Isozymes of Acid Phosphatase in Normal and Cancerous Human Prostatic Tissue

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SUMMARY

The supernatants of the homogenates from normal and cancerous human prostatic tissue run on polyacrylamide gel electrophoresis have 2 major electrophoretic bands when stained for prostatic acid phosphatase. The ratios of the electrophoretically distinguishable isoenzymes differ in normal and cancerous tissues. Similar distinctions between isoenzymes in normal and cancerous prostates are observed following column chromatographic separation or isoelectric focusing. The faster electrophoretic band can be separated by diethylaminoethyl cellulose column chromatography or by isoelectric focusing into at least five fractions with different electrophoretic mobilities. We could not find any differences in normal and cancerous tissues among these subfractions of the faster-moving electrophoretic band. Analysis by gel electrophoresis does not show association between these fractions after chromatographic or isoelectric separation of the prostatic acid phosphatase fractions. Quantitative, but not qualitative, differences in prostatic acid phosphatase isozymes occur in normal versus cancerous prostates.

INTRODUCTION

Prostatic acid phosphatase (EC 3.1.3.2) is one of the many phosphatases with clinical importance. The level of this enzyme is usually elevated in the serum of prostatic cancer patients and is reduced by 5 to 15 times in their prostatic tissue (13). Enzymatic methods have generally been used for the quantitation of prostatic acid phosphatase (1, 4, 8, 9, 14). However, the enzymatic activity of prostatic acid phosphatase is relatively unstable to temperature and pH changes (5, 7, 19). Therefore, we developed a radioimmunoassay for this enzyme (3, 6). Should normal or carcinomatous prostatic tissue contain at least 1 isoenzyme that is antigenically different from the others, the utilization of a radioimmunoassay could be greatly improved with the use of only that subgroup for the enzyme immunodiagnostics. Ostrowski et al. (12) and others (11, 16, 17) have shown that prostatic acid phosphatase activity in benign prostatic hypertrophy tissue consists of several different electrophoretic isoenzymes. This paper presents comparative data on the chromatographic and electrophoretic composition of prostatic acid phosphatase in normal and cancerous prostatic tissue.

MATERIALS AND METHODS

Materials. Naphthol AS-MX phosphate, Fast Red Violet LB salt, p-nitrophenyl phosphate disodium salt, and DEAE-cellulose were obtained from Sigma Chemical Co., St. Louis, Mo. Ampholyte solution, pH 3 to 6, was obtained from LKB S, 16125 Bromma 1, Sweden. All other chemicals were of reagent grade and were obtained from J. T. Baker Chemical Company, Phillipsburg, N. J.

Tissue Specimens. Normal prostate glands were obtained from autopsy, and cancerous prostatic tissue was obtained by surgery. Both normal and cancerous tissue were stored at −70°.

Enzyme Assay. A standard acid phosphatase assay was used (15). One-half ml of 15.3 mM p-nitrophenyl phosphate disodium salt in water and 0.5 ml of 0.09 M citrate buffer, pH 4.8, were preincubated at 37°. After the addition of 0.2 ml of enzyme sample, the mixture was incubated for 30 min at 37°. The reaction was stopped by the addition of 5 ml of 0.1 M NaOH. The reaction product was measured at 410 nm.

Isoelectric Focusing. The supernatants of the tissue homogenates were centrifuged at 4000 × g for 20 min. Isoelectric focusing was done according to the method of Svensson (18) with an LKB 8100 apparatus. The column capacity was 110 ml. During the run, usually 2 days in duration, glycerine water at 4° was circulated in the jacket. For stabilization of the protein bands, a sucrose density gradient (0 to 28%) was used. The starting voltage was 700 V. After the focusing was completed, 2-ml fractions were collected and analyzed for enzyme activity and pH.

Polyacrylamide Gel Electrophoresis. A Beckman Model R-113 electrophoretic apparatus was used. The gel concentration was 7.5% in a 12 mM potassium phosphate-citrate buffer, pH 6.8. Samples (5 μl) containing a small amount of sucrose to increase the density, were applied to the top of the polymerized gels. The assembly was cooled with cold running tap water. The current was 45 ma. After 3 hr, the gel was stained by the technique of Burstone (2) for acid phosphatase at 37° for about 4 hr. Protein was measured by the method of Lowry et al. (10).

