Isolation of Prostatic Acid Phosphatase-binding Immunoglobulin G from Human Sera and Its Potential for Use as a Tumor-localizing Reagent

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Abstract

A human immunoglobulin that binds prostatic acid phosphatases (PAP) was isolated from the serum of normal individuals by affinity chromatography using a PAP-Sepharose solid adsorbent. The yield of isolated protein, termed PAP-binding globulin (PAPBG), ranged from 4.7 to 16.3 μg/ml serum. As shown by immunoelectrophoresis, PAPBG is a γ-globulin of restricted electrophoretic heterogeneity. PAPBG was shown to bind radiolabeled PAP by radioimmune precipitation, and an association constant of 5.0 × 10⁴ M⁻¹ was calculated. As determined by immunofluorescence, PAPBG was shown to react with human prostatic tumor cell lines. No binding was detected to other tumor cells examined including those from cultures of human breast, thyroid, pancreas, or normal fibroblasts.

Introduction

Of considerable interest to tumor immunologists is the use of radiolabeled antibodies for diagnosis and/or therapy (5). Recent work with experimental animals has indicated that specific binding of radiolabeled antibodies to distinct tumor antigens in vivo can be achieved (4, 6, 9, 15, 17). Regarding tumor diagnosis in humans, difficulties encountered with the use of heteroantibodies (namely, sensitization) may complicate and limit their use. This is most acutely true in the use of such antibodies for serial monitoring of an individual patient over the course of several weeks or months. This problem may be overcome by removing species specificity of the heterologous globulin, and such an approach has been suggested concerning the therapeutic use of antifungal agent heteroantiserum (2).

Another alternative with precedent in the literature would be to use antigen-binding globulins separated from normal human serum. Serum globulins acting as distinctly binding antibodies have been isolated from normal sera of human and rabbit. Pressman and Korngold (16) have used immunoadsorption techniques to isolate normal serum globulins from rabbit serum which are capable of localizing the kidney, the lung, or the liver of rats. Similarly, Sela et al. (19) have reported the affinity isolation of cellular-binding antibodies from the normal globulins of several species, including human. More recently, Pressman et al. (14) have described the purification of carcinoembryonic antigen-binding globulins from normal human sera. In the present report, we describe the isolation of human (3, 11) PAP-binding globulins from sera of normal individuals.

Materials and Methods

Preparation of PAP and PAP-Sepharose Immunoadsorbent. PAP was purified to homogeneity from benign hypertrophic prostatic tissues, as described previously (11). The purified protein exhibited a single band upon polyacrylamide electrophoresis and was used to prepare a solid adsorbent and for radiolabeling.

Purified PAP (2.35 mg) was coupled to 1 g (3.5 ml) of CNBr-activated Sepharose 4B (Pharmacia, Piscataway, N. J.) at 4° for 18 hr at pH 8.0, in NaHCO₃ buffer (0.1 M containing 0.5 M NaCl). As measured by a quantitative enzyme assay (1), coupling efficiency was determined to be 97%. Charged gel was packed into a 0.9- x 10-cm chromatographic column and equilibrated with PB-NaCl buffer, pH 7.2.

One mg of the purified PAP was radiolabeled using 1 mCi of carrier-free Na¹²⁵I according to the procedure of McConahey and Dixon (12).

Isolation of PAPBG from the Sera of Normal Individuals.

The IgG fraction of NHS was isolated using (NH₄)₂SO₄ precipitation and DEAE anion-exchange chromatography (11). In each case, purity of the IgG was confirmed by IEP with 0.05 M barbital buffer, at pH 8.6. Purified IgG samples were dialyzed against PB-NaCl and passed through the affinity adsorbent at a flow rate of 0.5 to 1.0 ml/hr at 4°. Subsequently, the column was washed with PB-NaCl until the A₂₈₀ of the effluent was <0.01. PAPBG was eluted from the affinity adsorbent by the use of 3.5 M KSCN in PB-NaCl buffer and immediately dialyzed against H₂O and lyophilized. The concentration of human IgG was determined by A₂₈₀ measurement, using the extinction coefficient value of 13.8 (10 mg IgG per ml). Purity was confirmed by IEP analysis in each case.

Radioimmunoprecipitation Assay of PAPBG. The amounts of the following reactants, as indicated in Table 2, were mixed and then incubated at 37° for 2 hr in: (a) PAPBG (500 μg/ml) in NHS 20-fold diluted in borate buffer (0.1 M), pH 8.0; (b) borate buffer; and (c) ¹²⁵I-labeled purified PAP (420 ng/ml). Control mixtures containing normal IgG were used to correct for nonspecific precipitation of PAP.

