Acid Phosphatase from Rat Liver

PURIFICATION, CRYSTALLIZATION, AND PROPERTIES*

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SUMMARY

Rat liver acid phosphatase (EC 3.1.3.2) was separated into two highly purified fractions, differing in isoelectric point and $K_m$. One fraction was crystallized and proved homogeneous by ultracentrifugation and polyacrylamide gel electrophoresis. The molecular weights (100,000), substrate specificities, and pH optima of both enzymes were similar. Oxalate was a mixed type inhibitor to both enzymes. In the presence of dioxane, both enzymes exhibited sigmoidal inhibition curves by oxalate and by trypan blue.

Berthet and de Duve (1) and Berthet et al. (2) demonstrated the lysosomal nature of acid phosphatase (EC 3.1.3.2) activity in rat liver. Since then, there have been many studies (3) on lysosomes. However, chemical investigations have been handicapped by the need for characterization of purified preparations. Moore and Angeletti (4) separated acid phosphatase from rat liver by chromatography. Brightwell and Tappel (5) effected a 350-fold purification of acid phosphatase from rat liver. No crystallization of the enzyme from rat liver has been reported. This report describes the purification, crystallization, and certain unexpected properties of an acid phosphatase from rat liver.

MATERIALS AND METHODS

Male albino rats, CD strain, from Charles River Breeding Laboratories were used. Nucleotides, hexose phosphates, $p$-nitrophenyl phosphate, and DEAE-cellulose were purchased from Sigma. Imidazole, $\beta$-glycerophosphate, and 2-mercaptoethanol were bought from Distillation Products. Glycylglycine, ammonium sulfate of enzyme grade, 1-fluoro-2,4-dinitrobenzene, egg white lysozyme, and crystalline catalase were from Mann. Ampholine was from LKB Instruments, Inc. Reagents for disc gel electrophoresis were supplied by Canaleo. Reagent grade dioxane was purified by refluxing with KOH followed by distillation (6). Trypan blue, obtained from Allied Chemical Corporation, was purified by precipitation from hot aqueous solution by the addition of 4 volumes of ethanol (7).

Enzyme Assay

Enzyme activity during purification and for other experiments except kinetic studies was estimated by a minor modification of the method of Neil and Horner (8). Enzyme was incubated for 10 min at 37° with 0.1 M sodium acetate buffer, pH 5.0, and 2.54 $\mu$M NP as substrate, in a final volume of 0.5 ml. The reaction was started by the addition of enzyme and stopped with 1.5 ml of 0.25 N NaOH. The absorbance of the liberated $p$-nitrophenol was read at 410 nm. One unit of enzyme catalyzed the hydrolysis of 1 $\mu$ mole of NP per min. Specific activity is expressed as units per mg of protein.

Kinetic studies were performed by a modification of the method of Scott (9) in a Gilford model 2000 spectrophotometer at 315 nm with the cell compartment thermostatically controlled at 30°. In the study of trypan blue inhibition, measurements were made at 410 nm in 0.1 M Tris-acetate, pH 7.6. One activity unit is defined as the amount giving an increase of absorbance of 0.01 per min. Initial velocity was calculated on an Olivetti-Underwood Programma 101 electronic computer by the method of least squares. $V_{\text{max}}$ and $K_m$ of the Michaelis-Menten equation and standard errors were calculated by the method of Wilkinson (10) on the same computer. Fractional inhibition is expressed as $1 - v/V$, where $v$ is the velocity with inhibitor and $V$ is that without inhibitor.

Protein Concentration

Protein was estimated by the biuret procedure (11) with bovine serum albumin as standard.

Polyacrylamide Gel Electrophoresis

Disc electrophoresis was performed in 7.5% polyacrylamide gel. The gel buffer, pH 7.1, was 5 mM imidazole-acetate, 1 mM EDTA, and 0.1 M sodium acetate. The electrode buffer was 38.4 mM glycylglycine-5 mM imidazole, pH 7.1, with 1 mM EDTA.

The abbreviation used is: NP, $p$-nitrophenyl phosphate.
and 5 mM 2-mercaptoethanol. Spacer gel was not used, and sample layering was done according to the method of Hjertén, Jerstedt, and Tiselius (12). The gel was stained with Coomasie brilliant blue R 250 (13). The enzyme activity was stained according to the procedure of Barka and Anderson (14), with 7-bromo-3-hydroxy-2-naphtho-α-anisidide phosphate as substrate.

