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PROSTATIC BINDING PROTEIN: AN ANDROGEN-DEPENDENT MARKER FOR PROSTATE EPITHELIAL CELLS

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SUMMARY

Indirect immunofluorescent staining revealed that Prostatic Binding Protein, the major androgen-dependent protein in rat ventral prostate in vivo, is associated specifically with the epithelial cells in primary cell cultures derived from rat ventral prostate. The epithelial cells release Prostatic Binding Protein into the medium during primary culture. De novo synthesis of Prostatic Binding Protein is demonstrable during early phases of cell culture. Prostatic Binding Protein is an excellent marker for the identification of functional prostate epithelial cells and for the study of regulation at the cellular level of the synthesis and secretion of a major androgen-dependent prostate protein.

Several laboratories have independently reported that 15 to 45 per cent of the cytosol prepared from rat ventral prostate consists of a single protein of molecular weight 40,000 to 50,000 (1-5). The synthesis of the subunits of the protein and their mRNA's is strictly controlled by androgens in vivo. The protein and mRNA sequences which code for it appear to be specific to prostate tissue (6-8). Although the physiological role of the protein is not clear, it exhibits nonspecific steroid-binding activity and has been called "Prostatic Binding Protein" (PBP). In this communication, we report our findings that PBP is concentrated and synthesized in cultured epithelial cells from rat ventral prostate. PBP is an excellent marker for identification of functional prostate epithelial cells. It is also a good marker for study of the regulation at the cellular level of synthesis and secretion of an androgen-dependent protein from prostate under the defined and controlled conditions of tissue culture.

MATERIALS AND METHODS

Materials: Thirteen to 17 week old Sprague-Dawley rats were obtained from Taconic Farms (Germantown, NY). Collagenase Type I from Worthington

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Biochemical Corp. (Freehold, NJ). Medium F12K was purchased from Gibco (Grand Island, NY). Donor horse serum was from Flow Laboratories (McLean, VA). Goat fluoresceinisothiocyanate-(FITC)-labeled IgG against rabbit IgG was purchased from Cappel Labs. (Cochranville, PA). Staphylococcus aureus cells bearing Protein A on their surface (Pansorbin) were purchased from Calbiochem (LaJolla, CA). [^{35}S]methionine was from New England Nuclear (NEN) (Boston, MA).

Cell Culture. Primary cell cultures were established by enzymatic dissociation of the ventral lobes of prostates from 13 to 17 week old Sprague-Dawley rats. Minced prostate glands, free of connective tissue, were incubated in 7 ml per g wet weight tissue of a solution of 675 units per ml of collagenase in medium F12K and 5% horse serum for 1 hr at 37°C. Relatively homogenous aggregates of glandular cells were selected from the dissociation mixture by repeated settling at unit gravity at 4°C. Suspensions of cell aggregates containing 5 to 7 µg cellular DNA per ml of the culture medium indicated in the text were placed in 35 or 60 mm plastic Petri dishes. Cultures were incubated in a humidified atmosphere of 5 per cent carbon dioxide and 95 per cent air at 37°C and analyzed at the times indicated in the text. Details of the cell culture methodology will be described elsewhere.

Purification of PBP and Preparation of Rabbit Antiserum. PBP was isolated from rat ventral prostate by a modification of the method of Heyns and De Moor (9). Antiserum was raised in rabbits according to Heyns (10). In our hands, PBP purified by this procedure often contains trace amounts of rat albumin. To remove any traces of anti-rat albumin in the rabbit antiserum raised against the PBP preparation, all antiserum was routinely absorbed with 100 µg rat albumin per ml of antiserum before use. The rabbit anti-PBP serum was then demonstrated to be monospecific by 2-dimensional rocket immunoelectrophoresis (11).

