Crystal Structure of a Homolog of Mammalian Serine Racemase from Schizosaccharomyces pombe*  

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D-Serine is an endogenous coagonist for the N-methyl-D-aspartate receptor and is involved in excitatory neurotransmission in the brain. Mammalian pyridoxal 5'-phosphate-dependent serine racemase, which is localized in the mammalian brain, catalyzes the racemization of L-serine to yield D-serine and vice versa. The enzyme also catalyzes the dehydration of D- and L-serine. Both reactions are enhanced by Mg-ATP in vivo. We have determined the structures of the following three forms of the mammalian enzyme homolog from Schizosaccharomyces pombe: the wild-type enzyme, the wild-type enzyme in the complex with an ATP analog, and the modified enzyme in the complex with serine at 1.7, 1.9, and 2.2 Å resolution, respectively. On binding of the substrate, the small domain rotates toward the large domain to close the active site. The ATP binding site was identified at the domain and the subunit interface. Computer graphics models of the wild-type enzyme complexed with L-serine and D-serine provided an insight into the catalytic mechanisms of both reactions. Lys-57 and Ser-82 located on the protein and solvent sides, respectively, with respect to the cofactor plane, are acid-base catalysts that shuttle protons to the protein and solvent sides, respectively, with respect to the cofactor plane. The crystal-soaking experiment showed that the substrate serine was actually trapped in the active site of the modified enzyme, suggesting that the lysino-D-alanyl residue acts as a catalytic base in the same manner as inherent Lys-57 of the wild-type enzyme.

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The abbreviations used are: NMDA, N-methyl-D-aspartate; PLP, pyridoxal 5'-phosphate; SR, serine racemase; spSR, SR from S. pombe; spSRw, wild-type spSR; spSRm, modified spSR; AMP-PCP, 5'-adenylyl methylphosphonate.

5 The protein product is a bifunctional enzyme that catalyzes racemization and the \( \alpha,\beta \)-elimination reaction of D, L-serine as mammalian SR does. SR from S. pombe (spSR) comprises 322 residues (the N-terminal Met is removed in the purified enzyme) and one PLP per subunit, the subunit molecular weight being 34,917. The mammalian SR homolog, spSR, is an interesting target enzyme for the development of a novel therapeutic compound controlling the D-serine level because D-serine is the product of the postsynaptic membrane of the excitatory synapses (1–5) and is involved in excitatory neurotransmission and higher brain functions such as learning and memory (3, 6, 7). Stimulation of the NMDA receptor requires the binding of D-serine as well as the agonist L-glutamate. The major enzyme for D-serine synthesis from L-serine in the brain is considered to be pyridoxal 5'-phosphate-dependent serine racemase (SR) (8–10). D-Serine and SR are localized on protoplasmic astrocytes that have the \( \alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor. Glutamate released from presynaptic neurons approaches and activates the \( \alpha\)-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor, which in turn induces SR to produce D-serine and is followed by D-serine release from astrocytes that act on the NMDA receptor. Recently, it was shown that not only glia but also neurons synthesize and release D-serine involved in signaling (11). SR also catalyzes \( \alpha,\beta \)-elimination of water from D- or L-serine to form pyruvate and ammonia as well as the conversion of L-serine into D-serine and vice versa and is presumed to link D-serine synthesis and energy metabolism of astrocytes (12) and to control the D-serine level (13). Mg-ATP, which is fully bound to SR under physiological conditions, stimulates racemization and the \( \alpha,\beta \)-elimination reaction catalyzed by SR (12, 14).