After incubation, 50 μl of Pansorbin (Protein A-containing Staphylococcus aureus cells) (Calbiochem, La Jolla, Calif.) were added to each mixture and incubated for 30 min, and precipitates were collected by centrifugation. Precipitates were washed with PB-NaCl buffer and counted for radioactivity. Results were expressed as percentage of the applied radioactivity bound in the precipitate, corrected for nonspecific precipitation.

Assay for Enzymatic Activity. Acid phosphatase activity was determined colorimetrically by the method of Babson and Phillips (1), using α-naphthyl phosphate as the substrate. Prostate-specific acid phosphatase was detected by using rabbit anti-
PAP antiserum in a counterimmunoelectrophoresis procedure (3).

Indirect IF. The following cell lines were examined for PAPBG binding by IF: DU-145 (human prostatic adenocarcinoma) (13); LnCaP (human prostatic adenocarcinoma) (7); MCF-7 (human breast adenocarcinoma) (20); BT-20 (human breast adenocarcinoma) (10); PaCa-2 (human pancreatic adenocarcinoma) (21); TT-4 (human thyroid medullary adenocarcinoma) (isolated by J. S. Horoszewicz, S. S. Leong, and M. Friedman of this Institute); RT-4 (human bladder adenocarcinoma) (18); and BG-9 (human foreskin fibroblasts) (8).

Cytocentrifuge smears of the above cell lines in the logarithmic phase of growth were prepared and air dried. Slides were fixed in citric acid-sodium citrate (0.38 m) in 60% acetone for 30 sec and air dried. Slides were rehydrated in PB-NaCl buffer and incubated with PAPBG (500 μg/ml PB-NaCl) at 37° for 45 min. Slides were then washed 3 times with PB-NaCl for 10 min each. Secondary reagent (goat anti-human IgG conjugated to fluorescein isothiocyanate) at a dilution of 1:10 was applied for 30 min at 37° followed by repeated washing with PB-NaCl, as described above. Slides were mounted in 70% glycerol, pH 7.4, and examined with a Zeiss fluorescent microscope equipped with a BG-12 excitation filter and a 500-nm barrier filter. Photomicrographs were taken using Kodak Ektachrome type 64 film.

Results

Isolation of PAP-binding Globulin. The calculated recovery of PAPBG from 4 different sera ranged from 4.7 to 16.3 μg/ml serum (Table 1). Binding of PAPBG to the immobilized PAP was not due to nonspecific protein-protein interaction, since reapplication of nonbinding globulins to the affinity media resulted in the recovery of only 5 to 10% of the total binding globulins isolated from each serum source.

IEP of the PAPBG revealed a fast-migrating IgG of restricted electrophoretic mobility (Fig. 1) as compared to the IgG fraction from the same individual which did not bind to the PAP-Sepharose adsorbent. This phenomenon was not due to the effects of chaotropic ions in the affinity column buffer, inasmuch as precipitation experiments were performed (Table 2). After incubation with radioiodine-labeled PAP, the percentage of specific binding was determined. Results indicated that 10 μg of PAPBG bound approximately 9% of PAP over the 10-fold concentration range of PAP tested, from 30 to 300 ng/ml. Control mixtures used to correct for nonspecific precipitation of PAP contained, in place of PAPBG, the appropriate concentration of IgG isolated from normal human serum.

When the ability of PAPBG to bind to cultured human prostatic cancer cells was examined by indirect IF, a strong cytoplasmic staining pattern was observed (Fig. 1). Both lines of prostatic tumor origin (DU-145 and LnCaP) (7, 13) were positively stained using PAPBG, as were cells obtained from a bladder cancer cell culture (RT-4) (18). When the same prostatic cells were examined under dark-field illumination, each IF positively staining region was noted to be encapsulated by a distinct membrane which showed no fluorescent staining, suggesting that material which reacted with PAPBG was present in structures resembling storage or transport vesicles. No staining was observed on membranes or within nuclei when using the PAPBG reagent. Under the same IF conditions, prostatic cancer cells showed no staining with human IgG control. All other human cell lines examined for PAPBG binding by IF were negative. These included the following lines: breast cancer (BT-20 and MCF-7) (10, 20); pancreatic cancer (PaCa-2) (21); medullary thyroid cancer (TT-4); and foreskin fibroblasts (BG-9) (8).

Counterimmunoelectrophoresis revealed that, in addition to prostate cells, the RT-4 bladder tumor cells contained an acid phosphatase which reacted with rabbit anti-PAP antiserum (3).