Immunofluorescent Staining. Primary cultures of cells from rat ventral prostate were prepared on 9 x 22 mm glass coverslips instead of plastic Petri dishes. Cells on the coverslips were fixed for 10 min in cold acetone (-20°C). They were incubated for 30 min at room temperature in rabbit anti-PBP serum that was diluted 1:50 with phosphate buffered saline (pH 7.3) that contained normal goat serum at a dilution of 1:20. After washing thoroughly with the same solution without the rabbit antiserum, the coverslips were then incubated for 30 min at room temperature with a 1:20 solution of FITC-labeled goat anti-rabbit IgG. The unreacted second antibody was removed by thorough washing, and the coverslips were examined immediately for fluorescent staining with a Zeiss Photomicroscope III equipped for epifluorescence.

Immunoassay of [^{35}S]Prostatic Binding Protein. [^{35}S]methionine (50 µCi; 1081.3 Ci per mmol) was introduced directly into the culture medium. After 24 hr of incubation, the medium was collected and cells were removed from the medium by centrifugation at 250 x g. The cell-free medium was stored until use in analysis. Cells attached to the culture dish were harvested by scraping and pooled with the cells collected from the medium by centrifugation. After disruption by sonication, an aliquot of the cell extract was used to measure DNA content (12) and the remainder of the extract was used for analysis of PBP. The total PBP content of cell extract and culture medium was analyzed by rocket immunoelectrophoresis (11). Rabbit anti-PBP serum was mixed with agarose [0.20 % (v/v) antiserum] and the mixture was layered on a 9.4 cm x 9.4 cm glass plate. Each sample well contained 10 µl of cell extract or culture medium. Current was applied at 3 volts per cm for 5 hr. The plates were then rinsed for 16 to 18 hr in saline, stained with Coomassie Blue and then destained. Duplicate plates were treated for fluorography with "EN^{3}HANCER" solution according to the manufacturer's instruction (NEN). The fixed plate was exposed to LKB Ultrafilm for 9 days at -70°C. The [^{35}S]PBP in cell extracts and medium was quantitated by precipitation of the antigen-antibody

complexes with Staphylococcus aureus bearing protein A as the immunoadsorbent (13). Assay mixtures were carried out in Eppendorf tubes that contained 8.3 mM Tris-HCl (pH 7.4), 25 mM NaCl, 3.3 mM methionine, 0.0033% (w/v) NaN_2, 2 mg per ml ovalbumin, 75 µl of a 10% solution of S. aureus, 5 µl of normal or immune serum and 100 µl of sample in a total volume of 0.30 ml. After incubation for 30 min at room temperature, the [^{35}S]PBP-antibody-S. aureus complexes were collected by centrifugation in a Beckman 152 Microfuge. The pellets were suspended in 0.20 ml of buffer solution, precipitated with TCA and filtered on GF/C glass fiber filters. The filters were placed directly in 10 ml of Aquasol counting fluid (NEN) and counted by liquid scintillation at an efficiency of 90 per cent. Background was determined for each sample with normal rabbit serum in the reaction mixture and this value was subtracted from the values that resulted when anti-PBP serum was used. Results were expressed as cpm [^{35}S]PBP produced per µg cellular DNA. Specificity of the assay for [^{35}S]PBP was confirmed by competition experiments using purified cold PBP.

RESULTS

Indirect immunofluorescent staining with rabbit anti-PBP serum revealed that PBP was associated specifically with the epithelial cells in primary cell cultures of rat ventral prostate (fig. 1A,B). Although the intensity of fluorescence was reduced, PBP was still evident after 8 days of culture even after some deterioration of the epithelial cells was apparent (fig. 1C,D). The remaining PBP appeared to be concentrated around the nuclei of the cells. In contrast to epithelial cells, prostate fibroblasts (fig. 1E) did not display fluorescence at any time during primary culture (fig. 1F). Therefore, the presence of PBP is a specific marker for epithelial cells.

