Mechanistic Studies of Phosphoserine Phosphatase, an Enzyme Related to P-type ATPases*

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Phosphoserine phosphatase belongs to a new class of phosphotransferases forming an acylphosphate during catalysis and sharing three motifs with P-type ATPases and haloacid dehalogenases. The phosphorylated residue was identified as the first aspartate in the first motif (DXDXT) by mass spectrometry analysis of peptides derived from the phosphorylated enzyme treated with NaBH₄ or alkaline [¹⁸O]H₂O. Incubation of native phosphoserine phosphatase with phosphoserine in [¹⁸O]H₂O did not result in ¹⁸O incorporation in residue Asp-20, indicating that the phosphoaspartate is hydrolyzed, as in P-type ATPases, by attack of the phosphorus atom. Mutagenesis studies bearing on conserved residues indicated that four conservative changes either did not affect (S109T) or caused a moderate decrease in activity (G178A, D179E, and D183E). Other mutations inactivated the enzyme by >80% (S109A and G180A) or even by ≥99% (D178N, D183N, K158A, and K158R). Mutations G178A and D179N decreased the affinity for phosphoserine, suggesting that these residues participate in the binding of the substrate. Mutations of Asp-179 decreased the affinity for Mg²⁺, indicating that this residue interacts with the cation. Thus, investigated residues appear to play an important role in the reaction mechanism of phosphoserine phosphatase, as is known for equivalent residues in P-type ATPases and haloacid dehalogenases.

The reaction mechanism of many phosphatases or phosphotransferases involves the formation of a catalytic intermediate, in which the phosphate group derived from the substrate is bound to the side chain of a serine, histidine, cysteine (1, 2), or aspartate residue present in the catalytic site. The group of enzymes forming a phosphoaspartate intermediate comprises P-type ATPases (3) and a newly identified class of phosphotransferases further detailed below (4, 5), as well as other enzymes including bacterial protein phosphatases (6).

The function of P-type ATPases (e.g. Na⁺/K⁺ATPases and Ca²⁺ATPases) is to transport ions across membranes against an electrochemical gradient and at the expense of ATP (reviewed in Refs. 7 and 8). These enzymes are transmembrane proteins that covalently bind phosphate in P-type ATPases and as phosphomannomutases and an aspartate that is phosphorylated during the catalytic cycle. Lack of precise structural information (the resolution of the best structural models is 8 Å (9, 10)), due to the fact that ATPases, as well as membrane proteins, are difficult to crystallize, prevents the full understanding of the detailed mechanism by which the control of phosphoenzyme formation and hydrolysis is exerted by binding of ions and conformational changes.

The newly identified class of phosphotransferases is characterized by a conserved DXDX(T/V) motif close to the N terminus (5). It comprises at least 10 different enzymes that are typically soluble proteins acting either as monophosphate phosphatases (e.g. phosphoserine phosphatase and phosphoglycolate phosphatase) or as phosphomutases (β-phosphoglucomutase and eukaryotic phosphomannomutase) (4, 5, 11, 12). Formation of an acylphosphate intermediate was shown for these four enzymes (5, 13–15) and, in the case of phosphomannomutase, the phosphorylated residue was shown to be the first aspartate in the DXDX(T/V) motif (5). Results of site-directed mutagenesis of the two aspartates in phosphoserine phosphatase were compatible with this conclusion (5).

