Rapid Radioimmunoassay for Prostate-Specific Acid Phosphatase in Human Serum

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We describe a rapid radioimmunoassay for human prostatic acid phosphatase (EC 3.1.3.2) in serum, with use of monospecific antisera raised in rabbits against the primary highly purified acid phosphatase (pl 4.9) from human prostates, and with a second antibody—polyethylene glycol precipitation. This radioimmunoassay is sensitive and can be performed within 5 h. Concentrations of the immunoreactive acid phosphatase in sera of healthy men (n = 384) ranged from 0.3 to 3.6 μg/L (mean 1.94, SD 0.66 μg/L). Concentrations of the enzyme in sera of men with benign prostatic hyperplasia (n = 56) or with carcinoma of nonprostatic origin (n = 24) were identical with those of the reference group. Serum concentrations of immunoreactive prostatic acid phosphatase of patients with occult, nonmetastatic, and metastatic prostatic carcinoma varied from 1.7 to 9.3 (n = 9), 4.2 to 59.4 (n = 12), and 20 to 198 (n = 10) μg/L, respectively. The amount of immunoreactive prostatic acid phosphatase was unchanged for at least five days in serum stored at 4 °C.

Additional Keyphrases: cancer • reference intervals • prostatic hyperplasia • prostatic carcinoma

Several methods have been published for the measurement of serum immunoreactive prostatic acid phosphatase, but only radioimmunoassay, fluorescent immunoassay, and immunoadsorbent assay seem to be sensitive enough to detect the concentrations of prostatic acid phosphatase in the serum of disease-free males (1–6). Because circulating acid phosphatase is a mixture of isoenzymes originating from several tissues (7), an essential prerequisite for an organ-specific immunological determination of serum acid phosphatase is the availability of pure antigen. We have previously described a specific and sensitive radioimmunoassay for human prostatic acid phosphatase in serum (5), in which the antigen was purified to homogeneity from human prostatic tissue (8).1 The procedure, however, was rather lengthy and took four days to complete. We describe here further development and optimization of the method, which can now be completed in 5 h. We also give data on the application of the procedure for the determination of serum prostate-specific acid phosphatase in normal men, in patients with prostatic hyperplasia or carcinoma, and in men with other carcinomas.

Materials and Methods

Chemicals

Na$_{125}$I (spec. acty., 11–17 kCi/g) was from the Radiochemical Centre, Amersham, U.K. Sephadex gels (G-50 and G-200) and AH-Sepharose 4B were from Pharmacia, Uppsala, Sweden. Freund's complete and incomplete adjuvants were supplied by Difco Labs., Detroit, MI 48223; bovine serum albumin by Sigma Chemical Co., St. Louis, MO 63178; and p-nitrophenyl phosphate from Boehringer Mannheim GmbH, Mannheim, F.R.G. Diaflo PM 10 ultrafiltration membranes were from Amicon Corp., Lexington, MA 02173. Polyethylene glycol (Carbowax 6000) was from Fluka AG, Buchs SG, Switzerland. Other chemicals were from Merck AG, Darmstadt, F.R.G., and were of the highest purity available.

Serum Samples

The group of apparently normal men consisted of 199 young volunteers (ages 20–50 years) and of 196 older men (ages 51–75 years). Subjects who had previously experienced any form of urinary difficulty or urogenital disease were excluded. Only subjects whose prostate size was normal, as judged by rectal palpation, were accepted.

The group of patients consisted of men attending the Division of Urology, Department of Surgery, Oulu University Central Hospital, for treatment or check-up in connection with either benign prostatic hyperplasia (n = 56), carcinoma of the prostate (n = 22), or some other carcinomas (bladder, lung, liver, or colon, n = 24). For diagnosis and primary classification of the prostatic disease, patients were examined by rectal palpation of the prostate, isotopic bone scan, roentgenographic bone studies, and prostate biopsy. In the group of benign prostatic hyperplasia, we included only patients who had histologically proven hyperplasia in serial sections of the tissue after operation. Occult prostatic carcinomas (n = 9) were incidental histopathological findings after an operation for prostatic hyperplasia.