PBP appeared in the medium of primary cultures of prostate cells (fig. 2). Analysis by rocket immunoelectrophoresis revealed that the cultured cells secreted about 107 ng per µg cellular DNA on the first day of culture. A slight increase in medium content of PBP of about 14 ng PBP per µg DNA was detected on day 2 (fig. 2, slot 2). Interestingly, after a medium change at the end of day 2 (fig. 2, arrow), about 102 ng PBP per µg cellular DNA reappeared in the medium on day 3 (fig. 2, slot 3). However, the PBP content of the medium remained stable until another medium change after 5 days of culture (fig. 2, arrow). On day 6, 35 ng PBP per µg cellular DNA reappeared in the medium and no increase occurred on day 7 (fig. 2, slots 6,7). Fluorography of the immunoelectrophoretic patterns of culture medium after

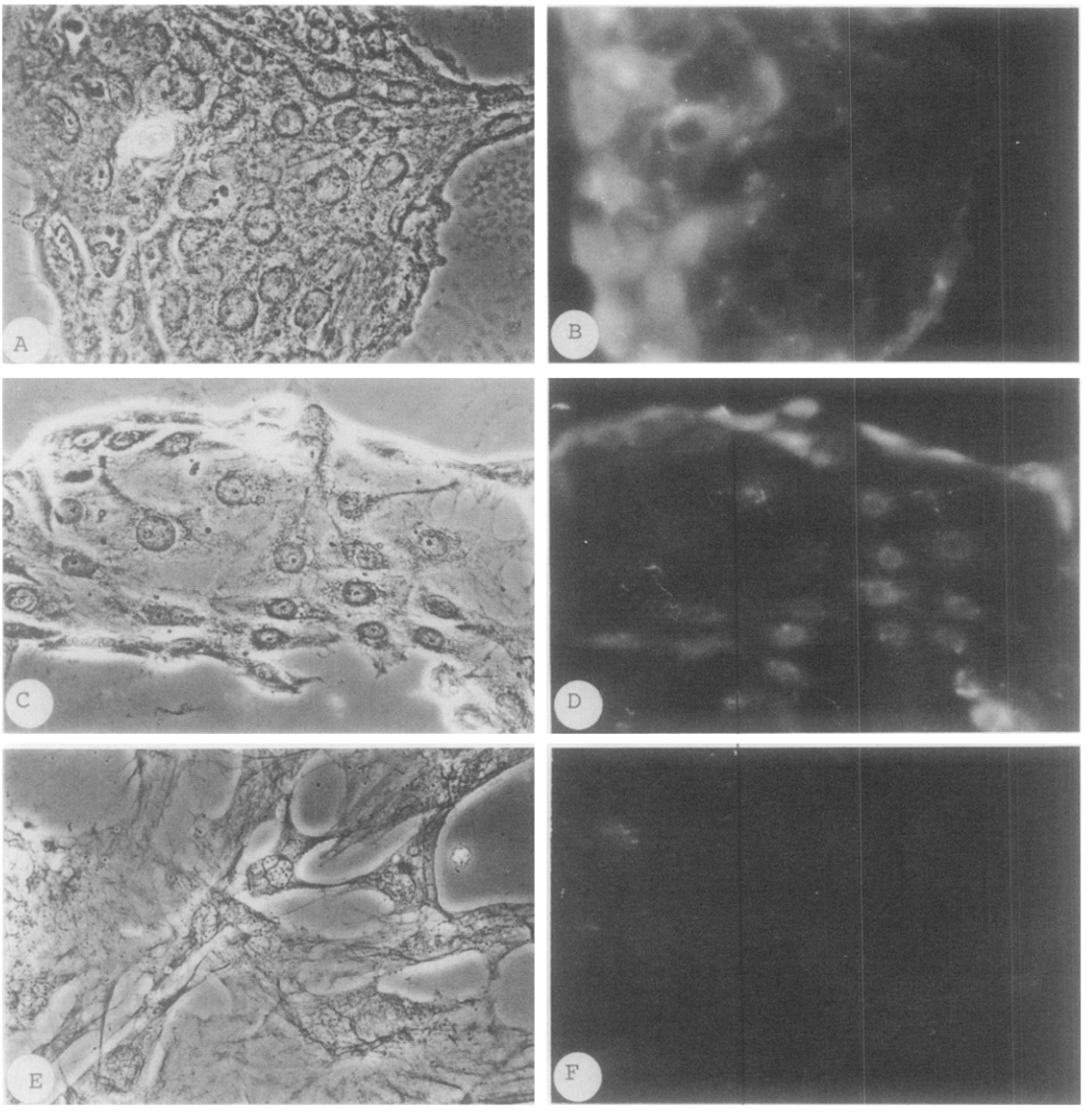

Fig. 1. Indirect immunofluorescent staining of PBP in cultured rat ventral prostate cells. A, C, E are micrographs taken under phase contrast optics. B, D, E are the same fields as A, C, E under epifluorescence optics. A and C show prostate epithelial cell colonies at day 5 and 8 of culture, respectively. E is a field containing prostate fibroblasts on day 8 of culture. Fields were magnified 320 times and exposed for equal times. Epithelial cells treated with normal rabbit serum instead of anti-PBP serum exhibited no fluorescence. Addition of purified PBP during incubation with anti-PBP serum reduced the fluorescence associated with the epithelial cells. Culture medium was F12K containing 5 per cent horse serum and 10 ng per ml epidermal growth factor (Collaborative Research, Waltham, MA).

