A Solid-Phase Fluorescent Immunoassay for Human Prostatic Acid Phosphatase

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ABSTRACT

Prostatic acid phosphatase was extracted from human malignant prostatic tissues by Tween 80 and precipitated with 40 to 75% of ammonium sulfate. The crude extract of prostatic acid phosphatase was further purified by a series of chromatographies with concanavallin A-Sepharose, diethylaminoethyl cellulose, and Sephadex G-100. A homogeneous prostatic acid phosphatase preparation with an 85-fold purification and a recovery of 38% of initial enzyme activity was obtained. Antiserum was raised by immunizing female rabbits with the purified enzyme preparation. The anti-prostatic acid phosphatase serum did not cross-react with the acid phosphatase of other human tissues. The anti-prostatic acid phosphatase immunoglobulin G (IgG) antibody was isolated by diethylaminoethyl cellulose and conjugated to CNBr-activated Sepharose 4B, which was then used in a solid-phase fluorescent immunoassay for serum prostatic acid phosphatase. Prostatic acid phosphatase in the serum was separated from other acid phosphatases by mixing serum with anti-prostatic acid phosphatase (IgG)-Sepharose 4B; the enzyme activity was subsequently measured by incubating prostatic acid phosphatase-IgG-Sepharose and a-naphthyl phosphate. The hydrolysis product, a-naphthol, is fluorogenic and can be quantitated with a spectrophotofluorometer at 340 nm (excitation) and 465 nm (emission). The sensitivity of this solid-phase immunofluorometric assay was 60 pg of prostatic acid phosphatase per ml, more sensitive than other immunoassays for prostatic acid phosphatase. Further, the antibody could be reused after the prostatic acid phosphatase was dissociated from IgG-Sepharose 4B.

INTRODUCTION

The acid phosphatase or orthophosphoric phosphohydrolases (EC 3.1.3.2) are ubiquitous cellular enzymes capable of hydrolyzing phosphate esters in an acidic environment. The first report regarding this enzyme occurred in 1924 (18). Serum acid phosphatase assay has been used as an aid in the diagnosis of prostate cancer since 1936 when Gutman et al. (13) first reported the relationship between serum acid phosphatase and prostate cancer. They found that serum acid phosphatase activity markedly increased in patients with prostate carcinoma, especially in those with bone metastasis. Elevated serum acid phosphatase activity has been reported in 70 to 90% of the patients with metastasized prostate cancer and in 5 to 30% of the patients who had no demonstrable metastasis (2, 20, 25). The serum acid phosphatase can also originate from platelets, leukocytes, RBC, and other tissues (26). Since diseases other than prostate cancer can also cause elevation of acid phosphatase activity (23, 26), it would be advantageous to develop a more specific assay method for the acid phosphatase of prostate origin.

The effectiveness of treatment in prostate cancer and the survival rate of patients depends, as in other cancers, upon an early diagnosis. At an early stage of prostatic tumor growth, the amounts of acid phosphatase released into the blood stream from the prostate may be very low. A sensitive assay for detecting minute amounts of acid phosphatase of prostate origin will play an important role in early diagnosis of this cancer. However, the measurement of serum acid phosphatase by conventional spectrophotometric methods has failed to detect prostate cancer at its early stages (26).

Recently, sensitive immunoassays have been reported for this enzyme by several investigators (4, 7, 8, 12). Although the counterimmunoelectrophoresis method is simpler, more sensitive, and more specific than the conventional spectrophotometric method for detecting serum prostatic acid phosphatase (7), it is, in practice, a semiquantitative method. The radioimmunoassay technique (4, 12) is a valuable quantitative method, but factors such as the expense of reagents, the potential health risks from isotope, and the instability of the labeled enzyme may limit its practical application. Therefore, we have developed a nonisotope, solid-phase fluorescent immunoassay method for prostatic acid phosphatase in which a combination of immunological, biochemical, and chemical approaches is used. This assay is not only more sensitive but also eliminates the disadvantages of radioimmunoassay or counterimmunoelectrophoresis. The technique is described in detail in this report along with the preliminary results of this assay in cancer patients.

