Complementary DNA and deduced amino acid sequences of porcine α_1-microglobulin and bikunin

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Analysis of complementary DNA for porcine α_1-microglobulin and bikunin indicates that both proteins result from proteolytic processing of a common precursor similar to that found in man. Complete primary structures of these proteins are deduced from the nucleic acid sequence and partially confirmed by peptide sequencing.

Proteinase inhibitor; Inter-α-(trypsin) inhibitor; Kunitz-type structure; α_1-Microglobulin; Bikunin

1. INTRODUCTION

Human α_1-microglobulin and bikunin are plasma glycoproteins known to result from a common precursor protein by intracellular proteolytic processing [1]. Both these proteins, α_1-microglobulin as well as bikunin, are also found in plasma protein complexes with IgA [2] and IgG [3], respectively. Furthermore, bikunin is the inhibitor subunit of the inter-α-(trypsin) inhibitor complex whose structure could be determined only recently [4–6].

In severe inflammation and in cancer increased levels of bikunin are observed in serum and urine. We explain these pathophysiological effects by an acute-phase-like increase of bikunin biosynthesis [5]. In order to study such an acute phase reaction in an animal species we first determined the structure of the common precursor protein of porcine α_1-microglobulin and bikunin.

2. MATERIALS AND METHODS

A porcine liver cDNA lambda gt11 library (Clontech Laboratories) was screened with an immunoselected polyclonal rabbit antiserum raised against human bikunin using the ProtoBlot immunoscreening system (Promega Biotec). Plaque-purified DNA was subcloned in plasmid pTZ19R (Pharmacia).

Both strands of the cDNA were entirely sequenced by the dideoxy-chain-termination method, using double-stranded DNA and T7 DNA polymerase (Pharmacia), nested deletions produced by the ExoIII-mung bean nuclease technique, and specific primers.

cDNA sequence data were compiled and analysed using the MicroGenie Sequence Analysis Program, version 5.0 (Beckman).

RNA was isolated from porcine liver by the LiCl/urea method and further purified by affinity chromatography on oligo(dT)-cellulose using a protocol described by Aviv and Leder [7]. Poly(A)-RNA was analysed by gel electrophoresis (1,4% agarose gel containing 6% formaldehyde) and transferred onto a nylon membrane (Biodyne A, Pall) by capillary blotting. The probe was labeled with ^{32}P using an oligo-labeling kit (Boehringer Mannheim). After a prehybridization step at 42°C for 3 h, hybridizations were carried out at 42°C overnight with fresh buffer (5 SSC, 10 Denhardt, 50% formamide and sonicated salmon sperm DNA at a concentration of 250 ag/ml [1 SSC: 0.15 M NaCl/0.015 M sodium citrate (pH 7); 1 Denhardt: bovine serum albumin, Ficoll, and polyvinylpyrrolidone, 0.02% each]). Filters were washed at high stringency (0.1 SSC, 0.1% SDS, 65°C). Autoradiography was performed overnight with Kodak X-Omat AR film at −70°C using a Dupont amplifier screen.

Porcine bikunin was isolated, reduced and carboxymethylated as previously described [8,9]. Tryptic peptides either were purified as described in [8,9] or subjected directly to subsequent HPLC runs on a Vydac C_{18}-column (50 min; 0.8 ml/min), firstly using a linear gradient (0–100% B) of buffers A (50 mM ammonium acetate pH 6.0) and B (30% A; 70% acetonitrile; pH 6.0), secondly using a linear gradient (0–70% D) of buffers C (0.1% trifluoroacetic acid) and D (20% C; 80% acetonitrile). Peptides were detected at 210 nm and/or 280 nm. Amino acid sequences were determined by solid-phase Edman degradation.

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3. RESULTS AND DISCUSSION

We obtained a single clone from immunological screening of about 40 000 recombinants of a porcine liver cDNA library. The 1.3 kb cDNA (Fig. 1) detects a prominent band of 1.4 kb and an additional species of 1.5 kb on northern blots of porcine liver poly(A)-RNA (Fig. 2 2). Therefore, we used the cloned cDNA as a probe for a second screening by plaque hybridization. However, all five additional clones contained significantly shorter inserts.