SR was first discovered in pupae of the silkworm Bombyx mori (15), which was followed by purification of the enzyme from a rat brain and cloning of the mouse and human genes (8, 9). The primary structure of mammalian SR is distinct from those of racemases from prokaryotes but is similar to those of fold-type II PLP-dependent enzymes (16–18). We have cloned and expressed the Schizosaccharomyces pombe gene homologous to human and mouse SRs, the sequence identities being 35.1 and 37.4%, respectively, in Escherichia coli. The protein product is a bifunctional enzyme that catalyzes racemization and the \( \alpha,\beta \)-elimination reaction of D, L-serine as mammalian SR does. SR from S. pombe (spSR) comprises 322 residues (the N-terminal Met is removed in the purified enzyme) and one PLP per subunit, the subunit molecular weight being 34,917. The mammalian SR homolog, spSR, is an interesting target enzyme for the development of a novel therapeutic compound controlling the D-serine level because D-serine is the product of...
an SR-catalyzed reaction. In our recent report, the active site of spSR was shown to be modified with its natural substrate serine by mass spectroscopic and x-ray studies (19). Interestingly, the catalytic lysine, which originally forms a Schiff base with PLP, is converted to a lysino-D-alanyl residue through the reaction with the substrate, serine (Fig. 1). The modified enzyme exhibits racemase (54% of the wild-type enzyme) and  , -elimination (68% of the wild-type enzyme) activities with the amino group of the D-alanyl moiety of the lysinoalanyl residue forming a Schiff base with PLP in place of the lysine (19). In addition, the mammalian SR seems to be possibly modified to have a lysinoalanyl residue at the active site, as observed in spSR (20).

Although the structure of modified spSR (spSRm) has been determined (19), the structure-function relationship of essential wild-type spSR (spSRw), the binding mode of activator MgATP, the catalytic base to shuttle protons to the substrate D-serine, and the substrate recognition of the modified enzyme have not yet been uncovered. We now report the three-dimensional structures of unliganded spSRw in the open form, spSRw-AMP-PCP in the open form, and spSRm-serine in the closed form.

EXPERIMENTAL PROCEDURES

Construction of a Mutant spSR and Enzyme Preparation—The S82A mutation was introduced into the plasmid pETSRI, which was used to express spSRw (19), using a QuikChange site-directed mutagenesis kit (Stratagene) according to the manufacturer’s recommendations. The primers used for the mutation were: 5′-GGTGGTTTTGACCTTTTCTGCTGGTA-ATCATGCTCACGCC-3′ and 5′-GGGTGAGCCTGATGAT-TACCAGCAGAGACGTCAAAAACC-3′. The entire coding region was sequenced to confirm the mutation. Expression of spSRw and the mutant spSR in E. coli and purification of the enzymes were performed as described elsewhere (19).

Enzyme Assays—Both the racemization and the  , -elimination (dehydration) of L-serine and D-serine were performed in a reaction mixture comprising 100 mm Tris-HCl (pH 8.0), 10 mm L- or D-serine, 20 mm PLP, and 1 mg/ml enzyme at 30 °C for 1 h. For the racemization assay, the amounts of L- and D-serine were determined as described previously (21). The limit of detection of our assay was 0.1 mmol/ml D- or L-serine in the reaction mixture. For the  , -elimination assay, the amount of pyruvate was measured with 2,4-dinitrophenylhydrazine as described previously (22).

RESULTS AND DISCUSSION

Overall and Subunit Structure—The asymmetric unit has one subunit, which comes from a dimer lying along the crystallographic two-fold axis (Fig. 2, A and B). The subunit comprises two domains, a large domain (1–55, 153–323) and a small domain (56–152). The surface area of the subunit interface was calculated to be 873 Å², of which 408 Å² is hydrophobic, 279 Å² is hydrophilic, and 187 Å² is ionic. We note that the enzyme forms a biological dimer, which is consistent with the molecular weight of 72,000 estimated on gel filtration through a Superose 12 column. The subunit interface consists of the large domain
residues only, although the small domain is adjacent to the large domain of the other subunit at the ATP-binding site. Eight of the 12 residues involved in the subunit interface are conserved or strongly conserved in human and mouse SRs. The molecule has a rectangular shape with a long groove formed at the domain and subunit interfaces connecting the top and bottom of the molecule (Fig. 2). The groove starts from the top of the molecule and subunit interfaces connecting the top and bottom or strongly conserved in human and mouse SRs. The molecule passes through the PLP binding site (active site) and of the molecule (Fig. 2). The groove starts from the top of the domain and subunit interfaces connecting the top and bottom (Fig. 2). The groove