Discussion

The PAPBG isolated from NHS may provide a useful reagent for the detection of PAP-containing tumors in humans. Radiolabeled PAPBG injected into tumor-bearing patients may behave as a tumor-localizing antibody where it should be possible to detect the fixed radioactivity by appropriate scanning techniques (5, 6). Use of PAPBG circumvents the sensitization problems which may be associated with the use of anti-PAP antibody raised in a foreign species to achieve the same purpose.

At a single PAPBG concentration, a constant percentage of PAP was bound over the 10-fold range of PAP added (Table 2), indicating a relatively small association constant where the

![Table 1](attachment:table1.png)

<table>
<thead>
<tr>
<th>Serum donor</th>
<th>Amount of serum (ml)</th>
<th>Blood group</th>
<th>Sex</th>
<th>IgG fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. L. M.</td>
<td>100</td>
<td>A, Rh+</td>
<td>F</td>
<td>385.5</td>
</tr>
<tr>
<td>M. C. W.</td>
<td>12</td>
<td>O, Rh+</td>
<td>M</td>
<td>44.5</td>
</tr>
<tr>
<td>B. R. S.</td>
<td>8</td>
<td>A, Rh−</td>
<td>F</td>
<td>29.3</td>
</tr>
<tr>
<td>L. D. P.</td>
<td>17</td>
<td>B, Rh+</td>
<td>M</td>
<td>44.4</td>
</tr>
</tbody>
</table>

* Portions of IgG fractions, as indicated, were treated by passage through a column containing 2.35 mg of prostatic acid phosphatase bound to 3.5 ml of CNBr-activated Sepharose 4B. After each IgG fraction was passed through the column, the column was washed, and the adsorbed protein (PAPBG) was eluted with 3.5 m thiocyanate.

![Table 2](attachment:table2.png)

<table>
<thead>
<tr>
<th>Amount added to affinity column (mg)</th>
<th>PAPBG isolated (μg/ml serum)</th>
<th>Total (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>385.5</td>
<td>15.4</td>
<td>1.54</td>
</tr>
<tr>
<td>44.5</td>
<td>7.50</td>
<td>0.09</td>
</tr>
<tr>
<td>29.3</td>
<td>16.25</td>
<td>0.13</td>
</tr>
<tr>
<td>44.4</td>
<td>4.71</td>
<td>0.08</td>
</tr>
</tbody>
</table>

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PAPBG is greatly in excess. An association constant for the binding of PAP by PAPBG was calculated to be approximately $5.0 \times 10^4$ M$^{-1}$, as follows: $K = \text{bound PAP}/[(\text{free PAP}) - (\text{PAPBG sites})]$. The concentration of PAPBG sites had a value of $1.9 \times 10^{-6}$, because $10 \times 10^{-6}$ g of PAPBG with a site molecular weight of $75 \times 10^3$ in $7.0 \times 10^{-6}$ I was used. The value of bound PAP/free PAP is 0.086/0.914 or 0.094, and the value of $K = 0.094/(1.9 \times 10^{-6}) = 5.0 \times 10^4$ M$^{-1}$. These calculations suggest that even the highest concentrations of PAP (M.W. 100,000) found in the circulation of some prostatic cancer patients (100 to 1000 g/liter) ($1 \times 10^{-11}$ to $1 \times 10^{-10}$ M) should not inhibit the deposition of radiolabeled PAPBG in the tumor-containing PAP.

Also of significance to experiments aimed at tumor localization is the matter of antibody specificity, and in this regard the PAPBG protein showed strong immunofluorescent binding to both prostatic cell lines examined. Binding also occurred with the use of RT-4 bladder cancer cells, which contain an acid phosphatase cross-reacting with rabbit anti-PAP antiserum, as detected by counterimmunoelectrophoresis. Other tumor cell lines examined, including those of origin from breast, pancreas, and thyroid, were not reactive with PAPBG at the level of IF sensitivity.

Results of the present experiments support earlier work indicating the occurrence of distinctly binding immunoglobulins in normal sera (14, 19). Using human serum globulins obtained from immunoadsorption procedures, possibly followed by further purification steps, may yield highly specific reagents for tumor localization in human patients which avoid the possible danger inherent in the use of heteroantibodies.

References

Fig. 1. Top, immunoelectrophoretic analysis of PAPBG (Wells A and C) and nonbinding IgG (Wells B and D) obtained from affinity chromatography using immobilized prostatic acid phosphatase. All troughs contain anti-human IgG antiserum. Anode to the right. Bottom, immunofluorescent staining of cultured human prostatic cancer cells by PAPBG. Staining of cells was limited to cytoplasmic structures of 0.2 to 2 μm in diameter, all noted to be membrane encapsulated, as determined by dark-field examination. No fluorescent staining of membranes of nuclei was observed.
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