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The domain structure of the inhibitor subunit of human inter-α-trypsin inhibitor reflects the exon structure of its gene

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The gene coding for the inhibitor subunit of the human plasma protein complex inter-α-trypsin inhibitor has been cloned. The exon structure of the gene corresponds with the organization of the protein in two distinct inhibitor domains. The exons coding for the inhibitor domains each comprise the complete information for a typical Kunitz-type proteinase inhibitor structure. In contrast to bovine aprotinin, the mature inhibitor protein contains amino acid residues flanking the inhibitor domains on both sides. These amino acid residues are encoded by additional exons.

Exon-intron junction; Kunitz-type structure; Aprotinin; Microglobulin, α_1-; Trypsin inhibitor; (Serum, Urine)

1. INTRODUCTION

Inter-α-trypsin inhibitor (ITI) [1] is a complex of three different types of subunits [2-5]. The smallest subunit previously called HI30 and thought to be a fragment of ITI carries the inhibitor activity and encloses two Kunitz-type inhibitor domains. The primary structure of the inhibitor protein is identical to that of the so-called serum trypsin inhibitor (STI) and urinary trypsin inhibitor (UTI). The inhibitor protein is released from a precursor protein composed of α_1-microglobulin and the double-headed proteinase inhibitor [6]. Only the latter becomes part of the protein complex ITI [2,3]. Renal filtration of STI leads to the occurrence of UTI in urine. In patients suffering from severe inflammations or cancer large amounts of UTI can be found. This may be due to an increase in biosynthesis of the precursor protein in an acute phase-like response.

In man, Kunitz-type inhibitor domains have been found recently in a lipoprotein-associated coagulation inhibitor [7] and in an amyloid precursor protein [8-10]. The former protein consists of three consecutively linked Kunitz-type inhibitor domains, whereas the latter contains only one such structural unit.

In order to obtain information on whether the domain structure reflects the structural organization of the gene and how Kunitz-type inhibitor proteins with multiple domains may have evolved, we studied the exon structure of the inhibitor subunit gene of human ITI and compared it to that of the bovine aprotinin gene.

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2. MATERIALS AND METHODS

High-M_r DNA was prepared from human leukocytes [11] and partially digested with restriction endonuclease *Sau*3AI. Fragments of 14-24 kb were enriched by centrifugation through a sucrose density gradient and used to construct a genomic library in EMBL3 λ cloning vector as described [12].

The genomic library was screened with a cDNA probe covering nearly the complete precursor of the inhibitor subunit of ITI. DNA of isolated genomic clones was prepared, digested with restriction endonucleases and subcloned into plasmid vector pTZ18R (Pharmacia).

Subcloned DNA hybridizing to complete cDNA was sequenced from both ends using the chain termination method

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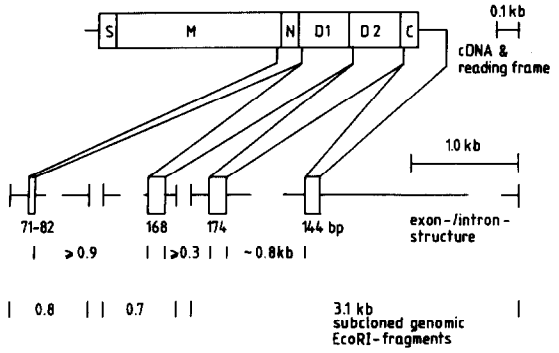

Fig.1. Exon structure of the inhibitor subunit of ITI. Different parts of the primary structure of the precursor protein deduced from the cDNA are shown in separate boxes termed S (signal peptide), M (mature α_1-microglobulin), N, D1, D2, and C (N-terminal peptide, domains 1 and 2, and C-terminal part of the mature inhibitor subunit, respectively).

[13]. Sequence data were collected and analysed using the MicroGenie^R Sequence Analysis Program, version 5.0 (Beckman).

3. RESULTS

Several clones could be isolated and characterized partially by restriction site mapping of their inserted genomic DNA. Some of these clones were found to hybridize selectively with one or both oligonucleotides designed for detection of DNA sequences coding for the N-terminal peptide (N, fig.1) of the inhibitor and the 3′-untranslated region. DNA of one of these clones contained three *Eco*RI fragments of 0.7, 0.8 and 3.1 kb (fig.1) hybridizing to the cDNA. The 3.1 kb DNA fragment comprises two complete exon sequences, one (174 bp) coding for nearly the complete second inhibitor domain, the other (144 bp) for the C-terminal part of the second domain as well as the 3′-untranslated part of the transcript (figs 1,2). The smaller *Eco*RI DNA fragments contain exons of 168 and 71–82 bp, coding for the first inhibitor domain and the N-terminal peptide of the subunit, respectively (figs 1,2).