We verified the identity of the porcine α_1-microglobulin-bikunin clone by comparison with amino acid sequences of peptides isolated from tryptic digests of porcine bikunin (Fig.1, underlined amino acids) and with the human α_1-microglobulin-bikunin clone [1].

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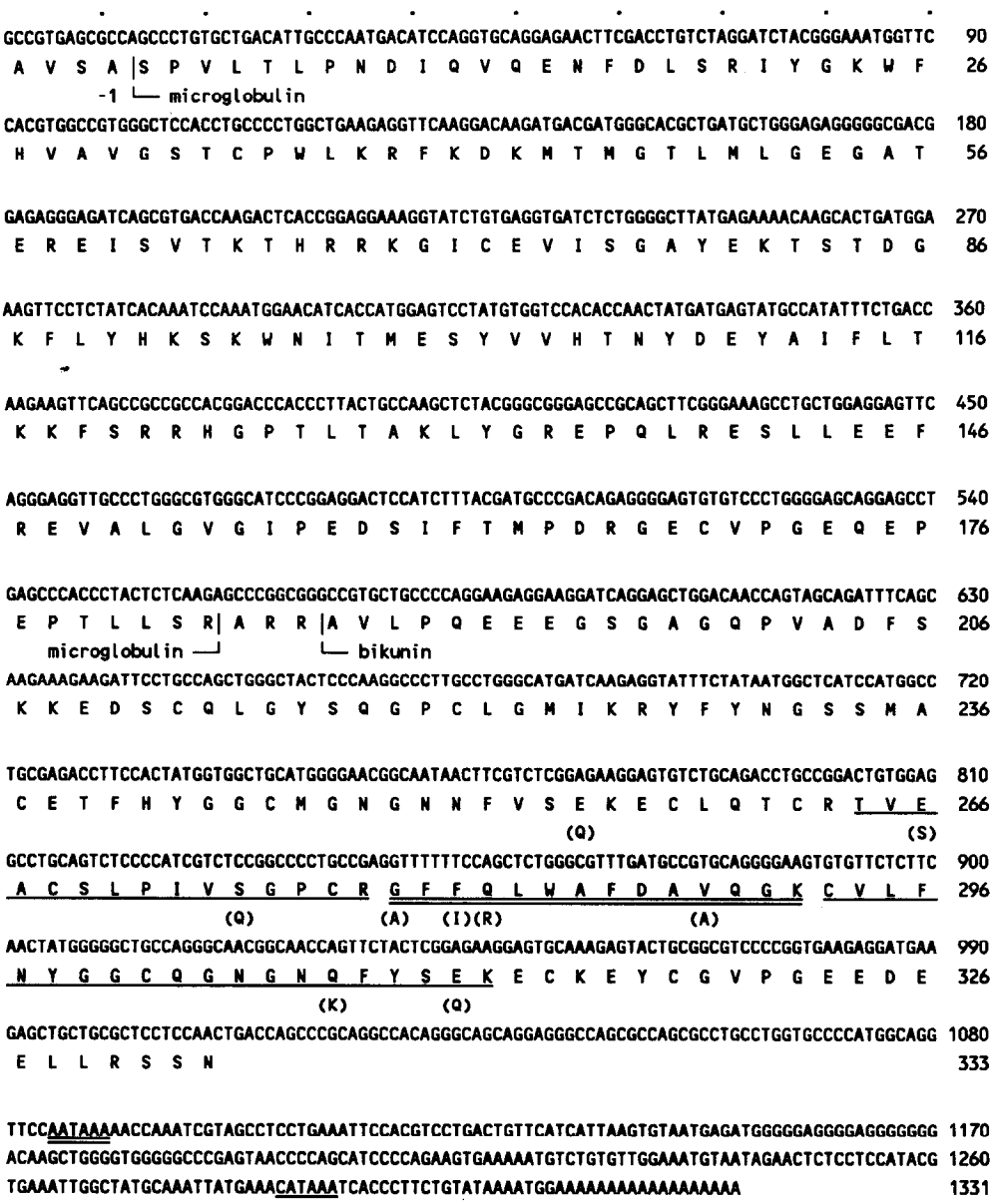

Fig. 1. Nucleotide sequence and deduced amino acid sequence (below) of cloned porcine α_1-microglobulin-bikunin cDNA. Deduced amino acid sequences that match those obtained from purified peptides of bikunin are underlined. Residues shown in brackets are taken from the literature [8]. Nucleic acid sequences underlined represent polyadenylation signals.