| TABLE 1 | Data collection and refinement statistics |
|---------|------------------------------------------|
| spSRw  | spSRw-AMP-PCP | spSRm-serine |
| Data collection | | |
| PDB ID | 1V71 | 1WTC | 2ZR8 |
| Space group | C2221 | C2221 | C2 |
| Unit cell | | |
| a | 69.47 | 69.43 | 59.54 |
| b | 148.17 | 148.09 | 72.88 |
| c | 64.20 | 64.66 | 64.81 |
| β | 90 | 90 | 101.73 |
| Diffraction data | Resolution (Å) | 1.70 | 1.90 | 2.20 |
| | Unique | 35,931 | 26,679 | 13,894 |
| | Completeness (%) | 97.4 (85)* | 100 (100)* | 99.2 (94.7)* |
| | Rmerge (%)* | 5.5 (30.3)* | 7.2 (25.8)* | 8.6 (28.6)* |
| | Rfree (%) | 28.1 (2.4)* | 7.1 (2.4)* | 15.2 (3.3)* |

* The values in the parentheses are for highest resolution shells (1.76-1.70, 1.97-1.90, and 2.28-2.20 Å) in spSRw, spSRw-AMP-PCP, and spSRm-serine, respectively.

Refinement

| Resolution limits (Å) | 10.0–1.70 | 10.0–1.90 | 10.0–2.20 |
| R-factor (%) | 21.96 | 22.84 | 17.05 |
| Rfree (%) | 24.85 | 26.63 | 21.68 |
| Deviations | Bond lengths (Å) | 0.006 | 0.007 | 0.006 |
| | Bond angles (deg) | 1.20 | 1.24 | 1.28 |
| Mean B-Factors | Main chain atoms (Å²) | 21.54 | 21.62 | 20.89 |
| | Side-chain atoms (Å²) | 25.13 | 23.79 | 23.99 |
| | Hetero atoms (Å²) | 32.91 | 29.88 | 29.06 |
| Procheck | Favored | 91.3 | 90.6 | 93.5 |
| | Additionaly allowed | 8.3 | 9.4 | 7.6 |
| | Generously allowed | 0.4 | 0.4 | 0.4 |
| | Disallowed | 0.0 | 0.0 | 0.0 |

The values in the parentheses are for highest resolution shells (1.76-1.70, 1.97-1.90, and 2.28-2.20 Å) in spSRw, spSRw-AMP-PCP, and spSRm-serine, respectively.

Open-Closed Conformational Change—PLP-dependent aspartate aminotransferases belonging to the fold-type I are known to show movement of the small domain to close the active site upon binding of the substrate (41–43). Ligand-induced movement of the small domain has also been observed in the fold-type II enzymes threonine synthase and O-acetylserine sulphydrylase, with the 25 and 15° rotation of the small domains, respectively (38, 39). Large domain Ca atom superimposition of spSRw onto spSRm-serine clearly indicates that the small domain undergoes a 20° rotation toward the large domain as compared with that of spSR without a change in the conformation of the large domain dimer to enclose the ligand in the active site formed at the domain interface (Fig. 2D). The molecular surface of the monomer unit in the closed form is reduced from 13,166 Å² in the open form to 12,455 Å². The domain movement plays an important role in the formation of the substrate recognition site and the catalysis of the enzyme.