4. DISCUSSION

Most small mammalian proteinase inhibitors belong to either the Kazal or the Kunitz family. The genomic organization of Kazal-type inhibitor domains is known to be highly conserved. Coding regions of functional domains have been shown to be interrrupted by two introns ([14] and references quoted therein). In the gene of aprotinin, the basic bovine pancreatic trypsin inhibitor (BPTI) which is considered as the prototype Kunitz proteinase inhibitor, and the gene of a homologous bovine inhibitor, introns do not split the coding region of the functional domains [15,16]. The exon structure of the inhibitor subunit gene of ITI is similar. Sequences coding for the complete conserved cysteine residue backbone of a typical Kunitz domain are flanked by introns (figs 1–3). In the case of

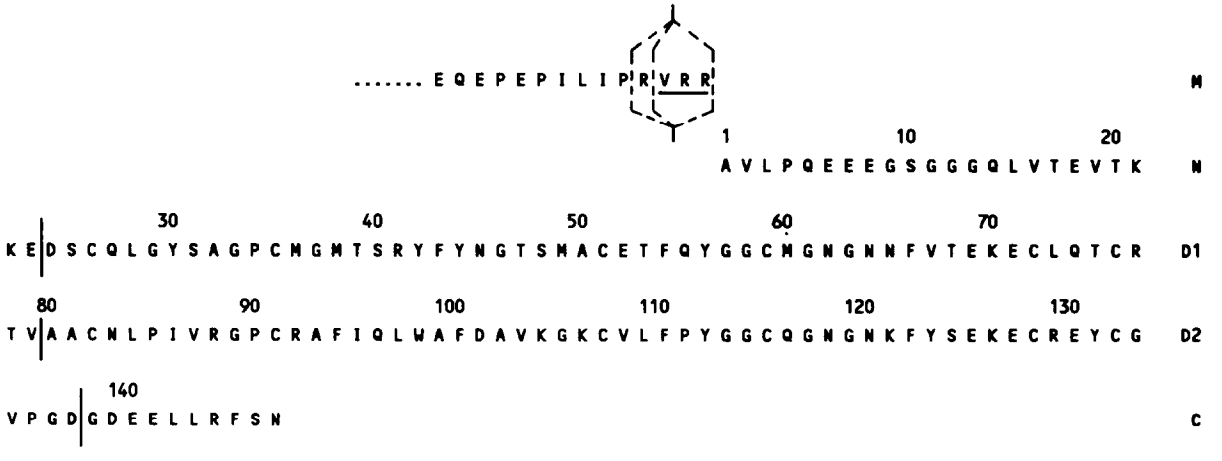

Fig.2. Primary structure of part of the precursor protein of the inhibitor subunit of ITI. Structural units are labelled as in fig.1. Residues which do not appear in the mature proteins are underlined. Regions encoded by distinct exons are separated by vertical lines. Dashed vertical lines mark an exon-intron border which cannot yet be defined clearly.

aprotinin the complete mature protein is encoded by only one exon whereas the mature inhibitor subunit of ITI contains at either side of the inhibitor domains additional amino acid residues which are encoded by their own exons.

The exon/intron junctions conform to the GT-AG rule (fig.3). The 5′-end of the exon coding for the N-terminal peptide cannot yet be determined exactly (fig.3) without knowledge of the 3′-end of the preceding exon coding for the C-terminal part of α_1-microglobulin. Four AG dinucleotides (fig.3) could be discussed as possible 3′-sites of the intron. In the case of splicing at the second AG dinucleotide, the proteolytically fragile linkage -R-V-R-R- (figs 2,3) between α_1-microglobulin and the inhibitor protein would be encoded by the already known exon, and in the case of splicing at the fourth AG dinucleotide this linker would be encoded by an α_1-microglobulin exon. Up to now, the first arginine residue of the linker sequence is regarded as the C-terminal residue of mature α_1-microglobulin [17].

According to the classification scheme of Patthy [18] all exons of the inhibitor subunit of ITI as well as those of aprotinin and its bovine relative are symmetrical and of class 1-1 (fig.3). This means that they have introns of the same phase class at both their ends. Such nonrandom intron phases are preconditions for insertion or duplication mechanisms by intronic recombination and a further hint at an evolutionary relationship between the representatives of Kunitz-type inhibitors.

