Immunological and Biochemical Evidence for Identity of Tartrate-Resistant Isoenzymes of Acid Phosphatases from Human Serum and Tissues

Kwok-Wal Lam,¹ Paul Lee,¹ Chin-Yang Li,² and Lung T. Yam³

We purified acid phosphatase isoenzyme 5b from a human spleen affected by leukemic reticuloendotheliosis and used it to produce a specific antiserum. The antiserum was used to show complete immunological identity among isoenzymes 5a and 5b in human serum, and 5b isolated from a giant-cell bone tumor and from the spleen of a case of Hodgkin’s disease. Acid phosphatase 5b in a giant-cell bone tumor was isolated for biochemical characterization. Its pH optimum and substrate specificity were very similar to those of isoenzyme 5b from human spleen.

Additional Keyphrases: enzyme activity • leukemic reticuloendotheliosis (hairy cell leukemia) • tissue source of isoenzymes

“Acid phosphatase” (EC 3.1.3.2) includes several protein species sharing similar catalytic activity toward phosphoesters in an acidic medium. They are separable into isoenzymes 1 to 5, numbered in order of their increasing electrophoretic mobility toward the cathode. Isoenzymes 1, 2, 3, and 4 are inhibited by tartrate; isoenzyme 5 is not (1). Tartrate-resistant acid phosphatase activity was recognized first as a specific biochemical marker that could be exploited in the histochemical diagnosis of leukemic reticuloendotheliosis, also known as hairy cell leukemia (1). The enzyme was isolated from a spleen infiltrated with hairy cells, and its biochemical properties (2, 3) were shown to differ clearly from those of the tartrate-sensitive prostatic acid phosphatases and the tartrate-resistant acid phosphatase of erythrocytes. Since then, the histochemical method for isoenzyme 5 (as it has been designated) has been evaluated thoroughly, and shown to be very useful for confirming the diagnosis of hairy cell leukemia (4).

An improved electrophoretic procedure separates isoenzyme 5 into 5a and 5b (5), both of which are present in serum of normal adults. Isoenzyme 5b activity is very high in hairy cells and giant-cell tumor of bone (6). It is also high in serum of normal children during physiological bone growth, Gaucher’s disease (7), and in malignancies metastasized to bone (6). Strong tartrate-resistant acid phosphatase activity was also observed in some polymorphous cellular infiltrates in the spleen from a case of Hodgkin’s disease (8). Here we describe our efforts to confirm the identity of isoenzyme 5 isolated from different sources.

Materials and Methods

Preparation of purified antigen and antiserum: Isoenzyme 5b was isolated from a spleen infiltrated with hairy cells, as previously described (2). The purified preparation had a specific activity of 200 U/mg of enzyme protein, as compared with 0.002 U/mg of starting material. About 50 μg of the purified enzyme was mixed with 0.2 mL of complete Freund’s adjuvant and injected subcutaneously into a rabbit. One month later, booster injections of 50 μg, dissolved in isotonic saline, were given intravenously every other day for two weeks.

Partial purification of acid phosphatase isoenzymes: Isoenzymes 5a and 5b were isolated from serum of normal adults as previously described (5). Isoenzyme 5b of giant-cell tumor (osteoclastic origin) was purified as previously described for that from spleen (2). Erythrocytes were sedimented from heparinized blood by mixing 20 mL of blood with 10 mL of a 30 g/L solution of gelatin. The gelatin caused the erythrocytes to settle within 30 min. The supernatant fraction—containing plasma, platelets, and leukocytes—was discarded. The erythrocytes were washed with 50 mL of isotonic saline, then hemolyzed in water and subjected to sonic disruption for 30 s. The solubilized proteins were absorbed on a column of diethylaminoethyl-Sepharose and eluted with a linear concentration gradient of sodium chloride (0 to 1 mol/L) in 200 mL of tris(hydroxymethyl)methylamine buffer (10 mmol/L, pH 8.0). Over 90% of the acid phosphatase activity was eluted from the column in one peak. After electrophoresis on acrylamide gel, which was the method used to classify the other acid phosphatases (1, 9), the activity was found to be tartrate-resistant and undetectable (7, 9) by the colorimetric reaction in which naphthyl phosphate is used as substrate.

The unit of enzyme activity is defined as micromoles of p-nitrophenyl phosphate hydrolyzed per minute at 37 °C in 1 mL of citrate buffer (0.1 mol/L, pH 5.0). The kinetic constants were determined by using 10 different substrate concentrations, ranging between 0.3 and 2 mmol/L, according to the method described by Bliss and James (10). Enzyme activity was calculated from the rate of inorganic phosphate formation detected by the method of Fiske and SubbaRow (11).

Immunodiffusion: Agarose was dissolved to give a concentration of 10 g/L, and 3 mL of the solution was layered on a microscope slide. Sample wells were cut 8 mm apart. A 10-μL sample (containing 10 μL of enzyme activity) was placed in each well, and allowed to diffuse overnight in a humid chamber at room temperature. The slide was placed in 200 mL of gently stirring isotonic saline for 6 h. The precipitin band was stained for acid phosphatase activity by exposure to 5 mg of 1-naphthyl phosphate and 5 mg of Fast Garnet GBC dissolved in 10 mL of citrate buffer (0.1 mol/L, pH 5.0). The enzyme staining was stopped after one hour by placing the slide in dilute (80 mL/L) acetic acid.

