Structural Characterization of the Rat Seminal Vesicle Secretion II Protein and Gene*

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The gene encoding rat seminal vesicle secretion II (SVS II) protein has been cloned from a rat genomic DNA library using a cDNA probe generated from rat dorsal prostate androgen-dependent mRNA. The cloned 7.3-kilobase pair genomic fragment contains ~5000 base pairs (bp) of the 5'-flanking region and the entire coding region of the SVS II protein within two exons. A sequence of 4156 bp of the rat SVS II gene has been determined, including 2037 bp of the 5'-flanking region, exon 1 (95 bp), intron 1 (236 bp), exon 2 (1171 bp), and 614 bp of the 3'-flanking region. The 5'-flanking region contains three conserved elements found in other seminal vesicle secretion genes (SVS IV–VI proteins) within 250 bp of the transcription start site as well as a glucocorticoid response element repeated 13 times within the SVS II protein and appears to be involved in the formation of the rat copulatory plug with the SVS II protein being the major component (Wagner and Kistler, 1987; Williams-Ashman, 1984). Clotting of the SVS II protein with itself and other SVS proteins is catalyzed by a transglutaminase that produces extensive covalent cross-linking between glutamine and lysine and the formation of γ-glutamyl-ε-lysine.

Recently, Dodd et al. (1986) identified a 260-bp cDNA in rat dorsolateral prostate that hybridized with an androgen-dependent 1.5-kb mRNA in both dorsolateral prostate and seminal vesicle. The cDNA clone, pRWB, was shown to select mRNA from total seminal vesicle or dorsolateral prostate encoding a protein with an Mᵣ of ~45,000. This protein was also the main immunoprecipitable translation product with antisera to the SVS II protein; and therefore, the pRWB cDNA was considered a useful probe for cloning the SVS II gene.

In a continuing effort to understand the molecular basis of androgen regulation, we have cloned and sequenced the rat SVS II gene using pRWB cDNA as a probe. Concomitant with the analysis of the SVS II gene, we isolated and characterized the rat SVS II protein. The strategy used and the evidence in support of the SVS II gene and protein sequence determinations are presented in this report. The results are remarkable in three ways. 1) The SVS II and IV–VI genes all share conserved elements in the 5'-flanking region potentially involved with androgen regulation. 2) Over 50% of the SVS

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† The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) J05443.

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1 The abbreviations used are: SVS, seminal vesicle secretion; bp, base pair(s); kb, kilobase pair(s); DDT, diethiothreitol; FAB, fast atom bombardment; PTC, phenylthiocarbamyl; PTH, phenylthiohydantoin; RP-HPLC, reverse-phase high performance liquid chromatography; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TPCK, tosylphenylalanyl chloromethyl ketone; SVP-1, seminal vesicle clotting protein 1.

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II protein is made up of a 13-amino residue repeat that appears to function as a transglutaminase substrate. 3) The SVS II protein exhibits little structural relatedness to any other known protein sequence, including another rodent protein of similar function.

**EXPERIMENTAL PROCEDURES AND RESULTS**

**Cloning and Characterization of SVS II Gene**—Rat dorsal prostate pRBW cDNA (Dodd et al., 1986) was used to screen a rat genomic DNA library in λ Charon 4A (Harris et al., 1983). Based upon SVS II amino acid sequence data, oligonucleotide probes were also prepared for screening purposes. From 1 of 10 genomic clones isolated, a 7.3-kb EcoRI fragment that hybridized to both the pRBW insert and the oligonucleotide probes was subcloned into plasmid pBS-SK (Stratagene), and a series of deletions were produced and subcloned for DNA sequencing and gene structure/function studies. The major deletions of the 7.3-kb EcoRI fragment subcloned for this study are presented in Fig. 1. Where overlaps were required, oligonucleotides (18–20-mers) were synthesized and used as DNA sequencing primers with the appropriate deletion plasmid. A restriction map and the sequencing strategy used in the analysis of the rat SVS II gene are presented in Fig. 2. A DNA sequence of 4156 bp of the SVS II gene including 2037 bp of the 5'-flanking region, exon 1 (96 bp), intron 1 (296 bp), exon 2 (1171 bp), and 614 bp of the 3'-flanking region are shown in Fig. 3. The DNA sequence 3' of position +1506 is 3'-noncoding and part of the second intron. Based upon homology, a 22-amino acid hydrophobic leader peptide is predicted from the DNA sequence (Fig. 3). Notably, the leader peptides in the SVS II and IV–VI proteins are preceded by a short, conserved 22-bp 5'-noncoding region (>80% identity). Based on a previously reported primer extension analysis of the SVS IV and V genes (Fawell et al., 1986), the major CAP protein is predicted to be at position +1 in the SVS II gene sequence (Fig. 3). The leader peptide plus 2 amino acids of the secreted protein are defined by exon 1; exon 2 defines the remaining 390 amino acids of the secreted SVS II protein.

