A proline-rich polypeptide is associated with prostatic binding protein, a major androgen-dependent protein described previously in the rat ventral prostate. This polypeptide has been purified. Its molecular weight estimated by gel filtration is about 8500, but a markedly lower value (3300) is obtained by sodium dodecyl sulfate-urea polyacrylamide gel electrophoresis. Isoelectric focusing on thin layer polyacrylamide gels yields two major forms with isoelectric points of, respectively, 7.75 and 7.05. The amino acid composition of proline-rich polypeptide is characterized by a high (19.5%) proline content and its NH₂-terminal amino acid is glycine.

Like prostatic binding protein, proline-rich polypeptide is a characteristic component of the rat ventral prostate and localized primarily in the intraluminal secretion of this gland. In intact adult male rats the cytosol of a whole gland contains 0.70 ± 0.15 (S.D.) mg of the polypeptide, as measured by radial immunodiffusion or 2.6 ± 0.5% (S.D.) of the total protein. This amount decreases gradually after castration and becomes undetectable after 8 days. Androgen treatment, on the other hand, results in a rapid stimulation, while estradiol and progesterone are ineffective. Proline-rich polypeptide is markedly more androgen-dependent than prostatic binding protein, and promises to be an interesting end point for studies on the mechanism of action of androgens.

The rat ventral prostate has been studied intensively as a target tissue for androgens (1). As previously shown (2–4), the epithelial cells of this organ secrete large amounts of a steroid-binding protein which we have called prostatic binding protein. This protein most probably corresponds to the α-protein described by Forsgren (6), the prostatic protein described by Lea et al. (6), and the estramustine-binding protein described by Forsgren et al. (7). PBP is composed of two different subunits (8): in each subunit two different peptide chains (components) are linked by disulfide bridges. One of these components (C₁) is present in both subunits. The other component (C₂ or C₃) is specific for each subunit, although there is marked sequence homology between C₁ and C₂ (9). The concentration (4) and synthesis (10) of PBP are controlled by androgens. This also is the case for the mRNAs encoding the three components of this protein (11, 12), which correspond to some of the androgen-dependent abundant mRNAs described by Parker et al. (13).

Up to now steroid-binding constitutes the most prominent functional characteristic of PBP. Indeed, this protein shows low affinity binding for several nonpolar steroids such as pregnenolone and 5α-dihydrotestosterone, but stronger binding for the synthetic estradiol derivative estramustine (7).

In this communication we describe and partially characterize a proline-rich polypeptide, which is found in the rat ventral prostate. This proline-rich polypeptide binds to prostatic binding protein and is more androgen-dependent than the latter.

**EXPERIMENTAL PROCEDURES**

**Materials—**Analytical grade reagents from Merck were used in this study. Sephadex G-50 and G-75, DEAE-Sepharose, and PD-10 columns were obtained from Pharmacia, DEAE-cellulose (DE-52) from Whatman, polyethylene glycol from Serva, and agaropectin (Indulbio A 37) from IBF. For polyacrylamide gel electrophoresis, electrophoresis-grade reagents from Aldrich were used. Electrophoretic transfer was performed on nitrocellulose membranes (SM 11336, pore size 0.45 μm) from Sartorius. Urea solutions were purified on mixed bed ion exchange resins (Amberlite MB3) and filtered through a small column of DEAE-Sepharose before use. Ultrafiltration cells from Amicon were used for the concentration of proteins.

**Animals—**All experiments were performed on Wistar rats. Castrations were done under ether anesthesia. Before removal of organs, rats were anesthetized with ether and bled to death via the carotid artery. Hormones were injected subcutaneously in oil solution.

**Purification of PBP and PRP—**PBP (actually PBP-PRP complex) was purified by DEAE-cellulose chromatography of prostatic cytosol at pH 6.5, followed by gel filtration on Sephadex G-75. PRP was purified from this complex by use of DEAE-Sepharose chromatography at neutral pH in the presence of 8 μm erythromycin. Both purification procedures are described in detail in the Results section.