MATERIALS AND METHODS

Malignant prostatic tissues were obtained from surgery or autopsy. Tissues were stored at −75° immediately until used. Serum samples from patients with prostate and other cancers were furnished by this institute. Normal sera were donated by healthy male volunteers.

1 Supported in part by USPHS Grants CA-15126 and CA-15437 from the National Cancer Institute through the National Prostatic Cancer Project, by Special Program Grant RD-1 from the American Cancer Society, and by USPHS Grant CA-23990, awarded by NCI.

2 To whom requests for reprints should be addressed.

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α-Naphthyl acid phosphate and α-methyl-D-mannopyranoside were purchased from Calbiochem, San Diego, Calif. Bovine serum albumin, Fast Red Salt B, and Fast Garnet GBC salt were obtained from Sigma Chemical Co., St. Louis, Mo. Con A-Sepharose, CNBr-activated Sepharose 4B, and Sephadex G-100 and G-200 were obtained from Pharmacia Fine Chemicals, Inc., Piscataway, N. J. Acrylamide and DEAE-cellulose were purchased from Bio-Rad Laboratories, Richmond, Calif. Guanidine hydrochloride was obtained from Aldrich Chemical Company, Inc., Milwaukee, Wis. Diaflo PM membrane ultrfilters were purchased from Amicon Corp., Lexington, Mass.

Preparation of Crude Acid Phosphatase from Prostatic Tissue. Prostatic tissues were weighed (102 g), minced, and mixed with 0.02 M sodium acetate buffer, pH 5.2, containing 0.01% Tween 80 (3 ml/g of tissue) and homogenized with an Omnimixer (Sorvall, Inc., Norwalk, Conn.). Tissues were blended for 5 min, 3 times at blade speed of 25,000 rpm with intermittent cooling times of 3 min. The homogenized materials were stirred overnight at 4°, followed by centrifugation at 13,000 rpm for 30 min (Sorvall RC2-B-superspeed centrifuge). The supernatant was dialyzed overnight against 0.02 M sodium acetate buffer, pH 5.2, and then concentrated to 70 ml in a concentrator fitted with a Diaflo PM-10 membrane ultrfilter under nitrogen pressure. The entire operation was carried out at 4°.

Purification of Prostatic Acid Phosphatase. The supernatant containing the crude acid phosphatase preparation from 102 g of prostatic tissue was brought to a 40% saturation of ammonium sulfate with mixing, settled for 3 hr at 0°, and then centrifuged at 13,000 rpm for 30 min. The supernatant was adjusted to 75% saturation of ammonium sulfate, mixed, kept at 0° for 3 hr, and centrifuged at 13,000 rpm for 30 min at 4°. The precipitate was dissolved in a minimum amount of starting buffer, pH 5.0 (1 mM each for CaCl2, MgCl2, and MnCl2, and 0.1 M each for NaCl and CH3COONa), dialyzed overnight against the same buffer, and then centrifuged at 13,000 rpm for 30 min at 4°. The supernatant (30 ml) was applied to a Con A-Sepharose column (2 x 40 cm) and incubated for 24 hr at 4°. The proteins not bound to the column were eluted with starting buffer (Chart 1, Peaks I and II). The acid phosphatase and other glycoproteins, bound to Con A, were eluted with a linear concentration gradient of 0.1 to 0.5 M α-naphthyl-D-mannopyranoside with the use of 500 ml of the above starting buffer in each reservoir (Chart I, Peak III).

The eluate containing acid phosphatase (Chart 1, Peak III) was dialyzed against 0.02 M phosphate buffer, pH 7.0, for 18 hr and concentrated to 3 to 4 ml with a Diaflo PM-10 membrane ultrfilter. This solution was applied to a DEAE-cellulose column (2 x 46 cm), preequilibrated with 0.02 M phosphate buffer, pH 7.0, and eluted with a linear gradient of 0 to 0.5 M NaCl in 0.02 M phosphate buffer, pH 7.0 to 6.0. Three fractions exhibiting acid phosphatase activity were obtained (Chart 2, Fractions I, II, and III). The Fraction II (Tubes 79 to 94) that contained the major protein peak was further purified by being passed through a Sephadex G-100 column (2.5 x 96 cm) and eluted with 0.01 M citrate buffer, pH 6.0. Acid phosphatase was separated into 1 major and 1 minor fraction (Chart 3). The first (major) fraction, containing the acid phosphatase with a molecular weight of 100,000, was rechromatographed on Sephadex G-100, and a homogeneous preparation was obtained. This purified acid phosphatase preparation was used for further experiments.

