Metabolism of Phosphoserine

III. MECHANISM OF O-PHOSPHOSERINE PHOSPHATASE*

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The partial purification and certain properties of O-phosphoserine phosphatase from chicken liver have been described (1), and a double displacement mechanism has been proposed:

\[
E + PS \rightleftharpoons EP + S \rightleftharpoons E + P_i
\]

In the above, \( E \) is the enzyme, \( PS \) is phosphoserine, \( I \) is the Michaelis complex of enzyme and \( PS \), \( II \) is the Michaelis complex of phosphoryl-enzyme and serine, and \( III \) is the phosphoryl-enzyme. A similar mechanism has been suggested for rat liver O-phosphoserine phosphatase by Borkenhagen and Kennedy (2). The present paper describes the inhibition of the hydrolytic reaction by serine and structurally related compounds, and establishes that the enzyme can catalyze the transfer of a phosphoryl group from \( n \)-phosphoserine to \( L \)-serine. The available evidence for the hydrolytic activity, exchange activity, and the inhibition by serine and related compounds will be summarized and evaluated in relation to the proposed mechanism.

Preliminary reports on the inhibition and the proposed mechanism have been published (3, 4). Independently of our own work, Borkenhagen and Kennedy (2) noted the inhibition of rat liver \( O \)-phosphoserine phosphatase by low concentrations of \( L \)-serine, and Schramm (5) found that the \( O \)-phosphoserine phosphatase from bakers’ yeast was inhibited by relatively high concentrations of \( L \)-serine.

EXPERIMENTAL PROCEDURE

The “phosphatase assay,” which has been described previously (1), is based on the release of orthophosphate from P-serine.1 Unless otherwise specified the final concentration of the components in the test system were 0.01 M \( \mathrm{MgCl}_2 \), 0.05 M succinate, 0.05 M acetate buffer, pH 5.90, and 0.01 M \( \mathrm{L} \)-P-serine. Incubations were carried out at 38° and aliquots were removed for orthophosphate analysis at zero time, 5, and 10 minutes. A unit of enzyme is that amount which will cause the liberation of 1.0 μmole of orthophosphate per ml of incubation mixture in 10 minutes.

The partially purified P-serine phosphatase which was used had a specific activity of 1.8 units per mg of protein, and was prepared according to the previously described procedure (1).

The “exchange assay,” which has been described previously (1), is based on the incorporation of serine-\( \mathrm{C}^4 \) into a P-serine pool. The assay mixture contained 10 μmoles of \( \mathrm{MgCl}_2 \), P-serine as indicated, 50 μmoles of Tris buffer, pH 7.12, 2.63 μmoles of uniformly labeled \( \mathrm{L} \)-serine-\( \mathrm{C}^4 \) (specific activity = 0.574 × 10⁶ c.p.m. per μmole), and enzyme in a total volume of 1.0 ml. The tubes were incubated at 38° and the procedure for terminating the reaction, isolating the P-serine by ion exchange chromatography, and determining the specific activity have been previously described (6).

Materials

\( \alpha \)-Methylserine and \( \alpha \)-hydroxyethylserine were kindly provided by Dr. E. E. Snell. \( \mathrm{L} \)-Alanine, \( \mathrm{D} \)-alanine, glycine, \( \mathrm{L} \)-threonine, \( \mathrm{DL} \)-allothreonine, \( \mathrm{L} \)-cysteine, \( \gamma \)-aminobutyric acid, \( \alpha \)-aspartic acid, \( \alpha \)-glutamic acid, and glycolic acid were obtained from the California Foundation for Biochemical Research. \( \mathrm{DL} \)-Homoserine was purchased from Nutritional Biochemicals Corporation. \( \alpha \)-Amino-isobutyric acid and \( \alpha \)-\( \alpha \)-amino-\( \alpha \)-butyric acid were obtained from the Amino Acid Manufacturing Company. \( \mathrm{DL} \)-Methionine and \( \mathrm{L} \)-histidine were purchased from Merck Chemicals Company. The sodium salt of 

