Androgen-dependent Protein from Mouse Vas Deferens

dDNA CLONING AND PROTEIN HOMOLOGY WITH THE ALDO-KETO REDUCTASE SUPERFAMILY*

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We report the cloning and sequencing of a 1225-base pair cDNA encoding an abundant protein from mouse vas deferens. An open reading frame of 948 nucleotides encodes a protein of 316 amino acids with a calculated mass of 35,965 Da. A high degree of homology was found between this protein and members of the aldoketo reductase superfamily, especially aldose reductase from human placenta (82%) and from rat lens (89%), suggesting that it could be an aldose reductase. Castration resulted in a marked decrease in the level of the 1.4 kilobase mRNA coding for the 35,965 protein, whereas administration of testosterone to castrated males resulted in a marked increase. Southern hybridization analysis of mouse genomic DNA revealed relatively simple patterns of bands.

The mammalian vas deferens is a single long tubule which emerges from the tail of epididymis, goes through the inguinal canal, and enters the abdominal cavity. Then, it reaches the back of urinary bladder where it is joined by the seminal vesicle forming the ejaculatory duct which traverses the prostate and empties into the urethra. It is generally believed that mammalian spermatozoa acquire the capacity to fertilize ova during transit through the epididymis (1). This process, called sperm maturation, is dependent on androgens and requires the synthesis of specific epididymal proteins (2). However, recent studies have shown that in the human the epididymis may not be as essential to spermatozoa development and fertility as it appears to be in most animals, since after vasoparidyomastomy the post-operative ejaculate contains normally motile sperm, suggesting that the vas deferens is probably involved in sperm maturation and survival (3). In the vas deferents of rodents, there is a variety of specific proteins, the role of which in the physiology of reproduction is unknown (4, 5). As recently shown, adult mouse vas deferens contains a large amount of a major protein (MVDP; mouse vas deferens protein) with an apparent molecular weight of 34,500 as determined by SDS-polyacrylamide gel electrophoresis (6). Androgens have been shown to be primary regulating factors of 34,500 gene expression at the protein and mRNA levels (6, 7). Antibodies against this protein have revealed that the secreted protein associates with the plasma membrane of spermatozoa from the deferent duct (8). However, its precise role remains to be elucidated. To gain further knowledge of the structure of this protein and to permit studies of its gene structure and of its regulation by androgens, a cDNA clone coding for this protein was isolated from a mouse vas deferens cDNA library. The nucleotide sequence of the cDNA has enabled the primary amino acid sequence of the protein to be deduced and considerable sequence similarity (65-82%) to frog lens ß-crystallin (9), bovine lung prostaglandin F synthase (10), human liver aldehyde reductase (11), rat lens aldose reductase (12), and human placental aldose reductase (11) have been revealed.

EXPERIMENTAL PROCEDURES

Animals—Mice of the Swiss strain (CD-1, Charles River, France) were used. When required, castration was performed under light ether anesthesia. Some castrated males were injected with testosterone heptate (75 µg twice daily). Excised tissues were frozen in liquid nitrogen and stored at -80 °C.

Total RNA and Poly(A)+ RNA—Total RNA were purified from vas deferens of adult male mice by the guanidinium isothiocyanate method (13). The total poly(A)+ RNA fraction was obtained by oligo(dT)-cellulose chromatography (14).

Isolation of MVDP Clones—A cDNA library was constructed from poly(A)+ RNA by using the cDNA synthesis kit (RPN1256) from Amersham Corp. Briefly, the first cDNA strand was primed with both oligo(dT) and random hexanucleotide primers, then copied with reverse transcriptase. The second strand was synthesized in the presence of RNaseH and DNA polymerase 1. Methylated cDNA with EcoRI linkers was ligated into pUC13 vector with T4 DNA ligase. Host bacteria (Escherichia coli DH5α from Bethesda Research Laboratories) were transformed according to Hanahan and Meselson (15) and screened by hybridization with a 900-bp MVDP cDNA probe obtained by immunoscreening of a λgt11 library (16). Membranes prehybridization was done for 1 h at 65 °C in a solution containing 6 x SSC (standard sodium citrate), 5 x Denhardt's, 0.5% SDS, and 100 µg/ml calf thymus DNA. Hybridization was performed overnight at 65 °C in the same solution containing 1.105 cpm/filter [32P]dATP probe labeled by the method of Feinberg and Vogelstein (17). Membranes were washed twice for 30 min at 65 °C in 2 x SSC, 0.1% SDS, and finally in 0.5 x SSC, 0.1% SDS at 65 °C for 10 min. Membranes were exposed to Kodak X-OMATS film intensifying screen at -80 °C for 2-4 days. Preparations of positive recombinant plasmid DNA were done using the alkaline lysis method (18).