Iterated sequence comparisons and position-specific iterated BLAST (PSI-BLAST) searches (16) starting from haloacid dehalogenase have shown that this enzyme shares three statistically significant motifs (Fig. 1) with the new class of phosphomonoesterases/phosphomutases and P-type ATPases (4, 11). The first of these motifs (DXDXT in phosphomonoesterases/phosphomutases, DRKTG in ATPases, and DXYGT in dehalogenases) contains an absolutely conserved aspartate, which covalently binds phosphate in P-type ATPases and in phosphomannomutases and an ε-hydroxy acid in haloacid dehalogenases (17). The second motif contains a strictly conserved serine or threonine, and the third motif contains a strictly conserved lysine residue followed, at some distance, by lesser conserved residues and a strictly conserved aspartate. Some of these residues have been mutated in P-type ATPases (reviewed in Refs. 7 and 18) and in Pseudomonas sp. haloacid dehalogenase (19) and shown to play an important role in catalysis. In addition, the three-dimensional structure of haloacid dehalogenase indicates that the three motifs line the catalytic pocket (20–22). This group of enzymes appears to be also related to phosphonoacetaldehyde phosphatases, with which they share the first motif (DWAGT), and possibly also part of the third motif (23).

We previously noted that the third motif was particularly conserved between phosphoserine phosphatases and P-type ATPases (15), all these enzymes showing the same GDGXXD sequence (Fig. 1), whereas the corresponding sequence in haloacid dehalogenase is SSXXXD (11). Another difference between ATPases and haloacid dehalogenases is that in dehalogenases, the covalent intermediate is hydrolyzed by attack of the γ-carbon of the aspartate (17), whereas in ATPases the attack occurs on the phosphorus atom (24). Nothing was known
in this respect for phosphoserine phosphatase.

As phosphoserine phosphatase may turn out to be an interesting model for the study of ATPases, the purpose of the present work was to explore further the reaction mechanism of this enzyme by 1) providing definitive identification of the phosphorylated residue, 2) determining the site of attack of the phosphoryl enzyme intermediate by water, and 3) investigating the effect of mutations of conserved residues of the second and third motifs, which have not yet been studied until now.

**EXPERIMENTAL PROCEDURES**

**Materials**—Glucose and Mes¹ were from Acros. Other chemicals were from Sigma or Merck. [1⁴NO₃]H₂O was from Cambridge Isotope Laboratories. Q-Sepharose, T₇ Thromosquence, [³²P]P₃, and l-[³⁵S]Cysteine were from Amersham Pharmacia Biotech. Pco polymerase and the restriction enzymes were from Roche Molecular Biochemicals. Trypsin, from Life Technologies, Inc., had not been treated with tosyl-L-phenylalanine chloromethylketone. Bactotryptone® and Bactoyeast® extract were from Amersham Pharmacia Biotech. Pwo polymerase and the restriction enzymes were from Roche Molecular Biochemicals. Trypsin, from Amersham Pharmacia Biotech.

**Site-directed Mutagenesis—**Site-directed mutagenesis was performed by using Pco DNA polymerase and “back-to-back” mutated primers as described (26). The polymerase chain reaction was directly performed on a PēT3α plasmid (27) containing the sequence of human phosphoserine phosphatase (15). The plasmids were re-circularized, amplified in *Escherichia coli* JM109, and checked by sequencing using T7 Thromosquence, fluorescent primers, and the LI-COR automated DNA sequencer 4000L.

**Expression of the Recombinant Proteins—**Bacteria BL21(DE3)pLysS (27) harboring the expression plasmids were grown aerobically in 0.5 liters of M9 medium at 37 °C until A₅₇₀ reached 0.5–0.6. The culture was then maintained on ice for 60 min before addition of isopropylthio-galactoside to a final concentration of 0.4 mM. Phosphoserine phosphatase was expressed during 2 h at 18 °C. Bacterial extracts were prepared as described (15) and centrifuged for 40 min at 20,000 × g and 4 °C. The resulting supernatant (25 ml) was diluted 3-fold with buffer A (25 mM glycine, pH 9, 1 mM dithiothreitol, 1 μM/μl leupeptin, 1 μM/μl antipain) and applied onto a Q-Sepharose column (1.6 × 10 cm). The column was washed with 100 ml of buffer A, and protein was eluted with a NaCl gradient (0–400 ml in 400 ml of buffer A). Phosphoserine phosphatase came out at a Na+ concentration of approximately 300 mM.