RESULTS

Inhibition of Hydrolytic Activity—Preliminary observations showed that the rate of dephosphorylation of \( \mathrm{DL} \)-P-serine under our experimental conditions was not linear after 15 minutes (1). It was later found that this nonlinearity was due to an accumulation of the product L-serine. Fig. 1 shows the relationship between L-serine concentration and activity. Under identical conditions L-serine inhibited the dephosphorylation of \( \mathrm{DL} \)-P-serine and \( \mathrm{L} \)-P-serine to approximately the same extent. Since the \( K_m \) of \( \mathrm{L} \)-P-serine is \( 5.8 \times 10^{-4} \) M in contrast to the \( K_m \) for \( \mathrm{DL} \)-P-serine which is \( 4.2 \times 10^{-4} \) M, it was concluded that the effect of L-serine on \( \mathrm{DL} \)-P-serine represented primarily the inhibition of

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1 The abbreviation P-serine is \( O \)-phosphoserine.
L-P-serine dephosphorylation. The reciprocal of the activity plotted against L-serine concentration (Dixon plot) (7) is linear in the range from 0 to 0.002 M. Fig. 2 compares L- and D-serine as inhibitors. The top curve shows the rate of L-serine dephosphorylation in the absence of added serine. Under identical conditions, D-serine (5.0 \times 10^{-4} M) inhibited the reaction 42%, whereas L-serine (5.0 \times 10^{-4} M) inhibited the reaction only 6%. The small inhibition observed with D-serine suggested that a significant inhibition would require higher concentrations. As illustrated in Fig. 3, D-serine inhibits the dephosphorylation of both L- and D-P-serine. The reciprocal of the activity plotted against D-serine concentration is linear in the range from 0 to 0.05 M.

A series of compounds were tested to determine further the specificity of the inhibition (Table I). Many compounds which are similar in structure to serine do not inhibit. Of those tested, only L-serine, D-serine, glycine, and L-alanine had a significant effect at a concentration of 0.01 M. In addition to the compounds shown in Table I, the following compounds were tested at a final concentration of 0.01 M and did not inhibit: ethanola-
mine, \( \alpha \)-methylserine, \( \alpha \)-hydroxymethylserine, L-cysteine, DL-
\( \alpha \)-amino-\( \alpha \)-butyric acid, \( \alpha \)-aminoisobutyric acid, \( \gamma \)-aminobutyric acid, L-histidine, DL-methionine, L-threonine, DL-allothreonine, \( \beta \)-alanine, L-glutamic acid, L-aspartic acid, glycolic acid, DL-glyceraldehyde. It may be concluded that the inhibition is specific for a carboxyl group, amino group (preferably in the L-configuration), and either a \(-CH_2\) or -OH attached to the \( \alpha \) carbon. The specificity pattern for the substrate (1) and inhibition are very similar. The specific inhibition by L-serine and structurally related amino acids appears to have no relationship to the general inhibition of alkaline and acid phosphatases by amino acids (8, 12).

In order to characterize the inhibition, the initial rates of hydrolysis were determined at several substrate levels in the presence of zero, 2.0 \times 10^{-4} M, and 5.0 \times 10^{-4} M L-serine. The results are presented in Fig. 4 in the form of Lineweaver-Burk plots (13). This type of inhibition data, where there is a change in intercept without change in slope, has been classified as uncompetitive by Ebersole et al. (14). It will become clear from the following discussion that the inhibition of P-serine phosphatase by serine is probably an apparent example of uncompetitive inhibition and, like glucose 6-phosphatase (15), the type of inhibition probably will not fit one of the classical types. In order to evaluate further the inhibition data, a reciprocal velocity expression was derived (16) by steady state treatment of the following mechanism:

\[
E + PS \xrightleftharpoons{k_1} EPS \xrightarrow{k_2} EP + S
\]

These symbols have been used previously in Equation 1 except for \( k_3 \), \( k_4 \), \( k_5 \), and \( k_6 \) which are velocity constants. The Michaelis complex of serine with phosphoryl-enzyme in Equa-
tion 1 has been omitted in order to simplify the derivation. It has been assumed that \( v = \frac{K_m}{[EP]} \) and that the rate of change of concentration equals zero for intermediates \( EPS \) and \( EP \).

