Comparison of Acid Phosphatase Isoenzymes of Human Seminal Fluid, Prostate, and Leukocytes

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We used ion-exchange column chromatography and electrophoresis on polyacrylamide gel to compare the acid phosphatase isoenzymes of prostate and leukocytes. The major isoenzyme of the prostate is band 2A; only a trace of band 2B was observed. However, the major isoenzymes of leukocytes are band 4 and band 2B, and only a small amount of band 2A was observed. The three isoenzymes isolated from leukocytes or prostate gland react to the antiserum prepared against the acid phosphatase isoenzyme of seminal fluid. Acid phosphatases of leukocytes other than the three isoenzymes mentioned above did not interact with the antiserum.

Additional Keyphrases: chromatography • electrophoresis • enzyme activity • radioimmunoassay

The high concentration of acid phosphatase in human prostate gland observed by Kutchter and Wolberts (1) drew wide attention to the diagnostic value of serum acid phosphatase for prostatic cancer. Radioimmunoassay (2) and counterimmunoelectrophoresis (3) have been recommended recently as highly sensitive and specific methods for quantitative analysis of prostatic acid phosphatase in sera. An immunohistochemical method based on the antiserum specific to prostatic acid phosphatase was used by Jobsis (4) to demonstrate the prostatic origin of a metastasis.

The specificity of immunohistochemical methods is dependent on the explicit location of a specific acid phosphatase isoenzyme in the prostate. It is important to understand the relationship of acid phosphatase isoenzymes of other tissues that are antigenically similar to the prostatic acid phosphatase, so that the source of false-positive results is recognized. In our previous study (5) by electrophoresis on polyacrylamide gel, acid phosphatase isoenzymes from different human tissues showed a marked difference between prostatic acid phosphatase and isoenzymes from all of the other tissues, except leukocytes (6–8). A preliminary immunohistochemical study in which antiserum specific to prostatic acid phosphatase was used showed binding of antibody to granulocyte. The characteristic of the acid phosphatase isoenzyme in the prostate is the intense band 2 isoenzyme. Electrophoretic mobility alone, however, is not conclusive evidence that band 2 of granulocytes is identical to that of the prostate. In the previous study, band 2 was also isolated from seminal fluid and was the only isoenzyme (5). The antiserum to seminal fluid acid phosphatase was used to identify the prostatic isoenzymes in extracts of prostate and leukocytes after separation on a DEAE-Sepharose column. The isoenzymes that share similar antigenicity were compared according to their electrophoretic mobility and chromatographic properties.

Methods
Purification of Acid Phosphatase from Seminal Fluid

Excess seminal fluid specimens from a fertility clinic were pooled, diluted five-fold with citrate buffer (0.05 mol/L, pH 3.7), and centrifuged for 10 min at 10 000 × g to remove the cells. The soluble proteins in the supernatant fraction were fractionated by ammonium sulfate precipitation. The proteins that precipitated at between 50 and 75% saturation of ammonium sulfate were dialyzed against citrate buffer (10 mmol/L, pH 5.0), then transferred to a CM-Sepharose column (2 × 30 cm). The column was washed with 50 mL of NaCl (0.1 mol/L), then eluted by a linear concentration gradient of NaCl (0.1–0.5 mol/L in 400 mL of elution buffer). The active fraction was again precipitated by ammonium sulfate (50–75% saturation). The precipitated protein was dissolved in 5 mL of tris(hydroxymethyl)methylamine (Tris) buffer (10 mmol/L, pH 8.0), and dialyzed overnight against the same buffer. The sample was absorbed on a DEAE-Sepharose column (0.9 × 60 cm) and eluted by a linear concentration of NaCl (0.1–0.3 mol/L in 400 mL of Tris buffer). The protein in the active fractions was precipitated by ammonium sulfate (50–75% saturation). The precipitated protein was dissolved in 0.5 mL of citrate buffer (0.05 mol/L, pH 5.0) and passed through a

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Sephadex G-25 column (0.9 × 60 cm) to remove the ammonium sulfate. The active fraction was stored at -45 °C.

