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AXEL ZWIRNER, JÜRGEN SEITZ and GERHARD AUMÜLLER

**BIOCHEMICAL AND IMMUNOLOGICAL RELATIONSHIPS  
OF PROSTATIC AND LEUKOCYTIC ACID  
PHOSPHATASES AND THEIR SUBCELLULAR LOCALIZATION\***

*Department of Anatomy and Cell Biology, Philipps-Universität, Robert-Koch-Str. 6, D-3550 Marburg, F.R.G.*

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Human acid phosphatases from various sources were purified by a new rapid procedure including chromatofocusing and gel permeation chromatography on an FPLC system.

Isoenzymes 2a and 4 (*M*, 105 000 and 76 000, respectively) are the prevalent acid phosphatases in prostate, leukocytes and bone marrow. Additional forms are proteolytic products formed during isolation procedure. The source of elevated acid phosphatase in blood serum in metastazing prostatic carcinoma can be either prostate itself, the tumour or, more likely, bone marrow cells destroyed during the invasion by the tumour cells.

Distribution of isoenzymes is not organ specific. It was found using an antiserum against isoenzyme 4 that immunoreactivity is confined to the specific secretory granules both of prostatic epithelium and leukocytes.

Our previous studies of acid phosphatases from different human organs resulted in the identification of three different groups of isoenzymes, none of which was restricted to the prostate [1]. Also, no specific inhibitor has been found that would selectively inhibit one particular enzyme without affecting the others. Hence,

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it was not surprising to find out that acid phosphatase isoenzymes extracted from prostate, seminal fluid and human leukocytes are immunologically identical [1]. Numerous isoenzymes of acid phosphatase from human prostate are not affected by the endogenous or exogenous proteases. The microheterogeneity of the enzymes is due to neuraminic acid residues present in different amounts in the acid phosphatase molecule [1]. Ostrowski *et al.* [2] described a stock molecule with pI of about 6.5 which is formed from different isoenzymes after degradation of sialic acid residues. According to Lam *et al.* [3] the sialic acid-deprived molecule is identical with isoenzyme 4.

In the present study we have purified acid phosphatases from various sources using a rapid FPLC method and we have found that depending on the method used the prevalent isoenzyme in the highly selected leukocyte preparations is either form 2a or form 4. This stresses the lack of organ specificity of this enzyme and is of particular interest with regard to genomic regulation of acid phosphatases synthesis in different organs.

#### MATERIALS AND METHODS

*Tissues.* Bone marrow was obtained from a knee joint removed during joint surgery; prostate tissue was obtained from specimens of benign prostatic hyperplasia. Neutrophil polymorphic nucleic leukocytes were enriched by gradient centrifugation from fresh blood (about 500 ml from a volunteer healthy donor).

*Analytical methods.* Determination of acid phosphatase, protein, isoelectric focusing, SDS-polyacrylamide gel electrophoresis, protein titration and incubation conditions were as described previously [1].

*Gel permeation chromatography and chromatofocusing with the FPLC system.* Tissue samples were homogenized in 0.025 M citrate buffer (pH 4.8). The homogenate was centrifuged for 30 min at 100 000 g and the supernatant was filtered through a membrane filter (0.2 µm pore size). Aliquots of 100 - 300 µl were injected into a Superose 12 column mounted on a Pharmacia FPLC apparatus and eluted with 35 ml citrate buffer. The flow rate at 6°C was 0.5 ml/min. Maximal pressure was 2.0 MPa. Fractions of 1 ml were collected and further assayed.

For chromatofocusing, using a Mono P column, samples were prepared as above. The buffer consisting of 0.025 M bis-Tris and 4% taurine was adjusted to pH 7.1 with 0.1 M HCl. After injection of the 200 - 500 µl samples, proteins of pI values above 7.15 were eluted with 4 ml of the starting buffer. Thereafter a pH gradient (range 7.15 - 3.85) was built up by injecting 46 ml of the elution buffer containing 10 ml polybuffer PBE 74 and 4% taurine adjusted to pH 3.85. Proteins were eluted according to their respective pI values. Residual proteins were washed out by repeated injection of 2 M NaCl.

*Immunological procedures.* Antibodies against prostatic acid phosphatases (105 000) and leukocyte phosphatase (105 000) were raised in rabbits using repeated

multiple intradermal injections of highly purified antigen, emulsified with Freund's adjuvant. Antisera were checked by immunoprint analysis through Western blotting [4] and used in a postembedding staining procedure for immunoelectron microscopy. The antibody-binding site was visualized with either ferritin-labelled anti-rabbit IgG or colloidal-gold-labelled protein A as a second immunoreagent (for details see [1]).

