Inhibition of Leukocyte Acid Phosphatase by Heparin

Lawrence R. DeChatelet, Charles E. McCall, M. Robert Cooper, and Pamela S. Shirley

Low concentrations of heparin (<1 unit/ml) inhibit by 80% the activity of leukocyte acid phosphatase. Leukocyte β-glucuronidase is less sensitive to heparin, and alkaline phosphatase and lysozyme are unaffected. The possible inhibitory effects of heparin should be considered in all measurements of leukocyte enzymatic activities.

For some time we have been concerned with the role of alkaline and acid phosphatases (orthophosphoric monooest- er phosphohydrolases, EC 3.1.3.1 and EC 3.1.3.2, respectively) in polymorphonuclear leukocytes, particularly as regards variation in disease states and factors that might regulate the activity within the cell (1-4). Here we demon- strate that relatively small concentrations of heparin profoundly inhibit leukocyte acid phosphatase, but have no effect on the alkaline-enzyme active.

Materials and Methods

Leukocytes were isolated from 50 ml of heparinized venous blood by sedimentation of the red blood cells with plasma gel (HTI Corp., Buffalo, N.Y. 14202) as previously described (1). The leukocyte pellet was washed 2× with saline (9 g/liter) and subjected to hypotonic lysis to remove contaminating red blood cells. These procedures were likewise presumed to remove traces of heparin present in the original blood sample. The cells were suspended in 5.0 ml of phosphate buffered saline, disrupted by sonication, and centrifuged at 27,000 × g for 15 min as previously described (1). The clear supernatant fluid was assayed im- mediately for enzyme activity.

Phosphatase activity of the sonicates was determined by mixing 0.50 ml of substrate solution (15 mmol of p-nitro- phenylphosphate per liter) with 0.50 ml of the appropriate buffer:

Acetate, 0.1 mol/liter, was used over the pH range 3.5 to 5.0.

Tris(hydroxymethyl)aminomethane-maleate (Tris- maleate), 0.1 mol of Tris acid maleate per liter (5) was used over the pH range 5.5 to 8.0.

2-Amino-2-methyl-1-propanol, 0.1 mol/liter, was used over the pH range 8.5 to 11.0.

The pH of the buffers was determined at 25°C although reactions were routinely incubated at 37°C.

Heparin was obtained from Sigma Chemical Co., St.

Louis, Mo. as the sodium salt (15 USP units/mg). It was dissolved in de-ionized water and added in a small volume to appropriate tubes. De-ionized water was added to all tubes to give a final incubation volume (after the addition of sonicate) of 1.50 ml. All tubes were equilibrated at 37°C for 10 min. A typical experiment was started by adding 0.10 ml of sonicate (containing 0.20 mg of protein) and stopped after 30 min by adding 10.0 ml of 0.1 molar sodium hydroxide. p-Nitrophenol released by the reaction was measured from its absorbance at 410 nm in a Model DB split-beam spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif. 92634); the instrument was zeroed on a control consisting of a replicate sample in which the NaOH was added before the sonicate. Absorbance values were converted to μmoles of p-nitrophenol by reference to a standard curve.

We assayed for β-glucuronidase activity (EC 3.2.1.31) by the method of Plaice (6), and for lysozyme (EC 3.2.1.17) by generally accepted methods (7). We estimated the protein content of sonicates by the biuret method of Gornall et al. (8), with bovine serum albumin as a stan- dard.

Results

The total soluble p-nitrophenyl phosphatase activity of the leukocytes was determined over the pH range of 3.5 to 11.0 in both the presence and absence of heparin. The re- sults (Figure 1) indicate that acid phosphatase activity is profoundly inhibited by as little as 0.50 unit of heparin per tube, while the alkaline phosphatase activity is not affected by a heparin concentration that is 1,000-fold greater. A similar inhibition of acid phosphatase activity was observed when citrate buffer and β-glycerophosphate were used as substrate (data not shown). In addition to inhibit- ing the activity of acid phosphatase, heparin consistently caused a shift in the pH optimum of the enzyme toward a more neutral pH value.

Figure 2 illustrates the effect of various concentrations of heparin on the activity of both serum and leukocyte acid phosphatase. Both activities are inhibited by hepa- rin, but the degree of inhibition is considerably different, the leukocyte activity being several fold more sensitive to a low concentration of heparin than the serum activity. The portion of the serum activity that is sensitive to heparin may represent the fraction of serum acid phosphatase that is of leukocytic origin, or it may simply represent one isozyme that is susceptible to inhibition by heparin, although not necessarily the same enzyme as that found in the leukocyte.

From the Departments of Biochemistry and Medicine, The Bowman Gray School of Medicine, Winston-Salem, N.C. 27103.

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Fig. 1. Effect of heparin on leukocyte phosphatase activity. Solid line, no additions; dotted line, heparin added. pH 3.5–5.0, 0.5 unit of heparin added/tube; pH 5.5–8.0, 1.0 unit of heparin/tube; pH 8.5–11.0, 500 units of heparin/tube

Fig. 2. Effect of heparin concentration on serum and leukocyte acid phosphatase activity. Solid line, serum enzyme; dotted line, leukocyte enzyme

It was of interest to determine whether other leukocyte lysosomal enzymes were susceptible to inhibition by heparin. Table 1 illustrates that relatively high concentrations of heparin have no effect on lysozyme activity, but do inhibit β-glucuronidase. The latter enzyme, however, is not nearly as sensitive to low concentrations of heparin as is the acid phosphatase.

