Primary structure and characterization of an androgen-stimulated autoantigen purified from mouse seminal-vesicle secretion

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A protein extract of mouse seminal-vesicle secretion was used to immunize mature mice (Balb/c) of both sexes. Results of Western-blot analyses for these secretory proteins indicated that only one minor protein component could be recognized by the autoantisera prepared from either autoimmunization of male mice or isoimmunization of female mice. The autoantigen was purified from seminal-vesicle secretion. The purified autoantigen retained the ability to induce autoantibody formation. The autoantigen has glycoprotein characteristics: the majority of the carbohydrate is N-linked and the remainder is O-linked. Rabbit antibodies to the autoantigen were used to isolate the corresponding cDNA from a mouse seminal-vesicle cDNA library. The primary structure deduced from the cDNA sequence was confirmed by direct amino acid sequence determination. The results indicate that the core protein consists of 131 amino acid residues. Analysis of the primary structure indicates that the autoantigen has two potential acceptor sites for the N-linked carbohydrate at Asn-12 and Asn-122, three potential phosphorylation sites for casein kinase II at Thr-55, Ser-68 and Thr-76, and three potential phosphorylation sites for protein kinase C at Thr-28, Thr-40 and Thr-124. The core protein and the carbohydrate portion together have a molecular mass of 19 kDa. Results from Western- and Northern-blot analyses for various tissues indicate that the seminal vesicle is the sole organ producing this autoantigen. Expression of this autoantigen gene was stimulated by testosterone.

INTRODUCTION

It is well-known that the seminal vesicles in adult mammals secrete a group of proteins that constitute the main proportion of seminal plasma. However, their biological significance remains obscure. A total of seven major proteins in mouse seminal-vesicle secretion (SVS) and five major proteins in rat SVS have been identified according to their molecular mass and distribution (Ostrowski et al., 1979; Mansson et al., 1981; Chen et al., 1987). The primary structures established for some of them show no correlation with the protein structures known thus far although their expression has been shown to be androgen-dependent. Much less progress has been made, however, in studying the minor protein components in SVS, except for a Kazal-type trypsin inhibitor reported recently (Lai et al., 1991). This is probably due to the difficulty in identification and purification of trace amounts of target proteins with unknown function.

This work reports on the characterization and cDNA cloning of a minor protein component purified from mouse SVS. It was found exclusively in seminal vesicles and was able to induce autoantibody formation. The autoantigen was shown to be a 19 kDa glycoprotein with a core protein consisting of 131 amino acid residues. Testosterone was found to stimulate the expression of this autoantigen gene.

EXPERIMENTAL

Materials

CM-Sephadex C-25, Sephadex G-75 and Protein A–Sepharose were purchased from Pharmacia (Uppsala, Sweden). Markers for isoelectric focusing and thin-layer ampholine PAGE were obtained from Sartorius G.m.b.h. A protein-pak DEAE 5 pw column and a Chemecopak 5 ODS-H column were obtained from Waters Co. (Millipore Corp., Bedford, MA, U.S.A.). *N*-Glycosidase F (glycopeptidase F), *O*-glycosidase (endo-α-*N*-acetylgalactosaminidase) and *N*-neuraminidase were obtained from Boehringer–Mannheim G.m.b.h. All of the reagents and enzymes used in the construction of the λgt11 cDNA library and the T7 DNA polymerase sequencing system were purchased from Promega (Madison, WI, U.S.A.). ^{125}I-labelled anti-rabbit IgG prepared from donkey, ^{125}I-labelled anti-mouse IgG prepared from rabbit and [α-^{32}P]dATP were obtained from Amersham Searle (Arlington Heights, IL, U.S.A.). Freund's adjuvants were from Sigma (St. Louis, MO, U.S.A.). All chemicals were of reagent grade.

Animals

Inbred Balb/c mice were supplied by the Animal Center, College of Medicine, National Taiwan University. They were housed under controlled lighting (14 h light, 10 h dark) at 21–22 °C and were provided with water and NIH 31 laboratory mouse chow *ad libitum*.

