Biochemical Homology between Rat Dorsal Prostate and Coagulating Gland

PURIFICATION OF A MAJOR ANDROGEN-INDUCED PROTEIN∗

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The anatomically distinct organs, rat dorsal prostate and coagulating gland, were found to display remarkable homology in protein composition, including two major androgen-dependent secretory proteins, referred to as dorsal proteins I and II. Dorsal protein I has been purified and found to be a dimer composed of two identical subunits with sedimentation coefficient 4.8 S, Stokes radius 32 Å, and M<sub>r</sub> = 71,000 (62,000 by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis). The dimer (6.8 S, 46 Å, M<sub>r</sub> = 150,000) dissociates at high ionic strength and can be partially reconstituted by removal of salt. Dorsal protein I is a basic protein (pI 9) with high lysine content and binds to phosphocellulose but not to DEAE-Sepharose. Schiff’s staining shows that it contains carbohydrate. Quantitative rocket immunoelectrophoresis using a rabbit antiserum indicates dorsal protein I is produced only in dorsal prostate and coagulating gland. The protein constitutes approximately 25% of total cytosol protein in both organs, yet makes up only 5% of coagulating gland luminal fluid and ejaculated seminal fluid. It was not detected in the rat dorsal prostate tumor (Dunning R3327H). Dorsal protein II is a larger protein (M<sub>r</sub> = 80,000 by SDS gel electrophoresis) with higher carbohydrate content. Under non-denaturing conditions, it has a Stokes radius of >200 Å, corresponding to M<sub>r</sub> of >300,000. Dorsal protein II represents a smaller proportional contribution of total cytosol protein than does dorsal protein I, yet is the predominant protein of coagulating gland fluid. These proteins will be useful probes for studies on androgen regulation of specific gene expression and prostatic secretory mechanisms.

The rat prostate gland develops from the embryonic urogenital sinus in response to androgens and at sexual maturity is composed of discrete lobes. Like other male accessory sex organs, the prostate complex secretes a variety of substances which enter the urethra to form the prostatic portion of the semen. The diverse nature and specificity of these secretion products exemplifies the biochemical heterogeneity among prostate glands. For example, the rat dorsal prostate and coagulating gland, sometimes referred to as the anterior prostate, are the major sites of fructose secretion (Humphrey and Mann, 1949; Mann 1964). The rat ventral prostate secretes high amounts of citrate, spermine and spermidine (Price and Williams-Ashman, 1961), and a specific protein, referred to as prostatein (Lea et al., 1979). The lateral prostate is the only major zinc-secreting portion of rat prostate (Gunn and Gould, 1957).

The goal of this work was to identify and purify a predominant protein of rat dorsal prostate for use as a probe in studies on androgen-induced gene regulation both in the dorsal prostate and the androgen-dependent dorsal prostate tumor (Dunning, R3327H). Electrophoretic and immunochemical analysis has revealed a striking protein homology between the two anatomically distinct organs, rat dorsal prostate and coagulating gland. Both organs contain two major proteins we refer to as dorsal proteins I and II. The smaller, dorsal protein I has been purified to homogeneity and characterized with respect to some of its physicochemical properties.

EXPERIMENTAL PROCEDURES

Materials

Reagents for electrophoresis were purchased from Bio-Rad. From Sigma, we obtained Trizma base (reagent grade), Coomasie brilliant blue G, catalase (bovine liver), chymotrypsinogen A (bovine pancreas), myoglobin type I (equine skeletal muscle), carboxypeptidase B (hog pancreas), and rat albumin (Fraction V). Myobacterium butyricum and Bordetella pertussis antigen were from Difco. Sephadex G-200, DEAE-Sepharose, and blue dextran 2000 were from Pharmacia. From Miles Laboratories we obtained bovine γ-globulins (Fraction II) and bovine albumin. Most reagent grade chemicals came from Fisher. Sprague-Dawley rats were from Zivic Miller, and Copenhagen Fischer rats bearing implanted Dunning tumors (R3327H) were from the Papanicolaou Cancer Research Institute, Inc. in Miami. Calcium l- (+)-lactate and ovalbumin were from Tridom Chemical Inc. Complete Freund’s adjuvant was from Grand Island Biological Co. Ampholine-polyacrylamide gel plates for thin layer gel electrophoresing, pH 3.5 to 9.5, were from LKB Instruments, Inc. Cellulose phosphate P11 was from Whatman.

