Biochem. J. (1979) **184**, 431–440
Printed in Great Britain

Androgen-Sensitive Spermine-Binding Protein of Rat Ventral Prostate

PURIFICATION OF THE PROTEIN AND CHARACTERIZATION OF THE HORMONAL EFFECT

By Gabriele MEZZETTI, Rueyming LOOR and Shutsung LIAO
The Ben May Laboratory for Cancer Research and the Department of Biochemistry, University of Chicago, Chicago, IL 60637, U.S.A.

(*Received* 25 *April* 1979)

The rat ventral prostate contains a cytosol protein that can non-covalently bind spermine much more tightly than spermidine or other natural diamines. The protein has been purified to homogeneity, as judged by electrophoresis in urea- and sodium dodecyl sulphate-containing polyacrylamide gels. The protein, with or without spermine bound to it, sediments at 3S in a sucrose gradient with or without 0.4M-KCl. The molecular weight of the protein is about 30000. Each molecule of the binding protein can bind one molecule of spermine. In the prostate of rats injected with cycloheximide, the protein appears to have a half-life of about 3.5h. The spermine-binding activity of an acidic fraction obtained by DEAE-cellulose chromatography of the prostate cytosol proteins is reduced by about 40–60% within 20–40h after castration. This effect is reversed very rapidly within 15–30min by intraperitoneal injection of 5α-dihydrotestosterone. The hormonal effect is androgen-specific and is not mimicked by dexamethasone or oestradiol-17β. The androgen effect was reduced significantly when rats were injected with cycloheximide or actinomycin D, suggesting that the acidic protein may be one of the earliest proteins induced by androgen in the rat ventral prostate.

We have reported (Liang *et al.*, 1978) that the cytosol fraction of the rat ventral prostate contains an acidic protein that binds spermine much more firmly than spermidine or putrescine. The spermine-binding activity of the prostate protein fraction decreases after castration, but this effect is reversed if the castrated rats are injected with an androgen. As the first step in understanding the mechanism involved in the hormonal regulation of the spermine-binding activity, we have purified the protein to homogeneity and characterized the polyamine-binding activity and the androgen effect in more detail. The present study indicates that the spermine-binding protein may be one of the earliest proteins induced or altered by the androgen in the rat ventral prostate.

Experimental

Materials

[*Tetramethylene*-1,4-^{14}C]Spermine tetrahydrochloride (sp. radioactivity 13.3mCi/mmol) was purchased from New England Nuclear. Cyproterone (6-chloro-17β-hydroxy-1,2α-methylenepregna-4,6-diene-3,20-dione) was kindly supplied by Schering A.G., and flutamide {2-methyl-*N*-[4-nitro-3-(trifluoromethyl)phenyl]propanamide}, from Schering Corp., Bloomfield, NJ, U.S.A. Cycloheximide and actinomycin D were obtained from Sigma. Steroids were products of Steraloid. Pure enzymes and proteins were purchased from Worthington and Pharmacia.

Abbreviation used: SDS, sodium dodecyl sulphate.

Manipulation of animals

Male Sprague–Dawley rats, from 60 to 90 days old, were purchased from Sasco Inc. (Omaha, NE, U.S.A.) or King Animal Laboratories Inc. (Oregon, WI, U.S.A.). Animal experiments were carried out in duplicate or were repeated at least three times to assure reliability. Rats were separated into groups of five animals each. Castration was performed under diethyl ether anaesthesia. For injection, hormones or antagonists were dissolved in 1ml of sesame oil and administered intraperitoneally. Control animals received an equal amount of solvent carrier. The animals were killed by cervical dislocation and the ventral prostate was dissected free of its capsule.

Preparations of prostate proteins for binding assay

All manipulations were carried out at 0–2°C. The ventral prostate was minced and homogenized in 4vol. of 40mM-Tris/HCl, pH7.5, in an all-glass Potter–Elvehjem homogenizer, and the homogenate

was centrifuged at 105000*g* for 1 h. The upper three-quarters of the supernatant were used as the cytosol fraction. This fraction (30–50mg of protein) was then chromatographed on a Whatman DE-52 DEAE-cellulose column (0.9cm × 10cm) that was equilibrated with the same buffer. The column was eluted stepwise, with the buffer containing 0.1, 0.2 and 0.3M-KCl. The protein fraction eluted at 0.3M-KCl was dialysed overnight in 40mM-glycine, pH 8.7, and assayed for spermine-binding activity.

For some experiments, the prostate was homogenized in glycine buffer, pH 8.7. Solid $(NH_4)_2SO_4$ was added slowly to the cytosol fraction until the salt concentration reached 50% saturation. The protein precipitate formed was then removed by centrifugation at 10000*g* for 15 min. The supernatant was saturated to 75% with respect to the salt. The precipitate formed was collected by centrifugation, dissolved in 40mM-glycine, pH 8.7, and dialysed overnight against 40mM-glycine, pH 8.7, with occasional changes of the dialysis medium. The dialysed preparation was used in the assay for spermine-binding activity.