**Immunization of Rabbits—**Two rabbits were immunized with PRP (about 0.4 mg) using the Vaitukatis technique of multiple intradermal injections (14) followed by a booster injection (about 0.2 mg) 6 weeks later. Clearly positive antisera were obtained from 8 to 20 weeks after the first injection.

**Radial Immunodiffusion of PBP and PRP—**PBP was measured as described previously (15). The assay of PRP was essentially the same, but with some modifications (see "Results"). In this case the immunodiffusion gel contained 1% (w/v) of agarose, 35 mg/ml of polyethylene glycol 6000, and 30 μl/ml of PBP antiserum in Veronal buffer (pH 6.0).

**Electrophoretic Techniques—**Polyacrylamide gel electrophoresis in the presence of SDS was performed according to Laemmli (16). Slab gels of 12 × 13 × 0.075 cm were used. Optimal separation was obtained on 15% acrylamide, 0.4% N,N'-methylenebisacrylamide gels. In some experiments the stacking gel was omitted (see "Results"). For the estimation of the molecular weight of PRP the method of Swank and Munkres (17) was used.
mental conditions, the purification procedure may thus lead to the purification of PBP-PRP complex (first peak) or of the cytosol is delipidated by acetone precipitation (3) before homogenization with an Ultraturrax mixer in 20 ml of 25 mM-[bis(2-hydroxyethyl)amino]methane-HCl buffer, pH 6.5, followed by ultracentrifugation (45 min at 200,000 × g). After adjustment of the ionic strength, the sample was applied at 4 °C to a DEAE-cellulose column (10.0 cm × 2.5 cm), equilibrated with 50 mM KCl in the same buffer. The column was eluted with 15 ml of this solution followed by a linear gradient (2 × 200 ml; 50 to 300 mM KCl) in the same buffer. Fractions of 10 ml were collected. Upper, the extinction at 280 nm (●) and the conductivity (○). lower, concentrations of PBP (●) and PRP (○) measured immunologically.

RESULTS

Different Forms of PBP in Prostatic Cytosol

When total prostatic cytosol is applied to a DEAE-cellulose column at pH 6.5, two major PBP-containing peaks are eluted from the column (Fig. 1). As will be shown in this paper the first and usually major peak (fractions 22 to 27 in Fig. 1) consists of a PBP-PRP complex, whereas the second peak (fractions 31 to 40) contains only PBP. In addition, partial dissociation of PBP in its subunits seems to occur in this second peak since the initial fractions of this peak show an excess of the S subunit and the final fractions an excess of the F subunit upon electrophoretic analysis. It should be noted that the relative proportions of the peaks are affected to some degree by the experimental conditions. Since PRP dissociates from PBP at alkaline pH (see below), a higher pH favors the second form. This is also the case when the cytosol is delipidated by acetone precipitation (3) before DEAE-cellulose chromatography. According to the experimental conditions, the purification procedure may thus lead to the purification of PBP-PRP complex (first peak) or of PBP without PRP (second peak).

For the purpose of the present study PBP (actually PBP-PRP complex) was purified by DEAE-cellulose chromatography of prostatic cytosol at pH 6.5 (see Fig. 1). The material of the first peak of PBP was concentrated on an Amicon YM-5 membrane and further purified by gel filtration on a Sephadex G-75 column.

A Small Protein (PRP) Is Bound to PBP and Dissociates from It at Alkaline pH

When this PBP (actually PBP-PRP complex) is submitted to gel filtration on a Sephadex G-50 column at pH 7.3, a single peak is obtained which is eluted close to the void volume of the column (Fig. 2). In a similar experiment, performed at pH 9.0, the major peak remains at the same position, but in addition a second much smaller peak is observed in the low molecular weight region. Analysis of both peaks by SDS-polyacrylamide gel electrophoresis reveals that the first peak still contains both subunits of PBP, or its three components observed after reduction of disulfide bridges. The second peak corresponds to a small protein, which migrates just behind the buffer front and which requires special precautions for staining (see below). Using appropriate staining conditions this small protein band is also detected in the original PBP sample. These data indicate that a much smaller protein is associated with PBP at neutral pH and dissociates from the latter at a higher pH. Because of its high proline content (see below), this protein is called proline-rich polypeptide in this paper.

