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PURIFICATION OF A HUMAN PROSTATE SPECIFIC ANTIGEN[1]

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ABSTRACT

Rabbit antiserum raised against the crude extract of normal human prostatic tissue contained antibodies to a prostatic tissue-specific antigen as shown by immunoprecipitation techniques. Using this antiserum a prostate antigen was detected in normal, benign hypertrophic, and malignant prostatic tissues, but not in other human tissues. The prostate antigen was purified to homogeneity from prostatic tissues and showed a single protein band on analytical polyacrylamide gel electrophoresis and isoelectric focusing. This report thus presents the first demonstration of the purification of a prostate-specific antigen that does not represent prostatic acid phosphatase.

Prostatic cancer is more prevalent at old age. Approximately 50 per cent of males over the age of 70 develop prostatic cancer (1). The high incidence of prostatic malignancy led to the search for markers to use for its detection. Prostatic acid phosphatase was identified more than 50 years ago as a useful marker associated with cancer of the prostate (2). Several reports (3–6) have also shown the occurrence of a prostate tissue-specific antigen(s). However, the lack of purified prostate antigen has hampered further study of its role in prostatic cancer. We describe the purification of a prostate-specific antigen. A preliminary report has been previously presented (7).

MATERIALS AND METHODS

Materials. Agarose (Type I:Low EEO), α-naphthyl phosphate, fast garnet GBC salt, Coomassie brilliant blue G, and Na_2 ethylenediamine tetraacetate were purchased from Sigma Chemical Co., St. Louis, Missouri. Coomassie brilliant blue R-250, DEAE-BioGel A, and the reagents used in polyacrylamide gel electrophoresis (PAGE) and isoelectric focusing were purchased from BioRad Laboratories, Richmond, California. Sephadex G-75, Sephadex G-100, and the molecular weight calibration kit were the products of Pharmacia Fine Chemicals, Piscataway, New Jersey. Freund adjuvants were obtained from Miles Laboratories, Elkhart, Indiana.

Human prostatic tissues (normal, benign hypertrophic, malignant) were obtained from St. Joseph's Intercommunity Hospital, Buffalo, New York during autopsy or surgery. Other human tissues were collected in this Institute and Buffalo General Hospital. Histology of each tissue was confirmed by pathologists.

Extraction of tissues. Approximately 10 g of tissues were minced and washed 3 times with 30 ml of physiologic saline, then mixed with 30 ml of 0.02 per cent (w/v) disodium ethylenediamine tetraacetate (EDTA)-0.1 M phosphate buffered saline (PBS), pH 6.8 (8). The mixture was placed in an ice-water-chilled blending chamber of Omnimixer (Sorvall, Inc., Norwalk, Connecticut) and subjected to three 5-min blendings at blade speed of 25,000 rev/min with an intermittent cooling time of 3 min. The homogenate was stirred overnight at 4 C, then centrifuged at 25,000 *g* for 30 min. The resultant supernatant constituted the crude tissue extract.

Purification of prostate antigen. The entire procedure was carried out at 4 C. The chromatographic fractions were concentrated with an Amicon concentrator fitted with a UM2 ultrafiltration membrane (Amicon Corp., Lexington, Massachusetts). Gel diffusion (9) was used to measure the presence of prostate antigen in various fractions of chromatography.

Pooled prostate tissues, 100 g, were extracted with 300 ml of EDTA-PBS as described above. Ammonium sulfate (60 g) was added to 285 ml of crude extract (35 per cent saturation), mixed for 30 min, and centrifuged (26,000 *g*, 30 min). To 295 ml of the resulting supernatant, 38 g of ammonium sulfate were added (55 per cent saturation), mixed for 30 min, and centrifuged. The precipitate was dispersed in 200 ml of 55 per cent saturated ammonium sulfate solution and centrifuged. The precipitate was washed twice and then dissolved in 20 ml of 0.01 M tris-HCl buffer, pH 8. The residual ammonium sulfate was removed by dialysis against 4 liters of the tris-HCl buffer for 48 hr with one change of buffer during dialysis.