Assays of spermine-binding activity

The spermine-binding activities of the fractionated prostate proteins were analysed by the gradient-centrifugation technique described previously (Liang *et al.*, 1978), or by a newly developed method in which free spermine was separated from the glutaraldehyde-fixed spermine–protein complex by polyacrylamide-gel electrophoresis. The latter method has the advantage of providing an assay for binding activity in the presence of a high concentration of spermine. For gradient centrifugation, the protein sample was mixed with [^{14}C]spermine in 0.3 ml of 40mM-glycine, pH 8.7. A portion (0.2 ml) of the mixture was layered on a linear sucrose (5–20%, w/v) gradient containing 40mM-glycine, pH 8.7, and centrifuged at 54000 rev./min in a Spinco ultracentrifuge with an SW-56 rotor for 22 h at 0–2°C. Fractions (0.2 ml each) were collected with an ISCO gradient fractionator and numbered from the top of the tube. For gel electrophoresis, the protein sample was incubated with the radioactive spermine in 0.15 ml of the glycine buffer for 15 min at 0°C. The polyamine–protein complex was fixed by the addition of 0.05 ml of freshly made glutaraldehyde solution, prepared at 0°C by mixing 0.67 ml of 2M-Tris/HCl, pH 7.5, and 1 ml of 50% glutaraldehyde, and by adjustment of the pH to 7 by addition of about 0.3 ml of 5M-NaOH. The fixed mixture was subjected to polyacrylamide-gel electrophoresis [12.5% (w/v) acrylamide, 0.4% bisacrylamide, 7.8M-urea and 1% SDS] by the method of Fairbanks *et al.* (1971). The electrophoresis was performed at room temperature (25°C) and at 4mA/tube (0.5cm × 12.5cm), until the tracking dye (γ-pyronin) migrated 10cm toward the anode. The gel was sliced into about 30 sections. The radioactivity of the gradient fractions or of the gel slices was determined in a scintillation counter by the procedure described previously (Liang *et al.*, 1978).

With both methods described above, the values obtained for duplicate tubes were within 10% of each other.

Purification of spermine-binding protein

The ventral prostates (42g) from male Sprague–Dawley rats (body wt. 350–450g) were homogenized in 4 vol. of 40mM-Tris/HCl, pH 7.5, in a Potter–Elvehjem homogenizer. The homogenate was centrifuged at 105000*g* for 1 h in a Spinco rotor. Solid $(NH_4)_2SO_4$ was added to the supernatant until the salt concentration reached 50% saturation. The precipitate formed was removed by centrifugation. The supernatant was then saturated to 80% with respect to $(NH_4)_2SO_4$. The protein precipitate was collected by centrifugation, dissolved in 40mM-Tris/HCl, pH 7.5, and dialysed thoroughly against the same buffer. The dialysed fraction was mixed with 100 cm^3 (bed volume) of phosphocellulose resin (P-11) that had previously been equilibrated with 40mM-Tris/HCl, pH 7.5. The unbound fraction, which contained the spermine-binding protein, was adjusted to 0.2M-KCl and then applied to a DEAE-cellulose column (1.6cm × 25cm) previously equilibrated with 0.2M-KCl containing 40mM-Tris/HCl, pH 7.5. The column was eluted successively with 0.25M-KCl and 0.3M-KCl (both in 40mM-Tris/HCl, pH 7.5). The latter fraction, which contained the spermine-binding protein, was dialysed against 40mM-Tris/HCl, pH 7.5. The dialysed fraction was mixed with concanavalin A–Sepharose 4B (1 cm^3 bed volume/5 mg of protein), which was previously equilibrated with 40mM-Tris/HCl, pH 7.5. The spermine-binding protein in the unbound fraction was dialysed against 40mM-glycine, pH 8.7, and then layered on a sucrose gradient [5–20% (w/v) sucrose containing 40mM-glycine, pH 8.7] and centrifuged at 54000 rev./min for 22 h in a Spinco SW 56 rotor. The binding protein sedimenting in the vicinity of 3S was collected with an ISCO gradient fractionator.

Other methods

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. The standard markers used in the estimation of the molecular weight of the binding protein, and their molecular weights, were: horse heart cytochrome *c*, 12000; bovine milk α-lactalbumin, 14400; soya-bean trypsin inhibitor, 20000; bovine erythrocyte carbonic anhydrase, 30000; rabbit muscle lactate dehydrogenase, 36000; chick ovalbumin, 43000; bovine

liver catalase, 60000; bovine serum albumin, 68000; The standard proteins used for the determination of the sedimentation coefficient were chick ovalbumin (3.6S) and bovine serum albumin (4.6S).

Polyacrylamide-gel electrophoresis of the prostate protein fractions was carried out in 8.5% acrylamide separation gel and 2.5% stacking gel without SDS at 2mA/gel, as described by Krakow (1971), or in the presence of 8.5% gel containing 1M-urea and 0.1% SDS by the modified procedure of Laemmli (1970). The protein samples for the latter procedure were dissolved in 1.0% SDS/5.0% β-mercaptoethanol, dialysed and boiled, before being applied to the gels as described by Kostraba *et al.* (1975).

Identification and quantification of polyamines were carried out by paper electrophoresis or t.l.c. of polyamines and of their dansyl derivatives and by measurement of their colour reaction with ninhydrin (Liang *et al.*, 1978) or fluorescence (Abdel-Monem & Ohno, 1975).

Results

Purification and characterization of spermine-binding protein

We have purified the androgen-sensitive spermine-binding protein to homogeneity by the method described in the Experimental section (Table 1). The initial purification steps included an $(NH_4)_2SO_4$ precipitation and phosphocellulose chromatography; these removed some proteins, which were difficult to eliminate by other methods. The major remaining contaminant was a secretory protein [which was named 'α-protein' by us (Fang & Liao, 1971; Chen *et al.*, 1979) or 'prostate binding-protein' by Heyns *et al.* (1978)] with its two subunits (A and B). As shown in Fig. 1(*a*), these components appeared as three major bands in SDS-free polyacrylamide-gel electrophoresis. These proteins could be removed easily since they were retained in DEAE-cellulose chromatography and eluted by 0.2M-KCl completely, whereas the spermine-binding protein required 0.3M-KCl for its elution. This step also removed a major protein that migrated to the same position as spermine-binding protein in SDS-free polyacrylamide-gel electrophoresis. After DEAE-cellulose chromatography, the active fraction contained a sharp protein band (S) and two diffuse bands (D_1 and D_2). Most of the D_1 and D_2 components could be removed by concanavalin A-Sepharose treatment. Final purification by gradient centrifugation was possible, since the spermine-binding protein sediments at 3S, whereas the D_1 and D_2 components, which do not bind spermine, sediment at 1.0–1.5S.