About 10 mL of blood was drawn from the antecubital veins of reference subjects and patients before rectal examination or eventual biopsy of the prostate. Blood samples were allowed to stand at room temperature for 30 min, and the serum collected by centrifugation (1500 × g, 15 min). The samples were stored at ~20 °C until analyzed.

Enzyme Purifications

Human prostatic acid phosphatases (enzymes with isoelectric points at pH 4.9 or 5.5) were purified as previously described (8, 9). Leukocyte preparations were obtained from the Finnish Red Cross Transfusion Service, Helsinki, Finland. Acid phosphatase from leukocytes was purified essentially by the same technique as that used for the prostatic enzyme.

Antisera

The antisera against human prostatic acid phosphatases were raised in rabbits as previously described (5). Antiserum against rabbit gamma-globulins, raised in sheep, was purchased from Oy Finnsorbertas Ab, Espoo, Finland.

Radioiodination of Acid Phosphatase

Preparation of $^{125}$I-labeled acid phosphatase was performed
as previously described (5), except that 10 μg of the purified enzyme protein was used in the labeling reaction and Sephadex G-200 was substituted for Sephadex G-100 in the purification of the tracer.

Radioimmunoassay Procedure

To 0.2 mL of the unknown samples and standards in 12 × 75 mm polypropylene tubes, add 0.2 mL of diluted anti-acid phosphatase serum [diluted with 50 mmol/L sodium phosphate buffer, pH 8.0, containing, per liter, 20 mmol of ethylenediaminetetraacetate (EDTA), 8 mmol of NaCl, and 1 mL of rabbit serum]. The usual dilution of the antiserum in the sodium phosphate buffer is 50 000- to 60 000-fold. Precipitate for 1 h at 20 °C, and then add to the reaction mixture approximately 50 000 cpm of 125I-labeled acid phosphatase in 0.2 mL of sodium citrate buffer (10 mmol/L, pH 5.5), containing, per liter, 1 mL of rabbit serum, 20 mmol of EDTA, 150 mmol of NaCl, and 8 mmol NaNO3. Incubate for 3 h at 20 °C, and then add 0.5 mL of a solution of 220 g of polyethylene glycol per liter of sodium phosphate buffer (50 mmol/L, pH 7.4), containing, per liter, 150 mmol of NaCl, 8 mmol of NaNO3, 5 μL of antirabbit gamma-globulin, and 10 mL of Tween-20 surfactant. Incubate the tubes for an additional 15 min at 20 °C and then centrifuge (3000 × g, 15 min) at 4 °C. Aspirate and discard the supernates and with a gamma counter count the radioactivity in the pellets containing the antibody-bound 125I-labeled acid phosphatase.

We assayed each unknown sample in duplicate; every series of assays also included duplicate acid phosphatase standard-curve-antibody tubes with the following concentrations of the purified nonlabeled enzyme: 0, 1, 3, 10, and 30 μg/mL. Both the standards and serum samples were diluted with acid phosphatase-free serum if necessary (see below). We calculated the concentrations of acid phosphatase in sera by using standard curves.

Other Procedures

To prepare serum free of human acid phosphatase (acid phosphatase-free serum), centrifuge (1500 × g, 15 min) heated (50 °C for 10 min) sheep serum and dilute with an equal volume of sodium phosphate buffer (50 mmol/L, pH 7.2), containing 20 mmol of EDTA and 8 mmol of NaNO3 per liter.

We performed immunoelectrophoresis as described previously (5). Polyacrylamide gel electrophoresis under denaturing conditions was carried out as described by Weber and Osborn (10).

