ABSTRACT

Acid phosphatase (phosphoric monoester hydrolase) was isolated from the Dunning R3327H prostatic adenocarcinoma, a slow-growing and hormone-sensitive rat prostate tumor histologically similar to well-differentiated human prostatic cancer. The enzyme was purified to homogeneity and characterized. In comparison with the acid phosphatase isolated from human malignant prostate, the acid phosphatase from the Dunning rat tumor was similar in molecular weight [100,000 ± 10% (S.D.)]. However, it possessed a single isoelectric point of 7.6 (human prostatic acid phosphatase showed multiple isoenzymes at 4.4 to 5.3); an electrophoretic mobility of 0.5 in reference to human prostatic acid phosphatase on 7.5% polyacrylamide gel, pH 8.5; an optimal pH of 5.0 with α-naphthyl phosphatase as the substrate in 0.1 M citrate buffer (human prostatic acid phosphatase, 5.5); and a $K_m$ (α-naphthyl phosphate) of $6.9 \times 10^{-4}$ M (human prostatic acid phosphatase, $4.4 \times 10^{-5}$ M). Furthermore, it did not cross-react with antisera raised against human prostatic acid phosphatase. These results show that the acid phosphatase of the Dunning R3327H prostatic adenocarcinoma is biochemically and immunologically distinct from human prostatic acid phosphatase and may be unique for this animal model of prostatic cancer.

INTRODUCTION

Several important therapeutic concepts of prostate cancer have been derived from studies utilizing animal models (5). Although evaluations of animal models alone are insufficient to determine the full clinical implication of a therapeutic approach due to the variations between human and animal cancer, study of the animal model can provide some unique opportunities that are difficult to obtain from human clinical studies within a reasonable period of time and ethical limitations. Investigation of an appropriate animal model is, therefore, of critical importance in searching for new effective treatment of human prostate cancer.

The Dunning R3327H rat prostate adenocarcinoma has been used as an animal model for human prostatic cancer (11). This rat prostate tumor was histologically similar to well-differentiated human prostatic cancer. The purpose of this study was to isolate and to characterize the acid phosphatase from Dunning R3327H prostatic adenocarcinoma and to compare its properties with acid phosphatase isolated from human malignant prostate (3, 7).

MATERIALS AND METHODS

Materials. The R3327H prostatic tumor grown in male Copenhagen × Fischer F1 rats was supplied through the National Prostatic Cancer Project by Dr. Norman Altman of the Papanicolaou Cancer Research Institute at Miami, Fla. This is a slow-growing and hormone-sensitive prostatic adenocarcinoma of the rat. The tumor was air shipped in a dry ice container to us. Bovine serum albumin, Fast red salt B, Fast garnet GBC salt, α-naphthyl phosphate, p-nitrophenyl phosphate, thymolphthalein monophosphate, phosphoryl choline, AMP, and α-methyl-D-mannopyranoside were purchased from Sigma Chemical Co., St. Louis, Mo. CON A-Sepharose and Sephadex G-200 were the products of Pharmacia Fine Chemicals, Inc., Piscataway, N. J. Acrylamide and DEAE-cellulose were purchased from Bio-Rad Laboratories, Richmond, Calif. Diaflo PM membrane ultrafilters were obtained from Amicon Corp., Lexington, Mass. Ampholine and isoelectrofocusing equipment were purchased from LKB Instruments, Inc., Rockville, Md.

Purification of Acid Phosphatase from Dunning R3327H Rat Prostate Tumor. Tumor tissues were thawed; minced; mixed with 0.02 M sodium acetate buffer, pH 5.2, containing 0.01% Tween 80 (3 ml/g tissue); and homogenized with Omnimixer (Ivan Sorval, Inc., Norwalk, Conn.). The purification procedure was similar to that for human prostatic acid phosphatase as reported previously (7). Briefly, acid phosphatase was extracted from Tween 80 solution by precipitation with 40 to 75% saturation of ammonium sulfate. The crude acid phosphatase preparation was purified first by an affinity chromatography on CON A-Sepharose followed by a DEAE-cellulose anion exchange column and finally with a gel filtration twice of Sephadex G-200.

Human Prostatic Acid Phosphatase. The purification of acid phosphatase and the production and specificity of antisera to purified human prostatic acid phosphatase have been reported elsewhere (3, 7).

Disc Electrophoresis on Polyacrylamide Gel. Gel electrophoresis was performed by the method of Davis (6) in a standard 7.5% polyacrylamide gel. Thirty μg of purified acid phosphatase with 25% sucrose were applied to the polyacrylamide gel column (5 × 70 mm). Electrophoresis was carried out in 0.05 M Tris-HCl buffer, pH 8.5, at 4° with a constant current of 5 mA/tube for 1 hr. The protein and enzyme activity staining was performed as previously described (3, 7).

