Rapid Communication

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Serine Racemase with Catalytically Active Lysinoalanyl Residue*

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Serine racemase synthesizes D-serine, a physiological agonist of the NMDA receptor in mammalian brains. *Schizosaccharomyces pombe* produces serine racemase (spSR) that is highly similar to the brain enzyme. Our mass-spectrometric and X-ray studies revealed that spSR is modified with its natural substrate serine. spSR remains partially active even though its essential Lys57 inherently forming a Schiff base with the coenzyme pyridoxal 5′-phosphate is converted to *N*(6)-(*R*-2-amino-2-carboxyethyl)-L-lysyl (lysino-D-alanyl) residue. This indicates that the α-amino group of the D-alanyl moiety of the lysino-D-alanyl residue serves as a catalytic base in the same manner as the ε-amino group of Lys57 of the original spSR.

Key words: D-serine, modification, pyridoxal 5′-phosphate, racemase, *Schizosaccharomyces pombe*.

Abbreviations: ATP, adenosine triphosphate; ESI, electrospray ionization; NMDA, *N*-methyl-D-aspartate; NMR, nuclear magnetic resonance; PCR, polymerase chain reaction; PLP, pyridoxal 5′-phosphate.

D-Serine, synthesized from L-serine by serine racemase in mammalian brains, acts as a physiological coagonist with glutamate at the glycine-binding site of the NMDA receptor (*1*, *2*). D-Serine plays an important role in the pathophysiology of schizophrenia: upon therapeutic trials, D-serine significantly improves the symptoms and cognitive deficits in patients with schizophrenia (*3*). We found that fission yeast *Schizosaccharomyces pombe* also has serine racemase (spSR) that is highly similar in sequence to the brain enzyme (*4–6*). Sequence identity of spSR to the mouse enzyme was 34% (Fig. S1, Supplementary Data). The gene encoding spSR was amplified by PCR and cloned into the pET21a(+) expression vector. Recombinant spSR was readily produced in a large quantity in *Escherichia coli* BL21(DE3)pLysS. spSR displayed absorption peaks at 280 nm and 410 nm with a shoulder around at 340 nm, similar to those of mammalian serine racemase (*4–6*). The absorbance at 410 nm was decreased by addition of L-serine with a concomitant increase of 315-nm absorbance (Fig. S2, Supplementary Data), suggesting the peak at 410 nm corresponds to a Schiff base complex of PLP with an active site Lys as seen in other PLP-dependent enzymes. The increase in the absorbance at 315 nm is most probably due to formation of intermediates including geminal diamines and enolimine tautomers of aldimine Schiff bases during reaction, as seen in reactions of other PLP-dependent enzymes (*7*). Kinetic analyses of purified spSR revealed that the enzyme catalyses racemization of L-serine and D-serine and also dehydration of L-serine and D-serine to form pyruvate and ammonia (Table S1, Supplementary Data). The racemase activity of spSR was slightly enhanced by addition of 1 mM Mg^{2+} (~110%) and 1 mM ATP (~140%). Thus, spSR shares its characteristics with mammalian brain enzymes (*4–6*): dual functions as serine racemase and serine dehydratase; bound cofactors consisting of PLP, Mg^{2+} and ATP. Since we noticed that spSR was partially inactivated during reaction, the enzyme dialysed against L-serine was analysed by ESI-mass spectrometric (MS) analysis. Concomitantly with the partial decrease in the activity, the molecular mass of $NaBH_4$-reduced spSR (*N*-(5′-phosphopyridoxyl)-spSR with a calculated mass of 35,147.65 Da) shifted from 35,148 to 35,235 Da as revealed by ESI-MS analysis (Fig. S3, Supplementary Data). The increment (87 Da) almost corresponds to the molecular mass of alanine moiety (86 Da). This indicates that spSR was covalently modified with dehydroalanine derived from serine while it was incubated with the substrate (discussed below in detail).

The MS data showed that ~97% of the total enzyme was the modified form (Fig. S3, Supplementary Data). The modification was not reversed by incubation in 20 mM potassium phosphate buffer (pH 7.2) without serine for several days at room temperature. The specific activities of modified spSR for L-serine racemization and L-serine dehydration were 54%, and 68%, respectively, of those of the original spSR. These results indicate that

*The coordinates for a structure have been deposited in the RCSB Protein Data Bank, accession number 2zpu.

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modified spSR essentially exhibits catalytic activities, and the remaining unmodified enzyme (present at ~3%) does not account for the most of the residual activity in the modified sample. Our results are reminiscent of alanine racemase, which is also modified with serine derivatives such as *O*-acetylserine (*8–10*). However, spSR is distinct from alanine racemase because modified spSR remains active, contrary to modified alanine racemase that is completely inactive (*8*, *10*).