Antiserum

The specific antibody against acid phosphatase was prepared from rabbit by subcutaneous injection with 1 mg of acid phosphatase purified from seminal fluid. The antigen was mixed with complete Freund's adjuvant before injection. Booster injections were given intravenously until there was optimum precipitation of 125I-labeled acid phosphatase with 10 000-fold diluted antiserum.

Leukocytes

The sample used in this study was obtained by leukopheresis from a patient with myelomonocytic leukemia; we used the continuous-flow Amino Celltrifuge (American Instrument Co., Silver Springs, MD 20910). The cells (20 mL of packed cells) were suspended in 100 mL of Tris sulfate (10 mmol/L, pH 8.0) and subjected to freeze-thawing five times. The solubilized enzymes were separated from the cell fragments by centrifugation at 20 000 × g for 5 min. The supernatant fraction was used for column chromatography.

Prostate Gland

The sample was obtained from a patient with benign hypertrophy. The tissue was homogenized and extracted as previously described (5). The extract was dialyzed against Tris sulfate (10 mmol/L, pH 8.0).

DEAE-Column Chromatography (Prostate or Leukocytes)

dDEAE-Sepharose was packed in a glass column, 0.9 × 60 cm, and equilibrated in Tris sulfate, 10 mmol/L, pH 8.0. After the sample was adsorbed on the column, the column was washed with 100 mL of NaCl (0.05 mol/L), then eluted by a linear concentration gradient of NaCl (from 0.05 to 0.20 mol/L in a total volume of 400 mL). All salt solutions were prepared in Tris sulfate (10 mmol/L, pH 8.0).

Analyses

Analyses of acid phosphatase activity by spectrophotometric and electrophoretic methods were described previously (9). Protein concentration was determined by the method of Lowry et al. (10), with bovine serum albumin as standard. Prostatic acid phosphatase was radiolabeled for us by New England Nuclear, Inc. (North Billerica, MA 01862). The incubation medium contained 0.2 mL of sample, 0.1 mL of 125I-labeled acid phosphatase (from seminal fluid), and 0.2 mL of antiserum. A second antibody against rabbit gamma-globulin was used to facilitate the precipitation of the primary antigen-antibody complex. A standard curve was constructed from known concentrations of purified seminal fluid acid phosphatase.

Results

Purity of Antigen and Antiserum

During the present purification study of acid phosphatase in seminal fluid, more than 90% of the acid phosphatase detected by spectrophotometry was recovered as one peak in each chromatographic step. These results confirmed our previous observation (6) that there is only one acid phosphatase isoenzyme secreted to the seminal fluid. The enzyme isolated from seminal fluid showed only one protein band (Figure 1A), coinciding with the location of the band for enzyme activity (Figure 1B). The antiserum produced by injecting the purified enzyme into a rabbit formed a single precipitin band with the crude extract of the prostate, or with seminal fluid (Figure 2A, B). The precipitin band had strong acid phosphatase activity (Figure 2C, D).
Multiplicity of Acid Phosphatase in Prostate Gland

Column chromatography of the crude extract of the prostate showed one dominant peak (Figure 3, peak III) and a small, yet distinct, peak (I). Peak II was a small peak between peaks I and III. Electrophoresis on polyacrylamide gel (Figure 4) showed that peaks I and III corresponded to bands 4 and 2A, respectively. Peak II had electrophoretic mobility slightly faster than that of peak III. The isoenzymes under the three peaks are designated as bands 4, 2B, and 2A, according to their electrophoretic mobility.

Multiplicity of Acid Phosphatase in Leukocytes

Column chromatography separated the acid phosphatases of leukocytes into more peaks than those of prostate. The major peaks (Figure 5, peaks I, III, and VII) were not detectable by the electrophoretic method. Peak II corresponded to band 3, the major acid phosphatase of most tissues (Figure 6, gel 38). Radioimmunoassays with antisera specific to band 2 of seminal plasma showed positive results for fractions from peaks IV, V, and VI, but were negative to fractions from all other peaks. Electrophoresis showed that peaks IV, V, and VI corresponded to bands 4, 2B, and 2A, respectively (Figure 6).