This hypothesis is confirmed when specific immunological methods are used for the assay of PBP and PRP. The immunoassay of PBP has been described previously (15), while the immunoassay of PRP is described in this paper (see below). As shown in the lower part of Fig. 2, both proteins are eluted concomitantly at pH 7.3. At pH 9.0 on the other hand, PBP is detected in the first peak, and PRP in the second peak.

Very similar results are obtained by ion exchange chromatography on DEAE-cellulose (Fig. 3). At neutral pH, purified PBP (actually PBP-PRP complex) is eluted from the column as a single peak, which contains both PBP and PRP as shown by the immunoassays. At pH 8.5, however, a small peak is already eluted at low ionic strength: this peak corresponds immunologically to PRP. It is followed by a larger peak, eluted at a higher salt concentration. This second peak consists of PBP without PRP.

It should be noted that a higher salt concentration is required for the elution of PBP without PRP than for the elution of the PBP-PRP complex. This also is the case at neutral pH, as shown by reaplication of the PBP peak obtained at pH 8.5 to a DEAE-column at pH 6.9 after buffer readjustment (Fig. 3, upper left: dotted line). Moreover, as already shown in Fig. 1, in total prostatic cytosol also the PBP-PRP complex is eluted before the peak of PBP without PRP.

Purification of PRP

Since the largest part of PRP is present in prostatic cytosol as a PBP-PRP complex (see Fig. 1), this preparation provides a suitable starting material for the purification of PRP. In fact, this step constitutes a 4.5-fold purification with respect to cytosol. In our initial experiments, PRP was purified from the PBP-PRP complex by DEAE-cellulose chromatography at pH 8.5 (see Fig. 2). The PRP peak was then concentrated on an Amicon YM-5 membrane and further purified by gel filtration on Sephadex G-50.

DEAE-Sepharose chromatography of PBP-PRP complex at neutral pH but in the presence of 8 M urea offers a suitable alternative and results at the same time in the purification of
the S and F subunits of PBP. As shown in Fig. 4, 3 major peaks are eluted. The first corresponds to PRP, the second to the S subunit of PBP, and the third to the F subunit of this protein. This figure also shows that the immunological reaction of PRP with its antiserum is conserved after contact with 8 M urea, and that the S and F subunits both react with the anti-PBP antiserum.

Table I and Fig. 5 summarize the purification of PRP. As shown in Table I, DEAE-Sepharose chromatography in the presence of 8 M urea forms the principal purification step, since it results in the separation of PRP and of the S and F subunits of PBP. Thereafter, urea was removed from the first peak by gel filtration on Sephadex G-25 and solid ammonium sulfate (0.3 g/ml) was added, resulting in precipitation of PRP. The precipitate was collected by centrifugation and redissolved in 2 ml of 50 mM Tris-HCl buffer, pH 7.4, containing 8 M urea. The presence of urea was required to completely dissolve PRP after ammonium sulfate precipitation, but urea could be removed thereafter without precipitation of PRP. This sample was applied to a Sephadex G-75 column (80 × 1.5 cm) run in 50 mM Tris-HCl buffer, pH 7.4, at a flow rate of 8 ml/h. As shown in Fig. 5, this step removes some weak protein bands of higher molecular weight from the electrophoretic pattern. Thereafter, the fractions corresponding to the PRP peak were combined and PRP was concentrated by
eluted almost completely from the gel. This problem was solved by staining the gel overnight in a staining solution consisting of 100 g of trichloroacetic acid, 90 g of sulfosalicylic acid, 250 ml of methanol, 656 ml of water, and 1 g Coomassie brilliant blue R250 (20) followed by short destaining (2 × 30 min) with 10% acetic acid. The color of the stained PRP-band had a different aspect than that of other protein bands. It was somewhat greenish blue and had a slightly luminescent aspect, when looked at in tangential light. Such metachromatic staining has been described for other proteins with a high proline content (21).