Disc Electrophoresis on Polyacrylamide Gel. Disc electrophoresis was performed by the technique described by Davis (9) in a standard 7.5% polyacrylamide gel. Twenty-five μg of purified enzyme, containing 25% of sucrose to increase the density of the tested solution, were applied to the top of a 5 x 70-mm polyacrylamide gel column. Electrophoresis was carried out in 0.05 M Tris-HCl buffer, pH 8.3, at 4°, with a constant current of 5 ma/tube for 1 hr. After electrophoresis the gels were pushed out of the glass tubes, and the protein was detected by staining (30 min) with 0.1% Coomassie blue R-250. The gel was destained with 5 to 10% acetic acid contained 5 to 10% methanol by volume. The enzymic activity of acid phosphatase was detected by staining in 0.1% α-naphthyl phosphate-0.1% Fast Garnet GBC salt in 0.1 M ammonium acetate buffer, pH 5.0. The stains in the gels were then scanned.

Assay of Enzymic Activity. Acid phosphatase activity in the chromatographic fractions was determined by the method of Babson and Phillips (1). This method used α-naphthyl phosphate as the substrate; the hydrolyzed product, α-naphthol, forms a stable colored complex with Fast Red Salt B in an alkaline condition. The absorbance was measured at 588 nm. One IU of acid phosphatase activity is defined as the amount of enzyme in 1 liter of sample that will hydrolyze the substrate at a rate of 1 μmol/min.

Determination of Protein Concentration. The method of Lowry et al. (17) was used. Bovine serum albumin was used as the standard.

Preparation of Antisera. The anti-prostatic acid phosphatase serum was raised by injecting the purified enzyme and a complete Freund's adjuvant into female rabbits as previously described (7).

Purification of Anti-Prostatic Acid Phosphatase (IgG). The method described by Harboe and Ingrid (15) was used. Briefly, rabbit anti-prostatic acid phosphatase serum (20 ml) was added to 10 ml saturated ammonium sulfate and thoroughly mixed. The mixture was kept in 0° for 3 hr and centrifuged at 2,000 rpm for 30 min; the precipitate was dissolved in a minimum volume of 0.0175 M sodium phosphate buffer, pH 6.3, and then dialyzed against the same buffer for 24 hr at 4°. The dialyzed solution was applied to a DEAE-cellulose column (1 x 40 cm) and eluted with 0.0175 M sodium phosphate buffer, pH 6.3. The IgG antibody eluted at the first protein peak.

Conjugation of Purified Anti-Prostatic Acid Phosphatase (IgG) to Sepharose 4B. This was carried out according to the manufacturer's procedure (Pharmacia). The CNBr-activated Sepharose 4B (1 g) was washed and reswelled on a sintered glass filter with 1 mM HCl. The purified anti-prostatic acid phosphatase (IgG) (5 to 10 mg protein per ml gel) was dissolved in 0.1 M NaHCO3 buffer, pH 8.5, containing 0.5 M NaCl and mixed with CNBr-activated Sepharose 4B gel suspension, in an end over end, for 2 hr at room temperature. The resulting mixture was applied to a column (2 x 46 cm) and eluted with 0.0175 M sodium acetate buffer, pH 5.2. The IgG antibody eluted at the first protein peak.

The abbreviations used are: Con A, concanavalin A; PBS, phosphate-buffered saline, containing 0.05 M NaH2PO4-Na2HPO4 and 0.14 M NaCl, pH 7.0.

Cancer Research Vol. 38
temperature. After incubation the excess of IgG was removed by means of the coupling buffer. The remaining active groups on Sepharose 4B were blocked by adding 1 M monoethanolamine solution, pH 9.0 (5 ml), with gentle mixing for 2 hr at room temperature. Finally, the excess blocking reagent was removed by washing first with acetate buffer (0.1 M, pH 4.0) containing 0.5 M NaCl and then with the coupling buffer. This IgG-Sepharose 4B conjugate was further washed with PBS and stored at 4° until used. The procedure resulted in coupling of about 98% of the IgG to the CNBr-activated Sepharose 4B, as determined by measuring immunoglobulin concentration before and after the coupling reaction by spectrophotometry at 280 nm.

