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FEBS Letters 342 (1994) 278–280

FEBS 13885

FEBS LETTERS

Cloning of cDNA for granzyme-like protein III, a novel serine proteinase from rat duodenum

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Received 25 February 1994

Abstract

We previously reported cloning of cDNAs which encode two granzyme-like serine proteinases (GLP I and GLP II) from rat duodenum. In this paper we present the cDNA sequence for a novel member of the granzyme-like protein family from rat duodenum, GLP III. The amino acid sequence deduced from the cDNA consists of 248 residues and shows 88.2% identity to GLP I and 50.6% identity to GLP II. Comparison of the amino acid sequence of GLP III with sequences of related proteinases reveals the location of the catalytic amino acid triad and enables the prediction of the substrate specificity. Despite close similarity to GLP I, GLP III is expected to demonstrate different substrate specificity due to a substitution of the Arg residue by Glu at the critical position. Northern blot analysis demonstrates that the GLP III transcript is present only in duodenum.

Key words: cDNA cloning; Duodenum; Granzyme-like protein; Serine proteinase

1. Introduction

Cytoplasmic granules of defence system cells have been shown to contain structurally related serine proteinases. These include granzymes of cytotoxic T-lymphocytes [1,2], mast cell proteases I and II [3–5], cathepsin G of polymorphonuclear leukocytes [6] and natural killer cell protease [7]. The actual physiological roles of these enzymes are not clear. Studies suggest that granzymes are involved in cytolytic events during T-cell interactions with target cells [2], while mast cell proteases and cathepsin G play an important role in allergic and inflammatory reactions [8] and in defence against pathogens [9]. Recently, a novel serine proteinase (duodenase) was isolated from bovine duodenum and characterized [10]. It was shown that the N-terminal amino acid sequence of duodenase has a high degree of homology with the sequences of granzymes, mast cell proteases, and cathepsin G. Investigations of duodenum proteolytic enzymes led us to the identification of cDNAs encoding two related serine proteinases named granzyme-like protein I and II (GLP I and GLP II) [11]. This paper describes the isolation and nucleotide sequence of a cDNA encoding a novel member of the granzyme-like protein family (GLP III). Tissue specificity of GLP III gene expression is demonstrated for several rat organs.

2. Materials and methods

For RNA isolation from rat duodenum the guanidine thiocyanate method was used [12]. Poly(A)$^+$ RNA was selected and used as a template for cDNA synthesis [13]. Double-stranded cDNA was ligated with *Sma*I-digested, dephosphorylated plasmid pSP64 and used for the transformation of *E. coli* MH I cells. The library was blotted onto a nylon membrane and hybridized overnight with ^{32}P-labeled multiprime probes.

For PCR amplification *Taq* DNA polymerase was used. The protocol was as follows: incubation at 94°C for 4 min and 30 cycles of denaturation at 94°C for 45 s, primer annealing at 60°C for 1 min and extension by enzyme at 72°C for 1.5 min. Aliquots of the amplification mixture were analyzed by electrophoresis on a 1.2% agarose gel stained with ethidium bromide.

The sequencing was performed on double- and single-stranded DNA templates according to the methods of Sanger et al. [14].

For Northern blot analysis poly(A)$^+$ RNA was electrophoresed on agarose-formaldehyde gels [13] and transferred to nylon membranes by vacuum blotting. Hybridization with ^{32}P-labeled cDNA probes was carried out in the presence of 50% formamide at 42°C overnight.

3. Results and discussion

3.1. Isolation of cDNA encoding GLP III

When a cDNA library, constructed from mRNA from rat duodenum, was hybridized with the ^{32}P-labeled fragment of GLP I cDNA [11] it was found that there is at

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Abbreviations: GLP I, II and III, granzyme-like protein I, II and III; RNKP-1, rat natural killer cell protease 1; GRAN B, granzyme B; PCR, polymerase chain reaction.