The fractions that were active according to radioimmunoassay were analyzed for proteins and for enzyme activity by electrophoresis. Band 2B had many inactive protein bands (Figure 7B). Band 4 had one dominant protein band (Figure 7F) that coincided with the band for enzyme activity (Figure 7E). The amounts of inactive proteins in band 4 appeared to be very small. The specific activity of all the three isoenzymes was very low (Table 1).

Comparison of Isoenzymes of Different Origin

The isoenzyme isolated from seminal fluid is a highly purified enzyme preparation (Figure 1) with high specific activity. Because seminal plasma does not have the other isoenzymes, it was used to isolate a purified antigen to produce the specific antiserum against one isoenzyme. However, the
antiserum reacted with three isoenzymes that can be separated by electrophoresis or column chromatography. The isoenzymes of prostate and granulocytes were not purified preparations. They had low specific activity (Table 1). The crude extract was subjected once to column chromatography so that the relationship of different isoenzymes in the crude extract could be visualized.

The antigenicity of different isoenzymes was compared by radioimmunoassay. Each isoenzyme was mixed with the radiolabeled isoenzyme 2A (seminal fluid), then incubated with the antiserum. Only 1.6 mU of isoenzyme 2A of the prostate was required to displace 50% of the radiolabeled isoenzyme from the antibody. The amounts of isoenzymes 2B and 4 required for 50% displacement are higher than that of isoenzyme 2A.

**Discussion**

The results described above showed that the acid phosphatase of seminal fluid has antigenicity similar to the three isoenzymes (bands 2A, 2B, and 4) of the prostate. The prostatic isoenzymes are also present in leukocytes. However, the proportion of isoenzymes of the prostate differs from that of leukocyte isoenzymes. More than 90% of the prostatic acid phosphatase is band 2A; band 2B is observed only in a trace amount (Figure 3). In leukocytes, however, bands 2B and 4 were each more abundant than band 2A.

The electrophoretic mobility of band 2B (Figure 5, fraction 84) was identical to that of band 3 (Figure 5, fraction 38). However, the two bands were well separated by column chromatography (band 4 elutes between them). We showed previously (5) that band 3 is the major acid phosphatase of other human tissues. In column chromatography to separate acid phosphatases of spleen, kidney, and platelets, band 3 of these tissues was eluted from the column in the same location as peak II from leukocytes but did not react with the antisera of prostatic acid phosphatase. Other laboratories (3, 11) have also reported the different antigenicity between prostatic acid phosphatase and the major acid phosphatase of other tissues. The similarity of the antigenicity of band 2B and that of the major prostatic acid phosphatase (band 2A), and the different chromatographic separation of band 2B from band 3 of other tissues, led us to designate the activity in peak V (Figure 5) as 2B instead of band 3.

The identity between peak IV (Figure 5) of granulocyte and peak I (Figure 3) of prostate was confirmed by their affinity to DEAE-Sepharose and their electrophoretic mobility: only one band was observed when a mixture of the two isoenzymes was subjected to electrophoresis. The distinction between 2A and 2B was indicated by the gradual disappearance of band 2B after fraction 84 and the later appearance of 2A after fraction 100 (Figures 5 and 6). The small difference in electrophoretic mobility of band 2A and 2B was visible when a mixture of the two isoenzymes was subjected to electrophoresis.

Acid phosphatase concentration in seminal fluid and prostate was very high in the starting material, and we quite easily obtained purified isoenzyme preparations. In leukocytes, however, the acid phosphatase activity was low in the starting material; we did not expect to obtain highly purified fractions with one column chromatography step. Staining for protein bands after electrophoresis (Figure 7) showed high contamination of inactive proteins (Figure 7B) in fraction 115 “isoenzyme 2A.” However, there was not much inactive protein in fraction 72 “isoenzyme 4” (Figure 7F). The amount of inactive proteins in fraction 72 was too little to account for its low specific activity (98 U/g). The data indicate that the leukocyte enzymes have much lower catalytic activity and affinity to the antisera than do those of the prostate.