Preparation of autoantisera to mouse SVS

The secretion collected from the seminal vesicles of 100 mature male mice (Balb/c, 10 weeks) was expressed directly into 100 ml of ice-cold PBS. After being stirred at 4 °C for 30 min, the solution was clarified by centrifugation at 14000 *g* for 30 min and passed through a glass filter to remove lipid.

Adult mice of both sexes were injected with mouse SVS extract subcutaneously three times, with an interval of 10 days between

Abbreviations used: SVS, seminal-vesicle secretion; SVA, seminal-vesicle autoantigen.

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The sequence data will appear in the EMBL/GenBank/DDBJ Nucleotide Sequences Databases under accession number M94179.

injections. Each injection consisted of 0.1 ml of the saline extract of mouse SVS (10 mg of protein/ml), which was mixed with an equal volume of complete Freund's adjuvant for the first injection or incomplete Freund's adjuvant for the last two injections. The same schedule was followed in the administration of PBS to the control animals. Autoantisera from autoimmunization of male mice or isoimmunization of female mice and sera from the control animals were collected by exsanguination using cardiac puncture.

The seminal-vesicle protein recognized by autoantiserum to mouse SVS was named seminal-vesicle autoantigen (SVA).

Purification of mouse SVA

The saline extract was subjected to CM-Sephadex C-25 chromatography (Chen et al., 1987). The eluate was then applied to a Sephadex G-75 column (2.5 cm × 75 cm), washed with 0.1 M ammonium acetate, pH 6.8, at a flow rate of 8 ml/h; fractions of volume 2 ml were collected. SVA appeared in fractions from peak I (see Figure 2a).

The peak-I fractions were resolved further by h.p.l.c. on a protein-pak DEAE 5 pw column (7.5 mm × 75 mm, 10 μm) equilibrated with 0.01 M ammonium acetate, pH 6.8. The column was eluted with a linear gradient of 0–60 % ammonium acetate (1.0 M) at a flow rate of 1.0 ml/min for 25 min (see Figure 2b).

Preparation of antibody to SVA-I

Antisera collected from immunization of rabbits with SVA-I were salted out by addition of 15 % (w/v) $(NH_4)_2SO_4$. The IgG fraction was then separated using a Protein A–Sepharose column.

SDS/PAGE, isoelectric focusing and protein blotting

Proteins were resolved by SDS/PAGE on a 15 % gel slab (6.5 cm × 10.5 cm × 0.075 cm) by the method of Laemmli (1970). Electrophoresis was conducted at 120 V for 1.5 h. The proteins on the gel were stained with Coomassie Brilliant Blue or periodic acid–Schiff's reagent, or transferred to a nitrocellulose membrane by the diffusion method (Bowen et al., 1980) at room temperature for 24 h. After transfer, protein blots were immunodetected by Western-blot procedures with the antisera or the antibody concerned.

Isoelectric focusing on a thin-layer ampholine PAGE plate (pH range 3–10) was performed using an LKB multiphor unit. The cathodic fluid consisted of 0.44 % Arg/0.06 % Lys, which was adjusted to pH 10 by the addition of ethylenediamine. The anodic fluid contained 0.33 % Asp/0.37 % Glu at pH 3.0. Focusing was conducted for 4 h at a constant power of 2.0 W with initial current of 10 mA and a maximum voltage of 1.7 kV.

Fragmentation of SVA-I

SVA-I was reduced in 6 M urea/5 mM dithioerythritol (pH 8.5) at 45 °C for 1 h. Alkylation of the reduced group was achieved by addition of iodoacetamide to a final concentration of 40 mM at room temperature for 20 min. The alkylated derivative was digested with trypsin in 0.05 % NH_4HCO_3 (pH 6.8) at 37 °C for 1 h. The trypsin digests were resolved by h.p.l.c. on a Chemecopak 5 ODS-H column (4.6 mm × 250 mm, 5 μm) using a linear gradient of 5–60 % acetonitrile in 0.1 % trifluoroacetic acid.