Results

Acid phosphatase isoenzyme purified to homogeneity from human leukocytes (isoelectric point, pI = 5.9) did not cross react with the antisera against the two purified prostatic acid phosphatases (pI 4.9 and 5.9) (Figure 1). Neither did it cross react in the present radioimmunoassay, at least when added in concentrations as great as 700 μg/L. The prostatic acid phosphatase purified from a metastasis originating from prostatic carcinoma had a pI of 4.9 and the same subunit relative molecular mass (54 000) as that of the subunits of the main prostatic acid phosphatase (pI 4.9) purified from hyperplastic prostatic tissue (8) and used in this radioimmunoassay. The purified acid phosphatase from the metastasis also cross reacted with the antiserum raised against the main prostatic acid phosphatase (Figure 1). The acid phosphatases with pI 4.9, whether originating from hyperplastic prostatic tissue or metastatic tissue, seem to be identical.

Figure 2 shows a standard curve for the radioimmunoassay of prostate-specific acid phosphatase (pI 4.9) in serum. With the antiserum dilution we used and with 0.2-mL serum samples, the lowest measurable acid phosphatase concentration was 0.2 μg/L. Whenever the concentration of the enzyme protein exceeded that of the highest standard used (30 μg/L), we diluted an aliquot of the sample with acid-phosphatase-free sheep serum and re-analyzed. Thus we were able to quantify concentrations at least 100-fold greater than those shown in Figure 2. The concentration of prostatic acid phosphatase, as measured by the present radioimmunoassay, was strictly proportional to the amount of serum added to the assay. Ten- to 200-μL portions of serum from a patient with advanced prostatic carcinoma were used in the assay; plotting immunoreactive acid phosphatase vs volume of the serum produced a straight line (not shown).

Increasing known amounts of prostatic acid phosphatase were added to a patient’s serum sample containing 2.2 μg of prostatic acid phosphatase...
prostatic acid phosphatase per liter. Analytical recoveries were 92 to 104% for five different prostatic acid phosphatase concentrations; as much as 20 μg of the enzyme protein per liter was added to the serum pool. Within- and between-assay coefficients of variation (CV) of the present radioimmunoassay, as evaluated by repeated measurements of acid phosphatase in three different serum pools, were 3–14% and 5–11%, respectively (Table 1).

Table 1. Within- and Between-Assay Precision of the Radioimmunoassay for Serum Prostatic Acid Phosphatase

<table>
<thead>
<tr>
<th>Sample</th>
<th>Within-assay (n = 10)</th>
<th>Between-assay (n = 10)</th>
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</thead>
<tbody>
<tr>
<td>Pool I</td>
<td>2.83 (0.40)</td>
<td>4.0</td>
</tr>
<tr>
<td>Pool II</td>
<td>5.78 (0.23)</td>
<td>4.6</td>
</tr>
<tr>
<td>Pool III</td>
<td>14.51 (0.47)</td>
<td>6.3</td>
</tr>
<tr>
<td>Pool IV</td>
<td>4.43 (0.50)</td>
<td>11.3</td>
</tr>
<tr>
<td>Pool V</td>
<td>9.63 (0.44)</td>
<td></td>
</tr>
<tr>
<td>Pool VI</td>
<td>15.32 (0.97)</td>
<td></td>
</tr>
</tbody>
</table>

Within-assay (n = 10) Pool I, Pool II, Pool III. Between-assay (n = 10) Pool IV, Pool V, Pool VI.

We also tested the influence of storage of serum samples on the amount of immunoreactive acid phosphatase. Samples could be stored at room temperature for 24 h, at 4 °C for at least five days, and at −20 °C for at least three months without any change in immunoreactive enzyme protein concentration. Repeated freezing and thawing up to four times had no effect on the assayable enzyme concentration.