Reactivity of IgG-Sepharose-bound Acid Phosphatase. The reactivity of IgG-Sepharose-bound acid phosphatase was studied as follows. Purified acid phosphatase (0.2 ml in PBS) was incubated with IgG-Sepharose (200 µl) at room temperature for 2 hr; the resulting Sepharose IgG-acid phosphatase was washed with PBS 3 times. The Sepharose-IgG-acid phosphatase was kept at 4°. Another aliquot, 0.2 ml of acid phosphatase, also was left at room temperature for 2 hr and then kept at 4°. Aliquots of 20 µl each were taken at 8-hr intervals and assayed for enzyme activity. Twenty µl of specimen were mixed with 1 ml of substrate (see below) and incubated at 37° for 15 min. The reaction was stopped by adding 0.1 M NaOH (2.5 ml), and the hydrolyzed fluorogenic product, γ-naphthol, was measured with a spectrophotofluorometer.

Solid-Phase Fluorescent Immunoassay for Prostatic Acid Phosphatase. Patient's serum (50 µl) or prostatic acid phosphatase was incubated with IgG-Sepharose 4B (50 µl) in polystyrene tubes (8 x 75 mm) in PBS for 2 hr at room temperature and then overnight at 4°. The prostatic acid phosphatase was bound to the IgG on the Sepharose 4B. After centrifugation and washing of the precipitate (3 times) with PBS, 1.0 ml of 3 mM γ-naphthyl phosphate (substrate) in 0.2 M citrate buffer, pH 5.6, was added to the acid phosphatase-IgG-Sepharose 4B, and this was incubated for 1 hr at 37°. The supernatant (0.8 ml) was transferred to a new tube containing 2.5 ml of 0.1 M NaOH. The enzyme activity was determined by an Aminco spectrophotofluorometer, with excitation at 340 nm and emission at 465 nm. A standard curve was established with various concentrations of γ-naphthol, and the protein was monitored by absorbance at 280 nm for γ-naphthol, and the protein was monitored by absorbance at 280 nm.

Reactivity of IgG-Sepharose-bound Acid Phosphatase. After the enzyme activity was measured, the prostatic acid phosphatase, which bound to the IgG-Sepharose 4B, was dissociated by the use of 5 M guanidine-HCl, pH 8.5, at room temperature for 30 min. The guanidine-HCl and prostatic acid phosphatase were removed by washing with PBS overnight. The dissociated and reactivated IgG-Sepharose can be reused (for at least 3 more experimental runs).

RESULTS

Homogeneity of Purified Prostatic Acid Phosphatase and Specificity of Anti-Prostatic Acid Phosphatase Serum. The human prostatic acid phosphatase was purified to homogeneity by the procedure described. After a series of chromatographies on a Con A affinity column (Chart 1), a DEAE ion-exchange column (Chart 2), and Sephadex G-100 gel filtration (Chart 3), a symmetrical protein peak exhibiting acid phosphatase activity was obtained. The homogeneity of the purified protein was confirmed by disc polyacrylamide gel electrophoresis, which demonstrated a single protein band superimposed on the enzyme activity band (Chart 4). The molecular weight of the prostatic acid phosphatase was estimated to be 100,000 by gel filtration by the use of Sephadex G-200 (Chart 5). With this procedure an 85-fold purification and 38% recovery of the activity was achieved (Table 1).

The anti-prostatic acid phosphatase serum, when reacted with prostatic acid phosphatase, gave 1 precipitin line on gel diffusion when stained for both protein and acid phos-
Sepharose 4B-bound Acid Phosphatase. The enzyme activity of acid phosphatase was studied with and without binding to IgG (anti-prostatic acid phosphatase)-Sepharose 4B. As shown in Chart 6, the acid phosphatase which bound to IgG-Sepharose exhibited enzyme activity. Furthermore, no loss of the enzyme activity was demonstrated for 48 hr, whereas the "free" acid phosphatase (not bound to IgG-Sepharose) lost about 64% of its enzyme activity during the same 48-hr period and 87% of activity after 96 hr. These results indicated that the IgG-Sepharose-bound acid phosphatase retained its catalytic reactivity at least for 48 hr under this experimental condition.