The dialyzed solution was centrifuged at 46,000 *g* for 30 min to remove any precipitate formed during dialysis. The supernatant, 23 ml, was applied onto a DEAE-BioGel A column (2.5 by 93 cm) preequilibrated with 0.01 M tris-HCl buffer, pH 8. The column was first washed with 420 ml of buffer, then eluted with 0 to 0.2 M NaCl gradient (1 liter of 0.01 M tris-HCl buffer, pH 8 in mixer and 1 liter of 0.2 M NaCl-0.01 M tris-HCl, pH 8 in the reservoir) at a flow rate of 6.5 ml per hr per cm^2. The effluent was collected (10 ml per fraction) and monitored for the prostate antigen. The prostate antigen-containing fractions were pooled and concentrated to 4.5 ml.

A portion (4.3 ml) of the concentrated solution was applied onto a Sephadex G-100 column (2.5 by 110 cm) preequilibrated with 0.01 M tris-HCl buffer, pH 8, which was eluted with the same buffer at a flow rate of 3.5 ml per hr per cm^2. The fractions containing prostate antigen were pooled, concentrated, and applied onto a Sephadex G-75 column (2.5 by 113 cm) preequilibrated with tris-HCl buffer; the column was eluted at a flow rate of 5.5 ml per hr per cm^2. Again, the fractions exhibiting prostate antigen reactivity were pooled and concentrated to 3.8 ml, of which 1 ml was subjected to further purification by a preparative poly-

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acrylamide gel electrophoresis (Shandon Southern Instruments, Ltd., Camberley, Surrey, England) as follows: 1 ml of specimen (4.5 mg of protein) was mixed with 1 ml of 50 per cent (w/v) sucrose solution and applied onto an annular 7.5 per cent gel column (cross-sectional gel area, 4.4 cm^2; height, 9.2 cm), followed by successive layerings of 2 ml of 10 per cent sucrose and 0.05 M tris-glycine buffer, pH 8.5. A constant current of 30 ma was first applied for 1 hr, and 80 ma thereafter. The tris-glycine buffer was continuously pumped at a flow rate of 14 to 15 ml per hr into the elution plate located at the bottom of the column to carry materials emerged from the column into a fractional collector. Fractions containing the prostate antigen, representing the final purified preparation, were pooled and concentrated to 0.5 ml.

Preparation of antisera. Female rabbits were immunized as described previously (10) with the crude extract of normal human prostatic tissue (for antiserum P_8), or with a partially purified prostate antigen (for antiserum P_{17}) obtained at Sephadex G-75 step (see above). Sera were collected, heat inactivated, and stored at −20 C until use. Absorption of the antiserum with normal female serum (NFS) or tissue extracts (10 mg of protein per ml) was carried out as previously described (9).

Immunoelectrophoresis. This was performed on a 9.5 by 10.2 cm agarose (0.65 per cent, w/v) plate (Immuno-Tech gammopathy kit, Behring Diagnostics, Sommerville, New Jersey). Barbital buffer, (pH 8.2, ionic strength 0.04) was used as the electrolyte and a constant voltage of 90 was applied for 1 hr. After electrophoresis and gel diffusion (20 hr), the plate was washed with 0.154 M saline for 2 days and stained first for acid phosphatase with a solution of α-naphthyl phosphate-fast garnet GBC salt in 0.1 M ammonium acetate, pH 5 (10), and then for protein with Coomassie brilliant blue R-250-perchloric acid solution (11).

Analytical polyacrylamide electrophoresis. Acrylamide gel (7.5 per cent) columns (50 by 60 mm) were made according to the company's instruction manual (Shandon Southern Instruments, Ltd., Camberley, Surrey, England), and 50 μl of sample (10 to 40 μg of protein in 25 per cent sucrose solution) were applied onto each gel column. Ten percent sucrose solution was then layered carefully above the sample solution, followed by layering 0.05 M tris-glycine buffer, pH 8.5, to the top of the gel-containing tube. Tris-glycine buffer was used in preparing the sucrose solutions and used also as the electrolyte. After electrophoresis with a constant current of 5 ma per tube for 40 min, the gels were stained for protein by Coomassie brilliant blue R-250-perchloric acid solution (11).

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. The method was essentially the procedure of Weber and Osborn (12). Sample (10 to 20 μg of protein) in 50 μl of 0.05 M tris-glycine buffer, pH 8.5, containing 250 μg of SDS and 2-mercaptoethanol each, was incubated at 37 C for 2 hr. After incubation the sample was mixed with an equal volume of 50 per cent sucrose, and 50 μl were subjected to the polyacrylamide gel (containing 0.1 per cent SDS) electrophoresis as described before. After electrophoresis, the gels were stained for protein with 0.5 per cent (w/v) Coomassie brilliant blue R-250 in ethanol-acetic acid-water (45:10:45, v/v) (13).