**Purification and Characterization of SVS II Protein**—The rat SVS II protein was purified according to Wagner and Kistler (1987) and subjected to direct microchemical analysis. The deduced structure of the rat SVS II protein is supported by Edman analysis covering ~85% of the total sequence (Fig. 3). The single pyroglutamyl-blocked polypeptide chain contains 392 residues corresponding to an M, of 43,116, in good agreement with the amino acid composition determined experimentally (Table 1). A summary of the peptide sequences determined by Edman degradation covering ~85% of the total sequence (Fig. 3) yields the sequence of 81 residues from seven lysyl peptides.

**Identification of the Blocked NH2-Terminal Structure**—The NH2-terminal pyroglutamyl-blocking group was determined by mass spectral analysis of the pyroglutamyl aminopeptidase digestion. The blocked NH2-terminal cysteine-bonded M1 peptide was purified by reverse-phase-HPLC (Fig. 10), and its amino acid composition was determined by phenylthiocarbamyl amino acid analysis (Table 1). The fast atom bombardment mass spectrum (Fig. 11) of the blocked M1 peptide (50...
pmol) recorded at low resolution (~700) over a mass range of 600–4000 exhibited an average (M + H)^+ signal at m/z 1741.7, an average (M + Na)^+ signal at m/z 1763.7 and, presumably from hydrolysis of the homoserine lactone generated during CNBr cleavage, an average (M + Na + Sb(HO)^+ signal at m/z 1781.8. Narrow mass range scans over the molecular ion region at higher resolution (~2000) yielded a monoisotopic (M + H)^+ signal at m/z 1740.7 and an (M + Na)^+ ion at m/z 1762.8 (data not shown). No sequence-informative fragments were observed. The molecular mass of the blocked M1 peptide calculated from the predicted sequence (QYGGTKGHFQSSSSGFMc) and amino acid composition is 1758.8 Da, a value 18.1 Da greater than the observed monoisotopic mass. Cyclization of the NH2-terminal Gln to pyroglutamic acid would yield the observed (M + H)^+ signal at m/z 1740.7. The identity of the blocking group and the sequence of the blocked M1 peptide were confirmed by enzymatic deblocking with pyroglutamyl aminopeptidase (Fig. 12) and Edman degradation (Fig. 5).

**DISCUSSION**

Genomic DNA clones for the rat SVS II gene have been isolated and characterized by sequence analysis. Concomitant with efforts to clone and sequence the SVS II gene, 85% of the rat SVS II protein was sequenced by Edman degradation; the deduced amino acid sequence corresponds exactly to that determined by direct analysis of the protein. Fast atom bombardment mass spectrometry and pyroglutamyl aminopeptidase digestion provided the data necessary to determine that the protein is N2-pyroglutamyl-blocked. The SVS II gene encodes an apparent leader peptide of 22 residues lacking in the secreted protein and also contains an intron of 239 nucleotides between residues 2 and 3 of the mature protein. Notably the combined use of protein chemistry, mass spectrometry, and molecular biology accelerated progress in this study. The results highlight the synergy of these methodologies. Isolation and direct characterization of the SVS II protein allowed: (i) the SVS II identity of the pRWB cDNA probe and then of the genomic clone to be confirmed; (ii) the structure of the secreted, post-translationally modified protein to be largely determined; (iii) the unambiguous establishment of the intron-exon borders in the gene; and (iv) the corroboration of the deduced SVS II protein sequence. Characterization of the SVS II gene: (i) revealed the existence of a leader peptide associated with the nascent polypeptide chain; (ii) allowed deduction of the complete SVS II protein sequence including the difficult-to-obtain overlapping sequences in repeating regions of protein structure; (iii) corroborated the COOH-terminal amino acid sequence; and (iv) revealed the location of conserved regions potentially involved in SVS II gene regulation.