Sequence analysis—The MVDP cDNA was digested with nuclease Bal31 according to Nixon (19). Briefly, 10 µg of pUC 13 vector containing the fragment to be sequenced was linearized at one end of the fragment with restriction endonuclease digestion, and 5 units of Bal31 exonuclease (Promega) was added. Every 30 s, a DNA sample was mixed with a STOP buffer (0.2 mM EGTA) and placed on ice. Ten samples were taken. The inserts were excised by a second restriction endonuclease digestion, and each point of the kinetic was subjected to electrophoresis analysis. Finally, the digested inserts were ligated to a linearized M13mp18 or M13mp19 vector. DNA was sequenced by the dideoxy-termination method (20) by using the M13 sequencing kit of Boehringer Mannheim. Reactions were carried out according to the manufacturer's instructions. Electrophoresis was performed at 70 watts constant power in 89 mM Tris borate, 2 mM EDTA. The abbreviations used are: MVDP, mouse vas deferens protein; bp, base pair(s); kb, kilobase(s); SDS, sodium dodecyl sulfide; EGTA, ethylenebis(oxayllylenenitrilo)tetracetic acid.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBankTM/EMBL Data Bank with accession number(s) J05663.
EDTA, pH 8.3. Gels were fixed, dried, and subjected to autoradiography. Sequence data were analyzed using BISANCE programs at CENITI 2 in Paris on a DPS8 Bull computer. Each segment of the cDNA was determined at least twice on both strands.

**Northern Blot Hybridization**—Total RNA was purified as described above. 10 μg of total RNA were electrophoresed in an 1% agarose, 2.2 M formaldehyde gel and transferred to a nylon membrane (Hybond N-Amersham Corp.) (21). Prehybridization was done for 1 h at 42 °C in a solution containing 6 X SSC, 5 X Denhardts, 0.5% SDS, 100 μg/ml calf thymus DNA, 50% formamide. Hybridization was performed overnight at 42 °C in the same solution containing 1.106 cpm/ml in vitro transcribed RNA probe (16). Membranes were washed three times in 0.1 X SSC, 0.1% SDS for 20 min at 65 °C. Autoradiographic exposure was usually for 24 h with one intensifying screen at −80 °C. The size of hybridizing mRNAs was estimated by migration of denatured 0.24 0.6 kb RNA ladder (Bethesda Research Laboratories) stained with ethidium bromide. A β-actin probe, labeled by the method of Feinberg and Vogelstein (17), was used as control.

**Southern Blot Analysis**—Total cellular DNA was isolated from liver according to the method of Bernhard et al. (22). DNA was completely digested with several restriction enzymes and subjected to electrophoresis on 0.8% agarose gel. DNA fragments were then transferred to Hybond N nylon filter. Prehybridization was done for 4 h at 65 °C in a solution containing 6 X SSC, 5 X Denhardts, 0.5% SDS, 100 μg/ml calf thymus DNA, and 100 μg/ml yeast RNA. Hybridization was carried out overnight at 65 °C in the same solution containing 1.106 cpm/ml in vitro transcribed RNA probe. Washes were at 65 °C, consecutively as follows: 2 X SSC, 0.1% SDS, 30 min; 2 X SSC, 0.1% SDS, 30 min; 0.1 X SSC, 0.1% SDS, 15 min. Autoradiographic exposure was usually for 4-5 days with one intensifying screen at −80 °C.