The reciprocal velocity expression is as follows:

\[
\frac{1}{v} = \frac{1}{V_{max}} + \left[ \frac{k_2}{k_1} \frac{I}{V_{max}} + \frac{K_m}{V_{max}} \right] \frac{1}{S}
\]
\[ v = \text{the velocity at any substrate concentration (S)} \]
\[ v = V_{\text{max}} \text{when (S) = \infty and I = 0} \]
\[ e = \text{total enzyme concentration} \]
\[ K_m = \text{the substrate concentration giving 50% of } V_{\text{max}} \]
\[ K_i' = \text{the inhibitor concentration giving 50% of } V_{\text{max}} \]

Inspection of Equation 3 shows that if 1/v is plotted against 1/S the ordinate intercept will be 1/V_{\text{max}} when I is zero, but when I is a finite concentration, the ordinate intercept will be 1/V_{\text{max}} \left(1 + I/K_i'\right). Furthermore, the slope will be \( K_i'/V_{\text{max}} \) when I is zero, but when I is a finite concentration, the slope will be \( (K_m + k_d/k_i'I/K_i') \cdot 1/V_{\text{max}} \). The observed changes in intercept with different levels of serine in Fig. 4 are in agreement with Equation 3. However, since the slope of the three lines in Fig. 4 shows no well defined change, the inhibition data are in agreement with the slope term in Equation 3 only if the term \( k_d/k_i \) is very small.

In summary, the kinetic study of the inhibition by L-serine is consistent with the proposed mechanism (Equation 2) and the reciprocal velocity expression (Equation 3). However, if further experiments can be carried out under conditions where a well defined slope change can be observed, this would make it possible to verify that the slope is a function of the term \( k_d/k_i - 1/K_i' \), and this would not only further substantiate a double displacement mechanism but would eliminate a single displacement mechanism (10). With glucose 6-phosphatase (15) it has been possible to eliminate a single displacement mechanism on a kinetic basis.

It was of interest to determine whether the inhibition by glycine and alanine was the same type as that observed for L-serine. This was tested as described in Fig. 4 by measuring the rates of hydrolysis at different substrate levels in the absence of 0.01 M \( \alpha \)-d-alanine and 0.01 M glycine. The reciprocal plots again showed a change in intercept whereas the slope remained essentially constant.

With Equation 3, \( K_i' \) for serine and structurally related inhibitors can be determined (Method a) from the ratio of intercepts obtained from a Lineweaver-Burk plot as follows:

\[
\frac{\text{Intercept-1}}{\text{Intercept-0}} = \frac{1}{V_{\text{max}}} \left(1 + \frac{1}{K_i'}\right)
\]

(4)

where Intercept-I is the intercept at a finite concentration of I (serine) and Intercept-0 is the intercept in the absence of I. Solving for \( K_i' \) there results:

\[
K_i' = \frac{\text{Intercept-0}}{\text{Intercept-1} - 1}
\]

(5)

Values for \( K_i' \) (Method a) may also be obtained from a Dixon plot (7). Equation 3 is rearranged into the following form:

\[
\frac{1}{v} = \frac{1}{V_{\text{max}}} \left(1 + \frac{K_m}{S}\right) + \frac{1}{V_{\text{max}}} \left(\frac{1}{K_i'} + \frac{k_d}{k_i} \cdot \frac{1}{SK_m}\right)
\]

(6)