Isoelectric focusing. The procedure was the same as that described previously (9) except that the gel size was 5 by 102 mm. After focusing, gels were stained with Coomassie brilliant blue R-250-perchloric acid solution (11).

Assay methods. Protein concentration was determined by the method of Lowry et al. (14), using bovine serum albumin as the standard. Acid phosphatase activity was measured by the method of Babson and Phillips (15) using α-naphthyl phosphate as the substrate, or an immunoassay described previously (10).

The concentration of prostate antigen was estimated by gel diffusion technique (9), in which the appropriately diluted antiserum was reacted with serially diluted prostate antigen-containing sample. One unit of prostate antigen was defined as the minimum amount of the antigen per milliliter that formed a visible precipitin line. This was a semiquantitative determination of prostate antigen and was used only for monitoring the degree of purification.

Determination of molecular weight. Gel filtration on a Sephadex G-75 column and sodium dodecyl sulfate polyacrylamide gel electrophoresis (see above) were used to determine the molecular weight of the purified prostate antigen. Marker proteins used in these experiments and their molecular weight were: ribonuclease A, 13,700; chymotrypsinogen A, 25,000; ovalbumin, 45,000; and bovine serum albumin, 67,000.

RESULTS

Demonstration of prostate specific antigen. As shown in Figure 1-*A*, an immunoelectrophoresis of the crude extract prepared from normal prostatic tissues and the antiserum P, an antiserum raised against the crude extract of normal prostate, resulted in three precipitin arcs. One of these arcs was formed by a normal human serum component, inasmuch as it disappeared after the antiserum P_8 was absorbed with normal female serum (NFS-P_8). Absorption of the antiserum NFS-P with various normal human tissue extracts (bone, kidney, intestine, liver and spleen, 10 mg each per ml) failed to abolish the two remaining precipitin arcs, one of which was identified as prostatic acid phosphatase inasmuch as it was stained with the α-naphthyl

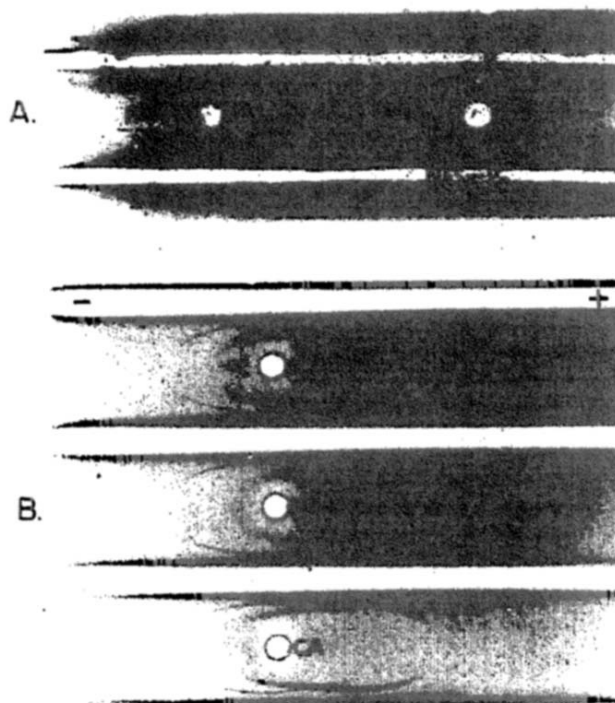

FIG. 1. *A*: Immunoelectrophoresis of the crude extract of a normal prostatic tissue. *Well at the left* (cathodic side): crude extract (5 μl containing 100 μg of protein). *Well at the right* (anodic side): blank as a control. *Upper trough*: rabbit antiserum (to the crude extract) P_8. *Lower trough*: NFS (normal female serum)-absorbed antiserum P_8. *B*: Immunoelectrophoresis of the crude extracts (100 μg of protein) of normal (*N*), benign hypertrophic (*BPH*), and cancerous (*CA*) prostatic tissues. *Troughs*: NFS-absorbed antiserum P_8.

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