The hydropathy profile of the SVS II protein (Fig. 13) exhibits the hydrophilic nature of the secreted protein as well as of the hydrophobic leader peptide and is similar to that of the SVS IV–VI proteins (Schifman et al., 1988). From secondary structure predictions (Fig. 13), the SVS II protein resembles the SVS IV protein in that it contains ~50% predicted \( \alpha \)-helical structure. Considerable predicted \( \beta \)-sheet structure is also present as in the SVS IV protein (Pan and Li, 1982), presumably due in part to the relatively high content of serine and basic amino acids. The higher \( \beta \)-sheet potential in the regions containing repetitive structure suggests that this may be the favored transglutaminase substrate conformation.

The SVS II protein contains significant internal repetitive structure. Over 50% of the protein (about residues 80–300) exhibits a 13-residue repeat with the consensus sequence "GSQLKSFQQQVQSSS" (Table 2). The positions of the underlined Gln, Lys, Ser, and Gly residues are over 90% invariant and are separated by a bulky, usually hydrophobic residue (e.g. Leu, Phe, and Val). The repeating structure complicated the direct protein sequence analysis by hindering the isolation of overlapping peptides. The repetitive structure in the SVS II gene is presented in Table 2 along with a 39-nucleotide consensus sequence. The repetitive structure degenerates considerably in both the 5’- and 3’-directions; but with appropriate deletions, the repeat structure can be seen within residues 80–300 of the coding region. Comparison of the 39-nucleotide consensus sequence with the remaining 5’- and 3’-coding regions of the SVS II gene using GenPro software revealed little identity. The SVS II gene appears to have evolved by internal duplication and rearrangement of a repeating unit.

Since the SVS II protein appears to be the major transglutaminase substrate in the formation of the rat copulatory plug (Wagner and Kistler, 1987), the repeating unit is probably involved in \( \gamma \)-glutamyl-\( \epsilon \)-lysine cross-linking. Spatial juxtaposition of the substrate residues for glutamine-lysine cross-linking may involve an antiparallel arrangement of the SVS II protein as proposed in Fig. 14. The abundant serine and glycine residues and the less abundant, but more bulky residues may contribute a favorable steric environment for cross-linking. A 24-residue repeating consensus structure (VTQGDSVKGRLQMKQDSLAFRS) reported for guinea pig seminal vesicle clotting protein 1 (SVP-1) (Moore et al.,...
1987) also contains invariant Gln, Lys, Ser, and Gly residues (underlined). The similarities between guinea pig SVP 1 and the rat SVS II protein consensus sequences are the dipeptide sequences GQ and VK and the 3 residues, including a bulky hydrophobic residue, separating glutamine and lysine residues (Fig. 14). Smaller repeating units can be found within the SVP-1 consensus sequence, and an antiparallel configuration could also potentially provide favorable conditions for glutamine-lysine cross-linking (Fig. 14). The SVS II protein and SVP-1 consensus sequences exhibit no significant structural similarity to several other known transglutaminase substrates such as those identified in human involucrin (Simon and Green, 1988) and the γ chain of human fibrin (Chen and Doolittle, 1971). Although SVP-1 and the SVS II protein most likely serve similar physiological functions in the formation of the vaginal plug following copulation (Williams-Ashman, 1984; Wagner and Kistler, 1987), based upon overall sequence comparison, these rodent proteins do not exhibit significant structural relatedness. Except for the 22-residue leader peptide, no evidence was found in the rat SVS II gene for a larger precursor such as the polypeptide precursor encoding guinea pig SVP-1 plus SVP-3 and SVP-4 (Hagstrom et al., 1989). Whether the SVS II and SVP-1 genes originated from a common ancestor is unclear; both were created by a process of duplication and perhaps evolved by convergent selection. It should be noted, however, that short oligonucleotide repeats have been proposed as the primordial source of all genes (Ohno, 1984). The SVS genes and proteins perhaps constitute a family. This possibility is suggested by the relatively high proportion of basic amino acids and eire in the rat SVS protein (Table 3), the conservation of 5'-noncoding regions in the SVS II and IV-VI genes (Table 4), the NH2- and COOH-terminal homology among the SVS IV-VI proteins, the common tissue-specific expression of the genes and protein functions all apparently related to reproductive physiology. Striking features of this possible family, however, are the limited sequence identity of the secreted proteins (McDonald et al., 1984; Schifman et al., 1988) and the lack of repetitive structure in all but the SVS II protein. Although the SVS IV-VI proteins show some conservation in amino acid sequence in the NH2- and COOH-terminal regions (Schifman et al., 1988), they exhibit no sequence similarity to the SVS II protein and lack the high glutamine content of the SVS II protein (Table 3).