Amino acid analysis and sequencing

Protein was hydrolysed with the vapour phase of 7.0 M HCl containing 10 % (v/v) trifluoroacetic acid and 0.1 % phenol at 158 °C for 30 min, by the method of Tsugita et al. (1987). The amino acid composition was determined by the conventional method of Spackman et al. (1958) on a Beckman system 6300 high-performance analyser. The amino acid sequence was determined by automated Edman degradation with a gas-phase microsequenator (477A protein sequencer with on-line 120A analyser; Applied Biosystems, Foster City, CA, U.S.A.).

Sugar analysis

The core protein and carbohydrate moiety of SVA were quantified by the modified Lowry method (Peterson, 1977) and the L-cysteine sulphuric acid assay (Chaplin, 1987) respectively. The glycoproteins were deglycosylated by treatment with trifluoromethanesulphonic acid at 0 °C for 2 h (Edge et al., 1981). *N*-Glycosidase F digests of glycoproteins were prepared by digestion with the enzyme at 37 °C for 24 h (Plummer et al., 1984). The *N*-glycosidase F-digested samples were digested further with *N*-neuraminidase at 37 °C for 5 h, followed by digestion with *O*-glycosidase at 37 °C for 24 h (Umemoto et al., 1977).

RNA isolation, cDNA preparation, cloning and analysis

Total cellular RNA was isolated from adult mice SV, and double-stranded cDNAs were prepared from the polyadenylated fraction by standard procedures (Sambrook et al., 1989). RNA samples were analysed by separation in denaturing 1.5 %-agarose/formaldehyde gel (Lehrach et al., 1977) followed by capillary transfer to nylon membrane and hybridization to a ^{32}P-labelled nucleotide probe (Sambrook et al., 1989).

The strategy for orientation-specific cDNA cloning in λgt11 was generally according to the procedure recommended by Promega. Double-stranded DNAs with one *Eco*RI terminus and one *Not*I terminus were prepared by standard procedures using a Riboclone cDNA synthetic system with a *Not*I primer adaptor and an *Eco*RI adaptor ligation system. The cDNAs were ligated into the digested *Eco*RI–*Not*I arms of the λgt11 *Sfi*–*Not* vector. Transfection of the bacterial host Y1090 (r^-) with the recombinant phage made all the cDNA molecules clone in the same orientation relative to the *lacZ* gene in the vector. The cDNA library efficiency was 3×10^5 plaque-forming units/μg of cDNA. Phages from the cDNA library in λgt11 were immunochemically screened using anti-(SVA-I) antibody. We screened 15 positives from 20000 plaques. Randomly chosen positives from different pools were plaque purified, and phage DNA was then prepared. The cDNA inserts were cleaved from the recombinant phage by *Not*I–*Sfi*I digestion. The *Not*I–*Sfi*I fragment was subcloned into a pGEM-11 Zf(−) vector and expressed in *Escherichia coli* strain JM109. Positive clones containing the cDNA inserts were confirmed by *Sfi*I–*Not*I digestion.

cDNAs were sequenced by the dideoxy T7 DNA polymerase technique using T7 promoter as the primer (Tabor and Richardson, 1987). Each base was determined at least three times.

The random-primed DNA probe was prepared by the method of Feinberg and Vogelstein (1983).

RESULTS

Identification of autoantigen in mouse SVS

The saline extract of SVS was resolved electrophoretically into

five visible protein bands that were defined in decreasing order of molecular size according to the nomenclature of Chen et al. (1987) (Figure 1, lane 2). A broad 30 kDa protein band between bands III and IV was recognized by antisera obtained from autoimmunization of male mice or isoimmunization of female mice with the saline extract of SVS (Figure 1, lanes 3 and 5). The protein, namely SVA, was a minor component of mouse SVS. None of the SVS proteins were immunodetected by sera from control animals of either sex (Figure 1, lanes 4 and 6). Among the SVS proteins, SVA was apparently the most effective at inducing autoantibody formation. The production of a high level of anti-SVA sera by isoimmunization suggests that SVA may be a non-self-antigen in females.