**Enzyme and Protein Assays—**Except if indicated otherwise, the hydrolytic activity of phosphoserine phosphatase was measured by the release of [¹⁴C]serine from [³⁵S]Phosphoserine as in (5) but with 1 mM phosphoserine. To study the inhibition by serine, the enzyme was measured by the release of [³⁵S]P (28) from 1 mM [³⁵S]Phosphoserine (29). The phosphatase reaction (incorporation of [³⁵S]serine into phosphoserine) and the phosphoenzyme formation were determined as described (15).

Protein was measured according to Bradford (30) with bovine gamma globulin as a standard. The NIH Image program (developed at the National Institutes of Health) was used to analyze SDS-PAGE gels stained with Coomassie Blue.

**RESULTS**

**Identification of the Phosphorylated Aspartate by Reduction with Borohydride—**A previous attempt (5) to identify the phosphorylated residue by reduction of the phosphorylated aspartate with tritiated borohydride, followed by digestion of the protein with trypsin and separation of the digested peptides was unsuccessful, presumably because the low degree of phosphorylation of the protein (about 7%) prevented the identification of a specific radioactive peak in the high pressure liquid chromatography elution profile. Knowing that the phosphorylated residue was most likely the first aspartate in the DAGD motif, we decided to omit separation of peptides by high pressure liquid chromatography and to analyze directly by tandem mass spectrometry the protein digests derived from the preparations of both non-phosphorylated/reduced and phosphorylated/reduced phosphoserine phosphatase. Prediction of the digestion pattern indicated that the positive ion corresponding to the phosphorylatable peptide (DVDSVIR) would have an m/z of 904 if not reduced and of 890 after reduction of phosphoserine to homoserine. An m/z 904 ion was indeed present in the electrospray ionization spectrum of the two digests, as well as a small amount of the m/z 890 ion (not shown). Upon fragmentation, this m/z 904 ion yielded two major ions with m/z 789 and 575 corresponding to fragments y7 (VDSTVIR) and y5 (STVIR) (not shown). We fragmented also the minor m/z 890 ion. In the case of the phosphorylated/reduced enzyme preparation, two major fragments of m/z 789 and 575 were also observed, showing that this peptide is identical to the m/z 904 ion, except for reduction of the first aspartate to homoserine.

¹ The abbreviations used are: Mes, 4-morpholinolmethanesulfonic acid; PAGE, polyacrylamide gel electrophoresis.
zyme was incubated without (A) tic/chymotryptic digest of phosphoserine phosphatase. The denatured phosphoenzyme in [18O]H2O should result in the disclosure of only one radiolabeled peptide of 789 kDa in the samples, indicating that only one aspartate had served the acylphosphate bond (14). Autoradiography of the gel analyzed by SDS-PAGE at low temperature, in order to pre-

The purified proteins were used to investigate their kinetic properties and their ability to form a phosphoenzyme (Table I). Mutant S109T had the same phosphatase activity as the wild type enzyme, and four other mutants (G178A, D179E, G180A, and D183E) still had between 15 and 78% of this activity. An intermediate value (6%) was found with mutant S109A, whereas the other mutants had only about 1% of the control activity (K158R and D179N) or no detectable activity (K158A and D183N). Significant increases in the $K_m$ for phosphoserine digestion with trypsin and chymotrypsin and analyzed by mass spectrometry. As for the experiment with NaBH$_4$ (see above), the $m/z$ 904 ion was found both for the control and for the partially phosphorylated protein. Its fragmentation yielded two ions ($m/z$ 789 and 575) whose masses correspond to the $y^7$ and $y^5$ fragments of the DVDSTVIR peptide (not shown). In both preparations, fragmentation of the $m/z$ 906 (904 + 2) ion yielded ions of $m/z$ 789, 790, and 791 as well as of $m/z$ 575, 576, and 577 (Fig. 3). However, the ratios of the abundance of the $m/z$ 789/791 and $m/z$ 575/577 were significantly higher in the case of the phosphorylated preparation than in the case of the control, indicating the presence of an excess of $^{18}$O in the aspartate residue that had been removed upon fragmentation. These results confirmed therefore that Asp-20 is the phosphorylated residue and showed that it was possible to measure $^{18}$O incorporation by mass spectrometric analysis of a crude digest.

