SPECIFICITY OF DOG INTESTINAL PHOSPHATASE ANTISERUM*

BY MAX SCHLAMOWITZ

(From the Research Biochemistry Section, Sloan-Kettering Institute for Cancer Research, New York, New York)

(Received for publication, July 3, 1953)

Alkaline phosphatases are known to occur in the various body tissues and in the blood serum. It may reasonably be said that the phosphatase of serum is a mixture of the enzymes contributed by the tissues in varying amounts. Under pathological conditions the contribution of phosphatase from particular tissues may be increased enormously. This fact has been utilized as an aid in clinical diagnosis. It could enjoy even greater use if the origin of the elevated serum phosphatase in disease could be established with certainty.

Many methods for a differentiation of the alkaline phosphatases from different tissues have been reported (2-8), based on differences in the response of the enzymes to activators and inhibitors. Such methods have elicited some differences. But the possibility exists (3) that the differences in response are related to differences in binding of the activators or inhibitors by components of the enzyme preparations other than phosphatases. The specificity often achieved with immunochemical reactions suggested that this approach might be of additional aid in differentiation.

The preparation of the phosphatase from dog intestine and the production of antibodies against it have been described (9). To be described below is the preparation of phosphatases from the intestine of dog (DIP-B), rat (rIP), rabbit (RIP), and bovine (BIP) origin, and from the kidney (DKP) and liver (DLP) of dog. A characterization of these enzymes in terms of the influence of pH, activators, and inhibitors on their activity and an immunochemical differentiation based on the specificity of the anti-dog intestinal phosphatase antibodies are also presented.

Materials and Methods

Preparation of Alkaline Phosphatases—The tissues investigated were the intestine of dog, rat, rabbit, and bovine1 origin, and the kidney cortex and

* A preliminary report of this work has appeared (1). This investigation was supported in part by a research grant (No. C-1351) from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

1 The bovine intestinal phosphatase was a commercial sample, prepared according to Schmidt and Thannhauser (10). The author is indebted to Dr. L. L. Lachat of Armour and Company for supplying this material.
liver of the dog, obtained from well nourished, adult animals under ether or
Nembutal anesthesia. The dog organs were exhaustively perfused in situ
with saline and water to minimize contamination of their phosphatases
with those present in blood. All of the enzymes were prepared by a pro-
cedure (11) which involved autolysis of the tissue and fractional precipita-
tion with ethanol. A typical preparation is presented here.

The tissue was blended with an equal volume of 50 per cent ethanol and
0.1 volume of a 1:1 mixture of ethyl acetate and toluene. The blended
tissue was autolyzed at room temperature for 70 hours, and the pH was
maintained at 6.8 to 6.9 by additions of a 3 per cent NaOH solution. The
mixture was then centrifuged, the supernatant solution cooled to 10°, and
its pH adjusted to 4.6 at 10°. After standing 1 to 2 hours at −10°, the
solution was clarified by centrifugation and the phosphatase precipitated

\[
\text{TABLE I}
\]

Relative Activity of Alkaline Phosphatase Preparations

<table>
<thead>
<tr>
<th>Phosphatase preparation</th>
<th>Relative specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIP-A</td>
<td>100</td>
</tr>
<tr>
<td>DIP-B</td>
<td>59</td>
</tr>
<tr>
<td>DLP</td>
<td>12</td>
</tr>
<tr>
<td>DKP</td>
<td>2</td>
</tr>
<tr>
<td>RIP</td>
<td>9</td>
</tr>
<tr>
<td>rIP</td>
<td>7</td>
</tr>
<tr>
<td>BIP</td>
<td>5</td>
</tr>
</tbody>
</table>

See the text for notation of the phosphatases.

by the addition of 1.5 volumes of ethanol at 4°.2 The precipitate was
collected by centrifugation, washed with ethanol, and dried in a vacuum
in the cold. The products obtained in this fashion were white to cream-
colored powders, partially soluble in water or saline.

The relative activities of the enzyme preparations are given in Table I.
The reference substance, DIP-A, is the material previously prepared from
dog intestine (9).

Anti-Dog Intestinal Phosphatase Antiserum—The pooled rabbit antiserum
prepared against dog intestinal phosphatase (DIP-A) (9) was used in this
study.

Determination of Protein Concentration and Phosphatase Activity—Analy-
ses of the enzymes for protein concentration and phosphatase activity were
carried out as previously described (9).