Methods

SDS-polyacrylamide Gel Electrophoresis—SDS slab gels of 9% polyacrylamide (10 x 13.5 cm with 3- to 5-mm stacking gel of 4% acrylamide) were prepared essentially according to Laemmli (1970), except that gels contained, in addition, 4 M urea and 1 mM EDTA and the electrophoresis buffer contained 0.096 M glycine, 25 mM Tris, and 0.1% SDS. Samples were diluted with at least an equal volume of 4.9 M urea, 2.5% SDS, 0.003% bromphenol blue, and 0.074 M Tris, pH 6.7. 2-Mercaptoethanol (1 to 3 µl) was added to each sample. Samples were heated at 70°C for 10 min and allowed to cool to room temperature. Sample wells were rinsed with 4 M urea, 0.1% SDS, and 0.15 M Tris, pH 6.8. This solution was removed from each well with a syringe directly before application of the sample. Gels were electrophoresed for 4 to 5 h at 100 volts. Gels were stained with 0.25% Coomasie blue in 50% methanol and 10% acetic acid and destained with 20% methanol and 10% acetic acid. Glycoprotein determination by periodic acid-Schiff staining of proteins was carried out as previously described (Dewald et al., 1974). Prior to drying, gels were soaked

∗The abbreviation used is: SDS, sodium dodecyl sulfate.
in 40% methanol and 10% acetic acid overnight and dried under vacuum on low heat.

Sucrose Gradient Centrifugation—Linear 5.4-ml gradients of 5 to 20% (w/v) sucrose in 1 mM EDTA and 50 mM Tris, pH 7.5, contained no KCl, 0.15, or 0.5 M KCl as indicated. Gradients were centrifuged in a Beckman SW 50.1 rotor at 44,000 rpm for 18 h at 2°C. Purified dorsal protein samples of 200 µg in 0.2 ml were applied. Sedimentation coefficients were determined by analyzing gradients containing 1 mg of each marker protein: myoglobin (29 S), ovalbumin (3.6 S), rat serum albumin (4.4 S), bovine γ-globulin (7 S), and catalase (11.3 S). Gradients were fractionated by suction from the bottom of the tube. Fractions containing 12 drops (0.18 ml) were analyzed for protein content by the method of Lowry (1951).

Preparation of Antiserum—A White New Zealand adult female rabbit was immunized according to the procedure of Vaitukaitis et al. (1971). Purified dorsal protein (300 µg in 45 µl of H₂O) was combined with 20 mg of M. butyricum, 2 ml of Freund's complete adjuvant, and 2 ml of sterile saline. The mixture was blended on ice in a Sorvall Omni-Mixer at increasing speeds for 4 min. The white colloidal suspension was transferred to a syringe and the contents injected into multiple intradermal sites in the form of rosettes in the shoulder and lower back of the shaved rabbit. B. pertussis antigen (1 ml) was injected separately at three sites, intradermally. Blood was collected immediately following immunization from an ear vein and used as control serum. A booster injection, administered 12 weeks after the first immunization, consisted of 130 µg of dorsal protein in 2 ml of Freund's complete adjuvant emulsified in the Omni-Mixer. The suspension was administered at four subcutaneous sites on the back. Two weeks later, the rabbit was bled from the ear with a syringe under suction. The blood clotted at room temperature for 3 h followed by overnight at 4°C. Serum was obtained by centrifugation and was stored at -20°C.