Tissue Extraction. The tissue extraction and purification procedures for prostatic acid phosphatase were carried...
out at 4°. Normal or cancerous tissue was thawed, minced, and extracted twice in a VirTis 23 homogenizer at top speed for 1-min intervals in 5 volumes of 0.05 M Tris-0.1 M KCl buffer, pH 6.5. The extract was centrifuged at 4000 x g for 20 min. The enzyme activity and the protein were determined from the supernatant. Greater than 95% of the total acid phosphatase activity in the crude homogenate is found in the supernatant after centrifugation at 4000 x g for 20 min.

**Chromatography.** The extract was passed through a Sephadex G-100 column (2.5 x 100 cm). Fractions (10 ml) were taken at a flow rate of 30 ml/hr with the use of 0.05 M Tris-0.1 M KCl buffer, pH 6.5, for elution. Enzyme activity and protein determination were carried out for each fraction. The fractions with enzyme activity were collected, dialyzed against 0.01 M phosphate buffer (pH 7.2), and adsorbed to a DEAE-cellulose column (1 x 40 cm) equilibrated in this buffer. After the column was washed with 0.01 M phosphate buffer, pH 7.2, the elution of the protein was carried out with a linear gradient made from 0.01 M sodium phosphate buffer, pH 7.2, and 0.175 M sodium phosphate buffer, pH 6.5, 250 ml each. Fractions (8 ml) were collected at a flow rate of 24 ml/hr; again enzyme activity and protein determination were carried out for each fraction.

**RESULTS**

Prostatic acid phosphatase activity in the supernatant of a homogenate of cancerous and normal prostate could be separated into 2 bands with different mobilities on polyacrylamide gel electrophoresis (Fig. 1, Slots 1 and 4). The slower band was quite narrow and appears to be less heterogeneous than the more quickly moving band. The latter was wider and seemed to contain several fractions of acid phosphatase; however, it was not possible to separate additional fractions from this band directly by gel electrophoresis. Visual observation suggested the ratio of acid phosphatase in the 2 electrophoretic bands in the supernatants of the extracts was different in normal and cancer tissue (Fig. 1). We also suspected that the faster band was a mixture of isozymes of different electrophoretic mobility.

Before attempting further electrophoretic or ion-exchange separation of potential isozymes, we first partially purified prostatic acid phosphatase from normal and cancerous tissue by chromatography on a Sephadex G-100 column. A typical elution pattern for both extracts is shown in Chart 1. There were 2 protein peaks; prostatic acid phosphatase eluted between the 2 major protein peaks. The recovery of the protein was usually 85 to 90%; the recovery of enzyme activity was usually 86 to 95%. Our pooled fractions from the Sephadex G-100 column generally contained about 30 to 40% of the initial protein.

Chromatography of the fractions from the Sephadex G-100 column with enzymatic activity on a DEAE-cellulose column resulted in 2 activity peaks. Chart 2 shows the prostatic acid phosphatase activity and the protein concentration in each fraction for normal and carcinomatous extracts. Table 1 shows the prostatic acid phosphatase content in a number of samples from normal and cancerous

![Chart 1](chart1.png)

Chart 1. Purification of normal (A) and cancerous (B) prostatic tissue on a Sephadex G-100 column. The centrifuged homogenate was eluted with 0.05 M Tris-0.1 M KCl buffer, pH 6.5. The flow rate was 30 ml/hr. O, prostatic acid phosphatase activity; ●, protein. Note difference in scales for A and B.

