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Primary Structure and Androgen Regulation of a 20-Kilodalton Protein Specific to Rat Ventral Prostate†

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ABSTRACT: Nuclear and cytosolic forms of a 20-kdalton rat ventral prostate protein were purified and partially sequenced from their N-termini. Isolated nuclei were treated with micrococcal nuclease and extracted in 0.6 M NaCl, and proteins were separated by affinity chromatography on Matrex gel green A, ammonium sulfate fractionation, and fast protein liquid chromatography on Superose 12. The 43 amino acid N-terminal sequence of the nuclear 20-kdalton protein was identical with the cytosolic protein except it lacked 7 N-terminal amino acids present in the cytosolic form. The DNA sequence of a full-length complementary DNA clone isolated from a ventral prostate gt11 library extended the N-terminal sequence of the cytosolic form by an additional nine amino acids from the predicted initiation methionine. The cDNA included the nucleotide sequence for the 43 amino acid N-terminal sequence of the purified 20-kdalton protein and predicted molecular weights of 16 686, 17 521, and 18 650, respectively, for the nuclear, cytoplasmic, and nonprocessed proteins. Northern blot analyses of reproductive tract tissue RNAs using the 20-kdalton protein cDNA as probe revealed a single mRNA species of 0.92 kb detectable only in extracts of rat ventral prostate. Expression of the 0.92-kb mRNA was androgen dependent since the mRNA was undetectable in extracts obtained 4 days after castration and was restored 16 h after restimulation with androgen.

Rat ventral prostate synthesizes two proteins in abundance; prostatein (Lea et al., 1979), known also as prostatic binding protein (Heyns et al., 1978; Heyns & Moor, 1977), α-protein (Chen et al., 1979), and estramustine binding protein (Forsgren et al., 1979), is androgen dependent, secreted into prostatic fluid, and binds several steroids with low affinity and high capacity. Prostatein has been reported to function in seminal fluid as a cholesterol carrier protein (Chen et al., 1982). The high concentration of prostatein and its dependence on androgen have made it a useful protein in studies on androgen action in the prostate (Wilson et al., 1981; Perry et al., 1985).

A second abundant protein of rat ventral prostate is a 20-kdalton glycoprotein identified by cell-free translation of ventral prostate messenger RNA and hybridization arrest of translation using cloned complementary DNA (Parker et al., 1978; Chamberlin et al., 1983). The 20-kdalton protein was subsequently purified and shown to be a glycoprotein secreted by rat ventral prostate (Chamberlin et al., 1983; Wang et al., 1986). It is androgen regulated, like prostatein, but does not bind dihydrotestosterone.

Secretory proteins of other regions of the rat prostate include a protein secreted by the dorsal/lateral prostate known as probasin (Matuo et al., 1982a) which has a calculated molecular weight of 18 936 and contains 160 amino acids (R. Matusik, personal communication). Dorsal prostate produces, in large quantities, an androgen-regulated protein referred to as DPI (Wilson & French, 1980).

In this report, we describe the purification of the 20-kdalton protein and partial N-terminal amino acid sequence analysis

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together with cloning and sequencing of its full-length complementary DNA. The protein was found to be present in cytosol and nuclei with the nuclear form lacking seven N-terminal amino acids found in the cytoplasmic form, but both lacking an additional nine N-terminal amino acids predicted by the full-length cDNA sequence.

Experimental Procedures

Materials. Reagent-grade chemicals were obtained from Sigma Chemical Co. (St. Louis, MO), Allied Fisher Scientific, and BDH Chemicals (Vancouver, British Columbia). Micrococcal nuclease was obtained from Sigma. Matrex gels were obtained from Amicon (Lexington, MA). The Bio-Rad (Bradford) protein assay and polypropylene Econo columns were from Bio-Rad Laboratories (Mississauga, Ontario). Ready-Solv was obtained from Beckman Instruments (Fullerton, CA).

Protein Purification. (A) Isolation and Extraction of Nuclei. Frozen rat prostate was purchased from Charles River (Wilmington, MA) and stored at −70 °C until used. The tissue was brought to room temperature over a 30-min period, stripped of connective tissue, blotted, and weighted. Approximately 30 g wet weight prostate was used in each isolation. Nuclei were isolated as described previously (Bruchovsky et al., 1987). Cytosol was centrifuged as 80000*g* for 90 min in a Beckman ultracentrifuge (Beckman Instruments, Palo Alto, CA) to remove particulate matter. Buffers used in the isolation of cytosol and nuclei contained 1 mM phenylmethanesulfonyl fluoride (PMSF).

Purified nuclei were disrupted by sonication, digested with micrococcal nuclease, and extracted in Tris buffer, pH 8.0, containing 0.6 M NaCl; details of these procedures have been reported elsewhere (Bruchovsky et al., 1987). Centrifugation of the solubilized fraction at 20000*g* for 20 min yielded a supernatant containing 138 ± 11 mg of protein (mean ± SEM, $n = 11$) in a final volume of 2 mL (nuclear extract).