Rocket Immunoelectrophoresis—Antiserum was used to quantitate dorsal prostate protein I by rocket immunoelectrophoresis, as previously described by Axelsen et al., (1973). A 1% agarose solution (300 mg in 30 ml) in the electrophoresis buffer, 2 mM diethylbarbituric acid (Na barbital), 0.4 mM Ca lactate, and 73 mM Tris, pH 8.6, is prepared by heating with stirring and then equilibrated at 50-55°C. The agarose is dissolved by heating with stirring and then equilibrated at 50-55°C for 30 min. Antiserum (100 µl) is added and the gel is formed between a coated (with 0.5% agarose in H₂O) and uncoated plate (11 x 20.5 cm). After cooling (30 min) at room temperature and removal of the uncoated plate, sample wells are prepared using a 2.5-mm, 5-µl template. Samples are appropriately diluted with electrophoresis buffer and applied with a Hamilton syringe under 50 volts to minimize diffusion. Electrophoresis continues overnight at 100 volts with cooling. Plates are soaked in saline (0.9% NaCl) for 30 min. The gel is dried and stained for 15 min in 0.3% Coomassie blue in 45% methanol and 10% acetic acid and destained in 45% methanol and 10% acetic acid. The relationship between rocket length and protein concentration was linear for rockets less than 25 mm in length.

Analytical Electrophoresis—Thin layer polyacrylamide gel electrophoresis was carried out on Ampholine (2.4%, w/v) polyacrylamide gel plates containing 5% polyacrylamide which have a pH range from 3.5 to 9.5. Samples (1 to 7 mg/ml) were saturated into application filters (approximately 15 µl) and applied to a cooled gel plate. The anode wick contained 1 m H₃PO₄ and the cathode, 1 n NaOH. All samples were electrophoresed simultaneously from anode and cathode. The voltage was increased at 10-min intervals from 200 to 950 volts, while the current decreased from 25 to 2 mA over a period of 2% h. Hemoglobin (pI = 6.8) and cytochrome c (pI = 10.65) were standards.

RESULTS

Anatomy of the Rat Prostate

The orientation of rat prostate lobes is illustrated in Fig. 1. Surrounding the neck of the bladder on the urethra are four large ventral lobes (two are visible in Fig. 1), two smaller lateral and two dorsal (or dorsocaudal) prostate lobes. Removed from the bladder region and lying contiguous on the urethra. Dorsal and lateral prostas share a common boundary that can be separated along a connective tissue plane of cleavage (Fig. 1B). Unlike other lobes of the prostate, but like the seminal vesicle, each coagulating gland (shown separated from seminal vesicle in Fig. 1B) has a lumen containing fluid of high protein concentration (~300 mg/ml).

Proteins of Rat Prostate

SDS-polyacrylamide gel electrophoresis of prostate cytosol proteins reveals a strong similarity between rat dorsal prostate and the dorsal and lateral prostas were partially separated. Indicated are the ventral (VP), dorsal (DP), and lateral (LP) lobes of the prostate, the urethra (U), seminal vesicle (SV), coagulating glands (CG), ductus deferens (DD), and urinary bladder (B).
(Fig. 2, Gel 2) and coagulating gland (Gel 3). In addition, their protein patterns clearly differ from ventral (Gel 9) or lateral (Gel 10) prostate or the Dunning tumor (Gel 5). Two proteins predominate in cytosols from dorsal prostate and coagulating gland, as well as in coagulating gland fluid (Fig. 2, Gels 4 and 7). These bands are also observed in a saline extract of ejaculated rat semen (Fig. 2, Gel 8). Purification and characterization of the $M_r = 62,000$ protein, referred to here as dorsal protein I, are described in this report. Preliminary studies on the larger protein of about $M_r = 80,000$, referred to as dorsal protein II, suggest that this protein is high in carbohydrate content, as revealed by dark staining with periodic acid-Schiff reagent. Chromatography of coagulating gland fluid on Seph- 
arrowleft
phoresis was with the following molecular weight marker proteins: ovalbumin, 43,000; carboxypeptidase A, 32,000; ovalbumin, 43,000; and bovine serum albumin, 66,000. Electrophoresis of a large amount of purified protein revealed only one band, indicating subunits of identical size. This fraction was used to prepare an antiserum by immunizing a rabbit. Further purification of the 46 kdalton peak fraction on phosphocellulose yielded a fraction containing the $M_r = 62,000$ protein contaminated with a protein of $M_r = 39,000$.