As shown in Fig. 5, PRP and the peptide components (C\textsubscript{1}, C\textsubscript{2}, and C\textsubscript{3}) of PBP could also be detected immunologically after SDS-polycrylamide gel electrophoresis, by electrophoretic transfer to nitrocellulose membranes, followed by incubation with rabbit anti-PRP or anti-PBP antiserum as first antibody and peroxidase-conjugated goat anti-rabbit IgG (Nordic) as second antibody (22).

A second problem concerns the lack of effective stacking of PRP in Laemmli-type systems (16). In 10% separation gels PRP moves just behind the buffer front and somewhat more rapidly than bromphenol blue. In 15% separation gels it moves somewhat behind the buffer front, but the band is diffuse because of ineffective stacking. In fact, the PRP band is better defined when the stacking gel is omitted.

For the estimation of the molecular weight of PRP the system of Swank and Munkres (17) was used, because this system is particularly suitable for proteins of low molecular weight. A value of 3300 was obtained, which is markedly different from the molecular weight (8500) obtained by gel filtration.

**Amino Acid Composition**—The amino acid composition of PRP is given in Table II. The most abundant amino acid is proline, which comprises 19.5% of all amino acids. The non-polar amino acids valine, leucine, and isoleucine account for 16%, the acidic amino acids for 12%, and lysine for 9% of all amino acids, whereas cysteine, serine, methionine, and arginine are absent. Assuming a molecular weight of about 3300, as suggested by polycrylamide gel electrophoresis, PRP contains 32 amino acid residues. The NH\textsubscript{2}-terminal amino acid, determined by the method of Chang et al. (19), is glycine.

**Isoelectric Focusing**—The presence of large concentrations of amphotolyte in the gel sets an additional problem for the staining and destaining of PRP after electrofocusing. PRP is not detectable when the gels are stained and destained as suggested by the manufacturer. After fixing in 10% trichloro-

<table>
<thead>
<tr>
<th>Amino acid composition of proline-rich polypeptide</th>
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<tbody>
<tr>
<td>Amino acid</td>
</tr>
<tr>
<td>------------</td>
</tr>
<tr>
<td>Cysteine</td>
</tr>
<tr>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Serine</td>
</tr>
<tr>
<td>Glutamic acid</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Phosphorylated</td>
</tr>
</tbody>
</table>

**Table I**

**Purification of PRP from PBP-PRP complex**

The initial purification consisted of PBP-PRP complex, prepared by DEAE-cellulose chromatography. PRP was separated from the subunits of PBP by DEAE-Sepharose chromatography in 8 M urea. After further purification by gel filtration on Sephadex G-75, PRP was concentrated by ammonium sulfate precipitation and redisolved in 8 M urea, which was then removed by gel filtration (final purification).

The concentration of total protein was measured by the Lowry method, the concentration of PRP by radial immunodiffusion.

**General Properties**—PRP is characterized by a high extinction at 280 nm. A solution containing 1 mg/ml of purified PRP according to the Lowry method (18) gave an extinction of 2.5 at that wavelength.

**Gel Filtration**—The elution position of purified PRP from a Sephadex G-75 column in 50 mM Tris-HCl buffer (pH 7.4) corresponds to a molecular weight of 8500. Bovine serum albumin, chymotrypsinogen A, cytochrome c, and aprotinin were used as markers.

**Polyacrylamide Gel Electrophoresis**—Gel electrophoresis of PRP in the presence of SDS poses some specific problems, probably related to its low molecular weight and high proline content. A first problem concerns the fixation and staining of PRP. During conventional staining with Coomassie brilliant blue in aqueous methanol (45%), acetic acid (10%), PRP is

![Fig. 5. SDS-polyacrylamide gel electrophoresis on 15% acrylamide gels. Lanes 1 and 2, electrophoresis of prostatic cytosol, followed by electrophoretic transfer to a nitrocellulose membrane and immunodetection (on adjacent zones) with anti-PRP antiserum (lane 1) or anti-PBP-antiserum (lane 2) and peroxidase-labeled second antibody (22). Lanes 3 to 5, electrophoresis at various stages of purification of PRP, followed by staining. Lane 3, partially purified PBP-PRP complex. Lane 4, PRP-peak after gel filtration on Sephadex G-75. Lane 5, final preparation of PRP. The arrows refer to the position of PRP and of the components of PBP (C\textsubscript{1}, C\textsubscript{2}, and C\textsubscript{3}) on lane 3. Due to a small difference in size of stained gel and immunoprint C\textsubscript{1}, C\textsubscript{2}, and C\textsubscript{3} are localized slightly more anodically on lane 2.