Molecular Weight

The molecular weight of the enzymes was estimated by sucrose gradient ultracentrifugation according to the method of Martin and Ames (15) and by Sephadex G-200 column chromatography (16). Bovine liver catalase and egg white lysozyme were used as standards.

Isoelectric Points

Isoelectric points were determined by isoelectric focusing according to the procedure of Vesterberg and Svensson (17), with the use of the apparatus of LKB Instruments, Inc., and Ampholine, pH range, 3 to 10.

Other Measurements

Phosphate was determined by the method of Fiske and SubbaRow (18). The ultraviolet absorption spectrum was measured with a Hitachi Perkin-Elmer UV-VIS spectrophotometer.

Enzyme Purification

The purification procedures are summarized in Table I. All procedures were carried out at 4°C. All solutions used contained 5 mM 2-mercaptoethanol and 1 mM EDTA, unless otherwise indicated. Rats were killed by decapitation and the livers were immediately homogenized with 50% glycerol in a Waring Blender for 3 min. The homogenate was centrifuged at 8000 rpm for 20 min, with the use of a GSA rotor in a Sorval RC-2 centrifuge. About 90% of the activity was recovered in the supernatant fraction.

Acid Treatment—The supernatant was adjusted to pH 5.0 with 1 M acetic acid. After 30 min of stirring, the precipitate formed was separated by centrifugation and discarded. The supernatant fluid was dialyzed overnight against 5 mM acetic acid buffer, pH 5.0. The precipitate was removed by centrifugation and discarded.

Ammonium Sulfate Fractionation—Solid ammonium sulfate was gradually added with stirring to 0.5 saturation (1.95 M). After 30 min of standing, the precipitate was removed by centrifugation. Additional ammonium sulfate was added to the supernatant fluid to 0.8 saturation (3.13 M). After 30 min the precipitate was collected and dissolved in a minimum quantity of 0.01 M sodium acetate buffer, pH 5.0.

Chromatography on Sephadex G-75—Ammonium sulfate fraction (200 units) was applied to a column, 3.5 x 75 cm, of Sephadex G-75 equilibrated with 0.01 M sodium acetate buffer. Elution with the same buffer gave a single peak of enzyme activity. Fractions containing the enzyme activity at levels above 0.15 unit per ml were pooled. The enzyme was precipitated by the addition of solid ammonium sulfate between 0.5 and 0.8 saturation. The precipitate was dissolved in 5 mM imidazole-acetate buffer, pH 7.1, and dialyzed against the same buffer.

Chromatography on DEAE-cellulose—The enzyme solution was then chromatographed on a column, 3.5 x 75 cm, of DEAE-cellulose which had been equilibrated with 5 mM imidazole-acetate buffer, pH 7.1. A linear gradient was achieved by the use of a mixing flask containing 1 liter of the starting buffer and a reservoir containing 2 liters of the starting buffer with 0.50 M NaCl. Fractions of 14 ml were collected. Two peaks of enzyme activity were obtained. One was not adsorbed by the column (Peak I). The other was eluted at 0.095 M sodium chloride (Peak II). These two peaks of enzyme activity were pooled separately and concentrated by precipitation with 0.8 saturated ammonium sulfate.

![Fig. 1. Dark field photomicrograph of crystalline acid phosphatase.](http://www.jbc.org/)

### Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total volume</th>
<th>Total protein</th>
<th>Total activity</th>
<th>Specific activity</th>
<th>Purification</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homogenate</td>
<td>1,612</td>
<td>211,000</td>
<td>4,320</td>
<td>0.020</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>pH 5 supernatant</td>
<td>2,881</td>
<td>21,700</td>
<td>2,420</td>
<td>0.112</td>
<td>5.6</td>
<td>56</td>
</tr>
<tr>
<td>Ammonium sulfate</td>
<td>278</td>
<td>18,500</td>
<td>2,296</td>
<td>0.124</td>
<td>6.8</td>
<td>53</td>
</tr>
<tr>
<td>Sephadex G-75</td>
<td>98.6</td>
<td>1,860</td>
<td>500</td>
<td>0.268</td>
<td>13.4</td>
<td>12</td>
</tr>
<tr>
<td>DEAE-cellulose Peak I</td>
<td>38.4</td>
<td>360</td>
<td>404</td>
<td>1.12</td>
<td>56</td>
<td>9.4</td>
</tr>
<tr>
<td>Hydroxylapatite</td>
<td>388</td>
<td>37</td>
<td>398</td>
<td>10.6</td>
<td>532</td>
<td>3.0</td>
</tr>
<tr>
<td>Crystallization I</td>
<td>0.8</td>
<td>7.3</td>
<td>130</td>
<td>18</td>
<td>885</td>
<td>3.0</td>
</tr>
<tr>
<td>Crystallization II</td>
<td>0.5</td>
<td>5.1</td>
<td>91.2</td>
<td>18</td>
<td>890</td>
<td>2.1</td>
</tr>
<tr>
<td>DEAE-cellulose Peak II</td>
<td>242</td>
<td>140</td>
<td>92.4</td>
<td>0.64</td>
<td>32</td>
<td>2.1</td>
</tr>
<tr>
<td>Sephadex G-200</td>
<td>206</td>
<td>29</td>
<td>78.4</td>
<td>2.68</td>
<td>134</td>
<td>1.8</td>
</tr>
<tr>
<td>Purification Recovery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chromatography on Hydroxylapatite and Crystallization of DEAE-cellulose Peak I Enzyme—The concentrated Peak I enzyme solution was dialyzed against 10 mM sodium succinate buffer, pH 6.0, without EDTA and without 2-mercaptoethanol, and applied to a column, 2.5 x 30 cm, of hydroxylapatite which

![Image](http://www.jbc.org/)

**Fig. 2.** Polyacrylamide gel electrophoresis at various purification steps. Gels 1 to 4 were stained for acid phosphatase activity; Gel 5 was stained for protein. A current of 4 ma per gel was applied to Gels 1 to 5 for 2 hours and to Gels 4 and 5 for 6 hours. Gel 1, homogenate; 2, DEAE-cellulose Peak II; 3, DEAE-cellulose Peak I; 4 and 5, crystalline enzyme.

**Fig. 3.** Sucrose density gradient centrifugation of crystalline acid phosphatase. Lysozyme (0.5 mg), catalase (0.04 mg), and crystalline acid phosphatase (0.006 mg), mixed in 0.1 ml of 0.1 M sodium acetate buffer, pH 5.0, were layered on a 5 to 20% linear sucrose gradient (4.6 ml) containing 0.1 M sodium acetate buffer, pH 5.0. After centrifugation at 35,000 rpm for 16 hours at 3° in a SW 39 rotor in a Spinco model L centrifuge, the solution was fractionated. The activities of lysozyme and catalase were assayed as described by Martin and Ames (15). The activity of acid phosphatase was assayed as described in the text. The tubes, containing 0.086-ml fractions, were numbered starting from the top of the sucrose gradient. The total number of fractions was 53. An approximate molecular weight of 100,000 was calculated according to the method of Martin and Ames (15) for crystalline enzyme.

**Fig. 4.** Isoelectric focusing of crystalline and P II enzymes. Of each enzyme, 0.6 unit was applied. The fractions were taken from a 110-ml column after isoelectric focusing for 30 hours with a final potential of 600 volts. Acid phosphatase activity was measured as described in the text. pH was determined at 4°. The isoelectric points were obtained by taking the pH values of the corresponding fraction at the maximum activity. Electrophoresis for 27 and 40 hours gave similar results.

**Table II**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative rate of hydrolysis</th>
<th>Inhibition with NP as substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystalline</td>
<td>P II</td>
<td>Crystalline</td>
</tr>
<tr>
<td>NP</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>ATP</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>ADP</td>
<td>0.20</td>
<td>0.27</td>
</tr>
<tr>
<td>AMP</td>
<td>0.33</td>
<td>0.35</td>
</tr>
<tr>
<td>β-Glycerol-P</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>α-Glucose-1-P</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>β-Glucose-6-P</td>
<td>0.15</td>
<td>0.14</td>
</tr>
<tr>
<td>Fructose-1,6-di-P</td>
<td>0.37</td>
<td>0.37</td>
</tr>
<tr>
<td>P1</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>Cyclic 3',5'-AMP</td>
<td>0.16</td>
<td>0.17</td>
</tr>
<tr>
<td>Cyclic 2',3'-AMP</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>Oxalate</td>
<td>0.22</td>
<td>0.22</td>
</tr>
<tr>
<td>L-(-)-Tartrate</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>Fluoride</td>
<td>0.37</td>
<td>0.37</td>
</tr>
</tbody>
</table>
TABLE III