#### RESULTS AND DISCUSSION

Comparison of gels stained for proteins and acid phosphatase activity subsequent to analytical isoelectric focusing revealed a multi-band distribution of the enzyme protein (Fig. 1A, B). The predominant proportion of proteins was represented by 2 - 3 bands eluted at pH 4.6 - 4.8. The remaining nine bands of isoenzymes displayed a very strong enzymatic activity while the protein staining was low due to a low protein concentration of the respective bands. Their molecular weight was 50 000 or 46 000, with respect, to a native molecular weight of about 106 000. These isoenzymes showed a very high pH stability. Using a protein titration technique it has been shown that the isoenzymes are active over a broad range of pH values and are not inactivated between pH 2.5 and 9.5. The titration plot at pH 2.4 is common for all isoenzymes. The neutralization of the neuraminic acid residues (incubation with *Clostridium perfringens* neuraminidase) with a  $pK$  of 2.2 i.e. below pH value of 2.4, resulted in the electrophoretic comigration of all isoenzymes. An antibody raised against secretory acid phosphatase from human prostate gave a nearly exclusive immunoreaction with the 50 000 subunit. The antibody preparation contained serum albumin to stabilize the enzymatic activity. Thus a distinct reaction can be seen with a protein at the albumin level on the nitrocellulose paper in addition to acid phosphatase.

Comparison of zymograms of prostate acid phosphatase and of the enzymes from other human tissues showed that the prostatic isoenzymes were also present in the other human organs tested, e.g. leukocytes or bone marrow. Therefore organ specificity of "prostatic" acid phosphatase is unlikely.

Elevation of serum acid phosphatase has long been regarded as an useful indicator in the diagnosis of metastatic carcinoma of the prostate [5]. As it has been pointed out by Lam *et al.* [3, 6] the occurrence of "prostatic" acid phosphatase in leukocytes may indicate that leukocytes can contribute to at least part of the acid phosphatase in serum detected by immunochemical methods. It is conceivable that during the invasion of bone marrow by metastasizing prostatic carcinoma leukocytes are destroyed and their content is released to the blood stream. The relatively low concentration of acid phosphatase in prostatic cancer cells [7], the increased serum levels in degenerative bone processes [8], the sometimes extreme circadian changes in serum levels in prostate cancer patients [9], and the detectable levels in

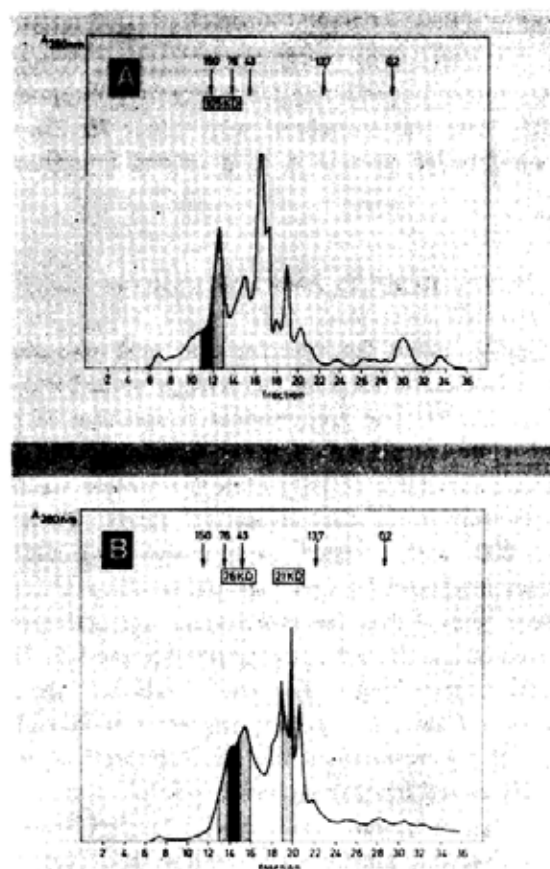


Fig. 1. Elution profile of acid phosphatase from human prostate (A) and from human neutrophil leukocytes (B) from the Superose 12 column. 0.6 mg and 0.96 mg protein, respectively, was applied. Buffer: 0.09% sodium citrate pH 4.8; elution velocity 1 ml/min; fraction size 1 ml. Reference proteins: IgG (M, 150 000), transferrin (76 000), ovalbumin (43 000), RNAase (13 700), tryptophan (200). Hatched and dotted areas: strong and medium activities of acid phosphatase, respectively.

serum of all prostatectomy patients and in women [10] are clearly in favour for the suggestion of a leukocyte-derived acid phosphatase, in all these cases.