Active Site of spSRw in the Open Form—The large deep groove of spSR formed at the domain interface is a part of the long groove connecting the top and bottom of the molecule (Fig. 2A). The groove embraces the cofactor PLP in the central region and is filled with water molecules. The active site structure of spSRw in the open form is shown in Fig. 3A. Many of the interactions between PLP and active-site residues reflect those observed in PLP-dependent fold-type II enzymes (37, 38, 44). The cofactor PLP forms a typical Schiff base bond (internal aldime) with the catalytic fold-type II enzymes and is similar in these SRs in which the substrate serine is catalyzed for Leu-187 (Met in human and rat), suggesting that the side chain at the N-terminal end of helix H9 forms a binding site for the phosphate group. The N-terminal loop (Ser-81–Ser-82–Gly-83–Asn-84–His-85) of α-helix H5, which is called the asparagine loop (44), resides on the O3’ side of PLP and acts as the recognition site for the substrate carboxylate in the closed form. The polar hydroyx group of Ser-308 forms a neutral hydrogen bond with the N-1 atom of the pyridine ring of PLP (37, 38, 45). The side chain of Asn-84 forms a hydrogen bond with the O3’ of PLP to play a role in fine-tuning of the electron state of the PLP-Schiff base conjugate together with the hydrogen bond to the N-1 atom (46–48). These active-site residues are completely conserved in human and rat SRs except for Leu-187 (Met in human and rat), suggesting that the side chain arrangements as well as the active site folds are quite similar in these SRs in which the substrate serine is catalyzed through the same mechanism.
A metal ion, which was assumed to be Mg\(^{2+}\)/H\(^{10}\) based on the height of the residual electron density, the atomic displacement parameter, and the coordination geometry, is located beside the tetra-glycine loop and coordinated by the carboxylates of Glu-208 and Asp-214 (Fig. 2C), the carbonyl group of Gly-212, and three water molecules (Fig. 4). spSR loses its activity on treatment with EDTA and recovers its activity on the addition of Mg\(^{2+}\)/H\(^{10}\) or Ca\(^{2+}\)/H\(^{10}\). However, the divalent cation is considered not to be directly involved in the catalytic action but in stabilization of the folding of the protein because the ion is located outside the active-site center. Mammalian brain SR was activated in the presence of divalent cations (12, 14). Glu-208 and Asp-214 are conserved in human and rat SRs, suggesting that a divalent cation is similarly bound to human and rat SRs. The Schiff base bond (C4' = N) is roughly coplanar with the pyridine ring of PLP (torsional angle of C3-C4-C4' = 32°) to form the conjugated \(\pi\)-system of the PLP-protonated Schiff base.

Active Site of spSRm—Serine in the Closed Form—In our previous report, it was shown that spSRm catalyzes the racemase and \(\alpha,\beta\)-elimination reactions, although the essential Lys-57
inherently forming a Schiff base with PLP in spSRw is converted to lysino-D-alanyl residue (19). This indicates that the α-amino group of lysino-D-alanyl residue acts as a catalytic base in the same manner as the ε-amino group of Lys-57 in spSRw (Fig. 1). To elucidate the interactions of spSRm with the substrate, a spSRm crystal soaked in a solution containing 10 mM L-serine was subjected to x-ray analysis. The omit electron density map exhibited the residual electron density corresponding to D- or L-serine or mixture of the two on the re-face side of PLP (Fig. 3B). The fact that spSRm forms a complex with serine is consistent with the residual activity of the modified enzyme.

The small domain rotates by 20° to the large domain as compared with that of spSRw to close the active site. The α-helix H5 and its N-terminal loop (the residues from Ser-81 to His-85) approach the PLP-lysinoalanine Schiff base with a large conformational change in the Ser-81–Ser-82–Gly-83 region forming the binding site for the carboxylate of the lysino-D-alanyl residue. The carboxylate interacts with Ser-81, Ser-82, Asn-84, and His-85 (Fig. 4). The negative charge of the carboxylate is partially compensated for by the dipole of α-helix H5.

