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CHARACTERIZATION BY cDNA CLONING OF THE mRNA OF A NEW GROWTH FACTOR FROM BOVINE SEMINAL PLASMA: ACIDIC SEMINAL FLUID PROTEIN *

F. Wempe, R. Einspanier [1] and K.H. Scheit

Max-Planck-Institut für Biophysikalische Chemie, Abteilung Molekulare Biologie, 3400 Göttingen, Germany

[1]Lehrstuhl für Physiologie der Fortpflanzung und Laktation, Technische Universität München, 8050 Freising, Germany

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Summary: A cDNA expression library in λgt11 prepared from cDNA derived of seminal vesicle tissue was screened by means of monospecific rabbit anti-aSFP IgG. The sequence of clone pTF21, containing an insert of 668 bp comprised an open reading frame from position 7 to 411 terminated by two stop codons. From this sequence a protein of 134 amino acid residues can be deduced. The mature aSFP was preceded by a signal peptide of 20 amino acids length. The protein sequence contains no signal for N-glycosylation. The molecular weight calculated from the amino acid sequence is 12922 Da. The start codon ATG is part of the sequence AAGATGA which fulfills the criteria of an initiation consensus sequence. The coding region was followed by 257bp of the complete 3′-untranslated region (3′UTR). A putative polyadenylation signal AATAAT, although not of the standard type, is observed at position 650. According to Northern analysis, aSFP mRNA is expressed in seminal vesicle tissue, ampulla and weakly in tissue of epididymis, but not in testis or other bovine tissue. aSFP is specified by a single copy gene. Attempts to detect homologies to known protein sequences were not successful.

In the bovine species, the seminal vesicle contributes significantly to the protein content of seminal fluid. For a review of hitherto identified secretory proteins of seminal vesicles in bulls, covering the literature up to 1988, see Shivaji et al. (1). Gene expression and cDNA cloning identified also a major basic protein constituent of bovine seminal plasma as bovine monocyte-chemoattractant protein-1 (2). Recently a new acidic protein *acidic seminal fluid protein* (aSFP) was purified from bovine seminal fluid to homogeneity (3). The purified aSFP displayed a pI of 4.8 and an apparent molecular weight of 14 kDa. Monospecific anti-aSFP IgGs were employed to characterize aSFP in bovine seminal plasma and seminal vesicle secretion by immuno blot analysis. Proteinchemical characterization of aSFP included amino acid analysis as well as determination of 23 amino acid residues of the N-terminal sequence of aSFP.

*The sequence data of pTF21 have been submitted to the EMBL/GenBank under the accession number M84603.

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According to this sequence, aSFP appears to represent a hitherto unknown protein. aSFP stimulated cell divison and furthermore progesterone secretion by bovine granulosa cells in vitro in a potent and dose dependent manner (3). In this contribution we report the isolation of an aSFP-specific cDNA clone from a bovine seminal vesicle cDNA expression library. A cDNA clone comprising the complete coding region allowed to deduce the amino acid sequence of aSFP. The expression of aSFP in bovine sex tissue and Southern analysis of genomic DNA of various species is likewise reported.

Materials and Methods

The preparation of monospecific anti-aSFP IgGs was described elsewhere (3). Oligonucleotides employed in sequencing of double-stranded DNA by the walking primer approach were synthesized by an Applied Biosystems model 318A synthesizer.

Total RNA and poly(A^+)RNA from bovine seminal vesicle tissue were prepared as described (4). cDNA synthesis from poly(A^+)RNA followed a modified protocol of Gubler and Hoffmann (5), using a commercial kit (BRL). A cDNA library in λgt11 was prepared employing the λgt11 cloning kit (Amersham) as reported earlier (2). Monospecific anti-aSFP IgGs were used to screen 0.75 x 10^5 plaques of the library by standard procedures. In short, the filters were incubated with a solution of 0.1μg/ml anti-aSFP IgG in phosphate buffered saline (PBS) (pH 7.5) containing 10% horse serum for 15 h at room temperature. Bound rabbit IgGs were detected by incubation with biotin labeled goat anti-rabbit IgG (Dianova) for 2h at room temperature, followed by reaction with alkaline phosphatase labeled streptavidin (Amersham) for 1.5 h at room temperature. Incubations were carried out in PBS containing 10% horse serum. Enzymatic activity was detected using 5-bromo-4-chloro-3-indolyl-phosphate and Nitro Blue tetrazolium chloride. The *Eco*RI inserts of positive clones were subcloned into pUC18 and sequences determined by double strand sequencing using the dideoxy chain-termination method of Sanger et al. (6); internal regions of the insert sequence were sequenced employing suitable synthetic primers. For sequencing a T7-DNA Polymerase kit (Pharmacia) was employed.