Discussion

Buruiana (9) has demonstrated that heparin can inhibit serum acid phosphatase and Buruiana and Hadaraj (10) have described a similar effect on the acid phosphatase of the erythrocyte. Considerably higher concentrations of heparin (e.g., 1.0 mg/ml) were used than in the present study. Similarly, Becker and Friedenwald (11) have described an inhibition of rat liver β-glucuronidase by heparin. The present communication demonstrates that leukocytic acid phosphatase is profoundly inhibited by heparin, while β-glucuronidase is considerably less sensitive to the compound. Neither lysozyme nor alkaline phosphatase is affected by relatively high concentrations of heparin.

Whether the inhibition of leukocyte enzymes by heparin is physiologically significant is a matter of speculation. The normal concentration of heparin in human plasma has been reported to be in the range of 0.10–0.25 units/ml of plasma (12), which could influence the acid phosphatase activity. However, concentrations of heparin within the leukocyte have never been determined, and it is not known whether the leukocyte is permeable to heparin.

The present results do have some important implications in terms of experimental methodology. Many workers homogenize leukocytes in the presence of heparin in concentrations as high as 500 units/ml (13). The results of acid phosphatase (and β-glucuronidase) assays run on a whole homogenate prepared in this manner would be of dubious significance. Other workers have reported that heparin added in vitro stabilized lysosomes as measured by a decreased release of enzyme activity from the granules (14). It is possible that heparin does not actually stabilize the granules, but simply inhibits the enzyme activity once it is released. Finally, the histochemical determination of leukocyte acid and alkaline phosphatase is commonly run on heparinized whole blood. Indeed, a widely used procedure for the histochemical determination of leukocyte acid phosphatase recommends the use of heparinized blood (15). It is quite possible that values obtained with this procedure would be spuriously low, and some other anticoagulant should be used.

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References

Simple Method for Determination of Urinary δ-Aminolevulinic Acid as an Index of Lead Exposure

Katsumaro Tomokuni and Masana Ogata

A simple method is described for the quantitative analysis of urinary δ-aminolevulinic acid. 2-Methyl-3-carbethoxy-4-(3-propionic acid) pyrrole, produced by the condensation of δ-aminolevulinic acid with ethyl acetoacetate, is almost completely extracted from an aqueous solution with ethyl acetate without resorting to ion-exchange column chromatography. The pyrrole is determined colorimetrically by treating an aliquot of the extract with a modified Ehrlich's reagent.

Additional Keyphrases: ethylacetoacetate condensation with δ-aminolevulinic acid · normal values · porphobilinogen · colorimetry · environmental hazard · toxicology of lead

Since Haegar-Aronsen (1) reported from animal studies that urinary δ-aminolevulinic acid (ALA),1 an intermediate product in porphyrin biosynthesis, increases after the administration of lead, the determination of urinary ALA has been considered to be one of the most reliable ways of assessing lead poisoning. Previously Mauzerall and Granick (2) described a method for determining urinary ALA by using two ion-exchange columns, a method recently modified by Davis and Andelman (3), who used two disposable ion-exchange columns. In these methods, porphobilinogen (PBG) and ALA are successively separated from the urine by ion-exchange resins. It has been suggested that PBG determination is unnecessary when urinary ALA is used for screening lead workers, because urine samples obtained from workers exposed to lead usually have little or no PBG present (1, 4, 5). Therefore, urinary ALA can validly be determined without first removing PBG in the routine screening of workers exposed to lead.

Both Williams and Few (4), and Sun et al. (5) reported a modification of the Mauzerall and Granick (2) procedure in which only one ion-exchange column is required. However, all these above methods, because of the ion-exchange column chromatography involved, are very time-consuming and so not very appropriate for use with large numbers of specimens. Wada et al. (6) described a simple method for determining urinary ALA, in which 2-methyl-3-carbethoxy-4-(3-propionic acid)-pyrrole (ALA-pyrrole) is complexed with Ehrlich's reagent, and the resulting colored compound is extracted with chloroform and measured colorimetrically.

We found that ALA-pyrrole produced by the condensation of ALA with ethyl acetoacetate is well extracted with ethyl acetate from urine, while the other Ehrlich-positive substances contained in the urine remain in the aqueous layer. Moreover, we found that the ALA-pyrrole forms a cherry-red colored compound by reacting with Ehrlich's reagent in ethyl acetate solution. These findings were used in developing a simple method for determining urinary ALA without ion-exchange column. The method is suitable as a rapid, inexpensive, and accurate procedure for the routine determination of urinary ALA in large numbers of specimens.

Materials and Method

Reagents

Acetate buffer, pH 4.6. To 700 ml of distilled water, add 57 ml of glacial acetic acid and 136 g of sodium acetate trihydrate. Add distilled water to 1 liter.

Ethyl acetoacetate.

Ethyl acetate.

Modified Ehrlich's reagent. To about 30 ml of glacial acetic acid in a 50-ml cylinder, add 1.0 g of p-dimethylaminobenzaldehyde, 5 ml of 60% perchloric acid, and 5 ml of distilled water. Dilute this mixture to 50 ml with glacial acetic acid.

δ-Aminolevulinic acid standard. δ-Aminolevulinic acid hydrochloride (Sigma Chemical Co., St. Louis, Mo. 63178) is used to prepare stock solution. ALA-HCl, 6.4 mg, is dilut-

1 Non-standard abbreviations used: ALA, δ-aminolevulinic acid; and PBG, porphobilinogen.

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From the Department of Public Health, Okayama University Medical School, 2-5-1 Shikata, Okayama, Japan.