Acid Phosphatase Assay. Acid phosphatase enzyme activity in chromatographic fractionation and isoelectric focusing was determined by the method of Babson and Phillips (1).

Preparative Isoelectric Focusing. This was performed similarly as reported previously (4, 13) using LKB 8100 ampholine and the manufacturer’s recommended procedure. The capacity

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1 The abbreviations used are: CON A, concanavalin A; P1, isoelectric point.
of the column was 110 ml, and 500 μg of purified Dunning tumor acid phosphatase were applied in this study. After electrophoresing for 24 hr with a voltage of 700 V, 1-ml fractions were collected with the aid of a peristaltic pump and were determined for acid phosphatase activity and pH.

**Immunoelectrophoresis and Double Gel Diffusion.** Immunoelectrophoresis was run on commercially available agarose plates (Behring Diagnostic) in a barbital buffer, pH 8.2, with 0.01 ionic strength. Double gel diffusion was performed in Petri dishes-containing 1% agarose in phosphate-buffered saline (0.02 M phosphate, 0.15 M NaCl, pH 7.0) (3, 7, 13).

**Other Analytical Methods.** Protein concentration was determined by the method of Lowry et al. (9) using bovine serum albumin as the standard. The pH optimum of purified acid phosphatase was determined using 3 mM α-naphthyl phosphate in 0.1 M sodium citrate with various pH (1, 14). Study of substrate specificity was performed as previously reported (14) using the following substrates: p-nitrophenyl phosphate; thymolphthalein monophosphate; phosphoryl choline; AMP; and α-naphthyl phosphate. K_m was calculated following the method of Lineweaver-Burk (8).

**RESULTS**

**Purification.** Acid phosphatase of Dunning R3327H rat prostate adenocarcinoma was purified to homogeneity by our previous procedure for human prostate acid phosphatase (7). Unlike the purification for human prostatic acid phosphatase, only one enzyme activity peak was constantly observed in the chromatographic profiles of CON A-Sepharose, DEAE, and Sephadex G-200 (Charts 1, 2, and 3).

**Chart 1.** CON A-Sepharose chromatography (2 x 40 cm) of the 40 to 75% ammonium sulfate-precipitated material from extract of Dunning R3327H prostatic tumor tissue. Elution was conducted initially with starting buffer, pH 5.0 (1 mM each for CaCl<sub>2</sub>, MgCl<sub>2</sub>, and MnCl<sub>2</sub>, and 0.1 M each for NaCl and CH₃COONa) and then with (arrow) a linear 0.1 to 0.5 M gradient of α-methyl-D-mannopyranoside (αMEM). The flow rate was 44 ml/hr, and 3-ml fractions were collected per tube. Acid phosphatase activity was monitored by absorbance at 588 nm (A), and protein was monitored by absorbance at 280 nm (•). Fractions-containing acid phosphatase activity (Tubes 135 to 165) were pooled and subjected to DEAE-cellulose chromatography.

**Chart 2.** Chromatographic profile of the DEAE-cellulose column (2 x 46 cm). Elution was carried out with a linear gradient of 0.02 M sodium phosphate, pH 7.0, to 0.02 M sodium phosphate, pH 8.0, containing 0.05 M NaCl. Flow rate was 25 ml/hr and 3 ml/tube. NaCl gradient (----) was monitored by a conductivity meter. Each fraction was assayed for acid phosphatase activity at 588 (A) and for protein absorbance at 280 nm (•). Fractions (Tubes 70 to 100) were pooled and further chromatographed on Sephadex G-200 gel filtration.
Table 1 summarizes the results of a typical purification of Dunning R3327H prostatic acid phosphatase, which demonstrates specific activity increasing from 6.6 to 1146 units/mg with a 175-fold purification and 28% recovery of the initial enzyme activity. Disc polyacrylamide gel electrophoresis showed a single enzyme activity band corresponding to a single protein band. The electrophoretic mobility was approximately 0.5 in reference to human prostatic acid phosphatase. Molecular weight of the purified enzyme was estimated to be 100,000 ± 10% (S.D) by Sephadex G-200 gel filtration using aldolase (M.W. 158,000), human prostatic acid phosphatase (M.W. 100,000), ovalbumin (M.W. 45,000), and chymotrypsinogen A (M.W. 25,000) as the marker proteins.

Characterization. The activity of Dunning tumor acid phosphatase was measured at various pH in 0.1 M citrate buffer containing 3 mM α-naphthyl phosphate as the substrate. Maximum hydrolysis was detected at pH 5.0 while human prostatic acid phosphatase occurred at 5.5 under identical substrate and buffer conditions as shown in Chart 4.