Purification and preliminary characterization of SVA

SVA was purified from SVS through a series of isolation steps (Figure 2; see the Experimental section). The eluate from CM-Sephadex C-25 chromatography of the saline extract of mouse SVS was resolved by gel chromatography on a Sephadex G-75 column (Figure 2a). It was resolved into two peaks. Only traces of SVA were immunodetected in peak-II fractions by the autoantisera. Peak-I fractions contained abundant SVA, as peak-I protein showed a single broad band with the same mobility as SVA on SDS/PAGE and was immunodetected by the auto-antiserum. It could also be stained with periodic acid–Schiff's reagent (not shown), indicating its glycoprotein nature. The molecular mass of SVA was estimated to be 22 kDa from the gel-chromatography partition coefficient. The ratio of carbohydrate to protein was determined to be 21:100 by weight. The peak-I fractions were further resolved by h.p.l.c. on a DEAE column into two major components, SVA-I and SVA-II (Figure 2b), final yields being 1.2% and 0.4% for SVA-I and SVA-II respectively. Both were glycoproteins recognized by the autoantiserum. However, they were indistinguishable on SDS/PAGE (Figure 3, lanes 1 and 2) although their isoelectric points, determined on a thin-layer ampholine PAGE plate (pH 3–10), were 6.1 and 5.9 (SVA-I and SVA-II, respectively) (not shown). Direct amino acid analysis of SVA-I and SVA-II showed that their amino acid compositions were almost identical.

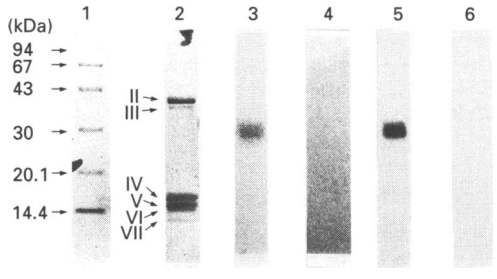

Figure 1 Immunochemical interaction between SVS proteins and SVS-induced autoantisera

The saline extract of mouse SVS (30 μg) was resolved by SDS/PAGE on a 15% gel slab. The standard protein markers (lane 1) and the SVS protein components (lane 2) were stained with Coomassie Brilliant Blue. The SVS proteins were defined as described by Chen et al. (1987). The SVS proteins on nitrocellulose membranes were immunodetected by Western-blot procedures with the autoantisera from autoimmunization (lane 3) and isoimmunization (lane 5), and with the control sera from male mice (lane 4) and female mice (lane 6).

Glycoprotein nature of SVA

Deglycosylation of SVA-I and SVA-II by treatment with trifluoromethanesulphonic acid reduced the molecular mass of both glycoproteins to 16.5 kDa. Both core proteins gave a sharp band and did not stain with periodic acid–Schiff's reagent on SDS/PAGE (Figure 3, lane 3). Exhaustive digestion of SVA-I with *N*-glycosidase F produced two protein components that appeared on SDS/PAGE as a sharp 18.5 kDa band and as a broad 24 kDa band (Figure 3, lane 4). It is probable that the 18.5 kDa band, which is slightly larger than the core protein, resulted from complete removal of the N-linked carbohydrate from the parent molecule. The majority of the carbohydrate conjugation in SVA-I appeared to be the *N*-linked moiety. The 24 kDa component was a partially digested product that still contained a portion of the N-linked carbohydrate. These data

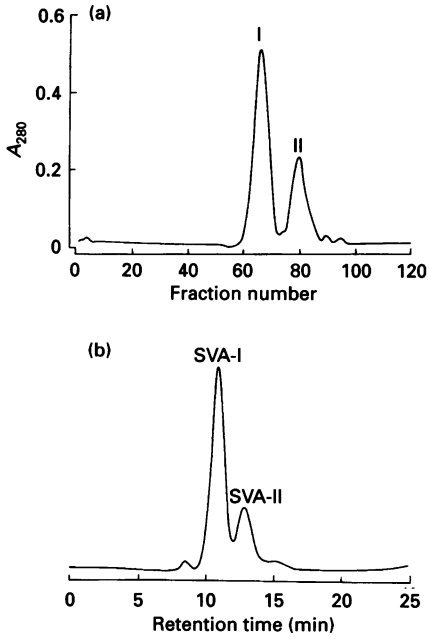

Figure 2 Purification of SVA from mouse SVS (see the text for details)

(**a**) Partial purification of SVA by gel chromatography on a Sephadex G-75 column. SVA appeared in peak-I fractions. (**b**) Separation of SVA-I and SVA-II by h.p.l.c. of peak-I fractions on a protein-pak DEAE 5 pw column.