(B) Chromatography of Nuclear Proteins. Matrex gel green A (2 × 1.5 × 10 cm column, 20-mL bed volume) was routinely used for preliminary chromatographic enrichment of nuclear androgen receptor and 20-kdalton protein (Bruchovsky et al., 1987). Unbound proteins were removed with 200 mL of 10 mM *N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (Tes) buffer, pH 8.5; retained protein was eluted with a linear salt gradient (0–2 M NaCl). Fractions between 1 and 2 M NaCl were pooled and brought to 40% saturation with ammonium sulfate. The precipitate was dissolved in 180 μL of 10 mM Tes, pH 7.2, containing 0.6 M NaCl.

Gel filtration was performed on a Pharmacia (Uppsala, Sweden) fast protein liquid chromatography system (FPLC) at 4 °C using a prepacked Superose 12 HR 10/30 column as reported before (Bruchovsky et al., 1987). Fractions 22–25 were pooled and precipitated with 4 volumes of acetone in silanized corex tubes. After at least 1 h at −70 °C, the mixture was centrifuged (12000*g* for 20 min), cold ethanol was added to the pellet, and the sample was stored at −70 °C. The precipitate recovered by centrifugation (12000*g* for 20 min) was dissolved in 200 μL of one of the following: 1% sodium dodecyl sulfate (SDS) in water for amino acid sequencing; Laemmli sample buffer (62.5 mM Tris buffer, pH 6.8, containing 5% 2-mercaptoethanol, 2.5% SDS, and 5% sucrose) for polyacrylamide gel electrophoresis; or phosphate-buffered saline for protein quantitation.

DNA Cloning. A rat ventral prostate cDNA library was prepared by Viskochil et al. (1983) in *Pst*I-digested pBR322. A complementary DNA clone encoding the 20-kdalton protein (20K-I) was verified by hybrid-arrested and hybrid-selected translation using previously described procedures (Paterson et al., 1977; Goldger et al., 1979) in a wheat germ cell-free translation system (data not shown). Plasmids containing the 20-kdalton protein DNA were purified as described (Viskochil et al., 1983) and sequenced by using both the chemical degradation method of Maxam and Gilbert (1980) and the dideoxy chain termination method of Sanger et al. (1977). A full-length cDNA clone (20K-II) was obtained by screening a rat prostate expression library prepared in λgt11 (Clontech Laboratories Inc., Palo Alto, CA) using as probe a 345 bp 5′ *Hae*III fragment of the partial 20K-I cDNA clone.

RNA Isolation and Analysis. Adult male Sprague Dawley rats (Zivic Miller, Zelienople, PA) were used and castrated through an abdominal incision under anesthesia (acepromazine/ketamine 1:1 mixture, 0.1 mL/100 g body weight). Tissues were removed immediately after decapitation, quick frozen in liquid nitrogen, and stored at −70 °C. Frozen tissues were pulverized in liquid nitrogen and homogenized in 4 M guanidine thiocyanate, pH 7.0. Total RNA was pelleted through 5.7 M cesium chloride (Chirgwin et al., 1979) and poly(A) RNA enriched by olido(dT)–cellulose chromatography (Aviv & Ledre, 1972). Samples of RNA were denatured and electrophoresed on 1.2% agarose gels (McMaster & Carmichael, 1977) together with molecular weight markers and ribosomal RNA. Samples were transferred to nylon membranes (Biodyne TMA, 1.2 μm; Pall, Glen Cove, NY) (Thomas, 1980), UV irradiated for 5 min, and baked for 2 h at 80 °C under vacuum (Church & Gilbert, 1984). Membranes were prehybridized for 4–16 h at 42 °C in 50% formamide, 5× SSC, 5× Denhardt's, 0.1% SDS, and 250 μg/mL denatured salmon sperm DNA (SSC: 0.15 M NaCl/0.015 M sodium citrate) and hybridized for 16–20 h at 42 °C in 50% formamide, 5× SSC, 1× Denhardt's, 20 mM sodium phosphate, pH 6.5, 0.1% SDS, 10% dextran sulfate, and 100 μg/mL denatured salmon sperm DNA. Hybridization buffer contained approximately 10^6 cpm/mL ^{32}P-labeled DNA probe prepared by nick-translation to approximately 10^8 cpm/μg of DNA (Rigby et al., 1977). Membranes were washed in 2× SSC and 0.1% SDS at room temperature (2 × 20 min) and in 0.1× SSC/0.1% SDS at 65 °C (2 × 30 min) and exposed to X-ray film with intensifying screens at −70 °C.

Other Analytical Procedures. Protein was measured by the method of Bradford (1976) using bovine γ-globulin as the standard. N-Terminal peptide sequencing was performed on an Applied Biosystems (Foster City, CA) gas-phase sequenator (Model 470A), and the phenylthiohydantoin (PTH)-amino acids were analyzed on an Applied Biosystems PTH analyzer (Model 120A). Amino acid sequence comparisons were done by using the FASTP program of Lipman and Pearson (1985), the NIH Protein Sequence Database of the Protein Identification Resource, and the BIONET National Computer Resource for Molecular Biology funded by NIH Grant P41RR01685. The method of Laemmli (1970) was used for polyacrylamide gel electrophoresis. Silver nitrate staining of gels was done by the method of Huekeshoven and Dernick (1985), and the periodic acid–Schiff stain (PAS) was used for detecting carbohydrate (Fairbanks et al., 1971). [1,2-^{3}H]-Dihydrotestosterone (specific activity 54 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Radioactivity was determined in a Beckman liquid scintillation counter using Ready-Solv scintillation cocktail.