The deduced porcine α_1-microglobulin-bikunin sequence extends for 337 amino acids. It is divided into four regions, corresponding to the domains defined by proteolytic processing of the primary translation product. Four C-terminal residues of the signal peptide sequence are encoded on the 1.3 kb cDNA (Figs 1,3). Cleavage of the signal peptide probably occurs at the same site as found in the human precursor protein [1] (Figs 1,3). This would lead to an N-terminal sequence which is also found in rat and rabbit α_1-microglobulin [10]. Further proteolytic processing occurs at Arg-186 and probably also at Arg-183 releasing the putative linker peptide A-R-R (V-R-R in human). Porcine α_1-microglobulin therefore would extend to residue 183 (Figs 1,3). The porcine bikunin sequence begins at residue 187 and terminates at residue 333 (Figs 1,4). Both termini were described earlier [8].

Human and porcine sequences of both proteins, α_1-microglobulin and bikunin, are highly conserved between the species (79% and 83%, respectively). Glycosylation of human as well as porcine bikunin occurs at Ser-10 and Asn-45. In porcine α_1-microglobulin the glycosylation site (N-X-S/T) at Asn-96 is conserved. The cysteine residues of both proteins are also strictly conserved. In bikunins, cysteine residues are responsible for the typical Kunitz-type structure of this proteinase inhibitor that is also known as an endothelial cell growth factor [11]. In the α_{2u}-globulin

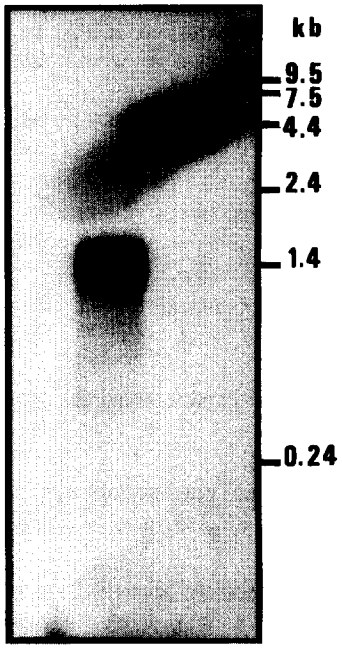

Fig. 2. Northern blot of porcine liver poly(A) RNA using porcine α_1-microglobulin-bikunin cDNA as a probe.

family, a family of transport proteins which α_1-microglobulin belongs to, residue Trp-25 and the region around residues 102–114 seem to be highly conserved and may be functionally important. The family comprises proteins like odorant-binding protein, α_1-acid glycoprotein (steroid ligands), retinol-binding protein, apolipoprotein D (binding cholesterol), β-lactoglobulin (binding retinol) and many others (see e.g. [12]).

It is notable, however, that several amino acid residues of the deduced porcine sequence differ from those reported earlier (residues shown in brackets in Fig. 1; [8]). Only some of these differences may be explained by well-known technical difficulties, e.g. decisions between Glu and Gln. In addition, unusual differences between the 3′-untranslated regions of porcine and human cDNA clones raised the question whether there might be two different genes. However, the following facts argue against such an assumption.