Figure 3 shows values for immunoreactive acid phosphatase measured by the present radioimmunoassay in serum samples from healthy men (ages 20–50 years and 51–75 years), men suffering from benign prostatic hyperplasia verified by histology after adenectomy, men with prostatic carcinoma, and men with prostatic carcinoma. The mean concentrations (and SD) for normal healthy men (n = 394) and for patients with benign prostatic hyperplasia (n = 56) were 1.94 (0.66) and 1.71 (0.76) μg/L, respectively. Values for patients with prostatic carcinoma were as much as 100-fold higher, with the following concentrations at different stages of the disease: T0 (= occult prostatic cancer), 5.02 (SD 2.59) μg/L (n = 9); T2 = T3 M0, 19.85 (SD 16.82) μg/L (n = 12); T3 = T4 M1, 94.3 (SD 66.17) μg/L (n = 10); for staging, see ref. 11.

Discussion

We have previously shown that our radioimmunoassay, based on the use of the main prostatic acid phosphatase (PI 4.9) purified from human hyperplastic prostatic tissue, is specific for human prostatic acid phosphatase (5). In the present work, we showed that prostatic acid phosphatase purified from a metastasis of prostatic carcinoma has the same isoelectric point, the same subunit composition, and the same cross reactivity with the antisera used. Therefore it probably makes no difference whether antigen for the assay is purified from carcinomatous or hyperplastic prostatic tissue. As with other acid phosphatases of nonprostatic origin (human spleen, erythrocytes, and synovial tissue), the acid phosphatase isolated from human leukocytes did not cross react with the antisera used.

The present radioimmunoassay is more sensitive than our previous one and allows measurement of immunoreactive acid phosphatase in sera of healthy men. Further, it is faster, and its precision and accuracy are satisfactory.

A uniformly low nonspecific binding of the 125I-labeled acid phosphatase was achieved. In the original radioimmunoassay (5), we used prostatic acid phosphatase-free serum from women to dilute the standards—to minimize variations in the protein concentrations of the standards and samples, and to avoid differences in nonspecific binding between standards and diluted serum samples or those samples analyzed without any predilution. In the present technique, we used diluted and heated sheep serum for the same purpose without encountering any problems in the assay. Differences in the properties of the immunological techniques published so far (1, 2) seem to be mainly due to the degree of success in the purification of the prostatic acid phosphatase, and in the optimization of the measurements. The purity of the main prostatic acid phosphatase, which we use, seems to be the highest of the currently available enzymes, when evaluated by its catalytic activity per milligram of enzyme protein (8). One indication of the differences between the various immunological techniques is the different reference values obtained for serum from normal men (1, 2). Lee et al. (3, 6) reported a solid-phase fluorescent immunoassay and an immunoabsorbent assay for human prostatic acid phosphatase; by their method, sera from 55 normal men contained 1.6–9.6 μg of prostatic acid phosphatase per liter (mean 5.6 μg/L). These values are very similar to those we obtained by our original radioimmunoassay technique (mean 6.0 μg/L from 53 normal men, range <1 to 10 μg/L) (5). After further optimizing our assay with respect to pH, EDTA and NaCl concentrations, and the specific activity of the tracer, we measured values ranging from 0.3 to 3.6 μg/L (mean 1.94, SD 0.66, μg/L) for 394 apparently normal men. In patients with histologically verified prostatic hyperplasia and in whom the presence of occult carcinoma was ruled out, we found serum prostate-specific acid phosphatase concentrations to be identical with those in healthy reference subjects.

The concentrations of serum immunoreactive prostatic acid phosphatase seem to reflect the degree of dissemination of prostatic carcinoma and were highest in the most advanced cases.

The present assay is simple and rapid to perform and allows handling of a large number of samples. Stability of the immunoreactive human prostate-specific acid phosphatase in serum makes it easy to mail frozen samples to centers performing the analyses.
We are grateful to Mrs. Maria Paloniemi for her expert technical assistance. This investigation was partly supported by a grant from the Finnish Cancer Foundation.

References