Results

Purification of the 20-kdalton Protein. Ventral prostate nuclear proteins were fractionated on a Matrex gel green A

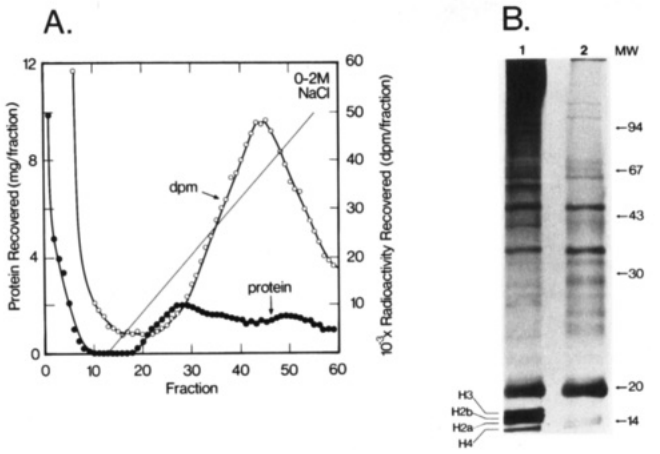

FIGURE 1: (A) Chromatography of a ventral prostate nuclear extract on Matrex gel green A. Ventral prostate nuclear extract containing 138 mg of protein was incubated with a 20-mL bed volume Matrex gel green A column for 30 min at 4 °C. The column was washed with 200 mL of Tes buffer, pH 8.5, containing 0.5 mM 2-mercaptoethanol and 5 mM EDTA and eluted with a 200-mL linear NaCl gradient (0–2 M). The protein (●) and radioactive (○) elution profiles are shown. Protein concentration was determined by using the Bradford assay. (B) SDS–polyacrylamide gel electrophoresis of ventral prostate nuclear extracts before and after chromatography on Matrex gel green A. The initial nuclear extract and the ammonium sulfate precipitate of Matrex gel green A column fractions 35–55 were desalted on Sephadex PD10 columns (Pharmacia), lyophilized, resuspended in Laemmli sample buffer (Laemmli, 1970), and heated for 5 min at 100 °C. Samples were electrophoresed on a 12% polyacrylamide gel and stained with silver nitrate as follows: 60 μg of initial nuclear extract (lane 1) and 60 μg of ammonium sulfate precipitate of Matrex gel green A fractions 35–55 (lane 2). Molecular mass markers were phosphorylase B (200 kdalton), bovine serum albumin (67 kdalton), ovalbumin (47 kdalton), carbonic anhydrase (30 kdalton), trypsin inhibitor (20 kdalton), and lysozyme (14 kdalton).

affinity column (Figure 1A). Analysis of the eluted proteins on silver nitrate stained polyacrylamide gels revealed numerous bands. The salt elution peak fractions 35–55 (the androgen receptor peak as determined by radioactive counts, Figure 1A) displayed a major 20-kdalton protein (Figure 1B, lane 2). Proteins lower than 20-kdalton molecular mass present in the unfractionated extract (Figure 1B, lane 1) corresponded to histones H2A, H2B, H3, and H4 (Matuo et al., 1982b) which, together with higher molecular mass proteins, were separated away during the chromatography step (Figure 1B, lane 2).

FPLC of the nuclear extract on Superose 12 yielded three major protein fractions (Figure 2A) which on polyacrylamide gel electrophoresis had a predominant 20-kdalton band beginning in fractions 20 through 24 (Figure 2B, lanes 3 through 5). Fractions 22–25 were combined to yield 20–50 μg of a homogeneous purified product (Figure 3). From approximately 140 mg of extracted nuclear protein, 20–50 μg of 20-kdalton protein was recovered. The androgen receptor copurified with 20-kdalton protein on Matrex gel green A (Figure 1) but was separated away on the FPLC column, with receptor eluting in fractions 12 through 14 (Bruchovsky et al., 1987).

N-Terminal protein sequence analysis of purified 20-kdalton nuclear protein resulted in the 43 amino acid sequence shown in Figure 4 beginning with an asparagine residue. A blank reading was obtained at amino acid position 33, suggesting the presence of a modified amino acid. Asparagine in position 33 was later confirmed by DNA sequence analysis (see below).