2 In the case of the liver phosphatase, ethanol was added to a concentration of
about 40 per cent instead of 70 per cent to reduce the contamination by non-phos-
phatase proteins.
Experimental

Comparison of Alkaline Phosphatases on Basis of Optimal pH—Measurements were made of the optimal pH for the activity of each enzyme preparation. Veronal buffers \((6.25 \times 10^{-2} \text{ M})\) of pH 9.1, 9.4, 9.7, and 10.0 at \(37^\circ\) were used. The pH of the activity mixtures was found to remain constant to within 0.15 pH unit over the 20 minute incubation period. The concentrations of substrate, buffer, and magnesium used are those already described (9). The first line of Table II summarizes the results of this experiment. It may be seen that the optimal pH for all of the enzymes, regardless of source or purity, is about 9.7 at \(37^\circ\), under the conditions used.

Comparison of Alkaline Phosphatases on Basis of Response to Activators

Table II

<table>
<thead>
<tr>
<th></th>
<th>DIP-A</th>
<th>DIP-B</th>
<th>DLP</th>
<th>DKP</th>
<th>RIP</th>
<th>BIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optimal pH</td>
<td>9.7</td>
<td>9.7</td>
<td>9.7</td>
<td>9.7</td>
<td>9.7</td>
<td>9.7</td>
</tr>
<tr>
<td>Activation by 0.0125 M Mg</td>
<td>1.3</td>
<td>1.4</td>
<td>1.8</td>
<td>2.4</td>
<td>1.3</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>% inhibition by</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0125 M cyanide</td>
<td>99</td>
<td>99</td>
<td>98</td>
<td>97</td>
<td>91</td>
<td>98</td>
</tr>
<tr>
<td>0.01 M fluoride</td>
<td>4</td>
<td>6</td>
<td>15</td>
<td>16</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>0.001 M &quot; taurocholate</td>
<td>6</td>
<td>7</td>
<td>51</td>
<td>57</td>
<td>9</td>
<td>24</td>
</tr>
<tr>
<td>0.001 M &quot;</td>
<td>4</td>
<td>3</td>
<td>12</td>
<td>26</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>0.001 M histidine</td>
<td>94</td>
<td>94</td>
<td>89</td>
<td>89</td>
<td>90</td>
<td>97</td>
</tr>
<tr>
<td>0.002 M &quot;</td>
<td>64</td>
<td>69</td>
<td>78</td>
<td>60</td>
<td>57</td>
<td>77</td>
</tr>
</tbody>
</table>

and Inhibitors—The influence of activators (Mg\(^{++}\)) and inhibitors (CN\(^{-}\), F\(^{-}\), taurocholate, and histidine) was tested on each enzyme. These are among the substances which have received the widest use in attempts to differentiate the alkaline phosphatases from different tissues (2-8). Incubation mixtures were set up at pH 9.7 in the usual manner (9), but included the activator or inhibitor at the concentration indicated in Table II. Activator and inhibitor solutions were adjusted to pH 9.7 at \(37^\circ\) before use. Measurement of pH at the end of each run showed that the pH of the incubation mixture had remained constant to within 0.15 pH unit. The pH, 9.7, used was that optimal for the system in the absence of inhibitors. For the purposes of this study it was not necessary to ascertain the influence, if any, of the inhibitors on the pH optimum.

The data of Table II show that with respect to activation by magnesium, all of the enzymes were affected to about the same degree (1.1- to 2.4-fold activation). Further, the use of cyanide and fluoride failed to show any
differences among the phosphatases used here. Similarly, the addition of histidine did not appear to show any significant differences among either the intestinal phosphatases of the different species or among the liver, kidney, and intestinal phosphatases of the dog at pH 9.7. In general, it may be said that, on the basis of their response to pH, Mg++, F−, CN−, and histidine, under the conditions tested, all of the enzymes, those from different tissues of the same species as well as those from the same tissue of different species, resemble each other very closely. Such differences as do occur are small. The differences observed for response to taurocholate appear somewhat greater.

Comparison of Alkaline Phosphatases on Basis of Their Precipitin Reaction with Anti-Dog Intestinal Phosphatase Antiserum—Precipitation of phosphatase activity by the antiserum against dog intestinal phosphatase

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Precipitation by DIP-A antiserum</th>
<th>Precipitation of DIP-A from phosphatase mixture by DIP-A antiserum</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIP-A</td>
<td>97-100</td>
<td>97</td>
</tr>
<tr>
<td>DIP-B</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>DLP</td>
<td>0</td>
<td>98</td>
</tr>
<tr>
<td>DKP</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>RIP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>rIP</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BIP</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

(DIP-A) was the criterion used to establish immunochemical similarities and differences among the enzymes.