The nucleotide sequence presented here has been submitted to the EMBL Nucleotide Sequence Databases under accession number X76996 (*R. norvegicus* mRNA for granzyme-like protein III).

SSDI 0014-5793(94)00270-6

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GTGGGACCTAGGTGGCCTTCCAGGGAAGATGAAGGTCCTCTTGCTCCTGCTGACCGTCTC     60
                                  M  K  V  L  L  L  L  L  T  V  S   -10

TCTGGCCCCCACGACAGAGGCAGGGGAGATCATCGGGGGACATGAAGCCAAGCCCCACTC    120
  L  A  P  T  T  E  A  G  E  I  I  G  G  H  E  A  K  P  H  S     11

TCGACCCTACATGGCCTATCTTCAGATCATGGATGAGGATTCTGGGAGTACGATGTGTGG    180
  R  P  Y  M  A  Y  L  Q  I  M  D  E  D  S  G  S  T  M  C  G     31

CGGTTTCCTTATACAAGAAGACTTTGTGCTGACTGCTGCTCACTGTTTGGGGAGCAAAAT    240
  G  F  L  I  Q  E  D  F  V  L  T  A  A  H  C  L  G  S  K  I     51

AACTGTCACGTTGGGGGCCCACAACATCAAAGAACAGGAGAAGATGCAGCAAGTCATCCC    300
  T  V  T  L  G  A  H  N  I  K  E  Q  E  K  M  Q  Q  V  I  P     71

TGTGGTGAAAATCATTCCCCACCCAGCCTATAATTCTAAGAAATACTCCAACGACATCAT    360
  V  V  K  I  I  P  H  P  A  Y  N  S  K  K  Y  S  N  D  I  M     91

GCTATTAAAGCTGAAGAGTAAGGCCAAGAGGACTAGAGCTGTGAAGACGCTCAGTCTGCC    420
  L  L  K  L  K  S  K  A  K  R  T  R  A  V  K  T  L  S  L  P    111

CAGAAGCAACTTCAAAGTGAAGCCAGGAGATGTGTGCAATGTGGCTGGTTGGGGAAAGCT    480
  R  S  N  F  K  V  K  P  G  D  V  C  N  V  A  G  W  G  K  L    131

GGGCCCAATGGGCAAATTCCCAGACAAACTGCAAGAGGTTGAGCTAACAGTACAGGAGGA    540
  G  P  M  G  K  F  P  D  K  L  Q  E  V  E  L  T  V  Q  E  D    151

TCAGGAGTGTGAGACTTACTTTAAAAAAGCTTACAACAAAGCCAATCAGATATGTGCGGG    600
  Q  E  C  E  T  Y  F  K  K  A  Y  N  K  A  N  Q  I  C  A  G    171

GGACCCAAAGATCAAACGTGCTTCCTTTGGGGGGGACTCTGGAGGGCCTCTTGTGTGTAA    660
  D  P  K  I  K  R  A  S  F  G  G  D  S  G  G  P  L  V  C  K    191

AAAAGTGGCCGCGGGCATCGTCGCCTATGGATCTAAAAATGGTTCAGCTCCAGAGGCCTT    720
  K  V  A  A  G  I  V  A  Y  G  S  K  N  G  S  A  P  E  A  F    211

CACCAAAGTCTCAACTTTCCTATCCTGGATAAAGGAAACTATGAAAAAGAGCTAACTACA    780
  T  K  V  S  T  F  L  S  W  I  K  E  T  M  K  K  S            228

AGAAGCAA                                                        788
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Fig. 1. The nucleotide sequence and protein translation of GLP III cDNA. Numbering of the mature protein begins at the N-terminal Ile residue common for serine proteinases of the granzyme family.

least one more cDNA which strongly hybridized with the probe but is clearly different from GLP I cDNA. The novel enzyme encoded by this cDNA was named 'granzyme-like protein III'. To determine the GLP III cDNA sequence, we attempted to use as primers for PCR amplification oligonucleotides AGAGAGCAAGGACAACACTCTCGACG and GGTGGTTAGTCAGGAAATGATCCACG corresponding to 5'- and 3'-untranslated regions of GLP I cDNA, respectively. After 30 PCR cycles, material of interest was separated, ligated with plasmid vector pSP64, and cloned. ^{32}P-labeled cDNA for GLP I was used as a probe for screening.