For Northern analysis, RNA was electrophoresed in a 1% agarose gel containing formaldehyde (7) and transferred to GeneScreen membranes (NEN). Hybridization of RNA blots followed the protocol of Khandjian (8). For Southern blot analysis genomic DNA was restricted, electrophoresed in 0.7% agarose and transferred to nitrocellulose membranes as described (9). Hybridization was performed at 60°C under stringent conditions following the protocol of Church and Gilbert (10). DNA probes were [^{32}P]-labeled by random priming to a specific activity of 1x10^9 cpm/μg employing a commercial kit (Amersham).

DNA and protein sequence analysis as well as sequence comparisons were performed using the computer program of the University of Wisconsin genetics computer group (11) in combination with the EMBL/GenBank DNA sequence- and NBRF protein sequence data base.

Results

The detection of the bioactive protein aSFP by immunological methods in the secretion of seminal vesicles indicated this accessory sex gland as a potential site for biosynthesis of aSFP

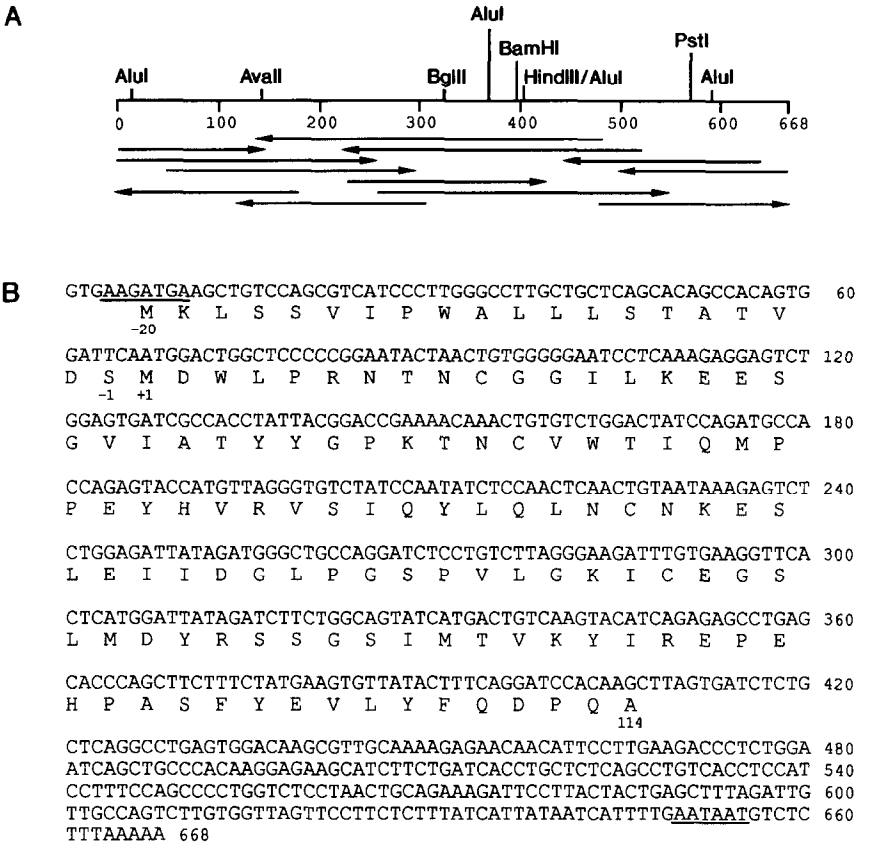

Fig. 1 cDNA sequence of pTF21. A: Sequencing strategy and restriction map of pTF21. The arrow heads within the map give the extent as well as the 5'-3'orientation of the sequenced fragments. B: Nucleotide sequence of pTF21 and deduced amino acid sequence of aSFP. Consensus sequences are underlined.

(3). A cDNA expression library in λgt11 prepared from cDNA obtained from poly(A+)RNA from seminal vesicle tissue was therefore screened by means of monospecific rabbit anti-aSFP IgG. Screening of 0.75 x 10^5 independent clones furnished 12 positive clones. Clone pTF21 containing an insert of 668 bp was sequenced. A limited restriction map, sequencing strategy as well as the DNA sequence of the insert of pTF21 are detailed in Fig. 1. The sequence contained an open reading frame from position 7 to 411 terminated by two stop codons. From this sequence a protein of 134 amino acid residues can be deduced. The amino acid sequence of the N-terminus of aSFP, determined by automated Edman degradation (3), was found within this deduced amino acid sequence and allowed the unambiguous identification of the reading frame of the aSFP mRNA. The mature aSFP was preceded by a signal peptide of 20 amino acids length. The protein sequence contains no signal for N-glycosylation. The molecular weight calculated from the amino acid sequence is 12922 Da and in reasonable agreement with the apparent molecular weight reported earlier (3); this also holds for the theoretically calculated pI value of 4.9. The start codon ATG is part of the sequence AAGATGA which fullfills the