The 3S protein migrated as a single band in urea- and SDS-containing polyacrylamide-gel electrophoresis (Fig. 1*b*). The molecular weight of the protein

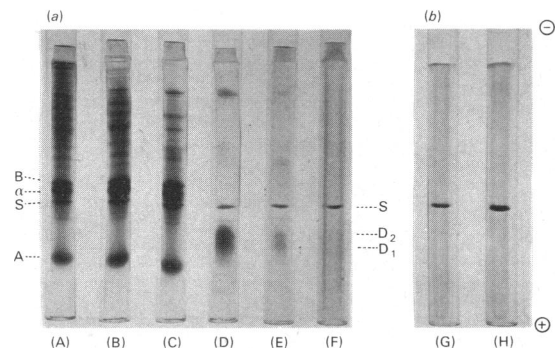

Fig. 1. *Polyacrylamide-gel electrophoresis of the prostate protein fractions obtained at different stages of purification as shown in Table* 1

Electrophoresis was performed in the absence (*a*) or presence (*b*) of SDS and urea. The amounts of proteins electrophoresed were: (A) total cytosol, 80μg; (B) $(NH_4)_2SO_4$-precipitated fraction, 80μg; (C) phosphocellulose fraction, 80μg; (D) DEAE-cellulose chromatographed fraction, 40μg; (E) concanavalin A-Sepharose-treated preparation, 30μg; (F), (G) and (H) gradient-centrifugation-purified binding protein, 20, 20 and 40μg repectively. For an explanation of the labelling of the bands on the gels, see the text.

Table 1. *Purification of the spermine-binding protein from rat ventral prostate*

Procedure	Total protein (mg)	Total activity (nmol of spermine bound)	Specific activity (activity/mg of protein)	Yield (%)	Purification (-fold)
Whole cytosol	929.3	167.3	0.18	100	—
$(NH_4)_2SO_4$ precipitation	460.0	165.7	0.36	99	2
Phosphocellulose chromatography	201.9	154.7	0.77	92	4
DEAE-cellulose chromatography	4.53	93.4	20.62	56	115
Concanavalin A-Sepharose fraction	2.20	57.6	26.18	34	145
Sucrose-gradient centrifugation	1.03	35.5	34.46	21	191

as determined by polyacrylamide-gel electrophoresis was about 30000 (Fig. 2*a*). This value is identical with that estimated by Sephadex G-200 gel filtration on a calibrated column (result not shown). Radioactive spermine fixed to the protein by glutaraldehyde and subjected to polyacrylamide-gel electrophoresis migrated to the same position as did the protein without spermine attached to it (Fig. 2*b*). During gradient centrifugation, the protein sediments at 3 S regardless of the concentration of KCl (0–0.4 M) or spermine (0–1 mM) in the sucrose gradient. [^{14}C]Spermine can

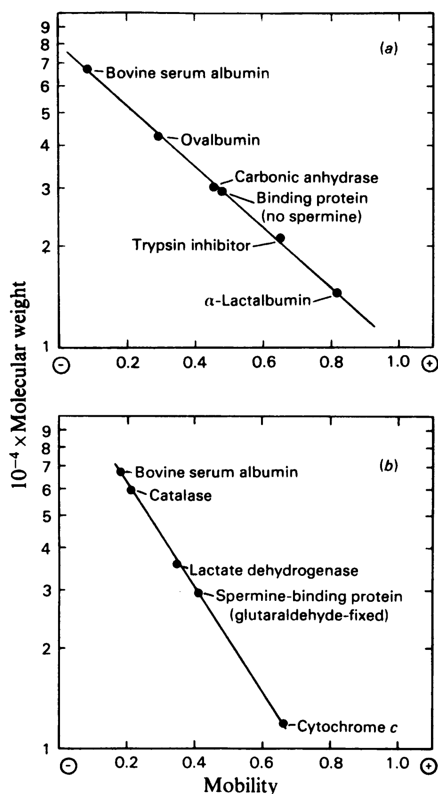

Fig. 2. *Determination of the molecular weight of the binding protein without (a) and with spermine fixed to it by glutaraldehyde (b)*

In (*a*), the protein purified by gradient centrifugation was subjected to SDS/polyacrylamide-gel electrophoresis. The protein was located by Coomassie Blue staining. In (*b*), the prostate cytosol protein fractionated by $(NH_4)_2SO_4$ was fixed with [^{14}C]-spermine by glutaraldehyde and subjected to SDS/polyacrylamide-gel electrophoresis as described in the Experimental section. The spermine-bound protein was located by measurement of the radioactivity of the gel slices. The mobilities of the binding protein and of the standard markers were compared, with the mobility of the tracking dye taken as 1.0.

dissociate completely from the purified binding protein in a medium containing high concentrations (>0.2 M) of KCl, or in an acetate buffer at pH 4 to 5. Since the dissociated radioactive ligand can be identified as spermine, the polyamine is bound to the protein non-covalently without prior conversion into another molecule.

The spermine-binding activity of the protein can be abolished completely by treatment with trypsin, but not with pancreatic ribonuclease or T_1 ribonuclease. The heat stability and the polyamine specificity of the purified protein are essentially identical with those of the crude protein fraction we described previously (Liang *et al.*, 1978).