Amount of Solid-Phase (IgG-Sepharose) Used for the Assay. In order to determine the minimum amount of IgG (anti-prostatic acid phosphatase)-Sepharose 4B necessary for the assay, various concentrations of acid phosphatase were incubated with a constant amount of IgG-Sepharose for 2 hr at room temperature and overnight at 4°. The enzymic activity of the acid phosphatase bound to IgG antibody was determined by the fluorometric technique as described. Results indicated that 50 μl of IgG (anti-prostatic acid phosphatase)-Sepharose (containing 0.28 mg of anti-prostatic acid phosphatase-lgG) would bind 45.6 ng of prostatic acid phosphatase. Therefore, a minimum amount of IgG (anti-prostatic acid phosphatase)-Sepharose (0.28 mg of IgG) was used in this solid-phase fluorescent immunoassay in order to measure the serum prostate acid phosphatase concentration up to 45.6 x 20, or 912, ng/ml without diluting the serum samples.

Optimal Incubation Condition of Solid-Phase Fluorescent Immunoassay. In order to determine the optimal incubation condition for the solid-phase fluorescent immunoassay, prostatic acid phosphatase was incubated with the IgG-Sepharose under different temperatures and at various time intervals. The enzyme activity was also assayed at different incubation times with the substrate, α-naphthyl phosphate. The results (Chart 7) revealed that incubation first at room temperature for 2 hr and then overnight at 4° afforded the best binding between prostatic acid phosphatase and IgG-Sepharose. The measurement of enzyme activity was best determined at 37° for 1 hr. In this manner the Sepharose 4B-bound Acid Phosphatase. The enzyme activity of acid phosphatase was studied with and without binding to IgG (anti-prostatic acid phosphatase)-Sepharose 4B. As shown in Chart 6, the acid phosphatase which bound to IgG-Sepharose exhibited enzyme activity. Furthermore, no loss of the enzyme activity was demonstrated for 48 hr, whereas the "free" acid phosphatase (not bound to IgG-Sepharose) lost about 64% of its enzyme activity during the same 48-hr period and 87% of activity after 96 hr. These results indicated that the IgG-Sepharose-bound acid phosphatase retained its catalytic reactivity at least for 48 hr under this experimental condition.

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Table 1

Preparation of homogeneous prostatic acid phosphatase from human malignant prostate tissues

<table>
<thead>
<tr>
<th>Steps</th>
<th>Total volume (ml)</th>
<th>Total protein (mg)</th>
<th>Total enzymic activity (units)</th>
<th>Specific activity (units/mg)</th>
<th>Relative activity</th>
<th>Recovered (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate from crude extract tissues&lt;sup&gt;c&lt;/sup&gt;</td>
<td>70</td>
<td>4,060</td>
<td>144,480</td>
<td>35.6</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>40-75% (NH₄)₂SO₄ precipitate</td>
<td>30</td>
<td>600</td>
<td>120,000</td>
<td>200</td>
<td>5.6</td>
<td>83.1</td>
</tr>
<tr>
<td>Con A-Sepharose</td>
<td>10.5</td>
<td>98.7</td>
<td>107,362</td>
<td>1,087.8</td>
<td>30.6</td>
<td>74.3</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
<td>6</td>
<td>44.4</td>
<td>74,724</td>
<td>1,682.9</td>
<td>47.3</td>
<td>51.7</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>4.5</td>
<td>29.4</td>
<td>65,412</td>
<td>2,236.2</td>
<td>62.8</td>
<td>45.3</td>
</tr>
<tr>
<td>Sephadex G-100</td>
<td>3</td>
<td>18.0</td>
<td>54,471</td>
<td>3,026.2</td>
<td>85.0</td>
<td>37.7</td>
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</table>

<sup>a</sup> The protein concentration was determined by the method of Lowry et al. (17). Bovine serum albumin was used as the standard.

<sup>b</sup> Enzyme activity was determined by the method of Babson and Phillips (1).