The substrate enters the water tunnel connecting the re-face side of the PLP-lysinoalanine Schiff base and the molecular surface and resides on the cofactor. The carboxylate of the substrate serine forms an end-on type salt bridge with the guanidino group of the N-terminal Arg-133 of α-helix H7 (49) and a hydrogen bond with the main-chain NH group of Ser-82 of the asparagine loop. Intriguingly, Arg-133, which is a key residue for recognition of the substrate in spSRm, is conserved in human and mouse SRs but not in other fold-type II enzymes. The amino group of the substrate forms a hydrogen bond with the main-chain C=O group of Gly-236 situated on the pyridine ring of PLP. The distance between the amino nitrogen of the substrate and the C4′ of PLP is 3.6 Å, suggesting that the cofactor PLP may form a new Schiff base (external aldimine) with the substrate serine to release the lysinoalanine residue (Fig. 1). The α-amino group of the lysinoalanine residue might serve as a base that shuttles protons to the substrate similarly to that of Lys-57 in spSRw. Thereby, the PLP-lysinoalanine Schiff base might play a critical role in the reactions catalyzed by spSRm.
FIGURE 5. Interactions of AMP-PCP with spSRw in the open form. The simulated annealing omit map is contoured at the 1.0σ level. AMP-PCP interacts with the small and large domain residues (gray sticks with red oxygen and blue nitrogen) of one subunit and the large domain residues (orange sticks) of the other subunit. Hydrogen bonds are shown by dotted lines (green), Met-53, Asn-84, Gln-87, Glu-281, and Asn-311 (blue sticks) form a hydrogen bond network (red) to link PLP and AMP-PCP. The magnesium ion is surrounded by β- and γ-phosphate and four water molecules. The water molecules are involved in direct hydrogen bonds with the neighboring amino acid residues.

Binding Mode of AMP-PCP—MgATP is a physiological activator and is considered to be fully coordinated to the ATP binding site of mouse SR in vivo (5, 12). The racemization and αβ-elimination catalyzed by mouse SR are stimulated upon the addition of MgATP. Similarly, in the presence of 1 mM MgATP, spSRw is activated by 1.2 and 1.8 times for racemization and αβ-elimination of l-serine, respectively. The lower stimulation observed for the racemization reaction might be due to the degradation of the produced d-serine through the increased αβ-elimination activity. To clarify the binding site of ATP and its interaction with spSRw, complex crystals of spSRw with AMP-PCP were prepared by soaking wild-type crystals in a solution containing 10 mM AMP-PCP.

MgAMP-PCP is bound to the groove formed at the intersection between the domain interface and the subunit interface (Figs. 2, A and B, and 5). The binding of MgAMP-PCP to spSRw in the open form does not induce a subunit conformational change, but, interestingly, changes the relative orientation between the two subunits. Each subunit rotates around the axis that goes through the adenyI rings of two AMP-PCPs to enlarge the width of the back groove (Fig. 2B). The ATP binding site is surrounded by Ala-115 (helix H6) and Tyr-119 (C-terminal loop of H6) of the small domain, Asn-25 (loop between H1 and H2), Phe-50, Asn-51, Lys-52, and Met-53 (loop between β-strand S1 and H3), and Asn-311 (loop between S8 and H14) of the large domain, and Ser-32*, Ser-33*, and Thr-34* (N-terminal part of H2) and Arg-275*, Met-276*, and Lys-277* (C-terminal part of H12) of the large domain of the other subunit (Fig. 5). The adenine ring of AMP-PCP is sandwiched by Ala-115 and the guanidino plane of Arg-275*. The O3′ of the ribose moiety interacts with the side chains of Asn-311 and Lys-277*. The triphosphate group interacts with Asn-25, Phe-50–Met-53, Tyr-119, Thr-31*–Thr-34*, and many water molecules. Mg2+ is coordinated by two oxygen atoms of the terminal pyrophosphate and four water molecules with octahedral geometry.

Out of the 14 residues interacting with ATP, 11 are conserved in human and rat SRs; the exceptions are Asn-25 (His in human and rat SRs), Thr-34 (Ile), and Met-53 (Thr). On the other hand, only 2 residues are conserved in threonine dehydratase from E. coli, and no residues are conserved in O-acetylserine sulfhydrylase from S. typhimurium or tryptophan synthase from E. coli. Possibly, ATP is coordinated to mammalian SR in a mode similar to that in spSR.