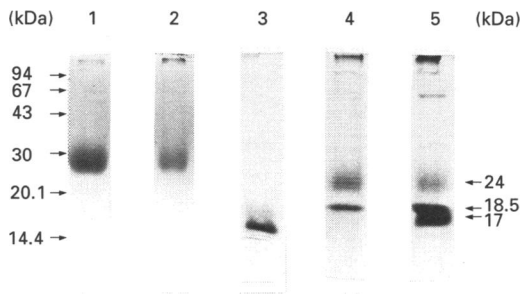

Figure 3 SDS/PAGE showing the glycoprotein nature of SVA

Proteins were stained with Coomassie Blue; lane 1, SVA-I (5 μg); lane 2, SVA-II (5 μg); lane 3, the chemically deglycosylated SVA-I (3 μg); lane 4, the *N*-glycosidase F-digested SVA-I (3 μg); lane 5, the *O*-glycosidase-digested sample of the *N*-glycosidase F-digested SVA-I (5 μg).

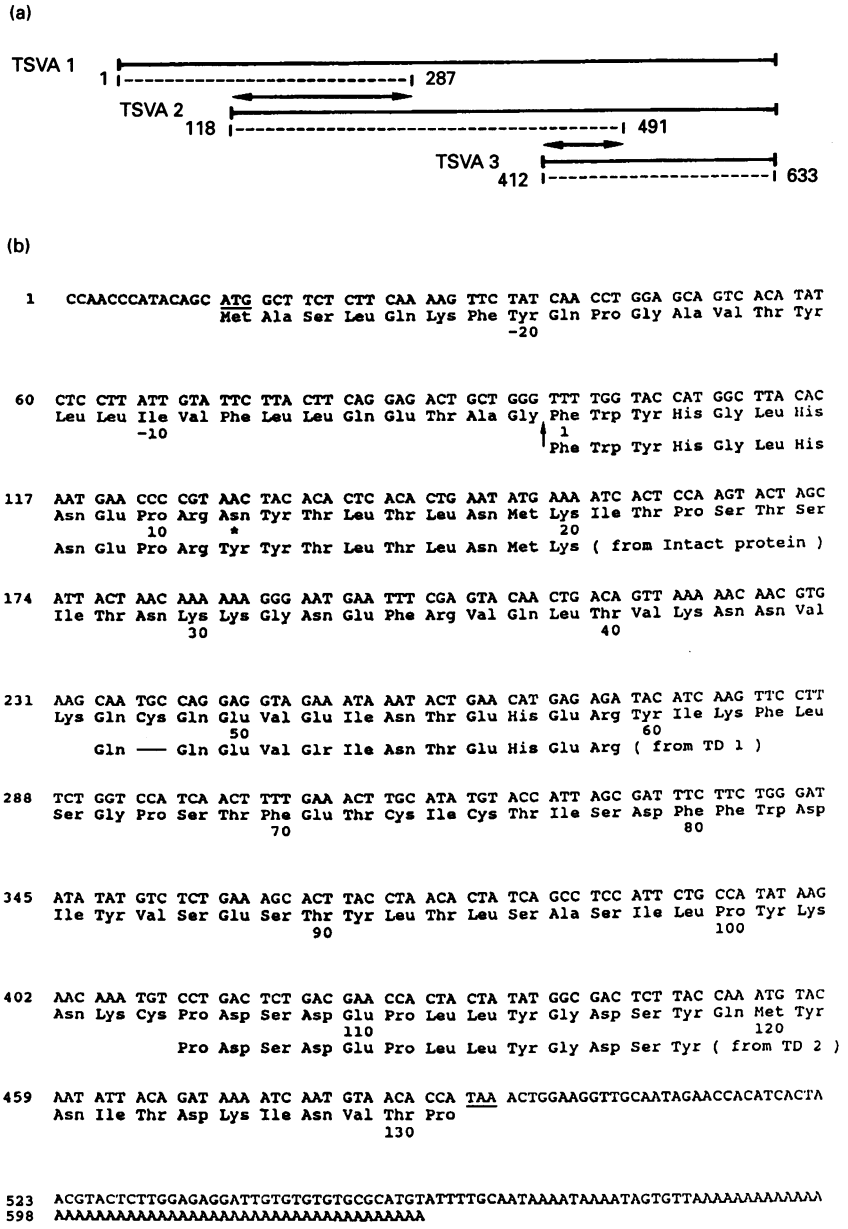

Figure 4 Determination of SVA primary structure by cDNA cloning and protein sequencing