With either the pure or the crude protein fraction protein binding of spermine is completed within 10 min after the protein and spermine are mixed together at 0–2°C. The binding sites in these protein preparations can be saturated at 5–10 μM-radioactive spermine (Fig. 3). The dissociation constant estimated from the Scatchard plot (Scatchard, 1949) obtained in such a study is approx. 0.25 μM. The binding activity is proportional, over a wide range of protein concentrations, to the amount of the protein added to each assay tube (Fig. 4). The results shown in Figs. 3 and 4 indicate that each protein molecule binds only one molecule of spermine.

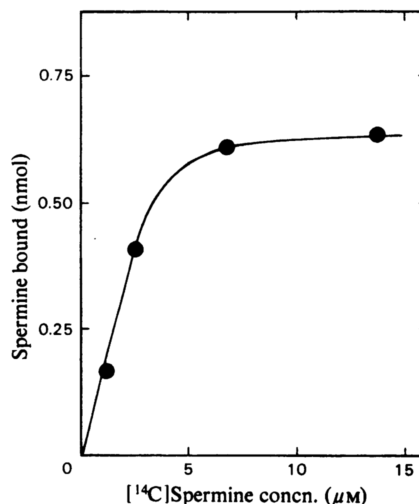

Fig. 3. *Spermine-binding activity of the gradient-purified binding protein*

The spermine-binding activity was assayed by the gradient-centrifugation method in the presence of different concentrations of [^{14}C]spermine. The amount of binding protein was 20 μg (0.67 nmol) per assay mixture. The amount of spermine bound to the protein was estimated from the radioactivity associated with the 3 S radioactivity peaks.

Appropriateness of the method for assaying the spermine-binding activity of prostate protein fractions

During the early phase of the present work, we studied the hormonal effects by comparing the spermine-binding activity of the crude prostate cytosol preparations. We soon found that the hormonal effect was seen more clearly if the cytosol proteins were fractionated either by $(NH_4)_2SO_4$ or by DEAE-cellulose column chromatography. This was apparently due to the removal of RNA and other proteins that might interfere with the binding assay.

Unless stated otherwise, we studied the hormonal effects described in the present paper by comparing the spermine-binding activities of the acidic protein fractions, which are retained firmly by a DEAE-cellulose column and which could be eluted from the column by 0.3M-KCl, but not by 0.2M-KCl. As described in the Experimental section, the whole-cytosol fraction was chromatographed directly on DEAE-cellulose without prior $(NH_4)_2SO_4$ precipitation or phosphocellulose chromatography (see Table 1). The latter steps would increase the time needed for processing of samples and would tend to yield unstable protein fractions that are not suitable for comparison. The DEAE-cellulose-chromatographed acidic protein generally contained about 5% of the total cytosol proteins and 90% of the spermine-binding activity in the original cytosol protein fractions. The recovery of the acidic proteins from the cytosol fractions of various groups of rats in the individual experiment was highly reproducible; the differences were within 10% of each other.

Like the pure protein, the partially purified protein can be saturated by 5–10μM-radioactive spermine. Since excess ligand does not affect the binding activity, we purposely used as much as 0.2mM-spermine in some of our experiments. The protein concentrations used in the individual assay tubes were within the ranges in which activities were proportional to the amounts of protein added to the assay tubes.

Since the rat ventral prostate is very rich in polyamines (Pegg *et al.*, 1970), some endogenous polyamines may contaminate the protein fractions and affect the binding assay. To test this, we added radioactive spermine or spermidine to the prostate homogenates and prepared the protein samples. No radioactivity was found when the protein fractions were collected by $(NH_4)_2SO_4$ precipitation or by DEAE-cellulose column chromatography and dialysed. The present study suggests that there is no covalent adduct formed between the protein and spermine or its oxidized products during the preparation of the

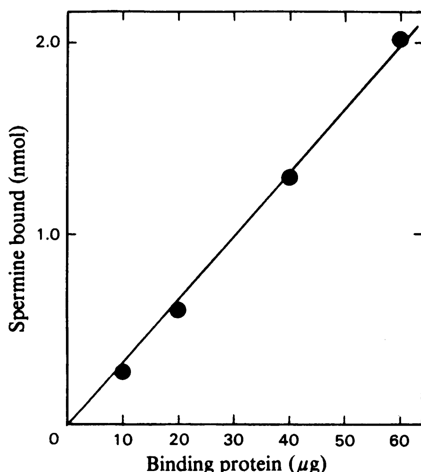

Fig. 4. *Stoicheiometry of spermine binding by the gradient-centrifugation-purified binding protein*

The spermine-binding activity was assayed by the gradient-centrifugation method in the presence of various amounts of binding protein. The concentration of [^{14}C]spermine in the assay tube was 74μM.

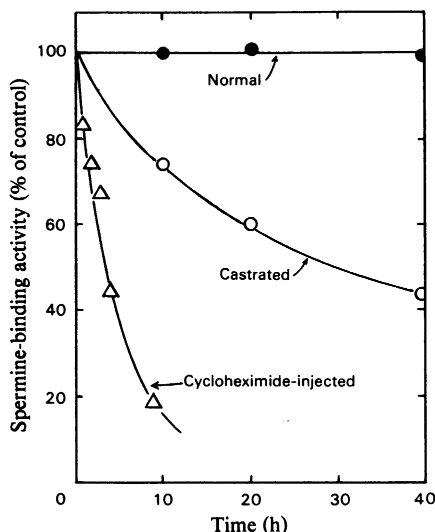

Fig. 5. *Effects of castration and cycloheximide injection on the spermine-binding activity of the prostate protein fraction*

Normal rats were castrated or injected with cycloheximide (3mg/rat) at 0h and killed at the time indicated on the abscissa. The cytosol fractions from the ventral prostate of these rats and of normal rats were chromatographed on DEAE-cellulose and assayed by the gradient-centrifugation method as described in the Experimental section. Each assay tube contained 40μg of DEAE-cellulose-chromatographed protein and 19μM-[^{14}C]spermine. The spermine-binding activity of the normal rats killed at 0h was taken as 100% for this comparison.