Precipitation of the cytosol fraction of ventral prostate with 40% ammonium sulfate followed by fractionation by FPLC resulted in isolation of a cytosolic form of the 20-kdalton protein. The purified protein was judged homogeneous on the

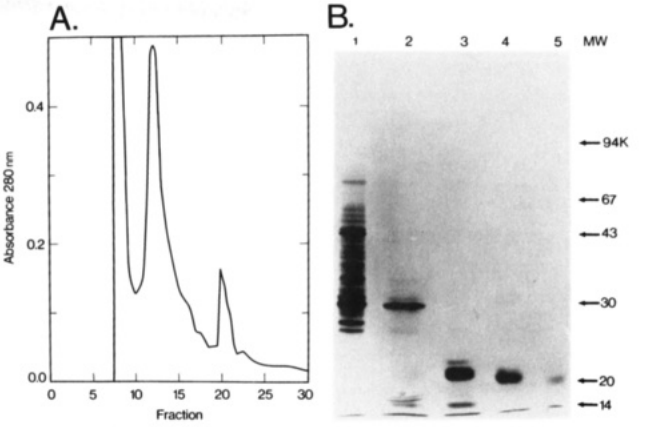

FIGURE 2: (A) FPLC of the partially purified prostate nuclear extract. Fractions 35–55 from the Matrex gel green A column were precipitated with 40% ammonium sulfate, resuspended in 10 mM Tes, pH 7.2, containing 0.6 M NaCl, and chromatographed on a Superose 12 HR 10/30 column equilibrated in the same buffer. Elution (1-mL fractions) of the applied protein (5 mg) was monitored by the absorbance at 280 nm. (B) SDS–polyacrylamide gel electrophoresis of FPLC fractions. Column fractions were desalted on Sephadex PD10 columns, lyophilized, resuspended in Laemmli sample buffer, and heated for 5 min at 100 °C. Samples (20–50 μg) were heated for 5 min at 100 °C, electrophoresed on a 12% polyacrylamide gel, and stained with silver nitrate. Lanes 1–5 correspond to FPLC column fractions 16, 18, 20, 22, and 24, respectively. Molecular mass markers as in Figure 1.

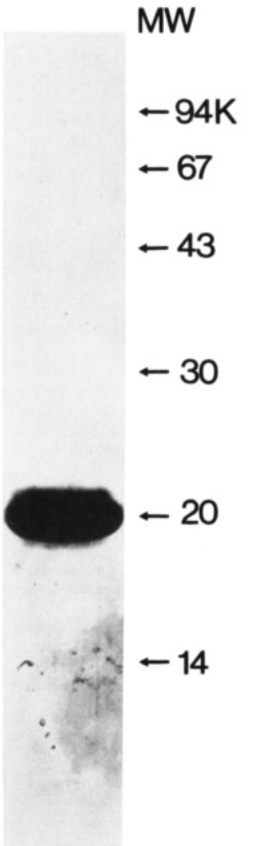

FIGURE 3: SDS–polyacrylamide gel electrophoresis of purified nuclear 20-kdalton protein. Fractions 22–25 from the FPLC column were pooled and precipitated with acetone. The precipitate was resuspended in Laemmli sample buffer and heated for 5 min at 100 °C. Purified 20-kdalton protein (20 μg) was analyzed on a 12% acrylamide gel and stained with silver nitrate. Molecular mass markers were as indicated in Figure 1.

basis of a single major band on an SDS–polyacrylamide gel (results not shown). N-Terminal amino acid sequence analysis revealed a sequence identical with the nuclear protein with the

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Cytosolic  Gly-Met-Glu-Ile-Phe-Glu-Lys-Asn-Phe-Ile-Asp-Lys-Asn-Lys-Leu-Lys-Asp-Val-Tyr-Asp-Val-Phe-Lys-Tyr-Leu-Tyr-

Nuclear                                Asn-Phe-Ile-Asp-Lys-Asn-Lys-Leu-Lys-Asp-Val-Tyr-Asp-Val-Phe-Lys-Tyr-Leu-Tyr-

Cytosolic  Asn-Thr-His-Ser-Ala-Asp-Thr-Tyr-Leu-Ser-Asn-Ile-Lys

Nuclear    Asn-Thr-His-Ser-Ala-Asp-Thr-Tyr-Leu-Ser-Asn-Ile-Lys-( )-Glu-Ser-Phe-Thr-Met-Asn-Ile-Trp-Gly-Phe
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FIGURE 4: N-Terminal amino acid sequences of 20-kdalton proteins isolated from nuclei and cytosol of ventral prostate. The 20-kdalton protein was purified from the nuclear and cytosolic extracts as described in the text and sequence determined as described under Experimental Procedures.

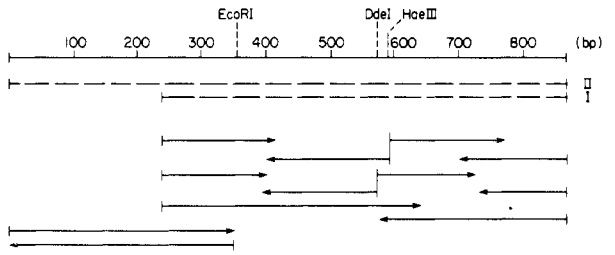

FIGURE 5: Sequencing strategy for 20-kdalton protein cDNA clones. Two cDNA clones were isolated: a partial cDNA from a pBR322 library prepared from ventral prostate RNA (clone I) and a full-length cDNA from a λgt11 expression library (clone II). The cDNA clones were isolated and sequenced as described under Experimental Procedures from the fragments shown. Restriction enzyme sites used in sequencing are illustrated.

exception of seven additional N-terminal amino acids beginning with a glycine residue (Figure 4).