The amino acid sequences of tryptic bikunin peptides which were purified by gel filtration, ion-exchange chromatography and subsequent HPLC according to the methods described earlier [8,9] clearly confirmed the sequence deduced from the cDNA (underlined peptides in Fig. 1). However, using these methods we failed to detect the very hydrophobic peptide shown in Fig. 5 (doubly underlined in Fig.1). The crucial step in its isolation was the application of HPLC without the prepurification steps recommended earlier [8]. As it is the only bikunin peptide containing a tryptophane residue it can be easily identified by its absorbance at 280 nm (Fig. 5). Obviously, in [9] the peptide in question escaped its isolation and was confounded with the

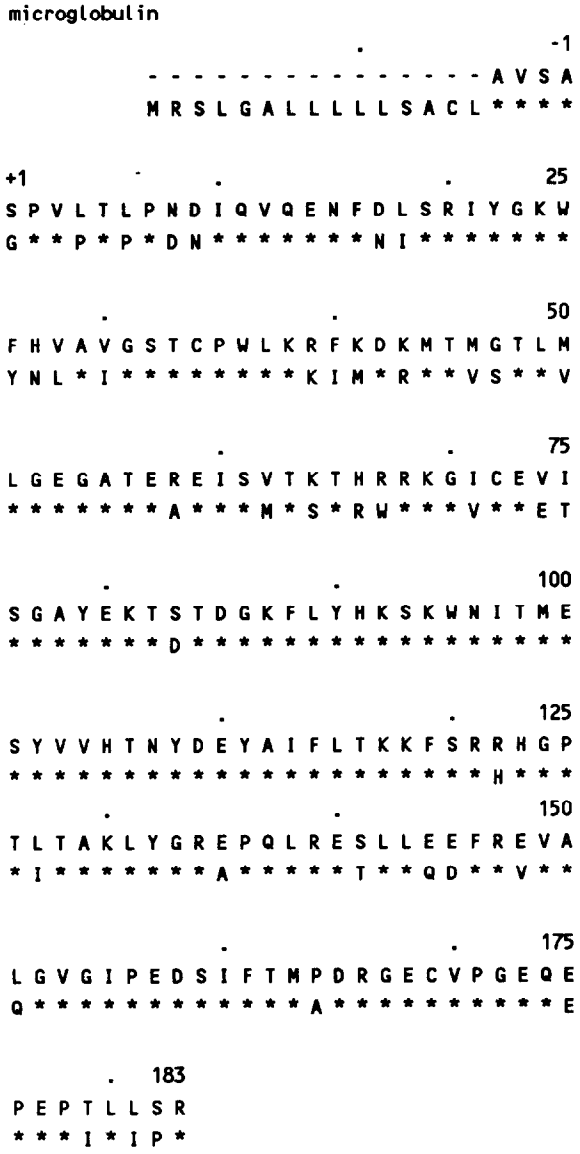

Fig. 3. Amino acid sequences of porcine (above) and human (below) α_1-microglobulin. Undetermined residues are marked by dashes, those identical to the porcine sequence are marked by stars.

simultaneously determined corresponding equine peptide.

Sequence analysis of an additional, hybrid selected 600 bp cDNA clone also confirmed the deduced bikunin sequence. The clone covers residues 518–1113 of the 1.3 kb cDNA followed by two additional A-residues just in front of the cloning linker sequence. Therefore, its 3′-untranslated region exactly terminates at the polyadenylation site already known from the human cDNA, 21–23 bp downstream of the AATAAA sequence at position 1085 (Figs 1,6). The 3′-untranslated region of the 1.3 kb cDNA covers 320 bp compared to 112 bp in the corresponding human cDNA [1]. In contrast to the human cDNA and the 600 bp porcine cDNA the AATAAA sequence at position 1085 is not used as a polyadenylation signal in the 1.3 kb