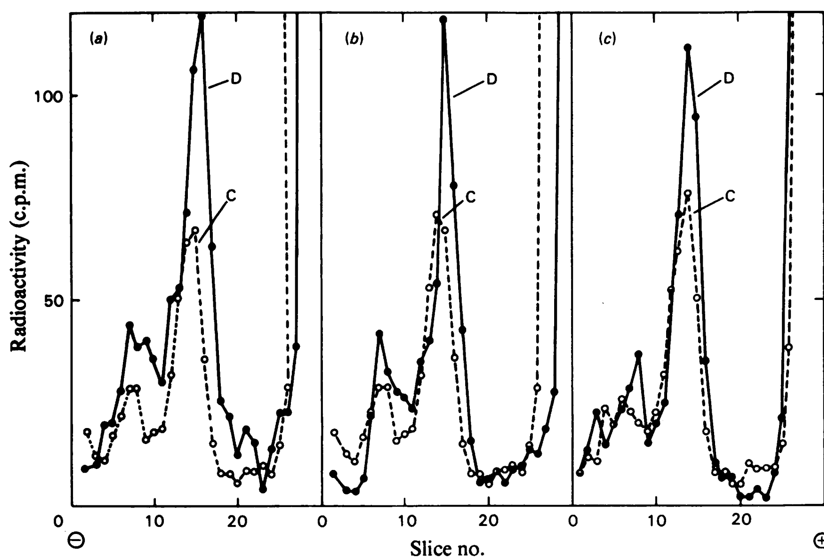

Fig. 6. *Effect of androgen on spermine-binding activity assayed by polyacrylamide-gel electrophoresis*
Rats were castrated 42h before they were killed. They were injected with 5α-dihydrotestosterone (D; 6mg/rat) at 30min (*a*), 60min (*b*) or 120min (*c*) before their ventral prostates were removed. The control groups (C) received only the solvent carrier. Prostate cytosol proteins fractionated by $(NH_4)_2SO_4$ were used for the assay. The radioactivities of the gel slices are shown in the Figure. The quantity of prostate proteins in each assay tube was 1.2mg. In this experiment, the concentration of [^{14}C]spermine in the incubation mixture was 180μM. The results were essentially the same when the spermine concentration was decreased to 20μM (results not shown).

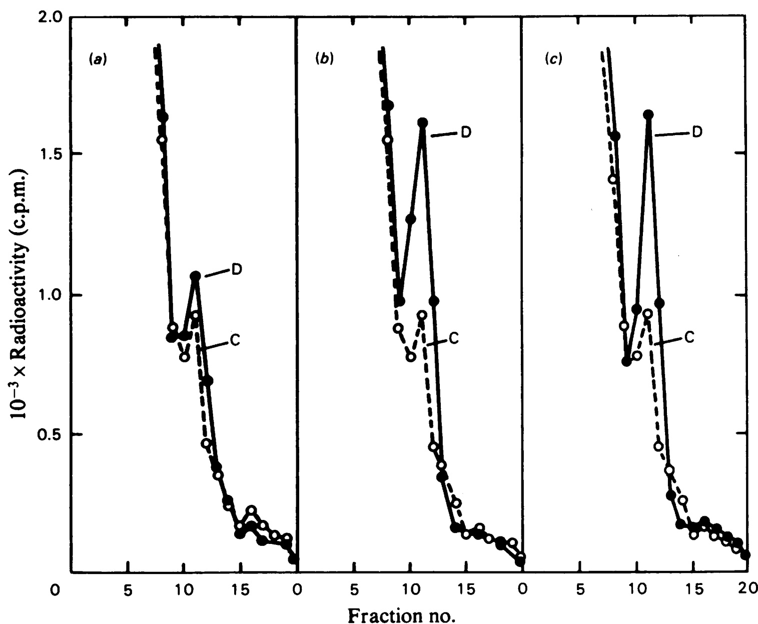

Fig. 7. *Effect of androgen on the spermine-binding activity assayed by gradient centrifugation*
Rats were castrated 42h before they were killed. They were injected without (C) or with 6mg of 5α-dihydrotestosterone (D) at 15min, (*a*) 30min (*b*) or 60min (*c*) before their ventral prostates were removed. The cytosol proteins fractionated by DEAE-cellulose column chromatography were used for the assay of the spermine-binding activity. The radioactivities of the gradient fractions are shown in the Figure. The concentration of [^{14}C]spermine in the incubation mixture was 11.3μM. The quantity of prostate proteins in each assay tube was 29μg.

protein samples. We also used colorimetric and fluorescence techniques to determine polyamines in these protein fractions. Calculations based on the limit of the sensitivity of the detection methods showed that our protein samples in the individual assay tubes did not contain more than 0.02 pmol of spermine, if any. Since our assay mixtures contained at least 2 nmol of radioactive spermine/tube, the endogenous polyamine could not interfere with the assay results.