Cloning and Sequencing 20-kdalton Protein cDNA. A partial 636 bp cDNA clone (20K-I) containing an open-reading frame for 108 amino acids in-frame with a TGA termination codon (Figure 5) was isolated from a pBR322 cDNA library prepared from rat ventral prostate poly(A) RNA (Viskochil et al., 1983). A 5′ 345 bp *Hae*III fragment of 20K-I cDNA clone was used to screen for a full-length clone in λgt11 expression library by filter hybridization. DNA sequencing was performed on both cDNA clones using the strategy diagrammed in Figure 5. Identity between nucleotide and N-terminal amino acid sequence (Figure 6, underlined amino acid sequence determined by protein sequencing) indicates that the clones are authentic for 20-kdalton protein. The amino acid sequence of 20-kdalton protein showed no significant homology with any other protein in available data bases. The nucleotide sequence extended the nuclear protein sequence to that of the cytosolic form and, in addition, contained nine additional N-terminal amino acids. The DNA sequence at the predicted initiation codon (ATG) was homologous to the consensus initiation sequence (Kozak, 1981) with a critical purine residue at position −3 (Kozak, 1984). The TGA stop codon and 57 adenine nucleotide residues on the 3′ end indicated that oligo(dT)-primed first-strand cDNA synthesis initiated at the 3′ end of the messenger RNA. A poly(A) addition signal (AATAAA) is present 25 nucleotide residues before the 3′ poly(A) region (shown underlined in Figure 6).

Sequence Characteristics and Charge Properties of 20-kdalton Protein. A hydropathic plot of the predicted amino acid sequence (Figure 7) reveals an overall high degree of hydrophilicity. An equivalent number of negatively and positively charged amino acids renders the protein a net neutral charge. There are an unusually high number of tyrosine (8% of total) and lysine (10%) residues. The N-terminal 7 amino acid extension on the cytoplasmic protein includes 1 of 4 hydrophobic regions that center also around amino acids 49, 96, and 127.

Tissue Distribution and Androgen Dependence. Total RNA isolated from reproductive tract tissues of intact male rats was analyzed on Northern blots using a ^{32}P-labeled cDNA fragment as probe. As shown in Figure 8, an intense 0.92-kb band

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        10        20        30        40        50        60        70        80        90       100       110       120
        •         *         •         *         •         *         *         *         *         •         *         *
CGTCTGAAGCTCCCCTTTCATATCTGAGAAGAAAAATGTGCAAAACCCTACATGGCACACTGCTCCTACTGGCCATCTTTGTCCTGTTTCTGAATTTCAGCCATGCAAACTGCAAAACGT
                                                                                                  MetGlnThrAlaLysArg

        *         •         *         •         *         •         *         *         •         •         •       240
ACCAGGAGAGGTATGGAAATTTTTGAGAAAAACTTCATAGACAAAAACAAACTGAAGGATGTATATGATGTCTTCAAATATCTTTACAACACGCACAGTGCTGACACATATCTCAGCAAC
ThrArgArgGlyMetGluIlePheGluLysAsnPheIleAspLysAsnLysLeuLysAspValTyrAspValPheLysTyrLeuTyrAsnThrHisSerAlaAspThrTyrLeuSerAsn
        cytosolic           nuclear
        •         •         *         *         *         *         *         *         *         •         *       360
ATAAAAAATGAGTCATTCACCATGAACATATGGGGATTTGGTGAAATTGAAATGGTTAAAACCAAATGTAGAAAGATTGATTCTGACTTCTACAAATGTTCTTTCCAGAGGGAATTCTAT
IleLysAsnGluSerPheThrMetAsnIleTrpGlyPheGlyGluIleGluMetValLysThrLysCysArgLysIleAspSerAspPheTyrLysCysSerPheGlnArgGluPheTyr

        •         *         *         *         *         •         •         *         •         •         *       480
AACCTAAAGCGGACTCCTGGCGAAACAATGTACTATATATCATTGCCTGGAAGCGTCAGATGCAGGAAGCTCCTATCTAAACTGGACAACTGTCCCTTCGAAGAACAAACAGAACAGCTG
AsnLeuLysArgThrProGlyGluThrMetTyrTyrIleSerLeuProGlySerValArgCysArgLysLeuLeuSerLysLeuAspAsnCysProPheGluGluGlnThrGluGlnLeu

        •         *         *         *         *         *         *         •         *         *         •       600
AAGAGAGAAATATGTTACTTTGTACTGTATCCTGACTATATTGAGCAAAATATACATGCTGTAAGATTCGATTGTTATACCAAATGAGACTCTGAGACTGACTGGCCCGTTACCCAACAC
LysArgGluIleCysTyrPheValLeuTyrProAspTyrIleGluGlnAsnIleHisAlaValArgPheAspCysTyrThrLysEnd