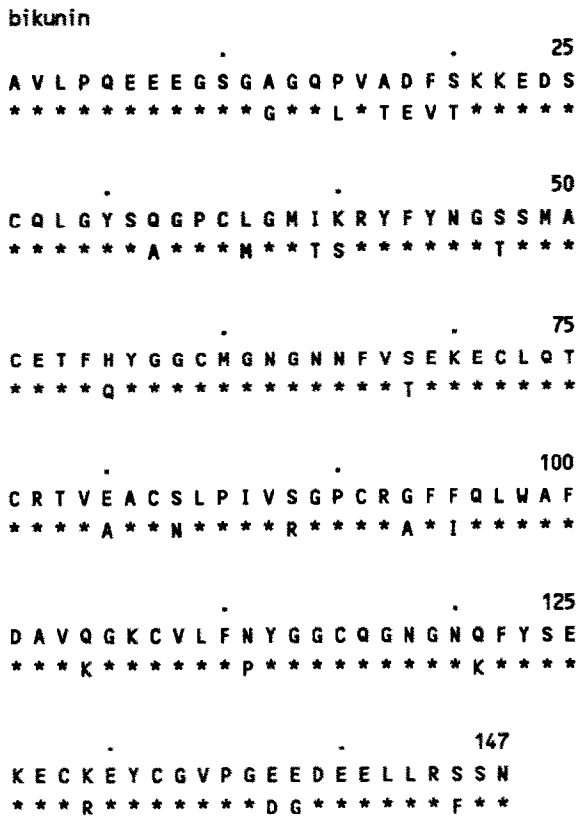

Fig. 4. Amino acid sequences of porcine (above) and human (below) bikunin. Residues identical to those of the porcine sequence are marked by stars.

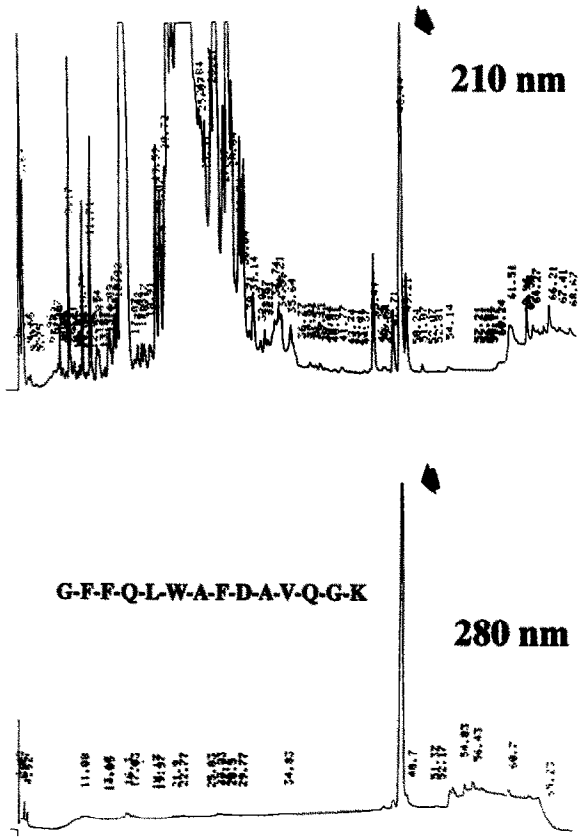

Fig. 5. HPLC runs of a tryptic digest of porcine bikunin; detection of peptides at 210 nm (above) and 280 nm (below). For the exact conditions see section 2.