It seems that no significant change in the cofactor or side-chain orientation occurs on binding of AMP-PCP in the active site of spSRw. However, the O3′ of PLP is linked to the ribose hydroxy groups and γ-phosphate of AMP-PCP through a hydrogen bond network formed by Met-53, Asn-84, Gln-87, Glu-281, Asn-311, and water molecules (Fig. 5). It should be noted that Asn-84 is a constituent of the mobile asparagine loop that recognizes the substrate carboxylate, and the side chain of Gln-87 interacts with the loop through water molecules. Upon binding of the substrate to the spSRw complexed with ATP, the enzyme changes its conformation from the open form to the closed form similar to that observed in spSRm-serine. In the closed form, the hydrogen bond network between PLP and ATP might be reorganized to fine-tune the active site structure, resulting in the stimulation of the enzyme activity. In effect, the side chain of Gln-87 is within direct hydrogen bond distances from the ribose hydroxy group of AMP-PCP and the side chain of Asn-84 if the large domain Ca atoms of the enzyme in the closed form are superimposed on those of spSRw-AMP-PCP. In addition, the adenine moiety of AMP-PCP interacts with the small domain of one subunit as well as with the large domain of the other subunit. The interactions of ATP with both domains would have effects on the mode of the open-closed conformational change (small domain movement) leading to the enhancement of the enzyme activity.

Structural Model of External Aldimine of spSRw—spSRw crystals soaked in a solution supplemented with 10 mM L-serine were subjected to x-ray structure analysis. However, the omit electron density map of the active site showed that no substrate or product was bound to the active site. The co-crystallization method was employed to produce the enzyme-substrate complex. X-ray analysis of the crystal thus obtained indicated that during the co-crystallization process in the presence of L-serine, which took much longer than the soaking method, spSRw was converted to spSRm.

To clarify the substrate recognition mechanism and the reaction mechanism, a computer graphics model of the external aldimine form of spSRw and L-serine was designed by utilizing the active site structure of the rat liver serine dehydratase complexed with O-methyl-L-serine in the closed form in which PLP forms a Schiff base with O-methyl-L-serine (external aldimine...
The external aldimine model with L-serine (Fig. 6). The external aldimine model directs its substrate D-serine. Lys-57 is considered to act as an acid-base catalyst to add a proton to, or abstract a proton from, the Cα atom of the substrate on the si-face side of PLP because the orientation of Lys-57 with respect to PLP is quite similar to those of fold type II enzymes such as serine dehydratase, threonine synthase, and tryptophan synthase (37, 38, 40). The external aldimine model with D-serine shows that Ser-82 is a candidate that shuttles protons to the substrate on the re-face side PLP (Fig. 6). To examine the involvement of Ser-82 in the catalysis, the S82A mutant was subjected to the enzyme assay. The assay showed that the racemase activity was not detected and that D-serine α,β-elimination activity of the S82A mutant was almost completely lost (<0.01% of the activity of spSRw), whereas the L-serine α,β-elimination activity of the mutant was retained ($K_m = 45 \text{ mm}, V_{max} = 450 \text{ nmol min}^{-1} \text{ mg}^{-1}$) as compared with that of wild-type enzyme ($K_m = 36 \text{ mm}, V_{max} = 870 \text{ nmol min}^{-1} \text{ mg}^{-1}$). A similar result was reported for the mutant as to the corresponding residue of serine racemase from Dicystostelium discoideum (50). It is thus reasonable to assume that Ser-82 is the proton carrier to the substrate Cα atom on the re-face side of the cofactor.

Mechanistic Implication of Wild-type Enzyme—The pyridine N-1 atom of the cofactor forms a hydrogen bond with the hydroxy group of Ser-308, acting as a hydrogen bond acceptor, as shown in the x-ray structures of threonine dehydratase (35), O-acetylseryne sulphydrylase (44, 51–53), threonine synthase (38, 54, 55), tryptophan synthase (40, 56), and serine dehydratase (N-1–H–S hydrogen bond). The involvement of the N-1 atom in the neutral hydrogen bond with the hydroxy group of Ser or Thr or the sulphydryl group of L-Cys as an acceptor is one of the characteristics of the fold-type II enzymes. In the fold-type I or IV enzymes, the positively charged protonated N-1 atom of PLP forms a salt bridge with the negatively charged carboxylate of aspartate or glutamate. The neutral hydrogen bond reduces the electron-withdrawing effect of the pyridine ring of PLP as compared with the salt bridge, destabilizing the quinonoid intermediate produced on Cα proton elimination from the substrate amino acid (37, 38, 45, 46). The reaction may proceed through the carbanion intermediate after substrate α-proton elimination rather than the quinonoid intermediate, which requires the protonated N-1 atom (Fig. 7).