(**a**) Construction of SVA cDNA. The overlapping regions (denoted by arrows) between sequences (dashed lines) determined from the cDNA inserts (solid lines) of TSVA 1, TSVA 2 and TSVA 3 were used to construct a 633 bp cDNA. The length of the solid and dashed lines is proportional to the number of nucleotides. (**b**) The nucleotide sequences of SVA cDNA and the deduced amino acid sequences. The initial and stop codons of the open reading frame are underlined. The cleavage point for the generation of the mature peptide is indicated by an arrow. The deduced SVA protein sequence was compared with the sequence derived directly from the protein and the trypsin-digested peptide fragments (TD 1 and TD 2) by Edman degradation. The deduced sequence and the sequence determined directly from the protein disagree at position 12, which is denoted by an asterisk.

suggest that SVA-I might have more than one acceptor site for the N-linked carbohydrate. Digestion of the *N*-glycosidase F-digested sample with *O*-glycosidase further reduced the molecular mass to 17 kDa, which is close to that of the core protein (compare lanes 3 and 5 of Figure 3). This suggests the existence of a minor amount of O-linked carbohydrate in addition to the N-linked carbohydrate in SVA-I.

Primary structure of SVA

Phages from the mouse seminal-vesicle cDNA library in λgt11 were screened immunologically with the rabbit anti-(SVA-I) antibody. A total of three randomly chosen positives designated TSVA 1–3 were used to construct a cDNA sequence corresponding to SVA (Figure 4a). Aligning the partial sequences determined from both TSVA 1 cDNA insert (approx. 650 bp) and TSVA 2 cDNA insert (approx. 500 bp) and the complete sequence of TSVA 3 cDNA insert (222 bp) led to the establishment of a 633 bp cDNA sequence. This cDNA sequence included a 5′-untranslated region of 14 bp, an open reading frame of 474 bp which encodes 158 amino acid residues and a 3′-untranslated region of 145 bp which ends with a polyadenylated

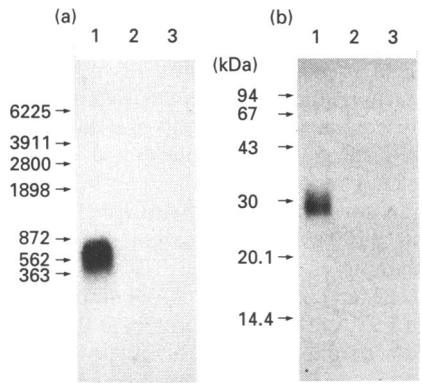

Figure 5 Tissue distribution of SVA

Northern-blot analysis of total RNA (**a**) and Western-blot analysis of protein extract (**b**) from seminal vesicle (lane 1), prostrate (lane 2) and coagulating gland (lane 3) are shown. Total RNA prepared from each tissue (100 μg) was run on a 1.5%-agarose/formaldehyde gel, transferred to a nylon membrane and probed with ^{32}P-labelled random-primed DNA to the cDNA insert of TSVA 3. Protein extract from each tissue (10 μg) was resolved by SDS/PAGE on a 15% gel slab, and SVA protein was detected by immunoblotting with rabbit anti-(SVA-I) antibody and anti-rabbit ^{125}I-IgG prepared from donkey. The nucleotide number of the RNA markers and the molecular masses of standard proteins are denoted on the left.