Effects of dioxane and oxalate on kinetic constants

Initial velocity was determined as described in the text. The reaction mixtures consisted of varying concentration of NP in the range of 0.0075 to 0.19 mM and the indicated amounts of dioxane and oxalate, in a total volume of 1.0 ml, in 0.1 M sodium acetate buffer, pH 5.0. Triplicate measurements were done at each substrate concentration. \( V_{\text{max}} \), \( K_m \), and standard errors were calculated as described in the text. Eight determinations were made under each experimental condition.

<table>
<thead>
<tr>
<th>Addition to reaction mixture</th>
<th>( K_m ) ± S.E.</th>
<th>( V_{\text{max}} ) ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crystalline P II</td>
<td>Crystalline P II</td>
</tr>
<tr>
<td>None</td>
<td>0.091 ± 0.007</td>
<td>0.047 ± 0.004</td>
</tr>
<tr>
<td>Dioxane (8%)</td>
<td>0.099 ± 0.008</td>
<td>0.042 ± 0.004</td>
</tr>
<tr>
<td>Dioxane (8%) and oxalate (1.5 mM)</td>
<td>0.25 ± 0.04</td>
<td>0.20 ± 0.02</td>
</tr>
<tr>
<td>Oxalate (0.6 mM)</td>
<td>0.24 ± 0.05</td>
<td>0.19 ± 0.04</td>
</tr>
</tbody>
</table>

Fig. 5. Inhibition of crystalline acid phosphatase by varying concentrations of oxalate or trypan blue in the presence and in the absence of dioxane. Initial velocity was determined as in Table III with 0.185 mM NP, varying concentrations of oxalate or trypan blue, and 1.20 absorbance units of crystalline enzyme in the presence or absence of 8% dioxane. P II enzyme gave similar results.

had been equilibrated with the same buffer. Stepwise elution with ammonium sulfate in the same buffer yielded a single peak of activity in the 0.1 M ammonium sulfate fraction. The pooled enzyme was then concentrated with 0.8 saturated ammonium sulfate to give 1% protein concentration in 5 mM imidazole-glycylglycine buffer, pH 7.1. Finely powdered ammonium sulfate was added gradually to this solution to 0.5 saturation. The solution became opaque and the precipitate was removed by centrifugation. The ammonium sulfate concentration was slowly increased to 0.55 saturation, at which point the solution was partly turbid. This solution was kept at 4°. Microscopic crystal formation was evident after 24 hours. Over a period of several days the crystal size increased. Recrystallization by the same method did not increase the specific activity. Fig. 1 shows the crystalline enzyme, photographed with the aid of a dark field condenser.

Chromatography on Sephadex G-200 of DEAE-cellulose Peak II Enzyme—The concentrated Peak II enzyme solution was applied to a column, 2.5 x 30 cm, of Sephadex G-200 which was equilibrated with 0.01 M sodium acetate buffer, pH 5.0, without EDTA and without 2-mercaptoethanol. The enzyme activity was eluted in a single peak, termed P II enzyme.

RESULTS

Polyacrylamide Gel Electrophoresis—Fig. 2 shows two zones of enzymatic activity in the crude homogenate. These zones were
The molecular weight was shown by Sephadex G-200 chromatography and by sucrose density gradient ultracentrifugation. When a mixture of these two enzymes was examined, a single peak of activity with the same sucrose gradient procedure. M II enzyme had the same molecular weight of 100,000 (Fig. 3). P II enzyme had the same zone of activity, coincident with a single line enzyme had one zone of activity, coincident with a single zone of protein.