<sup>c</sup> Obtained from 102 g of frozen human malignant prostate tissues and concentrated with Diaflo PM-10 ultrafilter under nitrogen pressure.

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**Sensitivity and Reproducibility of the Solid-Phase Fluorescent Immunoassay.** The sensitivity of this immunoassay was verified by performing various triplicate determinations of prostatic acid phosphatase, ranging from 45.6 ng to 3 pg in 50 μl of specimen. The results are presented in Chart 8. The prostatic acid phosphatase at 3 pg/50 μl could be detected by this immunofluorometric assay. This assay procedure was found to be reproducible, as a within-assay standard deviation of 6 pg/50 μl (10 determinations) and a between-assay standard deviation of 9 pg/50 μl (3 assays, 3 determinations in each assay) were obtained from a sample of 280 pg/50 μl, representing a coefficient of variation of less than 4%.

**Recycling of IgG (Anti-Prostatic Acid Phosphatase)-Sepharose.** As shown in Chart 9, the IgG (anti-prostatic acid phosphatase)-Sepharose, after the dissociation of acid phosphatase from the prostatic acid phosphatase-IgG-Sepharose, can be reused for the assay. Although a slight decrease of sensitivity occurred, it could be recycled at least 3 times without losing any appreciable binding to prostatic acid phosphatase.
Preliminary Results of Clinical Application. The results of an initial application of this newly developed immuno-fluororesay in testing sera from patients with prostate cancer and other tumors are shown in Table 2. The determination of serum prostatic acid phosphatase by this assay from a group of 30 apparently healthy male volunteers resulted in a mean of 5.619 ng/ml with a standard deviation of 2.110. A normal range of 1.399 to 9.839 ng/ml was thus determined. The serum prostatic acid phosphatase levels from 24 patients with all stages of prostate cancer were studied in this preliminary report. The enzyme was found to be elevated in 4 untreated patients with Stage A disease, in 2 of 5 with Stage B, and in 7 of 11 with Stage C, who were receiving standard estrogen therapy and/or radiation therapy, and in all 4 patients with Stage D disease receiving chemotherapy. On the other hand, the serum prostatic acid phosphatase levels were in the normal range in all 16 patients with other advanced tumors, such as cancer of the lung, breast, colon, rectum, stomach, and pancreas. These 16 specimens were randomly chosen from serum samples that had exhibited a highly elevated level of carcinoembryonic antigen (all had a value of greater than 15 ng/ml).

DISCUSSION

The solid-phase fluorescent immunoassay for human prostatic acid phosphatase described in this report primarily involves the immunological specificity of prostatic acid phosphatase and the inherent fluorescent property of α-naphthol, the enzyme hydrolysis product of prostatic acid phosphatase. Unlike other sensitive immunoassay techniques, such as the enzyme-linked immunoassay or double antibody radioimmunoassay, this technique does not require the application of a second enzyme or antibody. Further, the quantitation of prostatic acid phosphatase in this assay is based upon the biological (catalytic) activity of the enzyme and therefore differs from that of radioimmunoassay (4, 12), which measures the mass of the enzyme protein. The specificity of this assay is provided by the anti-prostatic acid phosphatase, raised against the purified enzyme from the prostate, which binds the prostatic acid phosphatase specifically; the assay is further characterized by the fluorescence of enzyme hydrolysis product, which permits the quantitation with a very sensitive spectrophotofluorometric technique (16). It represents a new report of an enzyme quantitation, which combines the immunological and biochemical as well as the chemical approach.

The purification procedure for acid phosphatase from human prostate tissue in this study is slightly different from our previous one (7). Because acid phosphatase is biochemically a glycoprotein (5, 6, 22), the initial step is the use of an affinity chromatography of Con A-Sepharose for its purification. Subsequent chromatographies on a DEAE ion-exchange column and Sephadex G-100 gel filtrations result in a homogeneous protein preparation exhibiting acid phosphatase activity. This purified protein preparation is used for further experiments in this study. The molecular weight of prostatic acid phosphatase has been determined to be around 100,000 (7, 22). The other protein, with a molecular weight 65,000 daltons (Chart 3), is not included in the present report. The nature of the "other" acid phosphatase is being investigated.