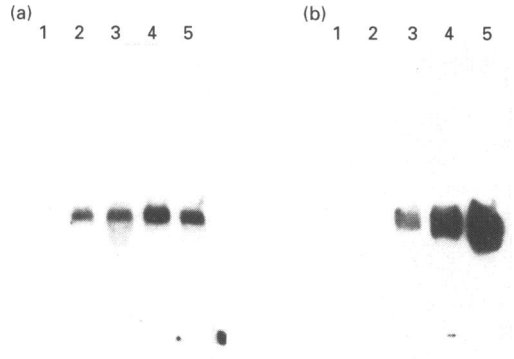

Figure 6 SVA synthesis in mice at different ages

Northern-blot analysis of total RNA (**a**) and Western-blot analysis of protein extract (**b**) from seminal vesicles of mice aged 3 weeks (lane 1), 4 weeks (lane 2), 5 weeks (lane 3), 6 weeks (lane 4) and 8 weeks (lane 5). Total RNA (100 μg) or protein extract (10 μg) was used for each experiment. Detection of SVA mRNA and protein was as described in Figure 5.

region (Figure 4b). The relationship of the cDNA sequence to SVA was confirmed unequivocally by the identification of the cDNA-deduced peptide sequence corresponding to that determined from direct amino acid sequence analysis. Automated Edman degradation of both SVA-I and SVA-II for 20 cycles gave reliable data. The same phenylthiohydantoin amino acid was identified for the two proteins at each corresponding cycle, suggesting that they may be microheterogeneous glycoproteins with very similar core proteins. The amino acid sequence determinations gave phenylalanine as the N-terminal residue and the amino acid sequence, FWYHGLHNEPRYYTLTLNMK, which is identical with the corresponding cDNA-deduced peptide sequence in all positions except for the 12th amino acid residue which was determined to be tyrosine from protein sequencing but asparagine from cDNA sequencing. Apparently, the post-translational cleavage occurs at the Gly-Phe peptide bond in the signal peptide containing 27 amino acid residues to produce a putative mature protein of 131 amino acid residues with a molecular mass of 15282 Da. The primary structures of the two peptide fragments resulting from the trypsin digest of SVA-I (denoted TD 1 and TD 2 in Figure 4) were established from the major phenylthiohydantoin amino acid detected in each cycle of automated Edman degradation, which confirmed residues 47–59 and 106–118 of the cDNA-deduced protein. This protein core together with the conjugated carbohydrate have a molecular mass of 19 kDa, which is much lower than the values estimated either from SDS/PAGE or from gel chromatography. The glycoprotein nature of SVA may account for this discrepancy. The cDNA-deduced primary structure of SVA was screened against the GCG database in GenBank and EMBL (Genetics Computer Groups). However, no significant similarity with the protein sequences was found in the database.

Tissue distribution of SVA

Both SVA-I and SVA-II could induce autoantibody formation. Rabbit anti-(SVA-I) antibody could recognize SVA-II. The antibody was used in the Western-blotting procedure on protein extracts from both sexual organs, such as ovary, uterus, vagina, testis, epididymis, seminal vesicle, coagulating gland, vas deferens and prostate, and non-sexual organs, including brain, heart, lung, thymus, liver, pancreas, duodenum, spleen and kidney, to examine the distribution of SVA in adult mice. The immunodetection experiment showed a large amount of SVA in the seminal vesicle but none in the other tissues (Figure 5b). The random-primed DNA probe to the cDNA insert of TSVA 3 was used in a Northern-blot hybridization on the total RNA of seminal vesicle, prostate and coagulating gland. The SVA mRNA was found exclusively in seminal vesicule (Figure 5a).

Testosterone induction of the expression of mouse SVA gene products

The level of SVA mRNA and protein in the seminal vesicles of mice at different ages was assessed (Figure 6). Mice at 3 weeks old were apparently not mature enough to produce either SVA mRNA or SVA protein. A considerable amount of SVA mRNA but almost no SVA protein appeared in 4-week-old mice. Thereafter, the expression of SVA gene to produce mRNA increased gradually and reached a maximum in 6-week-old mice. SVA protein appeared in 5-week-old mice; the older the mice, the more SVA protein was detected. These data demonstrate the modulation of SVA-gene expression during seminal-vesicle growth, which is well-known to be androgen-dependent.