Effect of castration and cycloheximide on spermine-binding activity of prostate protein fractions

As we described in our previous communication (Liang *et al.*, 1978), the spermine-binding activity of the prostate protein fraction was reduced considerably after rats were castrated. From 40 to 60% of the binding activity may be lost within 20–40 h after castration (Fig. 5). The remaining activity, however, was not very sensitive to castration. Whether this is due to the presence of a testicular hormone-insensitive class of the binding protein or to the residual supply of androgen from other sources (e.g. adrenal) is not clear.

Injection of the protein-synthesis inhibitor cycloheximide to normal rats can reduce the spermine-binding activity much more rapidly than castration. After the inhibitor was injected, about 50% of the activity was lost within 3.5 h, and more than 80% loss was seen after 9 h. If the cycloheximide effect is due primarily to inhibition of the synthesis of new binding-protein molecules, the observation may suggest that the protein has a short half-life in the prostate cells.

Rapid effect of androgen on the spermine-binding activity

When the $(NH_4)_2SO_4$-fractionated proteins were incubated with [^{14}C]spermine, fixed with glutaraldehyde, and analysed by polyacrylamide-gel electrophoresis, the protein-bound radioactivity migrated as a major and a minor (with a shoulder) peak, whereas the free spermine migrated to the anode end (Fig. 6). The major radioactivity peak was associated with a protein having a mol.wt. of about 30000. Whether the minor peak was due to glutaraldehyde-linked polymers of this protein has not been determined. The radioactivity in both peaks was higher for the protein fractions prepared from the prostates of the injected animals than for the fractions from the control castrated rats. The androgen effect could also be detected when the spermine-binding activity of the $(NH_4)_2SO_4$-fractionated or DEAE-cellulose-chromatographed proteins was analysed by gradient centrifugation (Fig. 7) at various protein concentrations (Fig. 8).

In at least six time-course experiments that we have performed, we have consistently observed that the androgen effect followed the pattern represented by the experiment illustrated in Fig. 9. The increase in spermine-binding activity was insignificant within 15 min after androgen administration to the castrated animals. This lag phase was followed by a period, up to 30 min, of a very rapid increase in the activity. Thereafter, the activity increased slowly and eventually reached a normal value (see also Figs. 6 and 7).

Steroid and tissue specificity

Besides being present in the ventral prostate, the spermine binding is found, although in lesser amounts, in other rat tissues that we have tested (Liang *et al.*, 1978). Injection of 5α-dihydrotestosterone (17β-hydroxy-5α-androstan-3-one) did not significantly enhance the spermine-binding activity of similar protein fractions from spleen or liver. In the ventral prostate, the hormonal effect appeared to be androgen-specific; injection of dexamethasone or oestradiol-17β did not result in any significant increase in spermine-binding activity (Table 2).

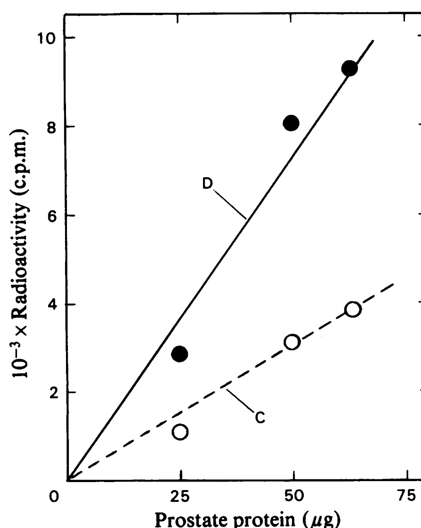

Fig. 8. *Androgen effect on the spermine-binding activity, assayed at different protein concentrations*

Rats were castrated 42 h before they were killed. They were injected without (C) or with 6 mg of 5α-dihydrotestosterone (D) at 0, 19 and 40 h after castration. The DEAE-cellulose chromatographed prostate proteins were assayed for the spermine-binding activity by the gradient-centrifugation method. The concentration of [^{14}C]spermine in each assay tube was 11.3 μM. The ordinate scale is the radioactivity associated with the 3 S peak area and the abscissa scale is the quantity of chromatographed prostate proteins used in the assay.

Effect of anti-androgens and antibiotics on the androgenic response

The spermine-binding activity appeared to be anti-androgen-sensitive. Treatment of normal rats for 42h with anti-androgens (either cyproterone or flutamide) reduced the spermine-binding activity by about 25–35%. The androgen effect was less pronounced when the castrated rats were injected with cycloheximide (Fig. 10) or actinomycin D (Fig. 11) at doses that respectively inhibited more than 95% of the incorporation *in vivo* of [^{3}H]leucine into the prostate proteins and more than 70% of the incorporation *in vivo* of [^{3}H]uridine into the prostate RNA.

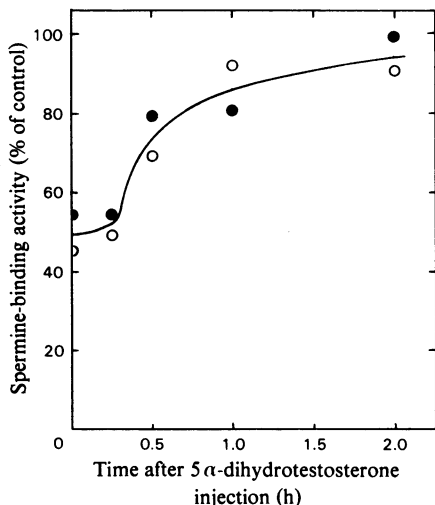

Fig. 9. *Rapid effect of androgen on the spermine-binding activity of the prostate protein fractions*
Rats were injected with 6mg of 5α-dihydrotestosterone 40h after castration and killed 0, 15, 30, 60 and 120min later. The DEAE-cellulose-chromatographed prostate proteins were assayed for the spermine-binding activity by gradient centrifugation for comparison. The activity exhibited by the prostate proteins obtained from normal rats was taken as 100%. Each assay mixture contained 40μg of prostate proteins and 18.5μM-[^{14}C]spermine. For operational convenience, the experiment was carried out in two sets (○ and ●) on two different days rather than in duplicate on the same day.