The substrate L- or D-serine approaches the re-face side of PLP with its α-amino group directed toward the Schiff base towards the PLP to induce the small domain movement forming a Michaelis complex. The amino group of the substrate must be in an unprotonated state to make a nucleophilic attack on C4’ of the PLP Schiff base to form an external aldimine (Fig. 7). This form is generated either through binding of the substrate with a free amino group or through deprotonation of the ammonium group after binding of the substrate with a protonated amino group. The nucleophilic attack produces an external aldimine.
to release the neutral side chain of Lys-57. The next step is the \( \alpha \)-proton elimination of the substrate. Judging from the PLP-L-serine external aldimine model, Lys-57 directs its side-chain amino group toward the \( \alpha \)-proton of the substrate L-serine, whereas Ser-82 directs its hydroxy group toward the \( \alpha \)-proton of the substrate D-serine. Ser-82 is one of the key residues for the enzyme to act as a SR because Ser-82 is conserved in mammalian SRs but not in other enzymes of fold-type II.

The reaction catalyzed by spSR is explained by the classic two-base mechanism (46, 57) (Fig. 7). The arrangement of two bases (Lys-57 and Ser-82) and the PLP-substrate Schiff base in spSRw is reminiscent of that observed in alanine racemase, although the catalytic lysine is on the re-face side of the cofactor (46). The neutral amino group of Lys-57 or the deprotonated hydroxy group of Ser-82 abstracts the substrate \( \alpha \)-proton of the PLP-L-serine or PLP-D-serine Schiff base, respectively, to yield the carbanion intermediate. The addition of the proton to the \( \alpha \) atom of the carbanion intermediate from the reverse side of PLP results in the racemization reaction. The hydroxy group of Ser-82 or the protonated amino group of Lys-57 adds its proton to the \( \alpha \) atom of the carbanion from the re- or si-face side to produce the PLP-D-serine or PLP-L-serine Schiff base, respectively.

After the carbanion intermediate is formed through \( \alpha \) proton elimination, the protonation on the substrate \( \beta \)-hydroxy group results in the \( \beta \)-elimination reaction, producing the cofactor-aminoacrylate Schiff base and a water molecule. The PLP phosphate is a possible candidate for the proton carriers to the substrate \( \beta \)-hydroxy group if the substrate hydroxy group is in a favorable position to form a hydrogen bond with the PLP phosphate, as shown in external aldimine models (Fig. 6) (37). Finally, the cofactor recovers the Schiff base with Lys-57 to release the aminoacrylate, which is nonenzymatically converted to pyruvate and ammonia. Alternatively, a portion of the \( \alpha \)-aminoacrylate intermediate is converted into spSRm, i.e. PLP-lysinoalanine Schiff base, by the Michael addition reaction of Lys-57 with the \( \alpha \beta \)-unsaturated product (Fig. 7). spSRm thus formed also participates in the overall catalytic cycle in the same manner as spSRw.

In summary, we have determined the three-dimensional structures of spSRw, spSRw-AMP-PCP, and spSRm-serine by means of x-ray crystallographic methods. On binding of the substrate, the small domain rotates toward the large domain with a local conformational change to close and complete the active site. The activator (ATP) is bound to the intersection between the domain and the subunit interface. Lys-57 and Ser-82 are considered to be acid-base catalysts that shuttle protons to the substrate \( \alpha \) atoms. The substrate serine trapped in the active site of spSR reasonably interacts with active site residues.

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**FIGURE 7.** The proposed reaction mechanism of spSRw and spSRm with the substrate L- or D-serine.
X-ray Structure of Serine Racemase

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