Summary of the Purification

An estimation of recoveries from the purification scheme
described above was obtained by rocket immunoelectrophoresis, as summarized in Table I. The apparent low recovery following DEAE-Sepharose may have been due to nonspecific adsorption of protein since little dorsal protein I was detectable in the 1 M KCl column wash. Sephadex G-200 (Fig. 3) effectively separated what appeared to be monomer from dimer (see below). Chromatography of the monomer on phosphocellulose separated away the seven remaining contaminating protein bands, as visualized on SDS-polyacrylamide gels. Approximately 5 mg of purified dorsal protein were recovered from 10 g of dorsal prostate taken from 35 rats.

Although not described here, dorsal protein I can also be readily purified from coagulating gland fluid by chromatography on Sepharose 4B, followed by the phosphocellulose step.

**Figure 3.** Sephadex G-200 chromatography of DEAE-Sepharose flow through of dorsal prostate cytosol. Dorsal prostate cytosol (30 ml) was chromatographed on a DEAE-Sepharose column. The flow through fractions were concentrated by lyophilization, resuspended in 1.5 ml of 1 mM EDTA and 50 mM Tris, pH 7.5, and applied to a Sephadex G-200 column (64.5 x 2.6 cm) equilibrated in the same buffer. Fractions of 40 drops (1.96 ml) were collected at a flow rate of 1 ml/15 min. Optical density was monitored by an ISCO scanner at 280 nm. Indicated are the void volume, as measured by blue dextran (V₀), and the elution of standard proteins γ-globulin, 52 Å, and ovalbumin, 27.3 Å, and peak fractions corresponding to dorsal protein dimer (46 Å) and monomer (32 Å).

**Figure 4.** Phosphocellulose chromatography of dorsal protein I monomer. Sephadex G-200 fractions 90 to 106 (see Fig. 3) of the 32 Å monomer were pooled, dialyzed against distilled H₂O overnight, and lyophilized. The protein (6 mg) was resuspended in 0.3 ml of distilled H₂O, dialyzed for 2 h against distilled H₂O, and applied to a phosphocellulose column (4 x 0.9 cm) prepared as described (see "Results," purification Step 4). A linear gradient from 0 to 0.3 M KCl in 1 mM EDTA and 50 mM Tris, pH 7.5, was applied while collecting 30-drop (1.1 ml) fractions. CI⁻ ion concentration was estimated with a CI⁻ electrode (Orion) as indicated (X).

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**Table I**

| Purification of rat dorsal protein I | Total protein | Purification Yield % |
|-------------------------------------|--------------|----------------------|
| Dorsal protein I                    | mg           | mg                   | fold |
| Dorsal prostate cytosol             | 92           | 418                  | 100  |
| DEAE-Sepharose flow                 | 28           | 93                   | 1.4  | 30   |
| through Sephadex G-200              | 14           | 33                   | 2.0  | 15   |
| Phosphocellulose*I* (eluted at 0.08 M KCl) | 5           | 5                    | 4.5  | 5    |

* The weight of dorsal protein I is defined in terms of rocket immunoelectrophoresis, where 58-mm rocket height ~1 mg of purified dorsal protein I. Total protein weight was estimated by the method of Lowry (1951).