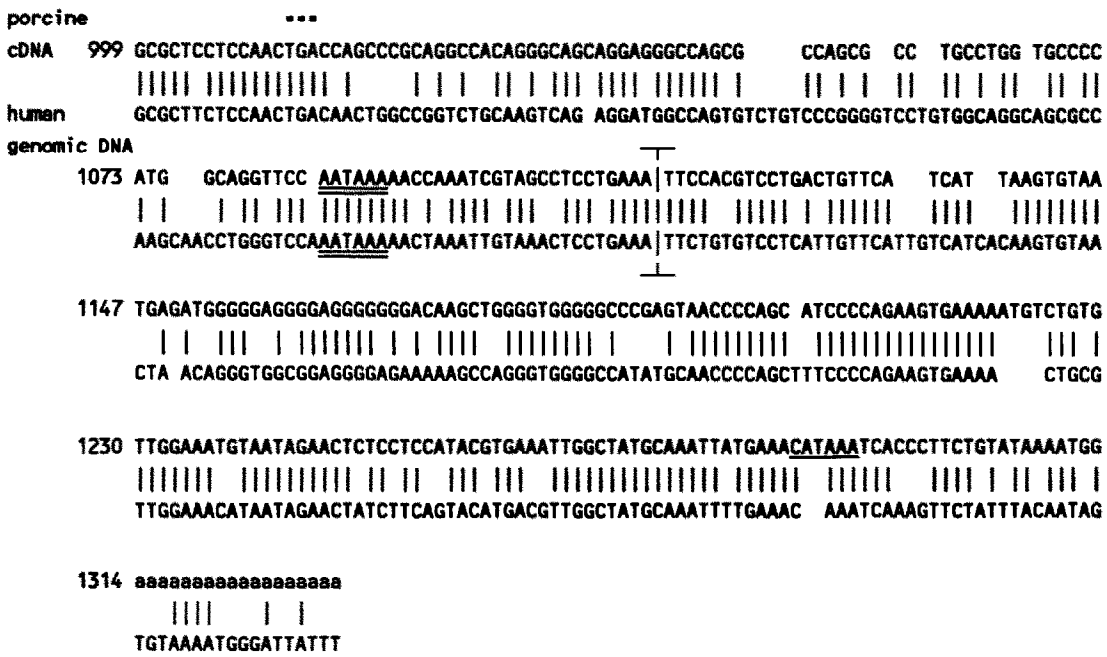

Fig. 6. Comparison of porcine cDNA sequence downstream from the stop-codon (■ ■ ■) with human genomic DNA sequence. Polyadenylation signals are underlined. The vertical broken line marks polyadenylation sites used in a human cDNA [1] and the 600 bp porcine cDNA. Poly(A)-tail residues of the 1.3 kb porcine cDNA are shown in lower case.

cDNA (Figs 1,6). However, residues 1314–1331 were clearly identified as a poly(A) tail when the 3′-untranslated region was compared to the corresponding human genomic sequence (Vetr, H. and Gebhard, W., manuscript in preparation) (Fig. 6). Obviously, in the 1.3 kb porcine cDNA, residues downstream position 1113 (broken vertical bar in Fig. 6) represent genomic DNA. The presence of two mRNA species in northern blots of porcine liver RNA therefore may reflect improper polyadenylation rather than transcription from different genes and, therefore, should not complicate studies of α_1-microglobulin-bikunin biosynthesis in the acute phase.

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REFERENCES

[1] Kaumeyer, J.F., Polazzi, J.O. and Kotick, M.P. (1986) Nucleic Acids Res. 14, 7839–7849.
[2] Tejler, L. and Grubb, A.O. (1976) Biochim. Biophys. Acta 439, 82–94.
[3] Salier, J.-P., Sesboüe, R., Hochstraßer, K., Schönberger, Ö. and Martin, J.-P. (1983) Biochim. Biophys. Acta 742, 206–214.
[4] Schreitmüller, T., Hochstraßer, K., Reisinger, P.W.M., Wachter, E. and Gebhard, W. (1987) Biol. Chem. Hoppe-Seyler 368, 963–970.
[5] Gebhard, W., Schreitmüller, T., Hochstraßer, K. and Wachter, E. (1989) Eur. J. Biochem. 181, 571–576.
[6] Enghild, J.J., Thøgersen, I.B., Pizzo, S.V. and Salvesen, G. (1989) J. Biol. Chem. 264, 15975–15981.
[7] Aviv H. and Leder P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408–1412.
[8] Hochstraßer, K. and Wachter, E. (1979) Hoppe-Seyler's Z. Physiol. Chem. 360, 1285–1296.
[9] Hochstraßer, K., Wachter, E., Albrecht, G.J. and Reisinger, P. (1985) Biol. Chem. Hoppe-Seyler 366, 473–478.
[10] Akerström, B., Lögdberg, L., Babiker-Mohamed, H., Lohmander, S. and Rask, L. (1987) Eur. J. Biochem. 170, 143–148.
[11] McKeehan, W.L., Sakagami, Y., Hoshi, H. and McKeehan, K.A. (1986) J. Biol. Chem. 261, 5378–5383.
[12] Pevsner, J., Reed, R.R., Feinstein, P.G. and Snyder, S.H. (1988) Science 241, 336–339.