Table IV

Desensitization of crystalline acid phosphatase to oxalate inhibition with fluorodinitrobenzene

For each experiment, 0.5 ml of crystalline enzyme (0.01 mg), in the indicated buffer, was stirred with 40 mmoles of fluorodinitrobenzene in the dark at room temperature. After the indicated period, the mixture was passed through a column, 1 X 15 cm, of Sephadex G-25. The control was prepared by an identical procedure but without fluorodinitrobenzene.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Incubation period</th>
<th>Inhibition with oxalate</th>
<th>Activity recovered after dinitrophenylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate (pH 8.1, Γ/2 = 0.1)</td>
<td>2 hrs</td>
<td>Ctrl 0.50</td>
<td>Fluorodinitrobenzene 0.30</td>
</tr>
<tr>
<td>Tris-acetate (pH 8.1, Γ/2 = 0.1)</td>
<td>1 hr</td>
<td>Ctrl 0.50</td>
<td>Fluorodinitrobenzene 0.00</td>
</tr>
<tr>
<td>Tris-acetate, with 30 mm oxalate</td>
<td>1/2 hr</td>
<td>Ctrl 0.50</td>
<td>Fluorodinitrobenzene 0.46</td>
</tr>
<tr>
<td>Tris-acetate, with 20 mm NP</td>
<td>1/2 hr</td>
<td>Ctrl 0.50</td>
<td>Fluorodinitrobenzene 0.23</td>
</tr>
</tbody>
</table>

* The activity of native enzyme is 1.00.

Fig. 6. pH-activity curves and oxalate inhibition in the presence and in the absence of 8% dioxane. Buffers were 0.1 ionic strength: glycine-acetate, pH 3.0 to 3.8; sodium acetate, pH 3.9 to 5.5; imidazole-acetate, pH 6.1 to 7.0; Tris-acetate, pH 7.6. Otherwise, conditions were as described in the legend to Table II except that 0.185 mM NP and 0.6 mM oxalate were used.

Discussion

The absorption spectrum of crystalline enzyme shows a maximum at 278 mµ with a slight shoulder at 283 mµ. The specific extinction coefficient, $E_{1%}$ at 278 mµ, was 4.7.

The isoelectric points of crystalline and P II enzymes were pH 7.7 and 4.5, respectively, by the method of isoelectric focusing (Fig. 4).

Substrate Specificity and Inhibitors—Table II shows the rate of hydrolysis of phosphate esters by both crystalline and P II enzymes. NP, 3-glycerophosphate, and fructose 1,6-diphosphate were hydrolyzed rapidly, in that order. Both enzymes were devoid of diphosphatase and pyrophosphatase activities. The activity of both enzymes with NP as substrate was almost completely inhibited by L-(+)-tartrate and fluoride. Adenine nucleotides and oxalate were also good inhibitors to both enzymes, but cyclic nucleotides did not inhibit activity at all. No specific metal requirement was evident, and the enzymes were not inactivated by EDTA. No alkaline phosphatase activity was noted in these two enzyme preparations.

Effect of Dioxane on Oxalate Inhibition—Kinetic studies with varying concentration of NP as substrate showed Michaelis-Menten hyperbolic curves for both crystalline and P II enzymes. Table III shows that $K_m$ values were different for the two enzymes. Oxalate showed mixed type inhibition (19) for both enzymes, shown by the $K_m$ and $V_{max}$ values in Table III.

A study of the effect of dioxane on oxalate inhibition showed several effects of interest. Fig. 5A shows that the addition of 8% dioxane to incubation mixtures containing substrate, enzyme, and various concentrations of oxalate produced striking kinetic changes. Both crystalline and P II enzymes gave hyperbolic plots of fractional inhibition against oxalate concentration. In contrast, similar studies in 8% dioxane gave sigmoidal curves. This difference is emphasized by Fig. 5B, which contrasts plots of the reciprocal fractional inhibition against the reciprocal oxalate concentration. Dioxane produced a clearly curvilinear plot. Cooperative binding of inhibitor can be expressed as $n$, the slope of the Hill equation. This value, calculated by the method of Tokata and Pogell (20) from these data, was 1.0 ± 0.065 in the absence of dioxane with either oxalate or trypan blue. This result is consistent with either a single binding site for inhibitor or multiple binding sites without interaction. In contrast, when reactions were carried out in dioxane-water, the Hill coefficient was 1.7 ± 0.16 with oxalate and 1.3 ± 0.11 with trypan blue. This result is consistent with the sigmoidal curves obtained in Fig. 5A, and suggests interaction between inhibitor sites.