**RESULTS**

**Nucleotide Sequence of the MVDP cDNA**—A cDNA library of 20,000 recombinant clones was constructed from mouse vas deferens poly(A)+ RNA and screened with a specific MVDP cDNA probe (see "Experimental Procedures"), yielding 11 clones. Plasmids extracted from each of them were digested with EcoRI, and the length of the cDNA inserts was determined by electrophoresis on agarose gel. The longest insert (clone 61) was mapped for cleavage sites by several restriction endonucleases (Fig. 1). To demonstrate the correspondence between this insert and MVDP mRNA, the clone 61 was characterized by mRNA hybrid selection and in vitro transcription experiments. The transcripts were translated in vitro and their products immunodetected with the anti-MVDP antiserum (18). Appropriate fragments of clone 61 insert were then subcloned into M13mp18 and mp19 and sequenced. Fig. 2 shows the nucleotide and deduced amino acid sequences for the clone FL. The cDNA contains 1225 nucleotides. It consists of a non-coding 5' fragment of 20 nucleotides, a coding segment of 948 nucleotides and a 3' non-coding region of 257 nucleotides. An eukaryotic consensus polyadenylation signal (AATAAA) is observed at position 1202 suggesting that the 3'-untranslated region of the cDNA is virtually complete. No poly(A)+ tail is observed. The first in-frame methionine codon is contained within the translation initiation consensus sequence, ACCATGG (23). This assignment predicts a translation product of 316 amino acid residues whose aggregate molecular weight is 35,965. The 3'-untranslated region contains more AT nucleotides (59%) than the total cDNA (51%) and an AT-rich sequence of 12 pb is observed at position 1062-1073. This sequence, frequently observed in the 3'-untranslated region of the mRNAs of transiently expressed genes has been involved in the selective mRNA degradation (24).

**Sequence Characteristics and Charge Properties of MVDP**—Fig. 3 shows the distribution of hydrophobic and hydrophilic segments along the MVDP sequence. The protein was found to have a net excess of basic residues which would give rise to slightly basic pi (7.55). This value is in agreement with the basic isoelectric point of about 8 determined by isoelectric focusing (6). There is unusually high leucine (10%) and low glycine (4%) content relative to the average amino acid distribution in proteins (26). The prediction of secondary structural domains (27, 28) shows that α-helices are predominant (not shown). An interesting particularity is the presence in positions 69-70, 90-91, and 242-243 of paired basic amino acids (Lys-Arg and Lys-Lys respectively) which could be important for proteolytic cleavage (29). No consensus sequence corresponding to potential N-glycosylation sites (Asn-X-Ser or Thr) are evident within the MVDP sequence. Of additional interest are the sequences around Ser-23, -87, -136, -264 and Thr-244, -307 which are similar to the sequences reported for casein kinase II phosphorylation sites (30). Similarly, some Ser or Thr residues surrounded by basic amino acids represent potential sites for protein kinase C phosphorylation (31). A search of current protein data bases reveals that the sequence of MVDP has significant homology with members of the aldo-keto reductase superfamily (11). A computer-assisted alignment of the amino acid sequence of MVDP with that of several members of the aldo-keto reductase superfamily is shown in Fig. 4. The results show that the conservation of residues is rather uniformly spread along the length of the protein. MVDP coding region shows a 69% (80%) and 71% (82%) identity with the rat lens aldose reductase (12) and human placental aldose reductase (11), respectively (percentages in parenthesis include conservative substitutions). MVDP also shows lower but significant similarities with human liver aldehyde reductase (11), bovine lung prostaglandin F synthase (10), and common frog lens α-crystallin (9) (51, 46, and 45% identity, respectively). A small similarity (30% identity) is also observed with the 2,5-diketo-6-gluconic acid reductase from Corynebacterium (32). Recently, the active NADPH-binding sites for both aldose reductase and aldose reductase have been reported (33). The sites include a tetrapeptide with the sequence Ile-Pro-Lys-Ser which is also observed in the MVDP sequence, within a hydrophobic segment in the carboxyl-terminal region (Figs. 2-4). Whereas within the first 140 bp of the 3'-untranslated region of cDNAs coding for MVDP, human placental aldose reductase (11), and rat lens aldose reductase (12) no similarity was seen, the following nucleotide sequence of MVDP cDNA showed 55-60% homology with the corresponding regions of the two other cDNAs.