**Properties of Purified PRP**

ammonium sulfate precipitation as already indicated. This step resulted in a further increase of specific activity. The final preparation was electrophoretically pure. The overall yield of PRP was 17.5% for an 8.4-fold purification with respect to PBP-PRP complex. The total purification with respect to prostatic cytosol was thus 37.8-fold.

**Table II**

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Amount recovered</th>
<th>Amount found</th>
<th>Nearest integer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>6.41</td>
<td>2.05</td>
<td>2</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>10.53</td>
<td>3.37</td>
<td>3</td>
</tr>
<tr>
<td>Threonine</td>
<td>19.52</td>
<td>6.24</td>
<td>6</td>
</tr>
<tr>
<td>Serine</td>
<td>6.12</td>
<td>1.95</td>
<td>2</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>6.53</td>
<td>2.09</td>
<td>2</td>
</tr>
<tr>
<td>Lysine</td>
<td>5.86</td>
<td>2.74</td>
<td>3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>5.63</td>
<td>1.80</td>
<td>2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8.50</td>
<td>2.72</td>
<td>3</td>
</tr>
<tr>
<td>Histidine</td>
<td>8.12</td>
<td>2.18</td>
<td>2</td>
</tr>
<tr>
<td>Alanine</td>
<td>7.87</td>
<td>2.81</td>
<td>3</td>
</tr>
<tr>
<td>Phosphorylated</td>
<td>1.93</td>
<td>0.62</td>
<td>1</td>
</tr>
</tbody>
</table>
acetic acid, however, two prominent bands, situated, respectively, at pH 7.75 and pH 7.05 become clearly visible without staining. A few additional, more acidic, weak bands appear when the gels are stained as described for SDS-gel electrophoresis after intensive washing of the gel with 10% trichloroacetic acid to remove the bulk of the ampholytes. The same bands can be visualized by immunofixation (23) on cellulose acetate strips impregnated with rabbit anti-PRP antiserum, indicating that they are related to this polypeptide.

**Immunological Assay of PRP**

Both rabbits immunized with purified PRP produced antisera to this protein. The antisera reacted with single immunoprecipitation lines to preparations of PRP and of PBP-PRP complex and to cytosol from the ventral prostate but did not react with preparations of PBP that did not contain PRP.

The specificity of these antisera is also shown by their use for the detection of antigens after sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by electrophoretic transfer to nitrocellulose membranes. Indeed, as shown in Fig. 5, the PRP antiserum specifically detects this polypeptide.

When these antisera were used for the measurement of PRP by radial immunodiffusion, the immunoprecipitation lines were not sharply outlined, possibly due to the small size of the antigen. However, in the presence of 3.3% polyethylene glycol 6000 and at 4 °C much sharper immunoprecipitation ranges were obtained, which could be easily measured. The lower detection limit of this technique was about 0.5 μg/ml. Linear standard curves were obtained, when the squared diameters of the immunoprecipitation rings were plotted as a function of the PRP concentration, which varied between 1 and 10 μg/ml for the standards. Samples of higher PRP concentrations were diluted within this range. Rat plasma (2%) was used as a diluent for samples and standards since too low total protein concentrations resulted in a relatively smaller response, probably because of losses by adsorption.

**Concentration of PRP in the Ventral Prostate**

As shown in Table III, the ventral prostate of an adult rat contains about 0.7 mg of PRP in the "cytosol" fraction. This value corresponds to 2.6 ± 0.5% (S.D.) of the total cytosolic protein or 8.4 ± 0.1% (S.D.) of the amount of PBP.