Mixtures containing antiserum (0.20 ml.) and enzyme (0.20 ml. containing the equivalent of 20 y of DIP-A) were incubated for 0.5 hour at 25°, 3.5 hours at 4°, and centrifuged to remove precipitates if any formed. The clear supernatant fluids were then analyzed for residual enzyme activity. The results of this experiment, carried out in duplicate, with appropriate controls, are shown in Table III.

The phosphatases from dog intestine, whether prepared by fractionation with acetone (DIP-A) or alcohol (DIP-B) were completely precipitated by the antiserum to the acetone-prepared enzyme, and hence were immunochemically indistinguishable. On the other hand, the phosphatases from the intestine of rabbit, rat, and bovine origin or from the liver or kidney of dog were not precipitated by the intestinal phosphatase antiserum.

The possibility existed that the enzymes were identical, but that failure to obtain precipitates with these enzymes was due to the presence of large...
amounts of immunochemically similar but enzymatically inactive antigens. Such conditions might produce a state of antigen excess and cause complete solubility of the precipitate (cf. (9)). Accordingly, to each of the enzymes, in amounts equivalent to 20 γ of DIP-A in activity, were added 20 γ of DIP-A. To these enzymes, in 0.2 ml. of saline, was then added an equal volume of the antiserum. After incubation as described above the mixture was centrifuged and the activity in the supernatant fluid was estimated in the usual manner. The percentage of DIP-A precipitated by its antiserum from the mixture was calculated from the results of these runs and from runs of each enzyme alone with the antiserum. The data, summarized in Table III, show that the presence of the other phosphatases did not interfere with the precipitation of DIP-A by its antiserum. Had the possibility considered above obtained, then DIP-A too would have become soluble. It may therefore be said that the phosphatases prepared from these tissues are different from the one isolated from dog intestine.

**DISCUSSION**

By means of the antibody specificity a differentiation has been made between the phosphatase from dog intestine and the intestinal phosphatases of other species (rabbit, rat, bovine) and also between this phosphatase and those derived from other organs (liver, kidney) of the same species. The enzymes differentiated in this manner were all similar in their response to agents affecting enzyme activity (pH, Mg**, **CN**, **F**, histidine).

An interpretation of these experimental results, i.e. immunochemical dissimilarity and enzymatic similarity of the phosphatases, may best be sought for in terms of enzyme structure. Speaking then in terms of the major phosphatase component of each enzyme preparation, if we assume that the catalytically active sites of the enzymes are different despite the similarities mentioned, the immunochemical differentiation has been made among enzymatically and perhaps functionally dissimilar alkaline phosphatases. On the other hand, if the similarity in response of the enzymes to pH, activators, and inhibitors reflects a similarity or identity of the active sites, then one must conclude that the immunochemical method has revealed structural differences in enzymatically and perhaps functionally similar phosphatases, but in portions of the molecule unrelated to catalytic activity. Since the physiological substrates and precise biological function of the phosphatases dealt with are unknown, this question cannot be resolved with finality.

3 The dog intestinal enzyme was shown to have approximately 12 per cent of a minor component (9).

4 Preparations of the phosphatases by a procedure which involved prolonged treatment and fractionation with ethanol served to minimize interference from the alcohol-labile hexosediphosphatase of Gomori (12, 13) and the hexose-6-phosphatase described by Swanson (14) and de Duve et al. (15).
SUMMARY

The alkaline phosphatases from dog intestine have been immunochemically differentiated from the phosphatases of rabbit, rat, and bovine intestine and from the phosphatases of the liver and kidney of dog. The species and organ specificity of these enzymes is thereby indicated. The implications of the immunochemical differences and of the similar effects of pH, activators, and inhibitors on these enzymes are discussed in terms of the structure-function relationship.

The author wishes to thank Dr. Oscar Bodansky for his continued interest and helpful criticisms in the course of this work.

BIBLIOGRAPHY

SPECIFICITY OF DOG INTESTINAL PHOSPHATASE ANTISERUM
Max Schlamowitz


Access the most updated version of this article at http://www.jbc.org/content/206/1/369.citation

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/206/1/369.citation.full.html#ref-list-1