        •         *         •         *         *         *         *         *         •         •         •       720
TTGCAGACATTGATTCTACTTCTGGAACTCTAATATGGAATCACCCTTCAGTATGGATGCTAGGAGTCACAGAGACATGCAGTCCAGTTATGTACACCTGAACAATTGTCTTCCTTTTAC

ATTTCACATACTTCTTTTAAATGAACCATTGTAAGCCTGCCATAACTAACCTATGCTTTGCTCAATAAAAGTACTGCCATTCATCTTGA57809
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FIGURE 6: Full-length cDNA sequence and corresponding amino acid sequence for 20-kdalton protein. Nucleotide sequence was determined from the two cDNA clones illustrated in Figure 5. Amino acid sequence determined by direct protein sequencing is shown underlined as is the poly(A) addition signal (AATAAA) and termination codon (TGA).

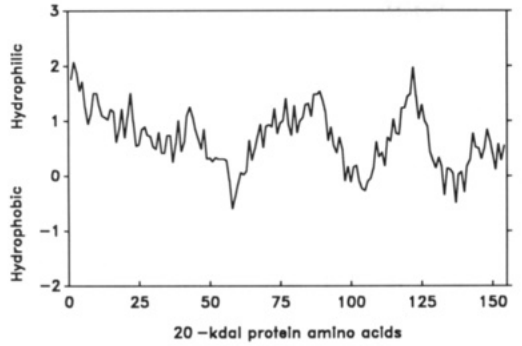

FIGURE 7: Hydropathic plot of 20-kdalton protein. The amino acid sequence deduced from the 20-kdalton protein cDNA nucleotide sequence was used in the protein structure analysis computer program prepared by the University of Wisconsin Genetics Computer Group (Devereux et al., 1983) with the algorithm of Kyte and Doolittle (1982) and an average window of 15 amino acids.

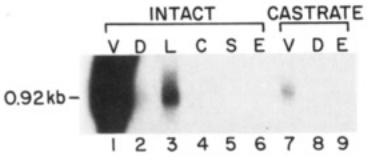

FIGURE 8: Tissue distribution of the 20-kdalton protein. Total RNA was isolated from ventral prostate (V), dorsal prostate (D), lateral prostate (L), coagulating gland (C), seminal vesicle (S), and epididymis (E). In lanes 1–6, RNA was isolated from tissues of untreated adult rats. In lanes 7–9, RNA was isolated from tissues of rats 4 days after castration. Total RNA (10 μg) was analyzed by electrophoresis and Northern blot hybridization using a ^{32}P-labeled 20-kdalton cDNA probe as described under Experimental Procedures. The size of the 20-kdalton mRNA was estimated by comparison with ^{32}P-labeled molecular weight markers derived from *Hin*dIII-digested λ DNA.

was observed with ventral prostate RNA (lane 1), indicating that the 20-kdalton protein mRNA is expressed at a high level in agreement with a high relative abundance of 20-kdalton protein in this tissue. There was no detectable hybridizing signal with RNA from dorsal prostate (Figure 8, lane 2), coagulating gland (anterior prostate) (Figure 8, lane 4), seminal vesicle (Figure 8, lane 5), or epididymis (Figure 8, lane 6). The less intense 0.92-kb signal from lateral prostate RNA (Figure 8, lane 3) likely resulted from incomplete separation of lateral and ventral prostate during tissue dissection. This tissue specificity to ventral prostate was supported by our inability to isolate 20-kdalton protein from liver and testis. Thus, it appears that expression of 20-kdalton protein mRNA is at a high level and specific to ventral prostate.

To investigate possible androgen regulation of 20-kdalton protein mRNA, rats were castrated, and RNA was isolated from various tissues 4 days later. There was a striking reduction in intensity of the 0.92-kb mRNA in ventral prostate 4 days after castration (Figure 8, lane 7), suggesting that expression of 20-kdalton protein mRNA is androgen dependent. Again, no hybridizing signal was observed in dorsal prostate or epididymis (Figure 8, lanes 8 and 9) when RNA was isolated from tissues of castrated rats.

The level of 0.92-kb mRNA diminished rapidly in the ventral prostate under androgen withdrawal (Figure 9A). Within 2 days of castration, the level of RNA was reduced by 70% and became almost undetectable by 4 days after castration. Androgen administration to rats 4 days after castration caused a reappearance of the 0.92-kb mRNA within 16 h of androgen stimulation (Figure 9B). Administration of cycloheximide 2 h prior to sacrifice of intact rats or rats 4 days after castration did not significantly alter the steady-state level of 0.92-kb mRNA (Figure 9C), suggesting that