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          *   *   *   *   *  *** * *  ********                 *****   * * **
GLPI      MNLLLLLLLTVSLAPTTEAAEIIGGHEADPHSRPYMAYLQYKNEDSRDTICGGFLIREDFVLTA
GLPII     MFLFLFFLVAILPVNTEGGEIIWGTESKPHSRPYMAFIKFYDSNSEPHRCGGFLVAKDIVMTA
GLPIII    MKVLLLLLLTVSLAPTTEAGEIIGGHEAKPHSRPYMAYLQIMDEDSGSTMCGGFLIQEDFVLTA
RNKP-1    MKLLLLLLLSFSLAPKTEAGEIIGGHEAKPHSRPYMAYLQIMDEYSGSKKCGGFLIREDFVLTA
GRAN B    MKILLLLLLTLSLASRTKAGEIIGGHEVKPHSRPYMALLSIKDQQPEA-ICGGFLIREDFVLTA

          ***  *  * *********  **   * *  **  **  *      ********  **     *
GLPI      AHCSGSKINVTLGAHNIKEQEKTQQVIPVVKIIPHPAYNAKTISNDIMLLKLKSKAKRTRAVK
GLPII     AHCNGRNIKVTLGAHNIKKQENT-QVISVVKAKPHENYDRDSHFNDIMLLKLERKAQLNGVVK
GLPIII    AHCLGSKITVTLGAHNIKEQEKMQQVIPVVKIIPHPAYNSKKYSNDIMLLKLKSKAKRTRAVK
RNKP-1    AHCSGSKINVTLGAHNIKEQEKMQQIIPVVKIIPHPAYNSKTISNDIMLLKLKSKAKRSSAVK
GRAN B    AHCEGSIINVTLGAHNIKEQEKTQQVIPMVKCIPHPDYNPKTFSNDIMLLKLKSKAKRTRAVR

             ***    **** ** *****            **** * **    *           *
GLPI      TLSLPRSNFKVKPGDVCYVAGWGKLGPMGKFPDKLQEVELTVQEDQECETYLKNAYDKANQIC
GLPII     TIALPRSQDWVKPGQVCTVAGWGRLANCTS-SNTLQEVNLEVQKGQKCQD-MSEDYNDSIQLC
GLPIII    TLSLPRSNFKVKPGDVCNVAGWGKLGPMGKFPDKLQEVELTVQEDQECETYFKKAYNKANQIC
RNKP-1    PLNLPRRNVKVKPGDVCYVAGWGKLGPMGKYSDTLQEVELTVQEDQKCESYLKNYFDKANEIC
GRAN B    PLNLPRRNVNVKPGDVCYVAGWGRMAPMGKYSNTLQEVELTVQKDRECESYFKNRYNKTNQIC

          * *    *   ******* **  ** *** *       *  **  * *  **  ***
GLPI      AGDPKIKCASFQGDSGGPLVCKKVAAGIVSYGRKDGSTPRAFTKVSTFLSWIEETMKKS
GLPII     VGNPSEGKATGKGDSGGPFVCDGVAQGIVSYRLCTGTLPRVFTRISSFIPWIQKTMKVLQQS
GLPIII    AGDPKIKRASFGGDSGGPLVCKKVAAGIVAYGSKNGSAPEAFTKVSTFLSWIKETMKKS
RNKP-1    AGDPKIKRASFRGDSGGPLVCKKVAAGIVSYGQNDGSTPRAFTKVSTFLSWIKKTMKKS
GRAN B    AGDPKTKRASFRGDSGGPLVCKKVAAGIVSYGYKDGSPPRAFTKVSSFLSWIKKTMKSS
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Fig. 2. Alignment of the predicted GLP III amino acid sequence with the sequences of related serine proteinases. Residues identical in all aligned sequences are marked by asterisks. The catalytic triad residues His, Asp, and Ser are indicated by ■. The residues determining the primary substrate specificity [15] are marked by ●. The alignment makes use of the following sequences: rat GLP I and II [11], rat GLP III (this study), RNKP-1 [7], murine GRAN B [16].