**Androgen Regulation of RNA Complementary to MVDP cDNA**—To study the androgen regulation of MVDP mRNA accumulation, total RNA was isolated from vas deferens of adult males: controls, castrated for 30 days and castrated for 30 days subjected to androgen treatment (75 μg of testosterone propionate twice daily for 2 weeks). The mRNAs were analyzed on Northern blots using a 32P-labeled cDNA. As shown in Fig. 5 an intense 1.4-kb band is observed indicating that the MVDP mRNA is expressed at a high level in agreement with a high abundance of the protein (7). There is a striking

![Fig. 1. Restriction endonuclease map and sequencing strategy of MVDP clones. The direction and length of each sequencing run are indicated by arrows.](http://www.jbc.org/)
**MVDP cDNA Sequence and Homologies**

**FIG. 2.** Full-length cDNA sequence and corresponding amino acid sequence of MVDP. The putative active NADPH-binding site was boxed. The consensus sequence flanking the AUG initiator codon and the polyadenylation signal are underlined. The nucleotide and the deduced amino acid sequences are numbered on the right and left, respectively.

**FIG. 3.** Hydrophilic plot of the amino acid sequence of MVDP. Hydrophilicity was calculated by the method of Hopp and Woods (25) with an average window of seven amino acids. Arrows indicate the position of the tetrapeptide NADPH-binding site.

**FIG. 4.** Alignment of the amino acid sequences (single-letter code) of MVDP with two other aldose reductase sequences from human placenta (hPAR) and rat lens (rLNAR). Sequences are numbered on the right. Identical residues present in the three sequences at a given position were boxed.

reduction in intensity of the 1.4-kb band 30 days after castration. Androgen administration restores the 1.4-kb mRNA within 14 days of androgen stimulation.

**Southern Blot Analysis of Mouse Genomic DNA—**To characterize the gene(s) that codes for MVDP mRNA, we analyzed mouse DNA by Southern blot hybridization with MVDP cDNA (clone 61) as probe. In the different digest two labeled bands of unequal intensity are observed. These two bands could be due either to two different genes or to the presence of internal restriction sites (inside introns) in the genomic fragment corresponding to the probe. The number of bands we observed is less than those described for the other aldose reductases (11, 35).

**DISCUSSION**

We have isolated and sequenced a full-length cDNA from a mouse vas deferens library which encodes MVDP, a major androgen-dependent secretory protein. A search of protein sequence data bases has revealed that the amino acid composition of MVDP shows significant homology with that of several members of the aldo-keto reductase superfamily (11). The members of the superfamily which give the highest homology with MVDP are the human placental aldose reductase (11) and rat lens aldose reductase (12). Highly conserved areas can be seen and overall identities as high as 70% are observed in spite of the differences in species and tissues. Aldose reductase (alditol NADP+ oxidoreductase, EC 1.1.1.21) is an NADP+ oxidoreductase in which the sequence at the NADPH-binding site is Ile-Pro-Lys-Ser (33). This sequence is conserved in MVDP. These degrees of similarity clearly suggest that these three proteins belong to the same superfamily with related structures and evolutionary origins. While we have not directly demonstrated the enzymatic nature of MVDP, the degree of homology between MVDP and both aldose reductases from human placenta and rat lens is such
that it is reasonable to conclude that they are functionally equivalent proteins. Aldose reductase catalyzes the reduction of a number of aliphatic and aromatic aldehydes to the corresponding alcohols and together with sorbitol dehydrogenase forms the sorbitol pathway, a minor route for carbohydrate metabolism. Although the physiological role of aldose reductase is still unclear, numerous studies suggest that this enzyme may play a key role in the etiology of some complications of diabetes mellitus (36). The biological function of MVDP is unknown; the homology of this protein with aldose reductases is still unclear, numerous studies suggest that this enzyme whose synthesis is dependent upon testicular androgens have been identified by molecular cloning as a secretory protease a bed oftestes and epididymis (11), human erythrocyte (42), rat lens (12), rabbit renal medulla (39), rabbit muscle (43), human psoas muscle (44), and sheep seminal vesicle (45), the effect of androgen on their expression has received little attention. To our knowledge there is no example of an androgen-regulated aldose reductase. Additional proteins in mouse sex accessory organs are androgen-inducible. For instance, two prostatic proteins whose synthesis is dependent upon testicular androgens have been identified by molecular cloning as a secretory protease and a sperm入境 binding protein (46, 47). Mouse seminal vesicle secretory protein IV, which is involved in the copulatory plug formation, has been recently isolated and its corresponding cDNA has been cloned and sequenced (48).

The characterization on nucleotide sequence of MVDP gene, determination of the enzymatic activity of the protein, and its possible role in sperm maturation and storage are the subject of future investigations.

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**REFERENCES**


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