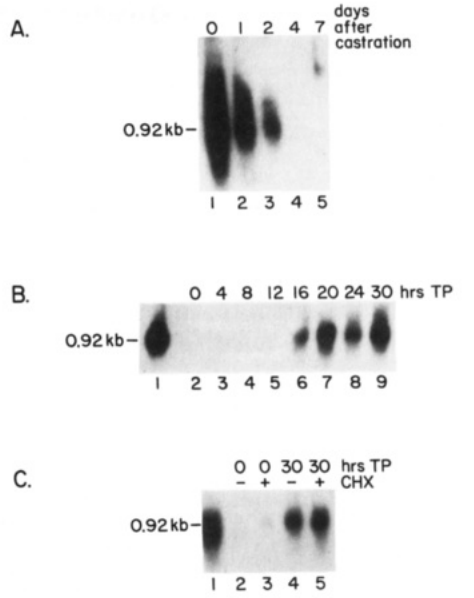

FIGURE 9: Androgen dependence of 20-kdalton mRNA levels in rat ventral prostate. (A) *Effects of castration*: Total RNA was isolated from ventral prostate of untreated adult male rats (lane 1) or from rats sacrificed 1 (lane 2), 2 (lane 3), 4 (lane 4), or 7 (lane 5) days after castration. Aliquots of total RNA (20 μg) were analyzed by Northern blot hybridization as described in Figure 7. (B) *Effects of androgen restimulation*: Poly(A) RNA was isolated from ventral prostate of intact rats (lane 1), from rats 4 days after castration (lane 2), and from rats 4 days after castration that received testosterone propionate (Boots Pharmaceuticals Inc., Shreveport, LA) (2 mg intramuscularly) 4 (lane 3), 8 (lane 4), 12 (lane 5), 16 (lane 6), 20 (lane 7), 24 (lane 8), and 30 (lane 9) h prior to sacrifice. Poly(A) RNA (2 μg) was analyzed by Northern blot hybridization as in Figure 6. (C) *Effects of cycloheximide*: Total RNA was isolated and analyzed (20 μg) from intact rats (lane 1), from rats 4 days after castration without (lane 2) or with (lane 3) cycloheximide treatment (20 mg intraperitoneally) 2 h prior to sacrifice, and from rats 4 days after castration that received testosterone propionate (TP) 2 mg intramuscularly 30 h prior to sacrifice without (lane 4) or with (lane 5) cycloheximide treatment as above. The Northern blot was analyzed as in Figure 6.

induction by androgen does not significantly influence the half-life of mRNA coding for the 20-kdalton protein.

Discussion

Nuclear and cytosolic forms of 20-kdalton protein were purified from rat ventral prostate and partially sequenced from their N-termini. The entire 20-kdalton protein sequence was obtained from a full-length cDNA clone. Correspondence between amino acid and nucleotide sequence confirmed the authenticity of the recombinant clone. Nuclear and cytosolic protein sequences were identical from amino acid residue 8 through residue 39, numbered from the N-terminus of the larger cytosolic form. The predicted amino acid sequence from the full-length cDNA indicates an additional nine N-terminal amino acids relative to the cytosolic form. A precursor/product relationship is possible considering that the two forms of the protein were specifically localized to the cytosolic and nuclear fractions. There was little or no apparent cross-contamination between cytosol and nuclei during protein purification based on N-terminal amino acid sequence determinations. Another possibility to explain the two forms of 20-kdalton proteins is that selective proteolytic cleavage occurred during isolation although PMSF was included in the buffers.

An abundant 20-kdalton glycoprotein has been reported previously in ventral prostate (Parker et al., 1978; Chamberlin et al., 1983; Wang et al., 1986) and appears to have some properties in common with the protein reported here. It was

regulated by androgen (Parker et al., 1978), did not bind androgen (Chamberlin et al., 1983), had a similar amino acid composition, and was specific to ventral prostate on the basis of mRNA (Carter et al., 1983) and protein (Chamberlin et al., 1983) analysis. Both 20-kdalton ventral prostate proteins differ from a protein of similar molecular mass described in dorsal/lateral prostate referred to as probasin (Matuo et al., 1982a). Probasin is not glycosylated (Matuo et al., 1982a) and has a different N-terminal protein sequence (R. Matusik, personal communication). Small amounts of probasin were detected, however, in nuclei of ventral prostate (Matuo et al., 1986), but nuclear localization was thought to result from mechanical disruption of the tissue (Matuo et al., 1985). Immunocytochemical staining with an antibody to the 20-kdalton protein is consistent with both nuclear and cytoplasmic localization of the protein in the ventral prostate (unpublished studies). Androgen-regulated nuclear proteins of similar molecular weight possibly related to or identical with the 20-kdalton protein described here have been detected by SDS–polyacrylamide gel electrophoresis (Kishimoto et al., 1982; Venkatraman et al., 1984) and shown to be at least partially localized to the nuclear envelope (Venkatraman et al., 1984) and nuclear matrix (Carmo-Fonseca, 1988).