![Chart 2](chart2.png)

Chart 2. Chromatography of prostatic acid phosphatase on DEAE-cellulose columns. Approximately 50 ml of normal (A) or cancerous (B) prostatic eluate from the Sephadex G-100 column were applied to a DEAE-cellulose column. A linear gradient of sodium phosphate buffer, 0.01 M (pH 7.0) to 0.175 M (pH 6.0), was used to elute the enzyme. O, acid phosphatase activity; ●, protein. Note difference in scales for A and B.
prostatic tissue and the ratio of the 2 peaks separated by DEAE-cellulose chromatography. The ratio of the area of Peak 1/Peak 2 for the enzyme activity was between 1:2 and 1:6 in normal prostatic tissue. In the cancer tissue extract, the ratio was between 1:7 and 1:14. The ratio of the 2 peaks was found to be the only difference in the elution profile from DEAE-cellulose of the extracts of normal and cancerous prostatic tissue. The acid phosphatase content in benign hypertrophic tissue is slightly higher than in normal prostatic tissue. In a separate series, the mean value for the acid phosphatase concentration of 21 benign hypertrophic prostatic tissues was 154,285 ± 89,691 Sigma units/g tissue. The mean value of acid phosphatase concentration in 7 additional cancerous tissues was 15,200 ± 13,094 Sigma units/g tissue.

Fig. 2 shows the electrophoretic mobility on polyacrylamide gel of the fractions from the DEAE-cellulose column of the normal tissue extract. When Fractions 32 and 45 were mixed, there was a clear separation of these fractions following electrophoresis. Their mobility coincided with their position prior to mixing. No association between these fractions could be seen. As the molarity of the buffer required to elute the protein from the column increased, the electrophoretic mobility of the fractions increased (Fractions 32 to 45). There is some variation in the number of fractions with different electrophoretic mobilities depending on individual tissues used for the DEAE-cellulose column.

Separation of prostatic acid phosphatase isozymes could also be achieved on an isoelectric focusing column with the use of Ampholine, pH 3 to 6. Usually, 4 or 5 bands of enzyme activity were found from both normal and cancerous tissue (Chart 3). The range of the isoelectric points for the 1st 3 or 4 bands was 4.1 to 4.8. The 1st peak from the DEAE-cellulose column corresponded with the isozyme with isoelectric point 5.5. This is the slowest migrating isozyme in polyacrylamide gel electrophoresis (Fig. 1). When the enzyme from the 1st peak of the DEAE-cellulose column was subjected to isoelectric focusing, only 1 peak showing enzyme activity was detected with an isoelectric point of 5.5 (data not shown). As the isoelectric points of the fractions increase, the electrophoretic mobility of the same fractions decreases. Only when the fractions of prostatic acid phosphatase with a major difference in isoelectric points were mixed and again subjected to electrophoresis did 2 bands with no association between the fractions result. When Fractions 39 and 49 were mixed and run on polyacrylamide gel electrophoresis, a clear separation could be observed. No association between these fractions was detected (Fig. 3).

**DISCUSSION**

Human prostatic acid phosphatase for biochemical study is usually extracted from benign hypertrophic prostatic tissue because of the ready availability of this tissue. It had previously been shown that prostatic acid phosphatase

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**Table 1**

The concentration of prostatic acid phosphatase and the ratio of isoenzymes in normal and cancerous prostatic tissue

<table>
<thead>
<tr>
<th>Sample</th>
<th>Prostatic acid phosphatase (Sigma units/g)</th>
<th>Peak 1/Peak 2</th>
<th>Prostatic acid phosphatase (Sigma units/g)</th>
<th>Peak 1/Peak 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>155,000</td>
<td>0.50</td>
<td>9,400</td>
<td>0.11</td>
</tr>
<tr>
<td>2</td>
<td>125,000</td>
<td>0.33</td>
<td>26,000</td>
<td>0.09</td>
</tr>
<tr>
<td>3</td>
<td>168,000</td>
<td>0.20</td>
<td>15,000</td>
<td>0.16</td>
</tr>
<tr>
<td>4</td>
<td>152,000</td>
<td>0.25</td>
<td>7,000</td>
<td>0.07</td>
</tr>
<tr>
<td>5</td>
<td>135,000</td>
<td>0.16</td>
<td>15,000</td>
<td>0.08</td>
</tr>
<tr>
<td>6</td>
<td>80,000</td>
<td>0.20</td>
<td>18,000</td>
<td>0.14</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>135,000 ± 31,000</td>
<td>0.27 ± 0.12</td>
<td>15,066 ± 6,700</td>
<td>0.11 ± 0.04</td>
</tr>
</tbody>
</table>