Anti-prostatic acid phosphatase serum is raised by immunizing female rabbits with the purified enzyme preparation. The antiserum demonstrates no immunological reactivity with extracts of other human tissues, which confirms

Table 2

| Prostate cancer | Other cancer
<table>
<thead>
<tr>
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<tr>
<td>Prostatic acid phosphatase (ng/ml)</td>
<td>Prostatic acid phosphatase (ng/ml)</td>
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<tr>
<td>Patient</td>
<td>Stage</td>
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<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
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<td>A</td>
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<td>23</td>
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<td>24</td>
<td>D</td>
</tr>
</tbody>
</table>

a Patients with metastasized cancer from primary tumor as indicated under "Primary."

b Staging of prostatic cancer is according to that of Whitmore as summarized in Ref. 7.

c Normal range of serum prostatic acid phosphatase: 1.399 to 9.839 (N, 30; mean, 5.619; S.D., 2.110).
our previous report (7) and the report of others (4). Therefore, the antiserum can be used directly for the isolation of IgG without further absorptions. Recently, we have succeeded in producing an anti-prostatic acid phosphatase serum in goats. The goat anti-prostatic acid phosphatase also exhibits this immunological specificity.

Studies have been conducted in the last 2 decades as to the effect of antibody binding on the catalytic activity of enzyme from which the antibody was produced. Some antibodies inhibit the catalytically active site of an enzyme (3, 14). On the other hand, some antibodies may stabilize the enzymes against pH and temperature inactivations or even activate the enzyme reactions (10, 19, 21, 24, 27). We have observed from our previous study that the human prostate acid phosphatase seems to have a different site for its antibody binding than for its enzyme activity (7). Foti et al. have also reported a similar observation (11). This is the basis for the development of our previous counterimmunoelectrophoresis technique as well as this solid-phase fluorescent immunoassay of this clinically important enzyme. Anti-prostatic acid phosphatase is conjugated to CNBr-activated Sepharose 4B to form a solid phase that specifically binds the acid phosphatase of prostate origin. This solid-phase anti-prostatic acid phosphatase not only separates prostatic acid phosphatase from other phosphatases and serum proteins but also stabilizes the enzyme. Subsequently, the activity of prostatic acid phosphatase is measured by the hydrolytic product, α-naphthol, which is fluorogenic and can be quantitated by spectrophotofluorometry with great sensitivity.

The sensitivity of this solid-phase immunofluorescent assay for prostatic acid phosphatase is 60 pg/ml of serum under the experimental protocol described. If a greater sensitivity is needed, it can be accomplished by increasing the volume of specimen assayed. The sensitivities of our counterimmunoelectrophoresis and the radioimmunoassay by Foti et al. are 20 and 10 ng/ml, respectively (7, 8, 12). This immunofluorooassay, therefore, provides a more sensitive tool for serum prostatic acid phosphatase determinations. This procedure is reliable, as supported by reproducibility studies of within-assay and between-assay. The solid-phase IgG (anti-prostatic acid phosphatase)-Sepharose can be reused. Furthermore, this assay does not require the use of isotope. The standard curve of this assay extends from 60 pg to 912 ng per ml of specimen. Therefore, it covers a much broader range than the radioimmunoassay.

The preliminary data presented in this report are encouraging, inasmuch as they indicate that this assay detects an elevation of serum prostatic acid phosphatase in patients with early stages of prostate cancer. Patients with other cancers are found to have normal levels of serum prostatic acid phosphatase.

Acknowledgments

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REFERENCES

Fig. 2. Immuneelectrophoretic study of the anti-prostatic acid phosphatase serum (troughs) with purified prostatic acid phosphatase and extracts of other human tissues. After immuneelectrophoresis the plate was washed and stained first for enzyme activity and then for protein. Another plate also indicated a negative reaction with extracts of: pancreas, 69.0 μg; kidney, 12.3 μg; and spleen, 20.3 μg (data not shown).

Fig. 1. Gel diffusion study of the anti-prostatic acid phosphatase (Well A) and the purified prostatic acid phosphatase (Well 2, 40 μg). Wells 1 and 3 were blank. After gel diffusion the plate was washed and stained first for enzyme activity and then for protein.
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