Our results confirm the previous finding of Parker et al. (1978) that the 20-kdalton protein of rat ventral prostate is glycosylated. The presence of carbohydrate was demonstrated by PAS staining of the protein (data not shown). Furthermore, the fact that amino acid 33 from the N-terminus of the nuclear protein was undetected during amino acid sequence analysis is consistent with a posttranslation modification. DNA sequence analysis indicated that the probable site of glycosylation is an asparagine residue at position 33 owing to the presence of the triamino acid code required for glycosylation of asparagine residues (Spiro, 1973), i.e., asparagine followed by a non-proline residue (position 34 in the 20-kdalton protein) and a serine residue (position 35). The anomalous electrophoretic migration of 20 kdalton for the 16 686 molecular weight nuclear form and 17 521 daltons for the cytosolic form is not completely accounted for either by carbohydrate modification or by the presence of acidic residues. In vitro translation of 20-kdalton protein in the wheat germ system where carbohydrate addition does not occur yielded a similar migrating protein (Parker et al., 1980; Wilson et al., 1981), although Parker and Scrace (1979) found the [^{35}S]-methionine-labeled protein synthesized in minced prostatic tissue to be slightly larger (22 000 daltons). Although the isolated 20-kdalton protein was not compared directly to the in vitro translated 20-kdalton protein, we assume that the former is equivalent to the 22-kdalton protein of Parker and Scrace (1979).

The estimated size of 20-kdalton mRNA (0.92 kb) by Northern blot analysis is in good agreement with earlier determinations (Carter et al., 1983; Parker et al., 1980) as is the restriction map of the cDNA (Carter et al., 1983). Changes in 20-kdalton mRNA levels following androgen withdrawal and replacement indicate that the male sex hormone regulates its expression acutely as it does prostatein (unpublished studies; Quarmby et al., 1987). The Northern hybridization study in 4-day castrated rats demonstrates an increase in the 0.92-kb mRNA within 16 h after testosterone treatment while earlier studies using solution hybridization with cDNA probes showed increases in mRNA within 1–4 h in 3- and 7-day castrated rats (Chamberlin et al., 1983; Parker et al., 1980; Zhang & Parker, 1985). It is interesting that at 16 h after testosterone restimulation there is a surge in prostatein mRNA (Kishimoto et al., 1982). The minimal effects of cycloheximide on mRNA levels in both untreated and testosterone-treated castrated rats suggest that testosterone increases 20-kdalton protein mRNA at least in part through an effect on transcription.

Since the 20-kdalton protein is recovered with androgen receptor during nuclear fractionation, a relationship between the two proteins is implied. In previous studies, it was found that androgen receptor cross-links to 20-kdalton protein following treatment of nuclei with formaldehyde (Foekens et al., 1987; Rennie et al., 1987). Association of androgen receptor with the C2 subunit of α-protein, the other major secretory protein of ventral prostate, has also been reported (Chen et al., 1979). Whether either of these proteins merely aggregates with the receptor during isolation or is truly an androgen receptor associated protein requires further study.

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Registry No. DNA (rat ventral prostate 20 000-molecular-weight androgen-dependent glycoprotein-specifying mRNA complementary), 121210-42-2; glycoprotein (rat ventral prostate 20 000-molecular-weight androgen-dependent precursor protein moiety reduced), 121210-46-6; protein (rat ventral prostate cytosol 20 000-molecular-weight androgen-dependent protein moiety reduced), 121210-44-4; glycoprotein (rat ventral prostate nucleus 20 000-molecular-weight androgen-dependent protein moiety reduced), 121210-45-5.

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Epidermal Growth Factor and Transforming Growth Factor α Bind Differently to the Epidermal Growth Factor Receptor

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ABSTRACT: Epidermal growth factor (EGF) and transforming growth factor α (TGFα) compete with each other for binding to the EGF receptor. These two growth factors have similar actions, but there are distinguishable differences in their biological activities. It has never been clear how this one receptor can mediate different responses. A monoclonal antibody to the EGF receptor (13A9) has been identified which has only small effects on the binding of EGF to the EGF receptor, but which has very large effects on the binding of TGFα to the EGF receptor; 5 μg/mL antibody has been shown to totally block 0.87 μM TGFα from binding to purified EGF receptor and to lower both the high- and low-affinity binding constants of TGFα binding to EGF receptor on A431 cells by about 10-fold. The 13A9 antibody causes a 2.5-fold stimulation of the tyrosine kinase activity of partially purified EGF receptor, compared to a 4.0-fold stimulation of the tyrosine kinase activity by EGF under the same conditions. The data suggest either that the antibody stabilizes a conformation of the EGF receptor which is not favorable for TGFα binding or that it blocks a part of the surface of the receptor which is necessary for TGFα binding but not EGF binding.

Transforming growth factor α (TGFα) is a 50 amino acid polypeptide found in the supernatants of a wide variety of tumor cell lines and virally transformed cells (Todaro et al., 1980, 1985; Ozanne et al., 1980; Roberts et al., 1980). The amino acid sequence of TGFα has 35% identity with the epidermal growth factor (EGF) sequence, which includes the conservation of all six cysteine residues (Derynck et al., 1984; Marquardt et al., 1984). Two-dimensional NMR studies provide evidence that in solution the two proteins have very similar polypeptide chain folds (Montelione et al., 1989). TGFα has been shown to compete with EGF for binding to

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