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**Chart 3.** Isoelectric focusing of the supernatant of the tissue homogenate of normal (A) and cancerous (B) prostates. A linear sucrose (0 to 26%) gradient containing 1% Ampholine (pH 3 to 6) was used for the separation. The focusing was carried out at 4°. After 36 hr, 2-ml fractions were collected. The activity of the enzyme (○) and the pH (●) were measured. Note difference in scales for A and B.
from benign hypertrophic tissue is not homogeneous (12). At present, the number of isoenzymes has not been defined. Sur et al. (17) reported 13 different electrophoretic bands on starch gel electrophoresis.

Reif et al. (13) were unable to detect isozymes of prostatic acid phosphatase unique to prostatic carcinoma. To date, 2 differences in prostatic acid phosphatase content of normal and carcinomatous prostatic tissue are known. The 1st difference, the decreased enzyme content of the carcinomatous prostatic tissue, is well known (13). Normal prostatic tissue contains approximately 5 to 15 times more prostatic acid phosphatase than carcinomatous tissue. Our data (Table 1) confirm this observation. The 2nd distinction, quantitative differences in the prostatic acid phosphatase isoenzymatic composition of normal and carcinomatous tissue, is described in this paper.

We were able to separate prostatic acid phosphatase in the supernatant of the extract of both normal and tumor tissue into 2 distinct fractions by polyacrylamide gel electrophoresis. Partial separation of the isoenzymes can be achieved by chromatography on DEAE-cellulose columns. The ion-exchange separation yielded 2 major forms of prostatic acid phosphatase. The ratio of these 2 enzyme fractions is different in extracts of normal and carcinomatous tissue. The percentage of the prostatic acid phosphatase activity eluting at a lower salt concentration and exhibiting a slower electrophoretic mobility was decreased in prostatic carcinomatous tissue. The 2nd fraction of the enzyme, eluting at a higher salt concentration, is composed of several isoenzymes of prostatic acid phosphatase with different electrophoretic mobilities.

The supernatants of the extracts analyzed by isoelectric focusing on Ampholine, pH 3 to 6, had 4 or 5 distinguishable peaks. Electrophoresis of these fractions on polyacrylamide gel showed that each isoelectric focusing fraction had a different mobility. It was not possible to show any association between the isoenzymes by electrophoresis. Ostrowski et al. (12) had shown that neuraminidase treatment of prostatic acid phosphatase decreased the enzyme electrophoretic mobility and heterogeneity as a result of removal of the sialic acid moiety from the molecule. It may be possible that the linkage of sialic acid to the protein is modified in carcinomatous tissue, resulting in a different acid phosphatase composition. Other posttranscriptional modifications may, of course, be responsible for the different chromatographic and electrophoretic profiles. Alternatively, different gene products, regulated differently in the transformed tissue, may yield different patterns of acid phosphatase.

ACKNOWLEDGMENTS

We express our appreciation to Dr. T. Malinin for the tissue samples and to R. R. Malvaez for his excellent technical assistance.

REFERENCES

Fig. 1. Polyacrylamide gel electrophoresis of prostatic acid phosphatase preparations. Left to right: supernatant of the tissue extract from prostatic tumor; 1st peak of enzyme activity to elute from DEAE-cellulose column; pH 5.5 fraction from isoelectric focusing; supernatant of the tissue extract from normal prostate.

Fig. 2. Polyacrylamide gel electrophoresis of fractions from a DEAE-cellulose column (Chart 2A). Left to right: Fractions 32 and 45 combined; Fraction 45; Fraction 32; Fraction 35; Fraction 40; Fraction 43. See "Materials and Methods" for details.

Fig. 3. Polyacrylamide gel electrophoresis of fractions from isoelectric focusing (Chart 3A). Left to right: Fraction 49; Fractions 39 and 49 combined; Fraction 39; Fraction 43; Fraction 46; Fraction 49; Fraction 53; supernatant of tissue homogenate from normal prostate. See "Materials and Methods" for details.
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