**Molecular Weight and Subunit Structure**

Sedimentation coefficients of the 32 and 46 Å fractions were 4.6 and 6.8 S, respectively, when determined by linear sucrose gradient centrifugation. As indicated, both fractions yielded identical bands of Mᵣ = 62,000 by SDS-polyacrylamide gel electrophoresis. Their hydrodynamic molecular weights were 71,000 and 150,000, as summarized in Table II. Frictional ratios of 1.16 and 1.3 were estimated for the 32 Å, 4.6 S and 46 Å, 6.8 S forms, respectively.

**Interconversion of the 32 Å, 4.6 S and 46 Å, 6.8 S Forms**

Addition of 0.5 M KCl to the sucrose gradient (Fig. 5A) or Sephadex G-200 (not shown) buffers caused a shift from 6.8 S, 46 Å to 4.6 S, 32 Å. Subsequent removal of salt by dialysis resulted in partial reconstitution of the 6.8 S form (Fig. 5B). At a salt concentration near physiological ionic strength (0.15 M KCl), the larger form remained 6.8 S, 46 Å. Both forms reacted to the same extent with antisera generated from the smaller 32 Å form. It is concluded that the 46 Å, 6.8 S, Mᵣ = 150,000 form is a dimer of two identical subunits of 32 Å, 4.6 S, Mᵣ = 71,000 (Mᵣ = 62,000 on SDS-polyacrylamide gels). Attempts to confirm the presence of identical subunits by partial NH₂-terminal sequence analysis were unsuccessful due to what appeared to be a blocked terminal amino acid.

The ratio of dimer to monomer in cytosol (in 50 mM Tris, pH 7.5, 1 mM EDTA, and 10% glycerol) is usually about 1 to 1 as judged on Sephadex G-200, although Fig. 3 shows significantly more dimer. The pooled dimer peak from Sephadex G-200 could be repeatedly rechromatographed in 50 mM Tris, pH 7.5, and 1 mM EDTA with no apparent dissociation. It is not clear whether both forms exist in vivo. Our inability to completely reconstitute dimer from monomer may be due to some alteration in the protein during dissociation and reassociation.

**Other Properties**

The purified dorsal protein I stains lightly with periodic acid-Schiff reagent, suggesting that it is a glycoprotein. It was not retained, however, on concanavalin A-Sepharose. Treatment with neuraminidase had no effect on monomer mobility in SDS-polyacrylamide gels.

The purified dorsal protein is basic in charge since it is not retained by DEAE-Sepharose and adsors to phosphocellulose at neutral pH. Analytical electrofocusing in 5% polyacrylamide gels indicates an isoelectric point of approximately pH 9. In agreement with its basic properties, amino acid analysis revealed an unusually high lysine content (Table III).

The dorsal protein precipitates between 40 and 60% satu-
ratel (NH4)2SO4 as determined by SDS-polyacrylamide gel electrophoresis or by rocket immunoelectrophoresis.

**Antiserum to Dorsal Protein I**

Antiserum to purified dorsal protein I formed a precipitin line of cathodal migration by rocket immunoelectrophoresis (Fig. 6). Crossed immunoelectrophoresis of dorsal prostate cytosol further indicated the specificity of the antiserum (Fig. 7). Typical rocket immunoelectrophoresis patterns are shown for an ejaculated seminal fluid-saline extract, coagulating gland fluid, and cytosols from dorsal prostate, coagulating gland, and ventral prostate (Fig. 8). The dark staining protein of coagulating gland fluid with anodal migration likely consists primarily of the high molecular weight glycoprotein complex (dorsal protein II); it displays no cross-reactivity with the antiserum.

**Tissue Distribution**

Rocket immunoelectrophoresis and SDS gel electrophoresis were used to determine the distribution of the dorsal protein in various organs of the rat. In cytosols from dorsal prostate and coagulating gland, the concentrations of dorsal protein were determined using the standard proteins ovalbumin (3.6 S), bovine y-globulin (52 A), and catalase (11.3 S) as markers. The S designates Svedberg units.