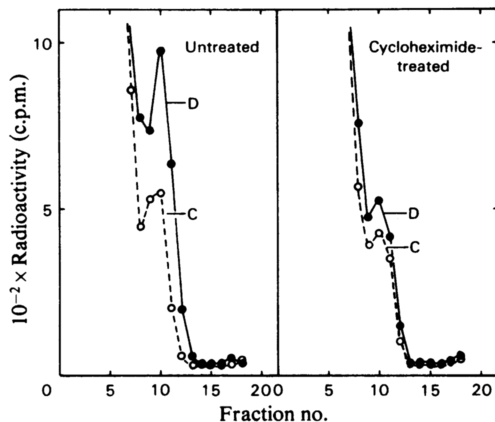

Fig. 10. *Effect of cycloheximide on androgen stimulation of the spermine-binding activity of the prostate protein*
All rats were castrated 42h before being killed and were injected with or without 3mg of cycloheximide 75min before being killed. They were divided into groups and injected without (C) or with 6mg of 5α-dihydrotestosterone (D) 1h before they were killed. The cytosol proteins were fractionated by DEAE-cellulose column chromatography and assayed for spermine-binding activity by the gradient-centrifugation method. The radioactivities associated with the gradient fractions are shown in the Figure. Each assay tube contained 48μg of prostate proteins and 11.3μM-[^{14}C]spermine.

Table 2. *Steroid specificity of the hormonal effect on the spermine-binding activity of the prostate cytosol protein*
Rats were castrated 42h before they were killed. They were injected with the solvent carrier only (control) or with 6mg of 5α-dihydrotestosterone, oestradiol-17β or dexamethasone at 19 and 40h after castration. The cytosol proteins were fractionated by DEAE-cellulose column chromatography and assayed for spermine-binding activity by gradient centrifugation. The assay tube contained 11μM-[^{14}C]spermine and 49μg of fractionated proteins.

Steroid injected	Radioactivity associated with 3 S protein peak (c.p.m.)	(% of control value)
Control	4443	100
Oestradiol-17β	3443	78
Dexamethasone	5440	122
5α-Dihydrotestosterone	10607	239

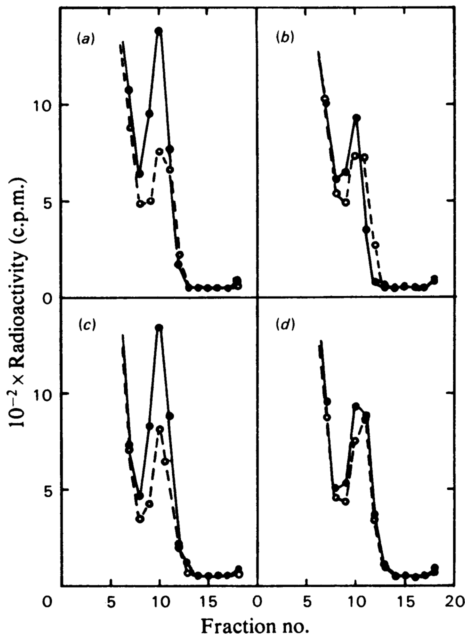

Fig. 11. *Effect of actinomycin D on androgen stimulation of the spermine-binding activity of the prostate protein*

All rats were castrated 42h before being killed and were injected with (●) or without (○) 6mg of 5α-dihydrotestosterone 30min (*a* and *b*) or 60min (*c* and *d*) before being killed. The rats in (*b*) and (*d*) were injected with actinomycin D (250μg/rat) 15min before the hormonal injection. The cytosol proteins were fractionated by DEAE-cellulose column chromatography and assayed for the spermine-binding activity by the gradient-centrifugation method. The radioactivities associated with the gradient fractions are shown in the Figure. Each assay tube contained 40μg of prostate proteins and 18.5μM-[^{14}C]spermine.

Discussion

It is not clear whether the protein binding described here plays a specific biological role in the ventral prostate. One can conjecture that the secretion and the intracellular translocation or compartmentalization of polyamines may be dependent on their binding to selective groups of cellular proteins. Alternatively, the intracellular transport and the cellular localization of certain acidic or phosphorylated proteins may be assisted by their binding to specific polyamines. The very rapid effect of androgen on protein binding of spermine then would greatly influence various cellular functions that may be affected by polyamines (Pegg *et al.*, 1970; Tabor & Tabor, 1976) or by acidic and phosphorylated proteins (Stein & Kleinsmith, 1975). Whether such a mechanism is involved in androgen-induced stimulation of protein phosphorylation (Ahmed & Ishida, 1971; Ahmed *et al.*, 1978), RNA synthesis (Liao, 1977), protein synthesis (Liang *et al.*, 1977) and chromatin alteration (Couch & Anderson, 1973; Mainwaring & Jones, 1975; Loor *et al.*, 1977) is worthy of further investigation.

It is also possible that we are simply assaying the androgen-sensitive acidic or phosphorylated proteins that can be detected conveniently by radioactive-spermine binding, and that the binding phenomenon does not represent a biologically important function. By using radioisotope pulse-labelling techniques, Gorski and his co-workers have shown that oestrogens can induce an acidic protein in the rat uterus (Barnea & Gorski, 1970; DeAngelo & Gorski, 1970). Previous attempts to show similar effects of androgen on rat ventral prostate by the Gorski technique were not successful. Therefore it is not clear whether the androgen-induced protein is similar to Gorski's oestrogen-induced protein.

