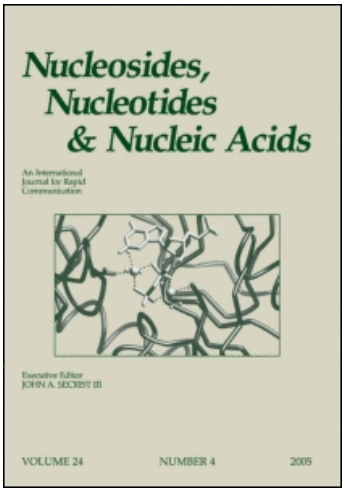

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Cloning and Sequence Analysis of a cDNA from Seminal Vesicle Tissue Encoding the Precursor of the Major Protein of Bull Semen

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CLONING AND SEQUENCE ANALYSIS OF A cDNA FROM SEMINAL VESICLE TISSUE ENCODING THE PRECURSOR OF THE MAJOR PROTEIN OF BULL SEMEN

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Abstract: A cDNA library derived from poly(A)$^+$RNA of bull seminal vesicle tissue was screened with a synthetic DNA hybridisation probe specific for the major protein of bull semen. A positive clone pMP17, containing a 680 bp insert, was sequenced. In combination with primer extension sequencing of poly(A)$^+$RNA, a DNA sequence of 700 bp was determined. This DNA encoded a reading frame for 134 amino acids, starting with an ATG and terminated by a TAG codon. This comprised 25 amino acids of a signal peptide followed by 109 amino acids with the known sequence of the major protein.

INTRODUCTION

We recently isolated the major protein with an apparent molecular weight of M_r 15 kD from seminal plasma as well as from seminal vesicle secretion of bull and proved by amino acid analysis and tryptic peptide mapping that the two proteins were identical (1). The protein chemical analysis indicated that the major protein was identical to the protein PDC109 purified from bull seminal plasma and sequenced by Esch et al. (2). Immunochemical studies by Aumüller et al. (3) revealed the regiospecific binding of the major protein to the fossa and the mid-piece of bull spermatozoa. Binding of gold-labeled major protein to subplasmalemmal sites at the mid-piece region of spermatozoa was demonstrated by electronmicroscopy and two membrane proteins which selectively bind the major protein were isolated from bull epididymal spermatozoa (3). Cell-free translation of poly(A^+)RNA from seminal vesicle tissue resulted in formation of a polypeptide of M_r 18 kD, immunoreactive with anti-major protein antiserum (1). Here

we report the sequence of a cDNA clone derived from poly(A)$^+$RNA of bull seminal vesicle tissue encoding the precursor of the major protein. Following a suggestion of Mbikay et al. (4) we propose that this bull seminal protein be referred to as seminal vesicle secretory protein of 109 amino acids (SVSP109).

RESULTS

To screen poly(A)$^+$RNA from seminal vesicle tissue for the presence of major protein-specific mRNA, a synthetic DNA probe was designed and synthesised on the basis of the known amino acid sequence of the protein (2). The amino acid sequence extending from residue 78 to 94 was chosen and a 51 nucleotide long DNA, complementary to the corresponding mRNA, synthesized. Northern analysis of poly(A)$^+$RNA from seminal vesicle carried out with the synthetic probe indicated the presence of a SVSP109-specific mRNA species of 750bp. Recombinant clones (10^3) of a cDNA library of bull seminal vesicle poly(A)$^+$RNA were screened by colony hybridisation using the radioactively labeled synthetic probe yielding 18 positive clones. A positive clone containing the longest cDNA insert (pMP17) was subjected to sequencing. The obtained sequence of the cDNA insert of pMP17 (Fig. 1) contains an open reading frame extending over 405 nucleotides and starting with a leucine residue. The derived polypeptide sequence includes in the C-terminal part the 109 amino acid residue long sequence of the protein PDC109 as published by Esch et al. (2). The N-terminus of this polypeptide sequence is extented by 23 amino acid residues of a signal sequence which was regarded as incomplete because of the missing methionine start codon. Hence, the PstI-insert of the pMP17 DNA did not represent the complete mRNA of the precursor for SVSP109. Sequence analysis by direct mRNA sequencing employing reversed transcriptase and a sequencing primer complementary to the region 99-118 of the cDNA-insert extented the known sequence of pMP17 by 20 bp. The extension comprised the missing start and a short untranslated 5'-region of the mRNA.

The M_r of the 134 amino acid residue precursor polypeptide of SVSP109 is 14. 850. The precursor sequence of 25 amino acid residues has a hydrophobic character and very likely constitutes a signal peptide, directing the protein towards the secretory pathway (5). The