**Table II**

| Molecular properties of dorsal protein I | Monomer | Dimer |
|------------------------------------------|---------|-------|
| Sedimentation coefficient (S)            | 4.6     | 6.8   |
| Stokes Radius (A)                        | 32      | 46    |
| Molecular weight                         | 62,000  | 71,000 |
| SDS-polyacrylamide gel                   | 50      | 150,000 |
| Hydrodynamic radius (A)                  | 1.16    | 1.3   |

*Determined on linear sucrose gradients as described under "Methods." Using the standard proteins ovalbumin (3.6 S), bovine y-globulin (52 A), and catalase (11.3 S) as markers. The S designates Svedberg units.

**Table III**

| Amino acid composition of dorsal protein I | % Concentration | Mol amino acid/mole protein |
|------------------------------------------|-----------------|-----------------------------|
| Lysine                                   | 13.42           | 68                          |
| Aspartic acid                            | 11.56           | 59                          |
| Asparagine                               | 11.56           | 59                          |
| Glutamic acid                            | 9.81            | 50                          |
| Glutamine                                | 8.87            | 45                          |
| Valine                                   | 8.52            | 43                          |
| Leucine                                  | 8.52            | 43                          |
| Threonine                                | 6.93            | 35                          |
| Serine                                   | 6.07            | 31                          |
| Isoleucine                               | 5.30            | 27                          |
| Glycine                                  | 5.08            | 26                          |
| Phenylalanine                            | 4.89            | 25                          |
| Alanine                                  | 4.30            | 22                          |
| Arginine                                 | 2.92            | 20                          |
| Methionine                               | 3.13            | 16                          |
| Tyrosine                                 | 2.85            | 14                          |
| Proline                                  | 2.60            | 13                          |
| Histidine                                | 2.41            | 12                          |
| Total                                    |                 | 506                         |

*If a Mr = 71,000 is assumed, then the per cent concentration is multiplied by 5.1 to determine moles of amino acid/mole of protein.

**Fig. 5.** Sucrose gradient centrifugation of the dorsal protein I monomer, dimer, and partially reconstituted dimer. Sedimentation coefficients of standard proteins are indicated (x--x) (see "Methods"). A, the 46 A dimer peak fractions from Sephadex G-200 chromatography were dialyzed against 1 mM EDTA and 50 mM Tris, pH 7.4, with (O--O) or without ( ) 0.5 M KCl and analyzed in sucrose gradients containing the same buffer (see "Methods"). B, the purified 22 A monomer peak fractions from Sephadex G-200 chromatography were dialyzed against 1 mM EDTA and 50 mM Tris, pH 7.5, for 3 h at 4°C and analyzed on sucrose gradients containing the same buffer (see "Methods").
were similar and ranged from 22 to 30% of total protein. The concentration of dorsal protein in coagulating gland fluid was surprisingly less than in cytosol and similar to that in saline extracts of seminal fluid (Table IV). Fluid could not be collected from dorsal prostate due to the absence of a luminal compartment. Separation of sperm plug from the primary ejaculate revealed negligible dorsal protein I in the plug and high amounts in the fluid portion containing the sperm. The low amount of dorsal protein I detected occasionally in lateral prostate (Table IV) is likely due to incomplete separation from dorsal prostate. The dorsal protein was undetectable in ventral prostate and in the Dunning dorsal prostate tumor (R3327H). It was also not detected in seminal vesicle cytosol or fluid, rat serum, or cytosols of rat testis, epididymis, liver, or kidney. No cross-reaction was noted with human seminal fluid.