Another striking feature of the SVS genes is the conserved sequences among SVS II and IV-VI in the 5'-flanking area. These homologous regions are presented in detail in Table 4. The 200 regions of the SVS II and IV genes have identical 13-bp sequences, and similar sequences are also found in this region of the SVS VI and V genes; the -200 region therefore appears to be a good candidate for a cis-control element, perhaps involved with androgen-mediated regulation. The -140 to -120 regions of the SVS II and IV-VI genes have inverted repeats with 8-12 bp loop structures. The initial analysis of the SVS IV and VI genes indicated little similarity in the loop, although both had a GC-rich stem structure. A similar GC-rich stem can be seen in the SVS II gene. Surprisingly, the loop has 10 out of 11 bp conserved between the SVS II, V, and VI genes. This palindromic structure is likely to also be part of a cis-control region for regulation of the SVS genes. Finally, in the -90 to -100 regions of the SVS II and IV-VI genes, there is a conserved DNA element exhibiting 12/13-bp identity. This third region of similarity in the SVS gene family shows some similarity in sequence and position to CAT box-like structures in a variety of other genes (Dyman and Tjian, 1985).

The identification of androgen-response elements devoid of glucocorticoid response has proven difficult. Since a glucocorticoid-response element sequence (GGTCCAatcTGTCCT) is observed in the SVS II gene at position -314, glucocorticoids also likely play a role in SVS II gene expression. Most glucocorticoid-response elements will respond to androgens in systems where the androgen receptor is present, e.g., MMTV-CAT constructions transfected into DDTI cells (Harris et al., 1988; Smith et al., 1990). With the SVS IV protein-CAT constructions and deletions, it was possible to separate a glucocorticoid response (positions -524 to -360) from an androgen response (positions -360 to -92) (Smith et al., 1990). Previous expression of the endogenous SVS IV gene in primary rat seminal vesicle epithelial cell cultures grown on extracellular matrix required both glucocorticoids and androgens. The availability of the SVS II genomic clone, the conservation of several elements in the 5'-flanking region with other androgen-dependent SVS genes, and the presence of a glucocorticoid-response element sequence in the SVS II gene make the SVS II gene system useful for studying the interplaying roles of glucocorticoid receptor, androgen receptor, and other trans-acting factors in transcriptional control of androgen-responsive genes.

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National Academy of Sciences. Biological Sciences

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Teng, C. T., and Harris, S. E. (1983) DNA (N. Y.) 2, 105-111.


EXPERIMENTAL PROCEDURES AND RESULTS

Materials. — Water was obtained from a Milli-Q apparatus. Buffer reagents were prepared fresh at least once a week. All other materials were obtained from Sigma Chemical Company. The following reagents were obtained from Fluka Chemical Company: All protein sequencing reagents and solvents were from Applied Biosystems. All internal standards were purchased from Sigma and CalBiochem. All proteins were obtained from Sigma. Restriction enzymes were from Pharmacia LKB Biotechnology, Inc., and Boehringer Mannheim Research Laboratories. All other chemicals and reagents were from the highest grade commercially available.


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Natl. Acad. Sci. U. S. A. 84, 6712-6714


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TABLE 3

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TABLE 4

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Small letters indicate non-identity of base pairs.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Summary of the SVI II peptide sequences determined by Edman degradation. The determined sequences of specific peptides are indicated. Peptides A, B, C, and D denote peptides generated by cleavage at tryptophan, lysine, and arginine residues, respectively. Peptides E to Q denote peptides generated with bromelain and ficin. Peptides A to Q were sequenced with an automated gas phase Edman degradation. QP denotes pyroglutamic acid.

Fig. 5

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Fig. 6: Primary separation of SVP II methylated peptides. Approximately 2.1 mg (50 nmoi) of a cyanogen bromide digest of SVP II protein (300 µl of 4 M urea) was injected onto a series of RPC-12 columns (column 1, 5.5 x 7.5 cm, 2.7 M acetonitrile, 72% acetic acid, 0.08% trifluoroacetic acid, 100 mM NaCl, and 72% methanol) at 0.5 ml/min using a gradient of 0-100% acetonitrile in 0.1% trifluoroacetic acid over 30 minutes. The eluate was pooled and lyophilized. The methionine residues were then removed using dithiothreitol (DTT) and 50% acetic acid at 72°C for 18 hours. The sample was then treated with 2% Pepsin at 37°C for 18 hours and the resulting peptides were separated by reversed-phase HPLC on a 10% C18 column (5 µm, 4.6 x 250 mm) using a gradient of 0-60% acetonitrile in 0.1% trifluoroacetic acid over 60 minutes.