Lack of Paracatalytic Incorporation of $^{18}$O from $[^{18}$O$]$H$_2$O—Now that we know that it was possible to measure incorporation of $^{18}$O into phosphoserine phosphatase, we decided to look for the fate of water in the enzyme-catalyzed hydrolysis of the phosphoenzyme. We expected that an attack on the carbon would result in the incorporation of $^{18}$O into the carboxyl group of aspartate if phosphoserine phosphatase was allowed to hydrolyze its substrate in $[^{18}$O$]$H$_2$O. After several catalytic cycles, both oxygen atoms of the carboxyl group would be replaced, leading to a mass increase of 4 daltons. By contrast, no $^{18}$O should be incorporated if water attacked the phosphorus atom. Lyophilized phosphoserine phosphatase (250 μg) was dissolved in 50 μl of $[^{18}$O$]$H$_2$O containing 5 mM MgCl$_2$, 25 mM Mes, pH 6.5, and 20 or 0 (control) mM phosphoserine and incubated overnight at 30 °C. The enzyme was precipitated with trichloroacetic acid, washed several times to remove $[^{18}$O$]$H$_2$O, and digested with trypsin. No ion of $m/z$ 908 (904 + 4) was detectable in the ESI spectrum, whereas the peak of 904 was clearly apparent (not shown). Furthermore, fragmentation of ions with $m/z$ 908 did not yield the characteristic fragments of 575 and 789 $m/z$, whereas fragmentation of the 906 and 904 ions yielded the same results as observed with the control enzyme (see above). It was checked that lyophilization did not inactivate phosphoserine phosphatase.

Site-directed Mutagenesis—To study the role of the residues conserved in ATPases and phosphoserine phosphatases, we have constructed and expressed 10 mutated proteins (Table I). Extracts have been prepared 13 h after the addition of isopro
pamylthiogalactoside, and SDS-PAGE analysis showed that the 10 mutants were expressed as soluble proteins of $\sim$25-kDa subunits (except for D183N, which appeared as a 27-kDa band, but was found by matrix-assisted laser desorption ionization mass spectrometry to have the expected subunit mass).

These proteins were chromatographed on Q-Sepharose at pH 9. Analysis of the fractions by SDS-PAGE and assay of phosphoserine phosphatase activity indicated that this chromatographic step clearly separated the mutants of human phosphoserine phosphatase from bacterial phosphoserine phosphatase (5). In all cases, the recombinant phosphoserine phosphatases were similarly pure, representing in the most purified fractions $\sim$20% of the material stained with Coomassie Blue.

The purified proteins were used to investigate their kinetic properties and their ability to form a phosphoenzyme (Table I). Mutant S109T had the same phosphatase activity as the wild type enzyme, and four other mutants (G178A, D179E, G180A, and D183E) still had between 15 and 78% of this activity. An intermediate value (6%) was found with mutant S109A, whereas the other mutants had only about 1% of the control activity (K158R and D179N) or no detectable activity (K158A and D183N). Significant increases in the $K_m$ for phosphoserine

![Figure 2](image-url)
were observed with mutants G178A (80-fold) and D179N (10-fold). The apparent affinity for Mg$^{2+}$ was increased for mutants S109A and K158R and decreased in the case of mutants D179E and D179N. Remarkably, the modification of the exchange reaction did not always parallel the change in phosphatase activity. The most striking example was mutant G180A, which had a slightly increased exchange reaction, whereas its phosphatase activity was decreased by 6-fold. In the case of mutant G178A, the decrease in the ratio of the two reactions is partly due to the fact that the phosphoserine concentration used in the exchange reaction (1 mM) was not saturating.