The occurrence of prostatic enzymes in leukocytes indicates that leukocytes can contribute to at least part of the acid phosphatase in serum detected by the immunochromatographic method. Perhaps prostatic acid phosphatase is also present in many other tissues in a very low concentration. The concentration in most tissues is so low that their interference with the diagnostic value of the immunochromatographic method is negligible. However, the amount of prostatic acid phosphatase in sera from apparently healthy men, as detected by immunochemical methods (2), is probably from leukocytes and other tissues rather than truly from the prostate.

Our previous studies (7, 8) show that band 2 appeared in the sera of patients with acute myelomonocytic leukemia. Therefore, sera of patients with acute granulocytic leukemia or monocytic leukemia, and pathologic fluids with high numbers of macrophages derived from monocytes, will give high values of prostatic acid phosphatase by immunochromatographic methods. The presence of prostatic acid phosphatase in granulocytes and monocytes also predicts a small binding of antiserum to these cells, which has been observed by the immunohistochemical method. Our data from immunohistochemical studies will be described in a future report.

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**Table 1. Comparison of the Isoenzymes in Granulocytes and from Prostate**

<table>
<thead>
<tr>
<th>Source of isoenzyme</th>
<th>Specific activity</th>
<th>Activity, mU, required to displace 50% of the tracer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seminal fluid</td>
<td>500.0</td>
<td>2.7</td>
</tr>
<tr>
<td>2A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2A</td>
<td>99.5</td>
<td>1.6</td>
</tr>
<tr>
<td>4</td>
<td>13.3</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Granulocytes

| 2A     | 0.15 | 39.8 |
| 2B     | 0.11 | 9.6  |
| 4      | 0.098| 9.5  |

U, micromoles of p-nitrophenyl phosphate hydrolyzed per minute in 1 mL of assay medium at 37 °C; specific activity, U per milligram of enzyme protein. Fifty-percent displacement of the tracer was determined by adding different amounts of unknown to the incubation medium.
Radial and Linear Thin-Layer Chromatographic Procedures Compared for Screening Urines to Detect Oligosaccharidoses

John McLaren and Won G. Ng

We describe a circular (radial) thin-layer chromatographic procedure for separating urinary oligosaccharides. Results were better than those obtained by a single linear development. Bands and specific patterns were finely resolved for various known oligosaccharidoses. The procedure provides a simple means of screening for these disorders.

Additional Keyphrases: inherited disorders • lysosomal storage disease

The term "oligosaccharidoses" refers to a group of genetic disorders in which there is a large excretion of oligosaccharides in the urine (1). The basic metabolic defects lie in a deficiency of lysosomal enzymes that degrade the carbohydrate moiety of glycoproteins and glycolipids (2–4). Some of these biochemical abnormalities can be readily demonstrated by thin-layer chromatographic (TLC) screening (5). Patients affected with fucosidosis, mannosidosis, and GM1-gangliosidosis have atypical and unique urinary oligosaccharide patterns, allowing these disorders to be distinguished from one another. Recently, Strecker and his associates have characterized the structures of many oligosaccharides found in the urine of patients affected with fucosidosis, sialidosis, and Sandhoff’s disease (6–8). More recently, an improved TLC procedure for detection of mannosidosis has appeared (9).

In this communication, we compare the radial TLC technique with the linear development technique for separating urinary oligosaccharides. Characteristic patterns were observed with urine of patients affected with GM1 gangliosidosis (infantile and adult types), fucosidosis, mannosidosis, sialidosis (types 1 and 2), mucolipidosis II (I-cell disease), and aspartylglycosaminuria.

Materials and Methods

Samples

Untimed urine samples were collected from eight normal children two to eight years old, from three ostensibly normal adults, and from patients with the various lysosomal storage diseases described. In all cases the biochemical defects were established. All urine samples were stored at −20 °C until chromatography.