Fig. 7: RP-HPLC of methylated peptides. Approximately 440 µg (130 nmoi) of cyanogen bromide digest of SVP II protein (500 µl of 4 M urea) was injected onto a Series of RPC-12 columns (column 1, 5.5 x 7.5 cm, 2.7 M acetonitrile, 72% acetic acid, 0.08% trifluoroacetic acid, 100 mM NaCl, and 72% methanol) at 0.5 ml/min using a gradient of 0-100% acetonitrile in 0.1% trifluoroacetic acid over 30 minutes. The eluate was pooled and lyophilized. The methionine residues were then removed using DTT and 50% acetic acid at 72°C for 18 hours. The sample was then treated with 2% Pepsin at 37°C for 18 hours and the resulting peptides were separated by reversed-phase HPLC on a 10% C18 column (5 µm, 4.6 x 250 mm) using a gradient of 0-60% acetonitrile in 0.1% trifluoroacetic acid over 60 minutes.

Fig. 8: RP-HPLC of tryptic peptides. Approximately 140 µg (5 µmol) of cyanogen bromide digest of SVP II protein (500 µl of 4 M urea) was injected onto a Series of RPC-12 columns (column 1, 5.5 x 7.5 cm, 2.7 M acetonitrile, 72% acetic acid, 0.08% trifluoroacetic acid, 100 mM NaCl, and 72% methanol) at 0.5 ml/min using a gradient of 0-100% acetonitrile in 0.1% trifluoroacetic acid over 30 minutes. The eluate was pooled and lyophilized. The methionine residues were then removed using DTT and 50% acetic acid at 72°C for 18 hours. The sample was then treated with 2% Pepsin at 37°C for 18 hours and the resulting peptides were separated by reversed-phase HPLC on a 10% C18 column (5 µm, 4.6 x 250 mm) using a gradient of 0-60% acetonitrile in 0.1% trifluoroacetic acid over 60 minutes.

Fig. 9: RP-HPLC of tryptic peptides. Approximately 1.5 mg (4.5 µmol) of cyanogen bromide digest of SVP II protein (500 µl of 4 M urea) was injected onto a Series of RPC-12 columns (column 1, 5.5 x 7.5 cm, 2.7 M acetonitrile, 72% acetic acid, 0.08% trifluoroacetic acid, 100 mM NaCl, and 72% methanol) at 0.5 ml/min using a gradient of 0-100% acetonitrile in 0.1% trifluoroacetic acid over 30 minutes. The eluate was pooled and lyophilized. The methionine residues were then removed using DTT and 50% acetic acid at 72°C for 18 hours. The sample was then treated with 2% Pepsin at 37°C for 18 hours and the resulting peptides were separated by reversed-phase HPLC on a 10% C18 column (5 µm, 4.6 x 250 mm) using a gradient of 0-60% acetonitrile in 0.1% trifluoroacetic acid over 60 minutes.

Fig. 10: Identification of the tryptic peptides. Positive ion FAB mass spectra of the tryptic peptides were obtained at a resolution of 3000 over a mass range of 500 - 2000, only the region displaying signals is shown.
Fig. 12. Purification of sperm (tac) aminopeptidase digested peptide NI. About 2.4 mg (1.6 mmol) of sperm NI aminopeptidase was cleaved with sperm (tac) aminopeptidase as described in Experimental Procedures and purified by RP-HPLC on a 3.9 by 15 cm reverse phase column (1.0x5 mm) using ammonium acetate/trifluoroacetic acid gradient. Above, analytical profile of peptide NI mixture. Addition of sperm (tac) aminopeptidase and below, purification of the de-blocked NI peptide after digestion with sperm (tac) aminopeptidase. About 95% yield of the de-blocked NI peptide was recovered.

Fig. 13. Secondary structure predictions by DVP tool. The hydrophobic profile was calculated according to Chou and Fasman, 1978 using a window size of 10 residues. Secondary structure predictions are according to Chou and Fasman, 1978 using a window size of 5 residues.
Structural characterization of the rat seminal vesicle secretion II protein and gene.
S E Harris, M A Harris, C M Johnson, M F Bean, J G Dodd, R J Matusik, S A Carr and J W Crabb


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