The mechanism by which androgen exerts its very rapid effect on the spermine-binding activity in the rat ventral prostate is not clear. As described in the Results section, the assay of the binding activity is apparently not complicated by the endogenous polyamines that may contaminate the prostate fractions, or that may be metabolized *in vitro* to products that form covalent adducts with the binding protein. However, we do not know whether androgen can affect the covalent binding *in vivo* of certain polyamine metabolites to the binding protein.

When the binding proteins are extensively purified (to over 70% purity as seen on polyacrylamide-gel electrophoresis) from the ventral prostate of the normal, castrated, or castrated and androgen-treated rats, and compared, they exhibited essentially identical spermine-binding activities regardless of the androgenic status of the animals. It is possible that factors that may affect the spermine-binding activity of the prostate protein are removed during the purification. Alternatively, the hormonal effect may be due to a rapid androgen-dependent induction of the spermine-binding protein. The latter possibility is plausible, since the protein appears to have a rather short half-life in the prostate and the effect is minimized by cycloheximide and actinomycin D. Direct evidence for an androgen-induced increase in the binding protein may be obtained if our attempt to raise antibodies against this protein is successful.

Since the protein may be a phosphorylated protein (Liang *et al.*, 1978), it is also conceivable that androgen induces another protein or enzyme, which may regulate phosphorylation of the binding protein and alter its spermine-binding activity. In three preliminary experiments we have observed an increase in the spermine-binding activity of the DEAE-cellulose-chromatographed acidic protein fraction after daily administration of dibutyryl cyclic AMP for 2 days to

the castrated rats. However, the protein is apparently different from the androgen- and cyclic AMP-regulated phosphoprotein reported by Liu & Greengard (1976), since the latter protein has a much higher molecular weight (54000). Although both the 5α-[^{3}H]dihydrotestosterone–receptor complex (Fang & Liao, 1971; Liao, 1977) and the [^{14}C]spermine–protein complex sediment at about 3S, the two proteins can easily be separated by $(NH_4)_2SO_4$ fractionation or DEAE-cellulose chromatography. We have not been able to show the interaction of these two complexes *in vitro*. When the two protein fractions were mixed together and analysed by gradient centrifugation in a low-salt medium (<0.05M-KCl), the receptor complex aggregated to larger forms, but the spermine–protein complex remained at the 3S position.

Since polyamine levels in the prostate do not change rapidly in response to androgen manipulation of rats (Pegg *et al.*, 1970), the highly androgen-sensitive spermine-binding protein in itself does not appear to play a key role in the synthesis or metabolism of polyamine. This assumption is in accord with the fact that this protein is prostate-specific and not found in other tissues, such as liver and kidney (G. Mezzetti, R. Loor & S. Liao, unpublished work), where polyamines are synthesized in abundant quantities.

We thank Ms. Stephanie Smythe and Ms. Karen Schilling for their excellent technical assistance. This work was supported by N.I.H. research grants AM09461 and American Cancer Society grant BC151.

References

Abdel-Monem, M. M. & Ohno, K. (1975) *J. Chromatogr.* **107**, 416–419
Ahmed, K. & Ishida, H. (1971) *Mol. Pharmacol.* **7**, 323–327
Ahmed, K., Wilson, M. J., Goueli, S. A. & Williams-Ashman, H. G. (1978) *Biochem. J.* **176**, 739–750
Barnea, A. & Gorski, J. (1970) *Biochemistry* **9**, 1899–1904
Chen, C., Hiipakka, R. A. & Liao, S. (1979) *J. Steroid Biochem.* **11**, 401–405
Couch, R. M. & Anderson, K. M. (1973) *Biochemistry* **12**, 3114–3120
DeAngelo, A. B. & Gorski, J. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **66**, 693–700
Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617
Fang, S. & Liao, S. (1971) *J. Biol. Chem.* **246**, 16–24
Heyns, W., Peeters, B., Mous, J., Rombauts, W. & DeMoor, P. (1978) *Eur. J. Biochem.* **89**, 181–186
Kostraba, N. C., Montagna, R. A. & Wang, T. Y. (1975) *J. Biol. Chem.* **250**, 1548–1555
Krakow, J. S. (1971) *Methods Enzymol.* **21D**, 520–528
Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685
Liang, T., Castañeda, E. & Liao, S. (1977) *J. Biol. Chem.* **252**, 5692–5700
Liang, T., Mezzetti, G., Chen, C. & Liao, S. (1978) *Biochim. Biophys. Acta* **542**, 430–441
Liao, S. (1977) in *Biochemical Actions of Hormones* (Litwack, G., ed.), vol. 4, pp. 351–406, Academic Press New York.
Liu, A. Y.-C. & Greengard, P. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73**, 568–572
Loor, R. M., Hu, A.-Li & Wang, T. Y. (1977) *Biochim. Biophys. Acta* **477**, 312–321
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
Mainwaring, W. I. P. & Jones, D. M. (1975) *J. Steroid Biochem.* **6**, 475–481
Pegg, A. E., Lockwood, D. H. & Williams-Ashman, H. G. (1970) *Biochem. J.* **117**, 17–31
Scatchard, G. (1949) *Ann. N.Y. Acad. Sci.* **51**, 660–672
Stein, G. S. & Kleinsmith, L. J. (1975) *Chromosomal Proteins and Their Role in the Regulation of Gene Expression*, Academic Press, New York
Tabor, C. W. & Tabor, H. (1976) *Annu. Rev. Biochem.* **45**, 285–306