We have therefore applied two different approaches to purify acid phosphatases very rapidly from different sources: chromatofocusing and/or gel permeation chromatography on Superose 12 using an FPLC system.

Depending on the freshness of the tissue used, the prostate yielded different proteins with acid phosphatase activity. By chromatofocusing two forms were isolated, one with pI ranging from pH 5.0 to 4.0 and another with pI at pH 6. On

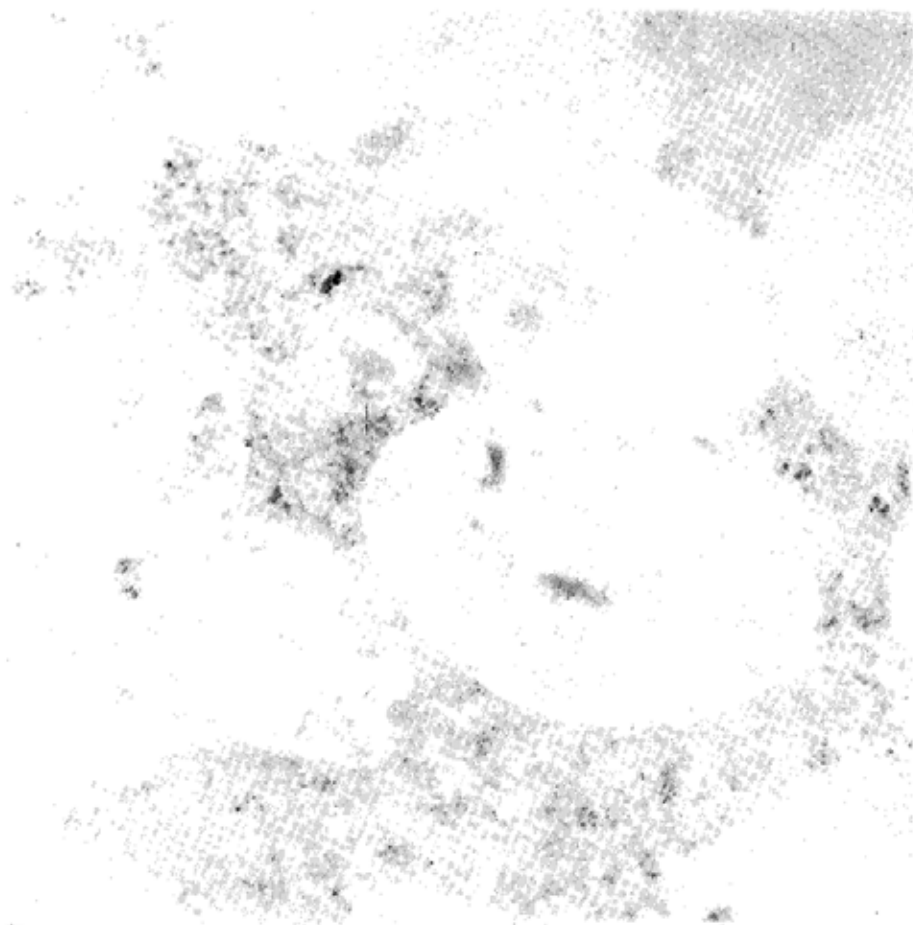


Plate 1. Immunoelectron microscopic localization of acid phosphatase in secretory granules of human prostate cells. Primary antibody against 105 000 raised in rabbit. Secondary antibody was ferritin-labelled ant-rabbit IgG. Magnification 41 000.



Plate 2. Ultrastructural localization of 105 000 antigen in neutrophil leukocytes. Magnification 41 000.

Superose 12 column the 105 000 form was obtained, when fresh tissue was used, and the 85 000 form from tissue samples stored frozen for more than 10 weeks. The storage obviously leads to proteolytic fragmentation without major changes in the net charge of the molecule.

When highly enriched neutrophil leukocyte preparations were analysed, acid phosphatase of a molecular weight of 76 000 (representing isoenzyme 4) was predominant, in addition to the enzyme of 105 000. As in the prostate, one minor fragment of 21 000 was found in the specimens after prolonged storage (Fig. 1A, B). Using an antiserum against the 105 000 isoenzyme (2a) we have localized the antigen at the ultrastructural level in prostatic secretory cells and in leukocytes. In both cases the immunoreaction was confined to the specific exocytotic granule, of the respective cells (Plates 1 and 2).

FPLC-analysis of acid phosphatase isoenzymes of blood serum in prostatic carcinoma and in bone marrow (from a 60 year old woman) indicated the prevalence of isoenzyme 2a (105 000) in bone marrow; the isoenzyme of reticulocytes (pI 6.9) was also present in considerable amounts.

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