A plot of 1/v against I should yield a linear relationship with \( 1/V_{\text{max}} \left(1/K_i' + k_d/k_i - 1/S \cdot K_i'\right) \) as the slope and \( 1/V_{\text{max}} \left(1 + K_m/S\right) \) as the intercept. The slope, under the conditions used, can be simplified since the substrate concentration is much higher than \( K_m \). With the use of the simplified terms for slope and intercept, \( V_{\text{max}} \) is calculated from the intercept and \( K_i' \) from the slope. When activity is plotted as % activity, e.g. Figs. 1 and 3, the estimation of \( K_i' \) simplifies even further since \( V_{\text{max}} = 1 \) and therefore \( K_i' = 1/\text{slope} \). With Methods a and b, \( K_i' \) has been evaluated for \( \alpha \)- and L-serine with \( \alpha \)- and L-P-serine as substrates, and these values are given in Table II.

### Table I

**Specificity of inhibition of O-phosphoserine phosphatase**

Table I gives the "phosphatase assay" used with 0.135 unit of enzyme preparation per ml in the presence of 0.01 M inhibitor.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. L-Serine</td>
<td>95</td>
</tr>
<tr>
<td>2. D-Serine</td>
<td>25</td>
</tr>
<tr>
<td>3. L-Alanine</td>
<td>46</td>
</tr>
<tr>
<td>4. D-Alanine</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5. Glycine</td>
<td>23</td>
</tr>
<tr>
<td>6. DL-Homoserine</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

### Table II

**Evaluation of \( K_i' \) for \( \alpha \)- and L-serine at pH 5.9**

The values of \( K_i' \) in Substrates 2 to 5 were calculated from the Dixon plots (7) (Method b) illustrated in Figs. 1 and 3, and the value for \( K_i' \) in Substrate 1 was calculated from the Lineweaver-Burk plots (Method a) illustrated in Fig. 4. The value for \( K_i' \) in Substrate 6 was taken from Fig. 5. Methods a and b are described in the text.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Method</th>
<th>Concentration range of</th>
<th>( K_i' )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>l-Serine</td>
<td>d-Serine</td>
</tr>
<tr>
<td>1. DL-P-serine</td>
<td>a</td>
<td>0.50 \times 10^{-4}</td>
<td>5.9 \times 10^{-4}</td>
</tr>
<tr>
<td>2. L-P-serine</td>
<td>b</td>
<td>0.59 \times 10^{-2}</td>
<td>2.9 \times 10^{-4}</td>
</tr>
<tr>
<td>3. D-P-serine</td>
<td>b</td>
<td>0.64 \times 10^{-2}</td>
<td>6.5 \times 10^{-4}</td>
</tr>
<tr>
<td>4. DL-P-serine</td>
<td>b</td>
<td>2.00 \times 10^{-4}</td>
<td>6.5 \times 10^{-4}</td>
</tr>
<tr>
<td>5. D-P-serine</td>
<td>b</td>
<td>2.00 \times 10^{-3}</td>
<td>6.8 \times 10^{-4}</td>
</tr>
<tr>
<td>6. L-P-serine</td>
<td>b</td>
<td>5.00 \times 10^{-4}</td>
<td>6.8 \times 10^{-4}</td>
</tr>
</tbody>
</table>

Fig. 4. Characterization of the inhibition by L-serine. The "phosphatase assay" with 0.135 unit of enzyme preparation per ml was used. DL-P-serine was used as the substrate; however, only the concentration of L-P-serine is plotted. •, in the absence of L-serine; ■, 2.0 \times 10^{-4} M L-serine; ▲, 5 \times 10^{-4} M L-serine.
The purified P-serine phosphatase from bakers' yeast (5) was kindly provided by Dr. M. Schramm. The following buffers were used in the determination of $K_i'$. Values for $K_i'$ were calculated by Method b.

* F. C. Neuhaus and W. L. Byrne, unpublished observations.
† F. C. Neuhaus and W. L. Byrne, unpublished observations.
‡ Not tested.