labeling for 24 hr with [^{35}S]methionine showed that the cultured epithelial cells were capable of de novo synthesis of PBP (fig. 2b). The intensity of the autoradiographic images associated with the rockets of immune precipitate

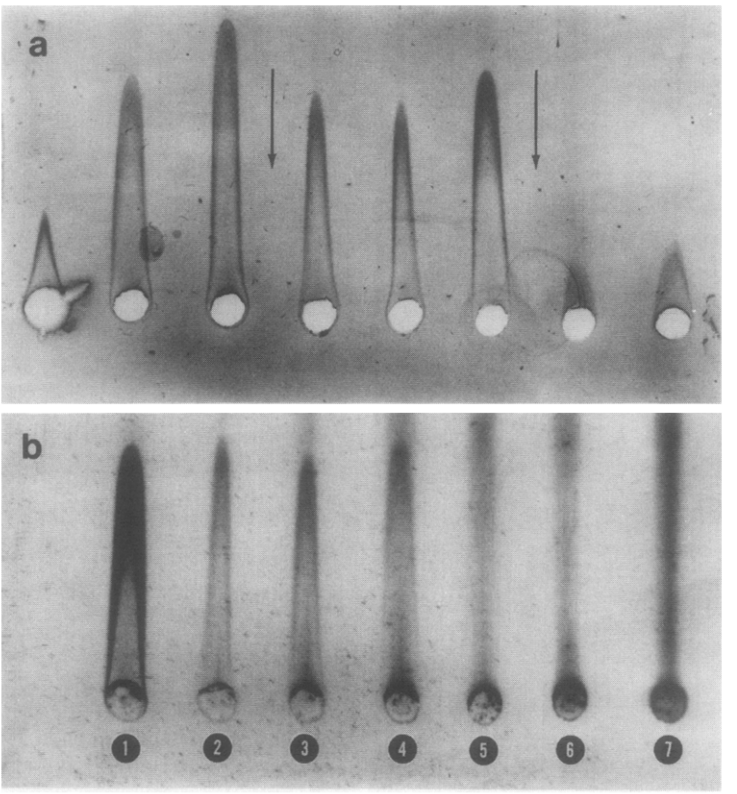

Fig. 2. Analysis of PBP in the culture medium by immunoelectrophoresis. Medium was collected from cell cultures that were prepared and labeled for 24 hr with [^{35}S]methionine as described in Materials and Methods. Immunoelectrophoresis was carried out as described in Materials and Methods. The numbered slots refer to cumulative days in culture at the time of collection of the medium for analysis. The rockets represent precipitates of PBP and antibody. Rocket height is proportional to the amount of PBP in the sample. Each sample represents the amount of PBP released into the culture medium by cells containing 24 pg DNA. The unnumbered slot at left in (a) contained 40 ng of purified PBP. Arrows denote a medium change. The plate in (a) was fixed and stained with Coomassie Blue dye. The duplicate plate in (b) was fixed and analyzed by fluorography as described in Materials and Methods. The culture medium was F12K containing 5% horse serum, 1.0 μM dexamethasone and 1.0 μM ovine prolactin.

decreased with increasing time of culture. No image could be distinguished from the background on days 5, 6 and 7.