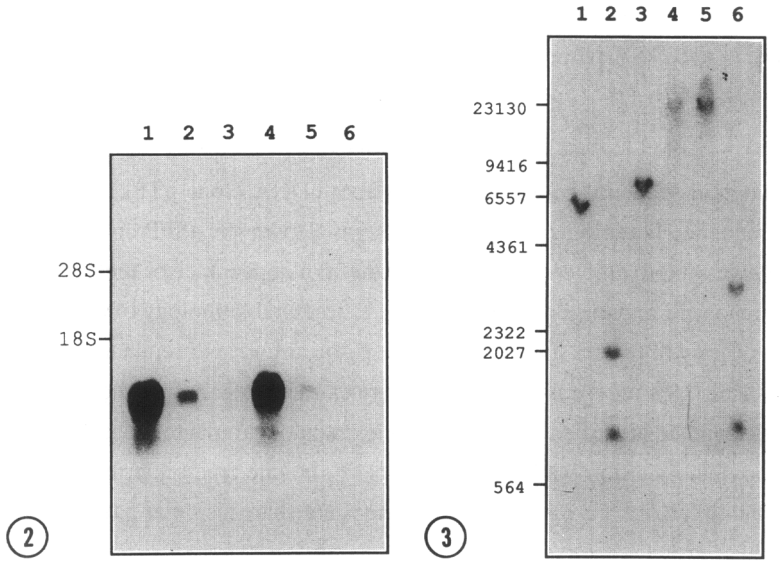

Fig. 2 Northern analysis of total RNA from male bovine sex tissue. Hybridization was performed with the 354 bp *Alu*I fragment of pTF21. Lane 1, seminal vesicle tissue (10µg); lane 2, seminal vesicle tissue (1µg); lane 3, seminal vesicle tissue (0.1µg); lane 4, ampulla (10µg); lane 5, total epididymal tissue (10µg); lane 6, testis tissue (10µg).

Fig. 3 Southern analysis of bovine genomic DNA. Each lane contains 10µg of restricted DNA. Lane 1, *EcoR*I; lane 2, *Pst*I; lane 3, *Hind*III; lane 4, *BamH*I; lane 5, *EcoR*V; lane 6, *Pvu*I. Hybridization was performed with the 354 bp *Alu*I fragment of pTF21.

criteria of an initiation consensus sequence (12-14). The coding region was followed by 257bp of the complete 3`UTR and a putative polyadenylation signal AATAAT at position 650 (15). The results of Northern analysis of total RNA of various bovine male sex tissue are depicted in Fig. 2. In tissue of seminal vesicle as well as in ampullae a similar strong expression of aSFP mRNA of approximate size of 800bp was observed. A very weak expression (approximately 5% of that observed in seminal vesicle tissue) appears to occur in epididymal tissue; no aSFP mRNA was detected in testis. Attempts to demonstrate aSFP mRNA in female bovine sex tissue, e.g. ovar and corpus luteum, as well as rumen and liver failed (data not shown; the limit of detection was estimated to <1% of that in seminal vesicle tissue).

Southern analysis of bovine genomic DNA, restricted with 6 different enzymes yielded generally one or two hybridizing fragments respectively. Because the cDNA insert of pTF21 possessed *BamH*I and *Hind*III sites, an 354 bp *Alu*I fragment of the cDNA insert lacking these sites was employed as hybridization probe. aSFP mRNA is apparently specified by a single copy gene. A difference between male and female bovine species was not observed (data not shown). A comparative genomic analysis employing a aSFP specific cDNA probe and stringend conditions of hybridization yielded no signals with human, rat, mouse, pig and rabbit DNA (data not shown); the existence of gene sequences with significant homology to bovine aSFP mRNA appears unlikely(Fig.3).

Attempts to detect any sequence homologies between the DNA sequence of the coding region of pTF21 and DNA sequences in a data base were not successful.

Discussion

The amino acid sequence of aSFP, derived from cDNA clone pTF21, apparently has no significant sequence homologies to known proteins. However, aSFP contains four cysteine residues, and we tried to employ the sequence pattern of appearance of these cysteine residues within the amino acid sequence of aSFP to detect a structural homology of aSFP with members of the group of growth factors. From the data base, the amino acid sequences of growth factor proteins which also feature 4 cystein residues were extracted. Only four members belonging to this subgroup could be identified currently: bovine granulocyte-macrophage colony-stimulating factor (16), bovine basic fibroblast growth factor (17), bovine type I interferon (18) and human B-cell stimulating factor 2 (19). aSFP however, does not share a similar pattern of Cys residues with any of these growth factors.

It was shown by others (for a review see Shivaji et al., 1990) as well as Derwenskus et al. (20) that bovine seminal plasma contains two basic proteins, seminal ribonuclease BS1 and seminalplasmin, with the potency to inhibit cell proliferation of lymphocytes. One protein component of bovine seminal plasma, aSFP, was shown to possess mitogenic properties (3). It may therefore depend on the ratio of concentrations of these proteins in semen, whether bovine seminal fluid will exhibit either net growth stimulating or lymphocyte inhibiting properties. To answer this question, the protein composition as well as the respective functions of particular semen samples with regard to growth stimulation and/or inhibition of lymphocyte proliferation should be determined. This, to our knowledge has not been investigated so far.

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