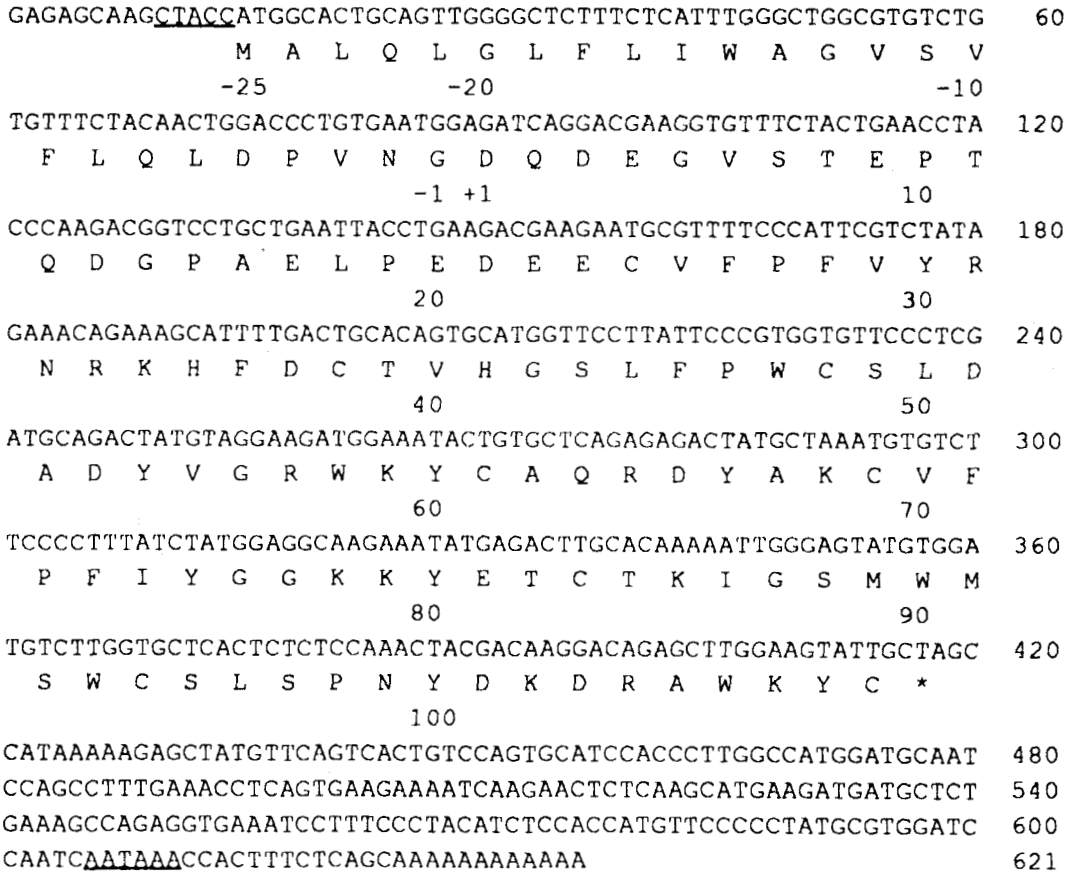

FIG.1 Nucleotides sequence of pMP17 and the deduced amino acid sequence for the precursor of SVSP109. The amino acid sequence numbering starts with +1 for SVSP109. The consensus sequence upstream of the ATG start codon as well as the polyadenylation signal are underlined. The arrow between +1 and -1 points to the site were the signal peptide (amino acid residues -25 to -1) is removed from the secreted SVSP109.

deduced amino acid sequence contained no consensus sequence indicative of N-glycosylation (N-X-S or N-X-T). The sequence immediately upstream the inition codon, CTACC, is highly homologous to the consensus sequence CCACC which controls translation efficiency of mammalian mRNAs (6; 7; 8). The cDNA-insert of pMP17 possesses a poly(A) tail and the putative poly-adenylation signal AATAAA, 13 nucleotides downstream of the poly(A) tail (9).

Northern analysis of seminal vesicle poly(A)$^+$RNA with the Pst1-insert of clone pMP17 furnished a mRNA species of 750 bp similar to the results obtained with the synthetic DNA probe.

Genomic DNA from an individual animal was analyzed by Southern blot after digestion with several restriction enzymes. Digestion with PstI and HindIII yielded four bands. In EcoRI digests, eight fragments were detected which reacted with the PstI-insert of pMP17. The results obtained by restriction with PstI and HindIII, in view of the fact that the cDNA probe itself contained no cleavage sites for both enzymes, could indicate the existence of more than one gene for SVSP109. The doubled number of fragments obtained by EcoRI restriction may result from cleavage by this enzyme within the gene.

DISCUSSION

It was firstly reported by Esch et al. (2) that protein PDC109 existed as a mixture of two polypeptide chains of identical sequence, possibly as the result of a partial oxidation of methionine residues. This observation was confirmed by Kemme et al. (1), Seidah et al. (10) and Manjunath et al (11). The amino acid sequence of a third protein of 115 amino acid residues, BSP-A3, recently published by Seidah et al. (10), displayed a 66% identity to the sequence of SVSP109. Seidah et al. (10) speculated that these two proteins arose via gene-duplication from an ancestral gene and that SVSP109 and BSP-A3 could either originate from a common protein precursor molecule or as the transcriptional products of two mRNAs. We show in this contribution that indeed the latter case is true, because the SVSP109-specific mRNA directs the synthesis of only one precursor polypeptide. Furthermore, we established the property of the major protein as a secretory product of bull seminal vesicle tissue. Baker (12) realized first a highly significant sequence similarity between SVSP109 and the collagen-binding domain of bovine fibronectin (13). This was verified also for protein BSP-A3 (10).

It remains to be shown whether the property of SVSP109 to specifically bind to bull epididymal spermatozoa is related to the fibronectin-like amino acid sequence.

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