Isoelectric focusing revealed that Dunning R3327H acid phosphatase exhibited a single PI value of 7.6 as compared to multiple PI values at pH 4.4 to 5.3 for human prostatic acid phosphatase (4).

The effects of various ions and organic compounds on the enzyme activities of Dunning tumor acid phosphatase are presented in Table 2. Hydrolysis of α-naphthyl phosphate by Dunning rat or human acid phosphatase at optimal pH, 5.0 or 5.5, was lightly activated by Zn²⁺ but moderately inhibited by Mg²⁺ and formaldehyde. Cu²⁺ and ethanol demonstrated a slight activation of Dunning acid phosphatase activity. Both enzymes were substantially inhibited by tartrate although Dunning tumor acid phosphatase showed a greater sensitivity to tartrate inhibition.

The Kₘ of Dunning tumor acid phosphatase using phosphomonoesters as the substrates as calculated by the Lineweaver-Burk method are shown in Table 3. Both Dunning tumor and human prostatic acid phosphatase had the highest affinity toward α-naphthyl phosphate among the substrates tested.

The immunological cross-reactivity of Dunning tumor acid phosphatase against anti-human prostatic acid phosphatase antiserum was studied by double gel diffusion (at 40 µg) and immunoelectrophoresis (at 20 µg). Dunning tumor acid phosphatase was shown to exhibit no immunological reactivity with the antiserum. The protein concentrations used were more than sufficient for precipitin formation inasmuch as human prostatic acid phosphatase at 20 and 10 µg, respectively, produced a clear and sharp precipitin line in each experiment.

**DISCUSSION**

The Dunning R3327H prostatic adenocarcinoma has been used as a suitable animal model for study of prostate cancer
as it fulfills many criteria of human prostate cancer (5, 11). Isolation and characterization of acid phosphatase from this commonly used animal prostate tumor, however, have not been described. By means of our previously reported purification procedures for human prostatic acid phosphatase, a homogenous acid phosphatase preparation has been isolated from the Dunning rat prostate adenocarcinoma as demonstrated by acrylamide electrophoresis showing a single protein band corresponding to a single enzyme activity band.

The molecular weight of Dunning tumor acid phosphatase is approximately 100,000. The binding to CON-A-Sepharose and its elution with α-methyl-mannopyranoside suggest that it is a specific interaction between this lectin and the enzyme possibly through a carbohydrate moiety and that the enzyme may be a glycoprotein similar in nature to human prostatic acid phosphatase (2, 7).

Study of substrate and inhibitor specificity reveals some characteristics of Dunning tumor prostatic acid phosphatase. The enzyme is capable of hydrolyzing many organic phosphates at acidic pH and shows preferential hydrolysis of α-naphthyl phosphate. Tartrate is shown to be the most effective inhibitor of this enzyme. The major physicochemical difference between Dunning and human acid phosphatases is the observation from isoelectric focusing study that Dunning rat prostate adenocarcinoma exhibits a single PI at 7.6 while human prostatic acid phosphatase possesses multiple PIs at pH 4.4 to 5.3 (4), suggesting that the Dunning enzyme may contain more basic amino acid residues in its molecule. This is further indicated by its slower mobility, 0.5 in reference to that of human prostatic acid phosphatase in 7.5% disc polyacrylamide gel electrophoresis at pH 8.5.

Acid phosphatase of normal rat prostate has been studied previously by other laboratories. Paul and Richardson (10) detected by electrophoresis and DEAE chromatography 2 acid phosphatase isoenzymes, referred to as α and β, in prostate glands of normal male rats. α-Acid phosphatase was shown to move toward the cathode at pH 5.0 and was inhibited by tartrate similarly to the presently purified Dunning rat tumor acid phosphatase. However, α-acid phosphatase was not bound to DEAE anion-exchange column. The presently reported Dunning rat tumor acid phosphatase was tightly bound to DEAE anion-exchange column. β-Acid phosphatase was shown to bind to DEAE column, but it was insensitive to tartrate inhibition. These results were confirmed by Vahna-Pertutti et al. (12) who designated after electron microscopy study these 2 isoenzymes as lysosomal and secretory acid phosphatase in normal rat prostate, respectively. These previously reported results along with our data, therefore, clearly show that the Dunning tumor acid phosphatase is different from normal rat prostatic acid phosphatases.

At present, it is not known whether the difference between acid phosphatase from normal rat prostate and Dunning rat prostatic adenocarcinoma is due to the different species of rat, age of animal, the different method of purification, or neoplastic transformation. Studies aimed at resolving these questions are underway in our laboratory.

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Purification and Characterization of Acid Phosphatase from Dunning R3327H Prostatic Adenocarcinoma

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