**Fig. 5.** Variation of $K_i'$ and $K_m$ with pH. The "phosphatase assay" with 0.135 unit of enzyme preparation per ml was employed. The following buffers were used in the determination of $K_m$: 0.05 M acetate and 0.05 M succinate; pH 4.48 and pH 5.90; 0.05 M Tris, pH 7.05. Values for $K_m$ were evaluated from Lineweaver-Burk plots. The following buffers were used in the determination of $K_i'$: 0.05 M acetate and 0.05 M succinate; pH 5.00; 0.05 M Tris, pH 7.20 and 8.10. The L-P-serine (L-PS) concentration for the determination of $K_i'$ was 0.005 M. Values for $K_i'$ were calculated by Method b.

Variation of $K_i'$ and $K_m$ with pH—If the inhibition by L-serine and the exchange of L-serine are related, the effectiveness of L-serine as an inhibitor ($K_i'$) might be expected to show the same variation with pH as described previously for the exchange curve (1). Values for $K_i'$ were determined at three pH values by Method b. Contrary to what was expected it was found that L-serine becomes a progressively better inhibitor, i.e., $K_i' > K_i'$ at pH 6, $K_i' > K_i'$ at pH 7, and $K_i' > K_i'$ at pH 8. Since it was observed that $K_m$ did not vary significantly with pH in the range 5.9 to 7.6, the change in $K_i'$ cannot be correlated with a change in $K_m$ for L-P-serine.

Exchange of L-serine-C$^{14}$ with L-serine—It has been established that D-serine and L-P-serine are both hydrolyzed at the same site on P-serine phosphatase (1, 2). Moreover, with either L-P-serine or D-P-serine as the substrate, either L- or D-serine inhibits the phosphatase activity. If the inhibition and exchange are related as proposed in Equation 1, it should be possible to transfer a phosphoryl group with any combination of donor (L- or D-P-serine) and acceptor (L- or D-serine). Since only L-serine-C$^{14}$ was available, it was decided to try the transfer of the phosphoryl group from D-P-serine to L-serine as shown in the following reaction:

$$D-P-serine + L-serine-C^{14} \rightarrow L-P-serine-C^{14} + D-serine$$

In Fig. 6, Curve A, the transfer of a phosphoryl group from D-P-serine to L-serine-C$^{14}$ is demonstrated. The initial rate of transfer was 34% of the rate of the exchange of L-serine-C$^{14}$ with L-P-serine (1). This calculation is based on the total counts exchanged during the first 5 minutes. When the log (100 minus % exchange) was plotted, it became evident that the incorporation was not first order as in the case of the exchange of L-serine-C$^{14}$ with L-P-serine (1). The enzyme appears to dephosphorylate preferentially the L-P-serine which is formed even though there is a high concentration of D-P-serine present. The preferential dephosphorylation of L-P-serine in the presence of a large pool of D-P-serine was tested by incubating 11 mmoles of L-P-serine-C$^{14}$ in the presence of 10,000 mmoles of D-P-serine. The incubation system was not inhibited with L-serine as in the case of the "exchange assay." In Fig. 6, Curve B shows that within 5 minutes 60% of the L-P-serine-C$^{14}$ was dephosphorylated, and this assay was performed under conditions where only 1 to 2% of the total P-serine was dephosphorylated. It can be concluded from this experiment that the L-P-serine-C$^{14}$ would be preferentially dephosphorylated, and that the reported rate for the transfer of a phosphoryl group from D-P-serine to L-serine represents a minimum value.
The possibility was considered in this experiment that the D-P-serine was contaminated with a trace of L-P-serine and that the formation of P-serine-C$^{14}$ was made possible by L-P-serine. However, it had been previously concluded from optical rotation measurements and d-amino acid oxidase studies on the enantiomers of P-serine that the maximum contamination of one enantiomer with the other enantiomer is less than 1% (10), and since P-serine phosphatase preferentially dephosphorylates L-P-serine in the presence of D-P-serine, preincubation of the enzyme, D-P-serine, and Mg$^{++}$ for 20 minutes in the absence of L-serine should, according to Fig. 6, Curve B, remove 90% of all the contaminating L-P-serine, if any, in the D-P-serine. If L-P-serine were the actual donor, preincubation would lower the L-P-serine content to the point where the final concentration would be sufficiently below the $K_m$ for L-P-serine so that the initial rate of incorporation of radioactivity into the P-serine pool would be smaller than in the nonpreincubated system (Curve A). This was tested by preincubating the enzyme, Mg$^{++}$, and D-P-serine for 20 minutes before adding the L-serine-C$^{14}$. Essentially the same rate (Curve C) was obtained with preincubation as was observed without preincubation (Curve A). It was concluded that the phosphoryl-enzyme, proposed in Equation 1, may be formed from either L-P-serine or D-P-serine.