Fig. 6 shows that dioxane shifts the pH optimum to higher values. At pH 5.0 there is a small (3% and 10% for crystalline and P II enzymes) increase in activity. Oxalate inhibition is significantly depressed in the presence of dioxane through the pH range shown in the figure. The presence of 8% dioxane had little effect on the glass electrode-determined pH of buffer mixtures. Table III shows that the presence of dioxane prevented change in $K_m$ and $V_{max}$ values, when compared with values determined without dioxane. The reduction in oxalate inhibition was not specific for dioxane. Similar results were obtained with acetone, isopropyl alcohol, dimethylformamide, and ethylene glycol monobutyl ether. However, these results were not studied in detail. The effects of dioxane and oxalate were reversible. When the reagents were removed by dialysis or passage through a Sephadex G-25 column, original enzyme behavior was restored. No change in molecular weight was noted by sucrose density gradient ultracentrifugation in the presence.
of 0.6 mm oxalate. Dioxane at a concentration of 8% also failed to affect sedimentation behavior significantly. Treatment with fluorodinitrobenzene desensitized crystalline enzyme to oxalate inhibition, as shown in Table IV. P II enzyme showed the same result. Dinitrophenylation did not affect the pH activity curves appreciably. Desensitization was best achieved in the presence of phosphate, which might be expected to protect the active site. The substrate, NP, also protected the active site and allowed desensitization. Lack of protection of the active site by oxalate is of interest. After dinitrophenylation, only one enzyme protein was obtained by acrylamide gel electrophoresis and DEAE-cellulose chromatography. We failed to show the concomitant presence of sensitive and desensitized enzymes.

**DISCUSSION**

Moore and Angeletti (4) separated three fractions of acid phosphatase from rat liver by DEAE-cellulose chromatography. Shibko and Tappel (21) found three fractions by DEAE-cellulose chromatography of the lysosomal extract of rat liver. Their extract yielded two zones by starch gel electrophoresis. Beckman and Beckman (22) found two zones of enzymatic activity by starch gel electrophoresis of human liver extracts. Although London, Sommer, and Hudson (23) reported a partial crystallization of prostatic acid phosphatase, the present paper describes the first crystallization of acid phosphatase from rat liver. Crystalline and P II enzymes have a molecular weight of 100,000. This is similar to the molecular weights reported for acid phosphatases from prostate gland (24, 25) and from cellular particles of human placenta (26). The specific extinction coefficient of the crystalline enzyme is higher than that reported for prostatic enzyme (27). The specific activity of the crystalline enzyme is nearly twice as high as that described by Brightwell and Tappel (5), whose preparation of liver acid phosphatase had hitherto been the most active reported. The molecular activity of the crystalline enzyme is 1,800 at 37°C. The crystalline enzyme is homogeneous by Sephadex G-200 chromatography, sucrose density gradient ultracentrifugation, and polyacrylamide gel electrophoresis. Constant specific activity after recrystallization is further evidence for homogeneity.

Prior studies had shown tartrate to be a competitive inhibitor of prostatic (28) and Neurospora crassa (29) acid phosphatases. Oxalate was clearly shown in the present studies to be a mixed type inhibitor. Both crystalline and P II enzymes showed classical hyperbolic kinetics with respect to substrate and oxalate inhibition. However, when hydrolysis was carried out in dioxane-water, sigmoidal inhibition kinetics with respect to enzymes (30). Inhibition of the enzymes by this dye showed kinetics similar to that of oxalate. Nonpolar solvents are expected to affect protein configuration by weakening the hydrophobic bonds (31) and by altering the ionic interactions in the protein molecule by increasing the pK values of the ionizing groups (32, 33). Solvents with a wide spectrum of dielectric constants affected oxalate inhibition in a similar way. Therefore, hydrophobic interaction with solvent with consequent configurational change is more likely to be responsible than changes in dielectric constant. The sigmoidal inhibition curves in dioxane-water and desensitization to oxalate suggest allosteric inhibition, but further study is needed for complete clarification.

These studies were carried out as part of a study on lysosomal activation. Most, if not all, of rat liver acid phosphatase is lysosomal in distribution (34). The enzymes studied here are similar with respect to pH optimum, substrate specificity, and behavior on electrophoresis and DEAE-cellulose chromatography to those isolated from lysosomes directly (21).

**REFERENCES**

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