**DISCUSSION**

**Identification of the Phosphorylation Site and Mechanism of the Phosphoenzyme Hydrolysis**—Our finding that the positive ion corresponding to the phosphorylatable peptide (DVDSTVIR) had an $m/z$ of 904 when not reduced and of 890 after reduction of phosphoaspartate to homoserine, as well as the fact that fragmentation of these two ions yielded identical y7 (VDSTVIR) and y5 (STVIR) ions, allows us to conclude that the phosphorylated aspartate in phosphoserine phosphatase is Asp-20. This conclusion is reinforced by our observation that upon alkaline hydrolysis of the control and of the phosphorylated enzymes in [18O]$\text{H}_2\text{O}$, an $m/z$ 906 ion was found which, upon fragmentation, yielded ions of $m/z$ 789, 790, and 791 as well as 575, 576, and 577 in different proportions in the two preparations. The presence of a DVDSTVIR peptide with $m/z$ 906 in the control lysate is indeed expected: the natural abundance of $^{13}$C (1.1%) and $^{18}$O (0.2%) allows one to predict that about 5.5% of the peptide will have a mass of 906 due to the presence of two $^{13}$C carbons and 3% because of $^{18}$O oxygen. The

### Table I

| Mutant | Hydrolytic activity | Exchange | Phosphoenzyme |
|--------|---------------------|----------|---------------|
|        | $V_{max}$ (µmol/min/mg) | $K_{m}$ for phosphoserine (µM) | $K_{m}$ for Mg$^{2+}$ (µM) | $K_{m}$ for serine (µM) | $V_{max}$ (µmol/min/mg) | ratio | mol/mol subunit |
| WT     | 2.7                | 20       | 100           | 500        | 0.78   | 0.29   | 0.012       |
| S109T  | 3.1                | 30       | 100           | 650        | 0.97   | 0.31   | 0.0105      |
| S109A  | 0.16               | 20       | <10           | ND         | 0.37   | 2.31   | 0.002       |
| K158R  | 0.03               | 30       | 10            | 820        | 0.06   | 2.00   | 0.0007      |
| K158A  | 0.01               | ND       | ND            | ND         | 0.01   | ND     | ND          |
| G178A  | 2.0                | 1700     | 100           | 600        | 0.11   | 0.06   | 0.001       |
| D179E  | 2.1                | 30       | 750           | 370        | 1.09   | 0.52   | 0.001       |
| D179N  | 0.015              | 200      | 1000          | ND         | 0.002  | 0.13   | ND          |
| G180A  | 0.41               | 10       | 100           | 900        | 1.1    | 2.68   | 0.0085      |
| D183E  | 1.7                | 40       | 50            | 500        | 0.24   | 0.14   | 0.0023      |
| D183N  | <0.01              | ND       | ND            | ND         | ND     | ND     | ND          |

*a* The phosphatase activity was measured through the release of $^{32}$P, from $[^{32}$P]phosphoserine.

were observed with mutants G178A (80-fold) and D179N (10-fold). The apparent affinity for Mg$^{2+}$ was increased for mutants S109A and K158R and decreased in the case of mutants D179E and D179N. Remarkably, the modification of the exchange reaction did not always parallel the change in phosphatase activity. The most striking example was mutant G180A, which had a slightly increased exchange reaction, whereas its phosphatase activity was decreased by 6-fold. In the case of mutant G178A, the decrease in the ratio of the two reactions is partly due to the fact that the phosphoserine concentration used in the exchange reaction (1 mM) was not saturating.