**Effect of Castration and Hormonal Treatment on PRP in the Ventral Prostate**

The concentration of PBP in the rat ventral prostate is regulated by androgens. This also is the case for PPR. Indeed, after castration the concentration of PRP drops gradually (Fig. 6). The first few days after castration this decrease occurs in parallel with the loss of PBP or of total protein. Thereafter, however, PRP is lost in a specific way and becomes unmeasurable (<0.5 μg/prostate) at 10 days after castration.

Opposite changes occur during androgen treatment of castrated rats (Fig. 6). After 1 day of treatment there is already a marked stimulation of PRP. At that time the total protein in the prostate remains at the same level, whereas the concentration of PRP shows a moderate but consistent decrease. This paradoxical decrease of PBP is probably due to an increased removal of the intraglandular stores of PBP, since the synthesis of PBP is then already stimulated (10). During the following days the total protein, PBP, and PRP increase further, but the rise is most prominent for the latter two, and for PRP in particular. After 7 days of treatment the ratio of PBP or PRP to total protein is close to the precastration value, but the ventral prostate has not yet regained its original weight and protein content. These data indicate that the effect of castration and of androgen treatment is more pronounced for PRP than for PBP. This is clearly shown by plotting the ratio of PRP to PBP (Fig. 6, below). This ratio drops from 0.084 to 0.000 after castration and increases rapidly during androgen treatment.

The effect of other steroid hormones on prostatic PRP in castrated rats was also studied. Estradiol (50 μg of estradiol benzoate daily for 8 days) or progesterone (2 mg daily for 8 days) have no effect on this parameter.

**Table III**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Weight (mg)</th>
<th>Total Protein (mg)</th>
<th>PBP (mg)</th>
<th>PRP (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control animals</td>
<td>8</td>
<td>387.3 ± 30.8</td>
<td>26.9 ± 3.1</td>
<td>8.28 ± 1.64</td>
<td>0.695 ± 0.146</td>
</tr>
<tr>
<td>After castration</td>
<td>4</td>
<td>51.2 ± 14.5</td>
<td>1.9 ± 0.6</td>
<td>0.08 ± 0.07</td>
<td>ND'</td>
</tr>
<tr>
<td>After castration plus androgen treatment</td>
<td>4</td>
<td>213.7 ± 38.5</td>
<td>11.9 ± 4.6</td>
<td>1.64 ± 0.17</td>
<td>0.154 ± 0.014</td>
</tr>
</tbody>
</table>

* Number of animals per group.

* The indicated parameters were measured in cytosol prepared individually from each prostate.

* Intact male rats 100 days of age.

![Fig. 6. Influence of castration and androgen treatment on PRP and PBP. Rats were castrated at the age of 100 days and left untreated or treated with daily injections of 5α-dihydrotestosterone (1 mg daily) starting on the 10th day after castration. Upper, the concentration in prostatic cytosol (as a percentage of total cytosolic protein) of PBP (—) and PRP (○); lower, the proportion (weight ratio × 100) of PRP to PBP. Each point corresponds to the mean ± S.D. of four rats.](http://www.jbc.org/)

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Prostatic Proline-rich Polypeptide

**PRP in Other Organs**

A number of other organs were examined for the presence of PRP by measurement of the cytosolic PRP content by radial immunodiffusion. Negative results were obtained from most organs, such as lung, liver, kidney, muscle, submaxillary gland, testis, and pancreas. Occasionally, traces of PRP (and PBP) were detected in the lateral prostate, the coagulation glands, seminal vesicles, and male rat bladder.

**DISCUSSION**

The function of PBP, the major secretory protein of the rat ventral prostate is still unknown. Its most prominent known functional characteristic is the binding of steroids: PBP binds several nonpolar steroids with a relatively low affinity (3), but shows strong binding for estramustine, a synthetic derivative of estradiol linked to nitrogen mustard (7). In this paper we present evidence that a proline-rich polypeptide binds to PBP in prostatic "cytosol." The fact that this PRP escaped detection in previous studies is not astonishing in view of the difficulties met in its electrophoretic demonstration.

This polypeptide is associated with PBP at neutral pH, as shown by gel filtration and ion exchange experiments. Indeed, in both cases PBP and PRP are eluted concomitantly at neutral pH, whereas separate PBP and PRP peaks are obtained under more alkaline conditions. Furthermore, the binding of PRP to PBP is reversible. Indeed, the recombination of the PRP and PBP peaks results at neutral pH in the formation of PBP-PRP complex as can be demonstrated by ion exchange chromatography or gel filtration.