We examined the influence of androgen on SVA gene expression in seminal vesicles after treatment with testosterone (5 mg/kg body weight per day, injected subcutaneously in corn oil) of mice (14 weeks) that had been castrated 3 weeks earlier. Induction of SVA mRNA and protein was observed after daily injection of testosterone for 8 days (Figure 7). Both SVA mRNA and SVA protein were undetectable in material from the castrates that had received daily injection of corn oil only. When compared with normal adults, considerable amounts of both SVA mRNA and SVA protein were present in material from animals that had received daily injection of testosterone in corn oil. The absence of mRNA and protein from control castrates and the increase seen after hormone administration indicate that the production of SVA is stimulated by testosterone.

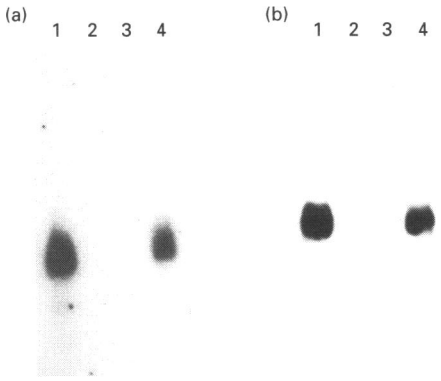

Figure 7 Demonstration of testosterone-dependence of mouse SVA synthesis

Northern-blot analysis of total RNA (**a**) and Western-blot analysis of protein extract (**b**) from mouse seminal vesicles of normal adult (lane 1), adults castrated 3 weeks previously (lane 2), adults castrated 3 weeks previously and receiving corn oil for 8 days (lane 3), and adults castrated 3 weeks previously and receiving testosterone in corn oil for 8 days (lane 4). Total RNA (100 μg) or protein extract (10 μg) was used for each experiment. Detection of SVA mRNA or SVA protein was as described in Figure 5.

DISCUSSION

Purification of a minor protein component from a mixture becomes feasible if a method of identifying the target protein is available. As mouse SVA can be recognized by the mouse SVS-induced autoantiserum, an immunochemical detection method was used to identify SVA during the isolation procedures. Of the mouse SVS proteins, SVA is the most effective at inducing autoantibody formation. This should not result from allograft because inbred Balb/c mice were used in this study.

Androgen-stimulated SVA is a 19 kda glycoprotein that has not been isolated and identified previously. It is different from the histocompatability complex antigens that could not be permanently tolerated in allograft. This autoantigen contains large amounts of asparagine (11 residues) and serine/threonine (25 residues in total), and a net excess of acidic residues over bases which gives rise to the slightly acidic isoelectric point. The positions of the four cysteines, Cys-48, Cys-73, Cys-75 and Cys-105, divide the polypeptide chain into three regions: an N-terminal region of 47 residues, a central region of 58 residues and a C-terminal region of 26 residues. The charged groups are distributed unevenly over the protein. At physiological pH values, the net charges are +6e in the N-terminal region, −3e in the central region and −5e in the C-terminal region. Moreover, two important structures are implicated from the primary structure. Firstly, Asn-12 and Asn-122, being part of the consensus Asn-Xaa-(Ser/Thr), are likely to be acceptor sites for N-linked carbohydrate (Marshall, 1972; Gavel and von Heijne, 1990). This may account for the change in molecular size during the *N*-glycosidase F digestion of SVA-I (Figure 3). Glycosylation of Asn-12 may lead to misidentification of phenylthiohydantoin amino acid during automatic Edman degradation, and this explains the discrepancy in the sequence analyses mentioned above. Secondly, SVA contains phosphate (Y.-H. Chen, unpublished work). Thr-55, Ser-68 and Thr-76 are the potential phosphorylation sites for casein kinase II (Pinna, 1990), and Thr-28, Thr-40 and Thr-124 are the potential sites for protein kinase C (Kishimoto et al., 1985).

Castration effects considerable involution of the seminal vesicles. Lack of SVA mRNA and protein in the seminal vesicles of castrates may be due to apoptosis of the sexual gland. The increase in SVA mRNA and protein after testosterone administration to castrates may result either from androgen-induced cell proliferation or from androgen-dependent gene expression. More studies are required to discover the function of this phosphorylated glycoprotein autoantigen.

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