Results

The antiserum formed a single precipitin band with isoenzyme 5b isolated from the spleen of a case of leukemic reticuloendotheliosis (LRE). This band had a strong acid phosphatase activity, hydrolyzing naphthyl phosphate to form a brown precipitate with Fast Garnet GBC (Figure 1). The enzyme activity in the precipitin band confirmed that the band resulted from interaction of antibody to an acid phosphatase. We used this isolated isoenzyme as a reference in comparing

¹ Departments of Ophthalmology and Biochemistry, Albany Medical College of Union University, Albany, NY 12208. Address communications to this author, at the Department of Biochemistry.
² Departments of Laboratory Medicine and Surgical Pathology, Mayo Clinic, Rochester, MN 55901.
³ Division of Hematology and Oncology, Veterans Administration Hospital, Louisville, KY 40202.

Received Oct. 31, 1979; accepted Dec. 7, 1979.
Fig. 1. Acid phosphatase isolated from different sources compared by immunodiffusion

the antigenicity of isoenzyme 5 isolated from different sources (Figure 1). Our immunodiffusion data show that isoenzymes 5a (Figure 1a) and 5b (Figure 1b) isolated from normal serum, 5b from giant-cell tumor (Figure 1c), and 5b from the spleen of a case of Hodgkin's disease (Figure 1d) are immunologically identical to isoenzyme 5b isolated from the reticuloendotheliosis spleen. The tartrate-resistant acid phosphatase of erythrocytes did not react with the antiserum. We also tested the reactivity of our antiserum to the tartrate-sensitive acid phosphatase (isoenzymes 2 and 4) of the prostate and to isoenzyme 3, the major isoenzyme of lymphocytes and other human tissues. None of these tartrate-sensitive isoenzymes formed a precipitin band with our antiserum.

Tartrate-resistant acid phosphatase activity in serum is only about 4 U/L. Thus it is difficult to obtain sufficient quantities of purified isoenzymes 5a and 5b from serum for biochemical characterization. We did isolate isoenzyme 5b from a giant-cell tumor of bone for biochemical characterization. The purified enzyme had optimal activity at pH 5.5 (Figure 2). It showed similar catalytic activity toward organic pyrophosphatases (ATP, ADP), inorganic sodium pyrophosphate, and monophosphoester of aromatic alcohols (1-naphthyl phosphate, and p-nitrophenyl phosphate) as compared to the enzyme isolated from human spleen described previously (2) (see Table 1). It was inactive toward monophosphoesters of alkyl alcohol such as glycerol phosphate and AMP. These characteristics are very similar to those of isoenzyme 5b of hairy cells (2).

Discussion

Tartrate-resistant acid phosphatase activity is present in erythrocyte, plasma, osteoclasts, and hairy cells. The erythrocytic isoenzyme is characterized by its low relative molecular mass (<20 000) (12) and lack of activity toward 1-naphthyl phosphate (13), characteristics that are quite different from those of the isoenzyme 5 of plasma (5, 14), osteoclasts, and hairy cells (2). Isoenzyme 5 has a relative molecular mass of about 70 000 and has similar reactivity toward 1-naphthyl phosphate and p-nitrophenyl phosphate (2). The biochemical properties of isoenzyme 5 of giant-cell tumor described in this report are very similar to those of plasma (2) and hairy cells (2), but differ from those described for the enzyme from erythrocytes (12).

The antigenic identity of isoenzyme 5 isolated from different sources is consistent with the biochemical data and confirms the identity of the isoenzyme 5 from serum, giant-cell tumor, spleen of Hodgkin's disease, and hairy cells. The tartrate-resistant acid phosphatases of human tissues clearly are not identical with isoenzyme 5. Although both hairy cells and osteoclasts have strong isoenzyme 5b activity, there are functional differences between these two types of cells. Acid phosphatase activity of the hairy cell is confined to subcellular granules (15) and does not leak out into the blood; in contrast, isoenzyme 5b of osteoclasts is released into the blood during pathological bone growth and in malignancies metastasized to bone (5, 6).

The presence of isoenzyme 5 in various tissues is of con-

![Fig. 2. Relation between buffer acidity and isoenzyme 5b activity](image)

| Table 1. Kinetic Properties of Acid Phosphatase Isoenzyme 5b Isolated from Osteoclast Bone Tumor |
|---------------------------------|-----------------|-----------------|
| Substrate                       | $V_m$           | $K_m$, mmol/L   |
| ATP                             | 11.1 ± 0.7      | 0.53 ± 0.1      |
| AMP                             | 0.0             | 0.0             |
| Pyrophosphate                   | 8.6 ± 0.8       | 0.38 ± 0.1      |
| 1-Naphthyl-PO$_4$               | 10.4 ± 1.0      | 1.41 ± 0.3      |
| p-Nitrophenyl-PO$_4$            | 18.4 ± 1.2      | 1.1 ± 0.2       |

$V_m$: micromoles of substrate hydrolyzed per minute, per milligram of enzyme protein.
considerable diagnostic significance if assays of this isoenzyme are to be properly performed and interpreted. Diagnosis of hairy cell leukemia is based on the detection of isoenzyme 5 in hairy cells, either by the histochemical study method or by electrophoretic separation of the acid phosphatases in the leukocyte. On the other hand, osteoclastic activity in young children and in patients with malignant diseases is best examined by assessing isoenzyme 5 activity in serum. Such assessment can now be made quantitatively by polyacrylamide gel electrophoresis (14). The present data demonstrating the specific immunological properties of isoenzyme 5, and that it is different from isoenzymes 2, 3, and 4, indicate that quantitative immunochemical assays would be useful for measuring the minute amounts of the isoenzyme in serum.

We are grateful to Mrs. Linda Lai and Mrs. Julia Chen for technical assistance. This study was supported by research grant GM 22960 from NIH and by a grant from the Medical Research Service of the Veterans Administration.

References