**Androgen Control**

Rocket immunoelectrophoresis was used to quantitate the purified protein in dorsal prostate and coagulating gland cytosol and fluids. Aliquots (5 μl) of each of the following were analyzed by rocket immunoelectrophoresis as described under “Methods.” 1, Ejaculated seminal fluid, 1.3 mg/ml (collected after decapitation of rats and homogenized in 10% glycerol, 1 mM EDTA, and 50 mM Tris, pH 7.5, and centrifuged at 100,000 × g); 2 to 3, coagulating gland fluid (approximately 300 mg/ml) diluted 1/200 and 1/80; 4 to 6, dorsal cytosol (22.6 mg/ml) diluted 1/50, 1/25, and 1/10; 7 to 9, coagulating gland cytosol (11.8 mg/ml) diluted 1/50, 1/25, and 1/10; and 10, ventral prostate cytosol (12.4 mg/ml) undiluted.

### TABLE IV

**Distribution of dorsal protein I in rat cytosols and fluids**

Dorsal protein I concentration was determined by rocket immunoelectrophoresis and total protein by the Lowry assay.

| Sample Type                          | Approximate % of total protein |
|--------------------------------------|-------------------------------|
| Dorsal prostate                      | 25                            |
| Coagulating gland                    | 27                            |
| Cytosol                              | 6                             |
| Fluid                                |                               |
| Ejaculated seminal fluid             | 5                             |
| Dunning dorsal prostate tumor (R3327H)| N.D.*                         |
| Ventral prostate                     | N.D.                          |
| Lateral prostate                     | <2                            |
| Seminal vesicle                      |                               |
| Cytosol                              | N.D.                          |
| Fluid                                | N.D.                          |
| Serum                                | N.D.                          |
| Testis, epididymis, liver, or kidney | N.D.                          |

*“Not detectable.”
The concentration of protein I increased linearly beginning near 20 days of age. This is near the time of onset of puberty when a surge in gonadotropin release stimulates androgen production. Following castration of adult rats, the concentration of dorsal protein I decreased to a low level by 4 weeks. The concentration of dorsal protein I in both glands is thus similarly dependent on androgen stimulation.

**DISCUSSION**

A major protein of rat dorsal prostate and coagulating gland cytosol, referred to as dorsal protein I, has been purified, characterized, and a specific antiserum prepared. The protein is a dimer composed of two identical subunits, each with a Stokes radius of approximately 62,000 (62,000 x g supernatants) were prepared from dorsal prostate (●) or coagulating gland (○). A rocket height of 56 mm corresponded to approximately 1 μg of pure dorsal protein. Total protein was measured by the procedure of Lowry (1951).

tosols from rats of differing ages (Fig. 9A) and in adult rats castrated for 2 or 4 weeks (Fig. 9B). The concentration of protein I increased linearly beginning near 20 days of age. This is near the time of onset of puberty when a surge in gonadotropin release stimulates androgen production. Following castration of adult rats, the concentration of dorsal protein I decreased to a low level by 4 weeks. The concentration of dorsal protein I in both glands is thus similarly dependent on androgen stimulation.

This relationship is consistent with the observation that coagulating gland secretion is strongly periodic acid-Schiff positive, while the cytoplasm reacts weakly (LeBlond, 1950).

The rat coagulating gland, first described by Walker (1910), is known for its secretion of the heat-labile enzyme, vesiculase (Gotterer and Williams-Ashman, 1957). This enzyme catalyzes coagulation of a protein secreted by the seminal vesicle through a transamidation reaction (Notides and Williams-Ashman, 1967; Williams-Ashman et al., 1972; Beil and Hart, 1973). Both dorsal proteins reported here salt out near 50% saturated (NH₄)₂SO₄, the same concentration used to recover vesiculase activity (Gotterer and Williams-Ashman, 1957). Although the enzymatic properties of dorsal proteins I and II have not been established, their high concentration in cytosol and fluid argues against an enzymatic function. Other proteins of the coagulating gland are involved in the formation of an intrauterine gel. Joshi et al. (1972) observed that, post coitus, the uterine contents of the rat form a viscous gel resulting from the interaction of two major glycoprotein components of coagulating gland fluid. The bulk of the gel consisted of a protein with high carbohydrate content and sedimentation coefficient of 17 S (similar to dorsal protein II). Gelation of this glycoprotein was dependent on the presence of a 4.7 S protein which contained less carbohydrate (similar to dorsal protein I).