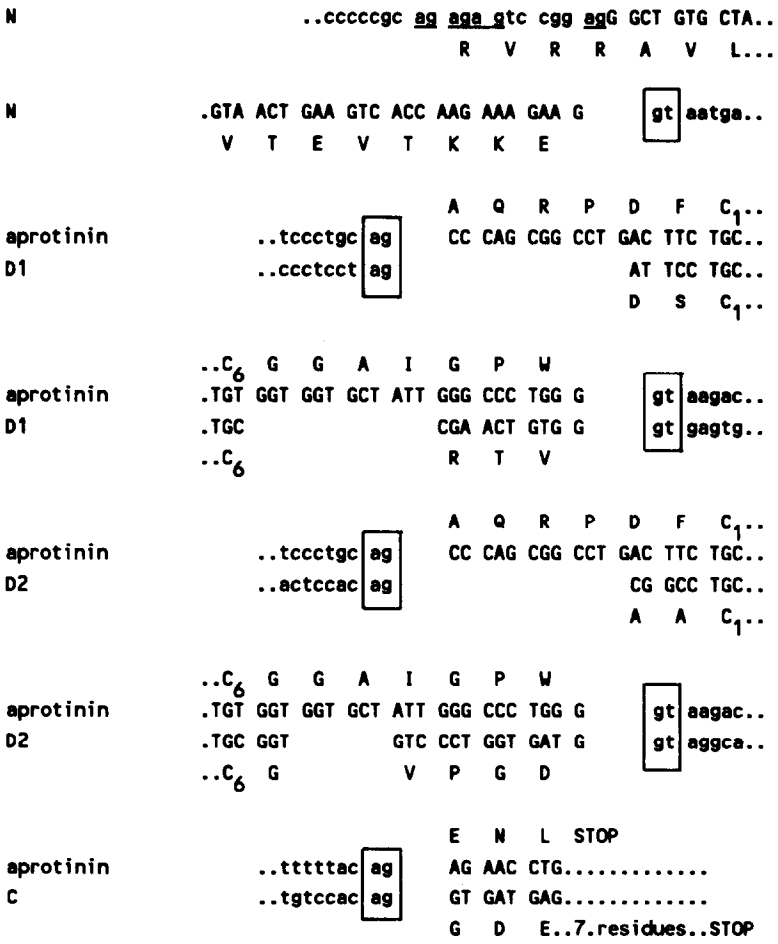

Fig.3. Exon-intron junctions compared to those of the aprotinin gene. Structural units are labelled as in fig.1. Intron sequences are shown in lower case. AG and GT dinucleotides at their ends are boxed. AG dinucleotides which cannot be appointed clearly to an exon-intron border are underlined.

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REFERENCES

[1] Gebhard, W. and Hochstraßer, K. (1986) in: Proteinase Inhibitors (Barrett, A.J. and Salvesen, G. eds) pp. 389–401, Elsevier, Amsterdam, New York.
[2] Schreitmüller, T., Hochstraßer, K., Reisinger, P.W.M., Wachter, E. and Gebhard, W. (1987) Biol. Chem. Hoppe-Seyler 368, 963–970.
[3] Gebhard, W., Schreitmüller, T., Hochstraßer, K. and Wachter, E. (1988) FEBS Lett. 229, 63–67.
[4] Gebhard, W., Schreitmüller, T. and Leysath, G. (1988) Biol. Chem. Hoppe-Seyler 369 S, 19–22.
[5] Gebhard, W., Schreitmüller, T., Hochstraßer, K. and Wachter, E. (1988) submitted.
[6] Kaumeyer, J.F., Polazzi, J.O. and Kotick, M.P. (1986) Nucleic Acids Res. 14, 7839–7849.
[7] Wun, T.-C., Kretzmer, K.K., Girard, T.J., Miletich, J.P. and Broze, G.J., Jr (1988) J. Biol. Chem. 263, 6001–6004.
[8] Ponte, P., Gonzalez-DeWhitt, P., Schilling, J., Miller, J., Hsu, D., Greenberg, B., Davis, K., Wallace, W., Lieberburg, I., Fuller, F. and Cordell, B. (1988) Nature 331, 525–527.
[9] Tanzi, R.E., McClatchey, A.I., Lamperti, E.D., Villa-Komaroff, L., Gusella, J.F. and Neve, R.L. (1988) Nature 331, 528–530.
[10] Kitaguchi, N., Takahashi, Y., Tokushima, Y., Shiojiri, S. and Ito, H. (1988) Nature 331, 530–532.
[11] Gautreau, C., Rahuel, C., Cartron, J.-P. and Lucotte, G. (1983) Anal. Biochem. 134, 320–324.
[12] Frischauf, A.-M., Lehrach, H., Poustka, A. and Murray, N. (1983) J. Mol. Biol. 170, 827–842.
[13] Chen, E.Y. and Seeburg, P.H. (1985) DNA 4, 165–170.
[14] Tan, F., Gippner, C. and Fink, E. (1988) Biol. Chem. Hoppe-Seyler 369 S, 51–54.
[15] Kingston, I.B. and Anderson, S. (1986) Biochem. J. 233, 443–450.
[16] Creighton, T.E. and Charles, I.G. (1987) J. Mol. Biol. 194, 11–22.
[17] Lopez, C., Grubb, A., Soriano, F. and Mendez, E. (1981) Biochem. Biophys. Res. Commun. 103, 919–925.
[18] Patthy, L. (1987) FEBS Lett. 214, 1–7.