The synthesis and secretion of PBP was quantitated by precipitation of [^{35}S]PBP-antibody complexes from cells and the culture medium with S. aureus bearing Protein A as the immunoadsorbent (fig. 3). Significant levels of [^{35}S]PBP were synthesized and exported to the medium on the first day of culture. The level of synthesis dropped rapidly on day 2 and fell to near

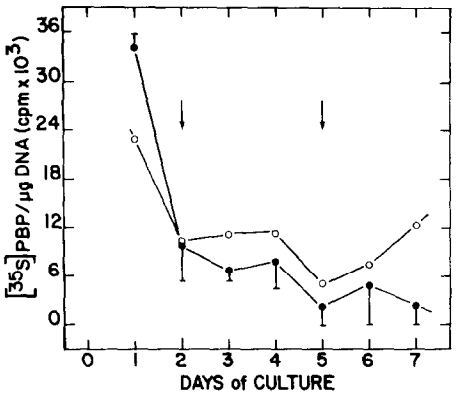

Fig. 3. Analysis of [^{35}S]PBP by immunoprecipitation. The same culture medium that was analyzed by electrophoresis in fig. 2 was analyzed for its [^{35}S]PBP content by reaction with anti-PBP serum and then precipitation with S. aureus containing Protein A as described in Materials and Methods. Each data point is the mean of duplicate cultures.

●, PBP in the culture medium;
o, PBP in the extracts of cells harvested from the same cultures from which the medium was collected and analyzed.

background levels by day 7. The cellular content of [^{35}S]PBP paralleled that in the medium except on day 1 and 7 of culture. These differences between cells and medium may reflect changes in secretory activity of the prostate epithelial cells and deserves investigation.

DISCUSSION

Up to 45 per cent of rat ventral prostate cytosol consists of a single androgen-dependent protein called Prostatic Binding Protein (PBP). The protein binds steroids (1,4), polyamines (3) and carcinogens (14) and the antiprostatic carcinoma agent, estramustine (5). A subunit inhibits the binding of androgen-receptor complexes to isolated DNA and chromatin in vitro (3). PBP also binds to the plasma membrane of spermatozoa (4). These properties and the abundance of PBP in prostate tissue and seminal fluid suggest that the protein probably plays a central role in rodent prostate function. Forsgren et al. (15) have shown that human prostate tissue contains a protein that is immunologically similar to rat PBP. Preliminary results in our labora tory based on steroid and carcinogen binding support their results.

In this report, we showed that PBP is stored, synthesized and secreted by cultured prostate epithelial cells. Although the majority of PBP associated with epithelial cells in primary culture is probably carried over in intracellular stores that were synthesized in the animal, de novo synthesis of PBP was demonstrable for up to one week in culture. Unlabelled PBP continued to appear in the culture medium even when de novo synthesis was declining and eventually undetectable. Presently, we attribute the decline of PBP synthesis in primary culture to inadequate cell culture conditions. Preliminary results suggest that the level of PBP synthesis and secretion can be modulated by a variety of extracellular conditions which include androgen, prolactin, glucocorticoids, polypeptide growth factors, culture matrix and nutrient concentrations.

These results demonstrate that PBP is an excellent marker for the study of regulation at the cellular level of the synthesis and secretion of a major androgen-dependent prostate protein. PBP is also a useful marker for the identification of functional prostate epithelial cells in vitro and in vivo and may be of value in the early detection of prostate cell hyperplasia or malignancy.

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