The nearly identical protein profiles observed in dorsal prostate and coagulating gland suggest that these organs may perform the same function in the rat, even though they are anatomically distinct and have a different overall gross structure. In agreement with this idea, earlier reports demonstrated similarities in their epithelial cells histochemically (Korenchevsky and Dennison, 1935), histologically (Price and Williams-Ashman, 1961; Brandes and Groth, 1961), and functionally (Humphrey and Mann, 1949; Mann, 1964). Some differences have been described, however, in the location of nuclei and organization of cisternal spaces (Korenchevsky and Dennison, 1935; Brandes and Groth, 1961). Dorsal prostate and coagulating gland appear to represent organs of unique embryological origin which have differentiated in nearly an identical manner. Each of the different lobes of rat prostate, including coagulating gland, is thought to arise out of the urogenital sinus from a separate epithelial bud (Price, 1963). These organs are of particular interest because they also relate embryologically to parts of the human prostate. Rat dorsal prostate is thought to be homologous to the outer region of human prostate (Price, 1963), the common primary site of prostatic carcinoma, while rat coagulating gland is homologous to the inner region of human prostate, the site of benign prostatic hypertrophy.

Dorsal protein I displays an androgen dependency characteristic of a secretory protein of the prostate or other accessory sex organs. Castration generally affects secretory processes initially, followed by histochemical and weight changes in the organ (Price and Williams-Ashman, 1961). The rather slow decline to near antigenically undetectable levels of dorsal protein I at 4 weeks postcastration may reflect, in part, the presence of stored pools of the dorsal protein within the cell. However, postcastrate regression of the coagulating gland, cytologically noticeable in 10 days, is slower than ventral prostate, observed by 2 days (Price and Williams-Ashman, 1961). All prostatic lobes are androgen dependent, although they atrophy at different rates in response to castration (Price and Williams-Ashman, 1961) or administered antiandrogens (Dahl and Kjaerheim, 1974). All can be subsequently maintained by administration of testosterone.

A goal of this work was to establish a marker that could be used in studies on androgen control of gene expression in the
dorsal prostate and Dunning tumor. The tumor (Dunning R3327H) is becoming a promising model for the study of prostate cancer. It is a well differentiated, androgen-dependent adenocarcinoma that is believed to have arisen spontaneously from rat dorsal prostate (Dunning, 1963). The tumor has been propagated by subcutaneous transplantation since 1961 at the Papanicolaou Cancer Research Institute in Miami. Like the normal dorsal prostate, it contains both androgen and estrogen receptors (Markland et al., 1978; Markland and Lee, 1979; Heston et al., 1979; Wilson and French, 1979). That the tumor is truly of dorsal prostate origin has been substantiated in part by an analysis of the activity and distribution of numerous enzymes (Smolev et al., 1977; Muntzing et al., 1978). The presence of the secretory form of acid phosphatase, an enzyme unique to the prostate, clearly supports at least its prostatic origin (Isaacs et al., 1979). SDS-polyacrylamide gel electrophoresis of tumor cytosol reveals, however, a pattern of proteins markedly different from normal dorsal prostate, coagulating gland, or other prostate lobes. Neither of the bulk proteins, dorsal proteins I or II, is a major protein in the tumor. Moreover, no antigenic cross-reaction of tumor cytosol or secreted fluid was detectable with antiserum to dorsal protein I by rocket immunoelectrophoresis or radioimmunoassay. Thus, the tumor displays a marked alteration of androgen-controlled gene expression associated with transformed epithelial cells that have remained well differentiated and androgen responsive.

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