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This conclusion is reinforced by our observation that upon alkaline hydrolysis of the control and of the phosphorylated enzymes in [18O]$\text{H}_2\text{O}$, an $m/z$ 906 ion was found which, upon fragmentation, yielded ions of $m/z$ 789, 790, and 791 as well as 575, 576, and 577 in different proportions in the two preparations. The presence of a DVDSTVIR peptide with $m/z$ 906 in the control lysate is indeed expected: the natural abundance of $^{13}$C (1.1%) and $^{18}$O (0.2%) allows one to predict that about 5.5% of the peptide will have a mass of 906 due to the presence of two $^{13}$C carbons and 3% because of $^{18}$O oxygen. The

**FIG. 3.** Fragment spectra of the $m/z$ 906 ion present in a trypic/chymotryptic digests of non-phosphorylated (A) or phosphorylated (B) phosphoserine phosphatase submitted to alkaline hydrolysis in [18O]$\text{H}_2\text{O}$. Isotopic clusters of the fragments at $m/z$ 575 and 789 confirm the incorporation of $^{18}$O at Asp-20.
Phosphoserine Phosphatase Mechanism

Summary of the effect of mutations in several enzymes of the dehalogenase superfamily

Residues in equivalent position according to Aravind et al. (11) are in the same row. Results are taken from the following references, this paper and Ref. 5 for phosphoserine phosphatase; Ref. 19 for haloacid dehalogenase; Refs. 33–35 for Ca\(^{2+}\)-ATPase. Abbreviations used are: WT, wild type; low exp., low expression.

| Phosphoserine phosphatase | Ca\(^{2+}\)-ATPase | Haloacid dehalogenase |
|---------------------------|---------------------|-----------------------|
| Mutation                  | Hydrolytic activity | Mutation | Ca\(^{2+}\) transport | Mutation | Activity |
|                           | % WT                | D351E                  | % WT                | D10E                  | % WT                |
| None (WT)                 | 100                 | 0                      | None (WT)           | 100                   |
| Motif 1                   |                     |                        | Motif 2             |                        |
| D20E                      | 0                   | D351N                  | 0                   | K151R                 |
| D20N                      | 0                   | T353S                  | 20                  | S175A                 |
| D22E                      | 50                  | T353A                  | 0                   | S176A                 |
| D22N                      | 0                   |                        |                      | K151R                 |
| Motif 2                   |                     |                        |                      |                       |
| S109T                     | 115                 | T625S                  | 79                  |                       |
| S109A                     | 6                   | T625A                  | Low Exp.            |                       |
| Motif 3                   | 1                   | G702A                  | 20                  |                       |
| K158A                     | <0.4                | D703E                  | 31                  |                       |
| K158R                     |                      | D703N                  | <5                  |                       |
| G178A                     | 74                  | G178A                  | 20                  |                       |
| D179E                     | 78                  | G178A                  | 31                  |                       |
| D179N                     | 0.6                 | G178A                  | <5                  |                       |
| G180A                     | 15                  | G178A                  | 20                  |                       |
| D183E                     | 63                  | G178A                  | <5                  |                       |
| D183N                     | <0.4                | G178A                  | <5                  |                       |

| Phosphoserine phosphatase | Ca\(^{2+}\)-ATPase | Haloacid dehalogenase |
|---------------------------|---------------------|-----------------------|
| Mutation                  | Hydrolytic activity | Mutation | Ca\(^{2+}\) transport | Mutation | Activity |
|                           | % WT                | D351E                  | % WT                | D10E                  | % WT                |
| None (WT)                 | 100                 | 0                      | None (WT)           | 100                   |
| Motif 1                   |                     |                        | Motif 2             |                        |
| D20E                      | 0                   | D351N                  | 0                   | K151R                 |
| D20N                      | 0                   | T353S                  | 20                  | S175A                 |
| D22E                      | 50                  | T353A                  | 0                   | S176A                 |
| D22N                      | 0                   |                        |                      | K151R                 |
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The distribution of these isotopes is random in the control peptide but not in the peptide where 18O has been incorporated during alkaline hydrolysis of the phosphoenzyme. Thus, elimination of the first aspartate removes a larger proportion of the +2 atomic mass unit excess, explaining the higher proportion of the m/z ions.