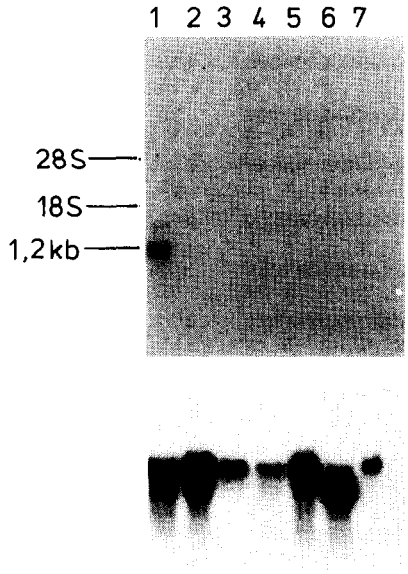

Fig. 3. Northern blot analysis of poly(A)$^+$ RNA from various rat organs. The cDNA fragment of GLP III was used as a probe; the same blot was reprobed with the actin cDNA probe (shown below). In the blot poly(A)$^+$ RNAs from various organs are presented in the following order: duodenum (1), lung (2), brain (3), liver (4), spleen (5), heart (6), and thymus (7). The size of the GLP III RNA transcript was determined by comparison to 28S and 18S ribosomal RNA bands.

Sequencing of several positive clones from three independent amplifications revealed that some of clones represent GLP III cDNA (Fig. 1).

3.2. Characterization of the GLP III amino acid sequence

Analysis of the cDNA-derived amino acid sequence showed that GLP III consists of 248 amino acid residues. It shows 88.2% identity to GLP I and 50.6% identity to GLP II. A high degree of identity (84,3%) was observed between GLP III and rat natural killer cell protease-1 (RNKP-1) [7]. Comparison of the amino acid sequence of GLP III with the sequences of related proteinases (Fig. 2) reveals the location of the catalytic amino acid triad and enables the prediction of the substrate specificity.

Interestingly, despite close similarity to GLP I, GLP III is expected to demonstrate different substrate specificity due to a substitution of the Arg residue by Glu at the critical position [15].

Most likely, GLP III has a preference for base residues at the P_1-position of the substrate, while GLP I has a preference for acid residues. Experimental data on bovine duodenase, an enzyme which is presumable an analog of rat GLP III and which also contains negatively charged residue at the critical position (Zamolodchikova et al., in preparation) show that duodenase demonstrates trypsin-like specificity [10].

3.3. Tissue specificity of GLP III gene expression

Northern blot analysis of GLP III gene expression in different organs revealed that the GLP III RNA transcript is present only in duodenum and is approximately 1.2 kb long (Fig. 3). Noteworthy, RNA transcripts of the GLP family members were detected in those organs where alien antigens are commonly encountered [11]. It seems that GLPs are involved in the organism protection and are located in defence system cells. Because GLP III has a high degree of identity to RNKP-1 one can suppose that natural killer cells can be the possible source of the enzyme. On the other hand, mucosal mast cells and cytotoxic intestinal intraepithelial lymphocytes also can be considered as a potential source of GLP III.

Acknowledgements: We thank D.Sci. L.M. Ginodman for critical reading of the manuscript, N.S. Bystrov for providing oligo-nucleotide probes, and J.M. Grondhal for his assistance in the preparation of the manuscript. This work was supported by the Human Genome Project of the Russian Academy of Science and by the Protein Engineering Project.

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