**DISCUSSION**

A schematic mechanism for P-serine phosphatase, based on Equation 1, is given in Fig. 7. The hydrolytic activity may be visualized in the following sequence of steps. After the binding of P-serine to the enzyme to give an enzyme (P-serine) complex (I), there is an attack on P-serine by a nucleophilic group (X) on the enzyme with a rupture of the phosphorus oxygen bond to give a phosphoryl-enzyme (serine) complex (II). The serine dissociates from II to give the phosphoryl-enzyme (III) and serine. This is then followed by a nucleophilic attack on the phosphoryl group of the phosphoryl-enzyme by water to give an orthophosphate and enzyme. The exchange reaction may be visualized by the binding of L-serine onto the phosphoryl-enzyme (III) to form the phosphoryl-enzyme (serine) intermediate (IV). The phosphoryl group on intermediate II is protected from or is no longer susceptible to a nucleophilic attack by water.

The description of the proposed mechanism does not differentiate between the D- and L-forms of P-serine and serine, and therefore assumes that the intermediates I and II are analogous and III is identical for both forms. The description also assumes that the intermediates I, II, and III are identical for the hydrolytic activity, exchange activity, and inhibition.

A close relationship between the activity observed with the D- and L-forms was indicated by the following observations. With either L-P-serine or D-P-serine as the substrate, either L- or D-serine inhibit the hydrolytic activity, and the $K_m$ for L- or D-serine is not significantly influenced by the choice of substrate. The ability of D-serine to accept a phosphoryl group has not been tested, but L-serine will act as an acceptor for the phosphoryl moiety of either L- or D-P-serine. The $V_{max}$ for the hydrolytic activity with D- or L-P-serine is identical, but the $K_m$ values are different. This comparison of $V_{max}$ and $K_m$ is not sufficient evidence to make a defined comparison of the velocity constants for the two enantiomers. However, this comparison is consistent with the mechanism in Fig. 7, and it could mean that the hydrolysis of intermediate III is the limiting step in the hydrolytic sequence.

A close relationship between the hydrolytic activity, exchange activity, and inhibition was indicated by the following observation. The $K_m$ for L-P-serine in the exchange reaction and $K_m$ for L-P-serine in the phosphatase reaction are identical. The hydrolytic activity and exchange activity require Mg$^{++}$. The $K_m$ for L-serine at pH 7.1 are almost identical. The maximum velocity in the exchange reaction essentially equals the maximum velocity in the hydrolytic activity. There is a quantitative relationship between the exchange activity, inhibition, and as in the case of glucose 6-phosphatase (15), the quantity of orthophosphate whose formation is prevented by the inhibitor (acceptor) approximately equals the quantity of inhibitor incorporated into the pool of substrate (donor).

