Scotte, M., Faye, L., Hiron, M., Lebreton, J. P., and Salier, J.-P. (1993) Human inter-alpha-inhibitor family in inflammation : simultaneous synthesis of positive and negative acute-phase proteins. Biochem. J. 292, 485-492
12. Daveau, M., Jean, L., Soury, E., Olivier, E., Masson, S., Lyoumi, S., Chan, P., Hiron, M., Lebreton, J. P., Husson, A., Jegou, S., Vaudry, H., Salier, J.-P. (1998) Hepatic and extra-hepatic transcription of inter-alpha-inhibitor family genes under normal or acute

inflammatory conditions in rat. Arch. Biochem. Biophys. 350, 315-323
13. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 18, 5294-5299
14. Lynas, C., Cook, S. P., Laycock, K. A., Bradfield, J. W. B., and Maitland, N. J. (1989) Detection of latent virus mRNA in tissues using the polymerase chain reaction. J. Pathol. 157, 285-289
15. Frohman, M. A., Dush, M. K., and Martin, G. R. (1988) Rapid production of full-length cDNAs from rare transcripts : amplification using a single gene-specific oligonucleotide primer. Proc. Natl. Acad. Sci. USA 85, 8998-9002
16. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74, 5463-5467
17. Diarra-Mehrpour, M., Bourguignon, J., Bost, F., Sesboue, R., Muschio, F., Sarafan, N., and Martin, J. P. (1992) Human inter-alpha-trypsin inhibitor: full-length cDNA sequence of the heavy chain H1. Biochim. Biophys. Acta 1132, 114-118
18. Chan, P., Risler, J.-L., Raguenez, G., and Salier, J.-P. (1995) The three heavy-chain precursors for the inter-α-inhibitor family in mouse: new members of the multicopper oxidase protein group with differential transcription in liver and brain. Biochem. J. 306, 505-512
19. Suzuki, H., Hamashima, N., Kimura, M., Ozawa, A., and Yasue, H. (1995) The sequence of porcine inter-alpha-trypsin inhibitor heavy chain H1 cDNA. DDBJ／EMBL／GenBank Nucleotide Database, D38754
20. Nakatani, T., Suzuki, Y., Yamamoto, T., and Sinohara, H. (1997) Molecular cloning and sequencing of cDNAs encoding three heavy-chain precursors of the inter-α-trypsin inhibitor in Syrian hamster : implications for the evolution of the inter-α-trypsin inhibitor heavy chain family. J. Biochem. 122, 71-82
21. Flahaut, C., Mizon, C., AumercierMaes, P., Colson, P., Bailly, C., Sautiere, P., and Mizon, J. (1998) Disulfide bonds assignment in the inter-alpha-inhibitor heavy chains-structural and functional implications. Eur. J. Biochem. 255, 107-115
22. Chen, L., Mao, S. J. T., McLean, L. R., Powers, R. W., and Larsen, W. J. (1994) Proteins of the inter-α-inhibitor family stabilize the cumulus extracellular matrix through their direct binding with hyaluronic acid. J. Biol. Chem. 269, 28282-28287

要　約

ウサギ・インター-α-トリプシンインヒビター重鎖1前駆体をコードするcDNAのクローン化と塩基配列の決定

鈴木淳夫、加藤博巳、入谷　明

ウサギの肝臓から、インター-α-トリプシンインヒビター重鎖1前駆体（HC1）をコードするcDNAを、RT-PCR（reverse-transcription polymerase chain reaction）法およびRACE（rapid amplification of cDNA end)法を用いて単離し、その塩基配列を決定した。単離したcDNAは906残基のアミノ酸翻訳領域を含む2,899塩基対であった。このアミノ酸配列をヒト、マウス、ブタ、およびハムスターのHC1と比較したところ、82, 79, 79, 79%であった。