A preliminary characterization of purified PRP has been performed. Its molecular weight estimated by gel filtration (8500) is more than twice the value (3300) obtained by SDS-gel electrophoresis. Although marked deviations from the true molecular weight have been observed for both techniques it seems more likely that purified PRP is present as a dimer under the conditions of gel filtration.

Isoelectric focusing of purified PRP on polyacrylamide gels yields a complex pattern with two major bands at pH 7.75 and 7.05. The complexity of this pattern is not due to impurities in the PRP preparation, since a similar pattern is obtained by immunofixation with anti-PRP antiserum. Furthermore, a single NH2-terminal amino acid (glycine) was detected in the purified protein. Whether the observed charge heterogeneity is caused by technical artifacts, such as an interaction with ampholytes or to real differences in primary structure remains to be elucidated. The isoelectric point of PRP in the neutral pH region explains why it is not retained on DEAE-cellulose or carboxymethylose at this pH. It may also be important for the binding of PRP to PBP. Indeed, this binding occurs at neutral pH, where PRP has no net charge and PBP, which has an isoelectric point of approximately 5.1, is negatively charged. At a more alkaline pH, both PBP and PRP are negatively charged and PRP dissociates from PBP.

In prostatic "cytosol" prepared at neutral pH two forms of PBP can be distinguished by ion exchange chromatography. The first and quantitatively major form consists of a complex of PBP with PRP, while PBP is not associated with PRP in the second form. The molar ratio of PRP to PBP in the PBP-PRP complex may be tentatively calculated on the basis of the concentrations of both proteins (both measured by radial immunodiffusion) in the complex. A value of 1.98 is obtained, when one assumes molecular weights of 50,000 and 3,500, respectively, for PBP and PRP. This value suggests that one mole of PRP is bound to each subunit of PBP, but it should be kept in mind that this type of calculation is subject to a large degree of imprecision. In gel filtration experiments, the differences in estimated molecular weights (47,900 versus 51,300) between PBP and PBP-PRP complex is much smaller. Both forms of PBP show qualitatively similar binding properties for neutral steroids and for estramustine, although there seem to exist some quantitative differences.2

As far as our study permits us to conclude, PRP is a characteristic component of the rat ventral prostate. Within this gland the largest fraction of PRP (and PBP) is probably localized in the extracellular secretion. Indeed, as for PBP, more than 97% of PRP is easily washed out after cutting the prostate gland in small pieces.

The function of PRP and of PBP is completely unknown in spite of their abundance in the rat ventral prostate. For this reason we can hardly speculate on the significance of their association. If PRP is the functionally important protein, PBP may act as a carrier or stabilizer for the latter, or, alternatively, PRP may affect the unknown function of PBP. Another interesting possibility could be that the role of PBP is related to the processing, assembly, or secretion of PRP, which is a complex multichain protein. Such a hypothesis may explain why PRP is associated primarily with newly secreted protein as suggested by the castration and androgen substitution experiments.

Proline-rich proteins or polypeptides have been found in other secretory fluids, such as colostrum, milk and parotid gland saliva (24–26). The physicochemical properties of these proteins, however, are different from those of rat prostatic PRP. Whether a functional relation exists is hard to evaluate at the present time.

But even without a clear understanding of its function, PRP promises to be an interesting marker for the action of androgens on the prostate. Indeed, the changes of the concentration of PRP in the rat ventral prostate, after castration or androgen treatment, are quantitatively much more important and more rapid than for PBP. For this reason we intend to study the effect of androgens on the rate of synthesis of PRP and on its mRNA.

**Acknowledgments**—We thank Maria Hertogen, Ria Brepoels, and Valere Feytons for their skilful technical assistance and Bernadette Minten and Marie-Paule Vander Auwera for their excellent secretarial work. The advice and comments of Dr. H. Van Baelen and the continuous stimulative interest of Prof. P. De Moor were greatly appreciated.

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