The effect of pH on the exchange activity, phosphatase activity, and inhibition by L-serine has been studied. With respect to the phosphatase activity, 50% of the maximum activity was observed at pH 4.2 and 7.8 whereas in the case of the exchange reaction, 50% of the maximum exchange was realized at pH 6.4 and 7.9. One possible explanation for the difference in response of the hydrolytic activity and the exchange activity with pH involves the Michaelis complex of serine and phosphoryl-enzyme (II). Serine can complex with the phosphoryl-enzyme to give intermediate II over a wide pH range, as judged by inhibition, and therefore it seems likely that the effect of pH does not involve the formation of intermediate II. By elimination, this suggests that the conversion of intermediate II to I is the step which is markedly influenced by pH. A proposal which would explain this effect of pH is that the proper ionic form of II is required for the conversion of intermediate II to I. The conversion of II to I is required for the exchange activity.

$^3$Unless otherwise specified the data cited in this discussion are taken from the present paper or Paper II of this series (1).
but not for the phosphatase activity. Consideration of the data for the rate of the exchange reaction with pH indicates that the proposed ionic form could be assigned a pKₐ of 6.4, and a pKₐ of 6.4 suggests the secondary ionization of the phosphoryl group in intermediate II.

The proposed mechanism for P-serine phosphatase includes three intermediates. Intermediate I is justified since the omission of this intermediate leads to a velocity equation which would predict competitive inhibition (15, 16). Intermediate II is consistent with the inhibition of the hydrolytic activity by serine and related compounds, and it has been used to rationalize the influence of pH on the exchange activity. A double displacement mechanism, based on intermediate III, is justified (17) by the rapid exchange activity, and by the similarity of substrate specificity and inhibitor specificity. A double displacement mechanism is also supported by consideration of an analogous phosphatase, glucose 6-phosphatase. Kinetic studies of the glucose inhibition of glucose 6-phosphatase have eliminated a single displacement mechanism (15). The exchange of L-serine with the serine moiety of L-P-serine, under conditions where there was no detectable incorporation of P³² labeled Pᵢ into P-serine, is consistent with intermediates I, II, and III, and justifies the omission of an arrow indicating the synthesis of the phosphoryl-enzyme from enzyme and Pᵢ. Preliminary experiments on the nature of the postulated groups, X and ⊛, (Fig. 7) have not been rewarding, but the effect of pH on the hydrolytic activity and the exchange activity suggests that the ⊛ group may have a pKₐ of 7.8 to 7.9. A single serine specific site involving ⊛ and X has been proposed based on the substrate and inhibitor specificity.

Table III summarizes the observations of several groups of workers on P-serine phosphatase from different sources. Hydrolysis of P-serine, inhibition by serine, and exchange of L-serine-C³⁴ have been observed in each case. The common properties of the enzymes from the different sources suggests that they all have an analogous mechanism. However, there is a quantitative difference in their sensitivity to serine inhibition. The enzyme preparations from chicken and rat liver are inhibited by low concentrations of L-serine whereas relatively high concentrations of serine are required to inhibit the enzyme from bakers’ yeast and Escherichia coli.

**SUMMARY**

1. The inhibition of phosphoserine phosphatase by serine is described. In addition to L-serine and D-serine, the structural analogues, L-alanine and glycine, were found to inhibit. The Kᵢᵢ (the concentration of inhibitor which gives 50% inhibition) for L-serine is 6.5 × 10⁻⁴ M and the Kᵢᵢ for D-serine is 2.7 × 10⁻² M, with L-phosphoserine as the substrate. Essentially the same values are obtained with D-phosphoserine as the substrate.

2. Phosphoserine phosphatase can catalyze the transfer of a phosphoryl group from D-phosphoserine to L-serine in addition to the previously described exchange reaction between L-phosphoserine and L-serine.

3. A mechanism for phosphoserine phosphatase which correlates the hydrolytic activity, exchange activity, and inhibition is proposed, and the supporting evidence is outlined. A double displacement mechanism, based on a phosphoryl-enzyme intermediate, has been proposed.

**Acknowledgment**—We would like to thank Professor G. W. Schwert and Dr. Louis F. Hass for their helpful advice.

**REFERENCES**

Metabolism of Phosphoserine: III. MECHANISM OF O-PHOSPHOSERINE PHOSPHATASE
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