This Asp-20 residue of phosphoserine phosphatase is in equivalent position to the phosphorylated aspartate in eukaryotic phosphomannomutase and ATPases and to the esterified residue in haloacid dehalogenases (5, 11). That there is no equivalent position to the phosphorylated aspartate in eukaryotic phosphomannomutase and ATPases and to the esterified residue in haloacid dehalogenases (5, 11). Site-directed mutagenesis of Motif 3

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| D179N                     | 0.6                 | G178A                  | <5                  |                       |
| G180A                     | 15                  | G178A                  | 20                  |                       |
| D183E                     | 63                  | G178A                  | <5                  |                       |
| D183N                     | <0.4                | G178A                  | <5                  |                       |

The 80-fold increase in the K\(_m\) for phosphoserine observed when Gly-178 was mutated to alanine suggests that this residue participates in substrate binding. Such is also the case for Asp-179 since mutations of this residue to asparagine (though not to glutamate) resulted in a marked increase in the K\(_m\). Both D179N and D179E mutations decreased the apparent affinity for Mg\(^{2+}\) suggesting that Asp-179 also participates in binding of this indispensable cation, as recently proposed (32) on the basis of a structural homology between haloacid dehalogenase and CheY, a Mg\(^{2+}\)-dependent enzyme that also forms a phosphoprotein.

Considering that the assays were performed in the presence of 1 mM Mg\(^{2+}\), the low activities of mutant D179N and D179E are partly explained by the use of a subsaturating concentration of the divalent cation. The effect of the G180A mutation to decrease the affinity for serine suggests that the Gly-180 residue participates in the binding of this product of the reaction.

Comparison with ATPases and Dehalogenases—Table II compares the effect of mutations to those of residues in equivalent position (Fig. 1) in Ca\(^{2+}\)-ATPase (33–35) and Pseudomonas haloacid dehalogenase (19). Site-directed mutagenesis of the first motif had indicated previously that Asp-20 could not be substituted by glutamate or asparagine without complete loss of activity, whereas the replacement of Asp-22 by glutamate (though not by glutamate) resulted in a marked increase in the K\(_m\). Both D179N and D179E mutations decreased the apparent affinity for Mg\(^{2+}\) and Mg\(^{2+}\)-dependent enzyme that also forms a phosphoprotein.

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superfamily as a serine or a threonine, and it is therefore not surprising that the conservative change S109T does not result in a loss of activity. The presence of the hydroxyl group on this residue seems particularly important since the S109A mutation resulted in a ~17-fold decrease in activity, an effect that is much more marked than the ~3-fold decrease observed for the equivalent mutation in haloacid dehalogenases. In the latter enzyme, Ser-118 was shown to form a hydrogen bond with the substrate carbonylate (22). By analogy, Ser-109 could bind the phosphate group in the substrate in phosphoserine phosphatase, as suggested by Ridder and Dijskstra (32). However, this interpretation does not agree with the absence of effect of the mutations affecting this residue on the $K_m$ of the enzyme for phosphoserine (Table 1).

The almost complete loss of activity of phosphoserine phosphatase observed when Lys-158, the first conserved residue in phosphoserine phosphatase or the equivalent residue in Ca$^{2+}$ATPase by asparaginase abolishes the formation of the phosphoenzyme indicates that this aspartate plays a major role in this process (33).

In conclusion, the results of these and of our previous mutagenesis experiments indicate that the three conserved motifs play an important role in phosphoserine phosphatase, and that it has been previously shown for haloacid dehalogenase and, to some extent also, for ATPases. This is in keeping with the fact that these residues are known to form the catalytic site in the case of haloacid dehalogenase, the only enzyme of the superfamily for which the detailed three-dimensional structure is available (20–22). The stringent requirement for these residues is indicated by the fact that in most cases even what can be considered as a conservative change caused a reduction in activity. On the basis of the finding of low but significant homologies between dehalogenases and ATPases, it has been previously proposed that the active site of ATPases can be modeled on the dehalogenase fold (11). Our results indicate that phosphoserine phosphatase may be a better model than dehalogenase both with respect to the type of reaction catalyzed and to the residues involved in catalysis.

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