We determined the three-dimensional structure of modified spSR by X-ray method (Table S2, Supplementary Data). The 2*Fo*–*Fc* map calculated at 1.7 Å resolution clearly exhibited the residual electron density corresponding to PLP at the active site. The quality of the omit electron density map reached the point at which the molecular skeleton was identified from the shape of the electron densities contoured at the 1-σ level. In most PLP enzymes analysed thus far by X-ray crystallography, PLP was bound to the ε-amino group of the lysyl residue at the active sites. However, modified spSR has extra electron density bridging the gap between the C4' atom of PLP and the ε-amino group of Lys57, which are otherwise directly linked to each other in the native spSR. The alanyl moiety of serine with *R*-configuration at Cα clearly fits the residual density (Fig. 1). This indicates that Lys57 was converted to *N*(6)-(*R*-2-amino-2-carboxyethyl)-L-lysyl (lysino-D-alanyl) residue **1** forming a Schiff base with PLP, as shown in Fig. 2. Thus, the α-amino group of the D-alanyl moiety of lysino-D-alanyl residue derived from serine probably serves as a catalytic base in the same manner as the ε-amino group of Lys57 of the original spSR (Scheme 1). It is worth noting that the X-ray structure implies that there are still spaces adequate to bind an additional serine in the active site of modified spSR, consistent with its catalytic activity.

According to the preceding studies of other PLP enzymes, we can delineate the reaction mechanism of spSR with serine as shown in Scheme 2. PLP forms internal aldimine **3** with Lys57 at the active site of unmodified spSR. Serine replaces lysine to produce external aldimine **4** or **5** with PLP (Scheme 2). Then, the α-proton of the substrate is removed from aldimine **4** or **5** by a catalytic base to form carbanion intermediate **6**. When the proton is returned to the Cα carbon from the opposite side of the planar structure, the racemization of serine is accomplished. However, if the β-hydroxyl group of carbanion intermediate **6** is subsequently removed, then α-aminoacrylate intermediate **7** is formed. Free α-aminoacrylate is produced by transaldimination with Lys57 and then hydrolysed to form pyruvate and ammonia. The reactions of alanine racemase and other PLP enzymes with β-substituted alanine are proposed to proceed in the same manner (*10*). However, they are modified with nascent α-aminoacrylate before hydrolysis in order to be inactivated by forming structure **2**. spSR is distinct from these enzymes because it is catalytically active even after modification with serine. Moreover, modification occurs at a completely different position in spSR: the Cβ carbon of α-aminoacrylate is linked to the ε-amino nitrogen of Lys57. It is reasonable to assume

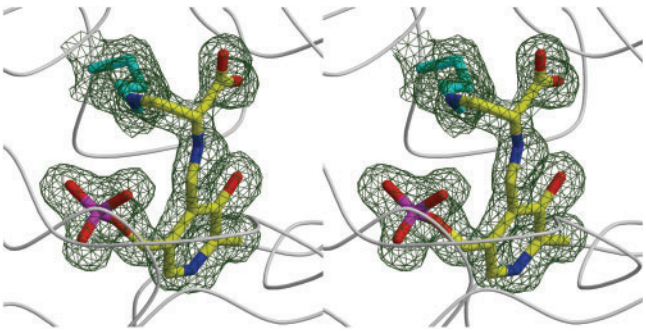


Fig. 1. **Stereoview of the omit electron density map (1.7 Å resolution, contour level 1.0 σ) for the PLP-D-Ala Schiff base moiety in modified spSR.** The lysino-D-alanyl residue (with Lys57 in cyan) forming a Schiff base with PLP is shown.

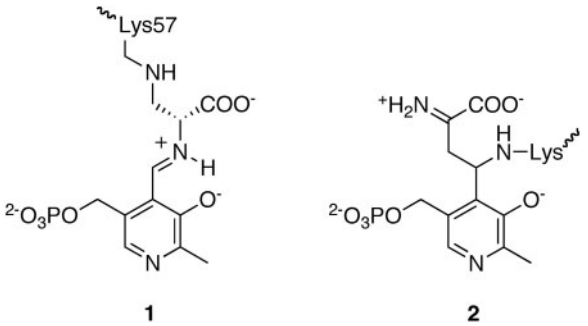
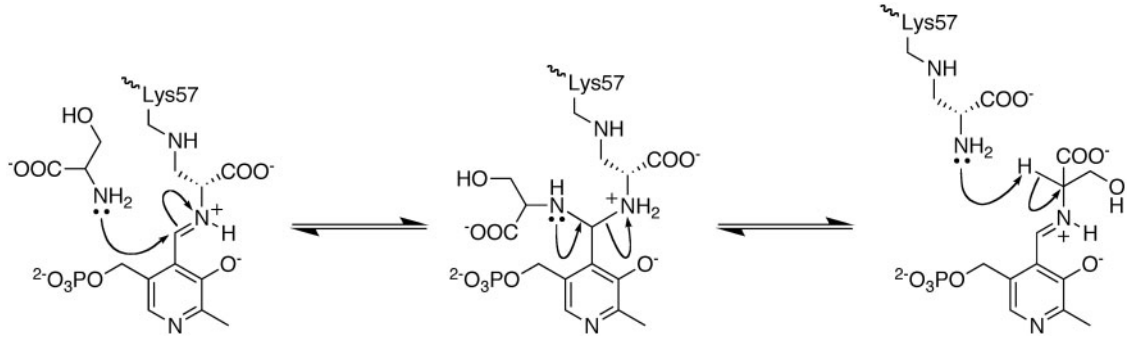


Fig. 2. **The enzyme active site structure of modified spSR 1 and inactivated alanine racemase 2 (*10*).**



Scheme 1. **Proposed mechanism of the reaction catalysed by modified spSR.**

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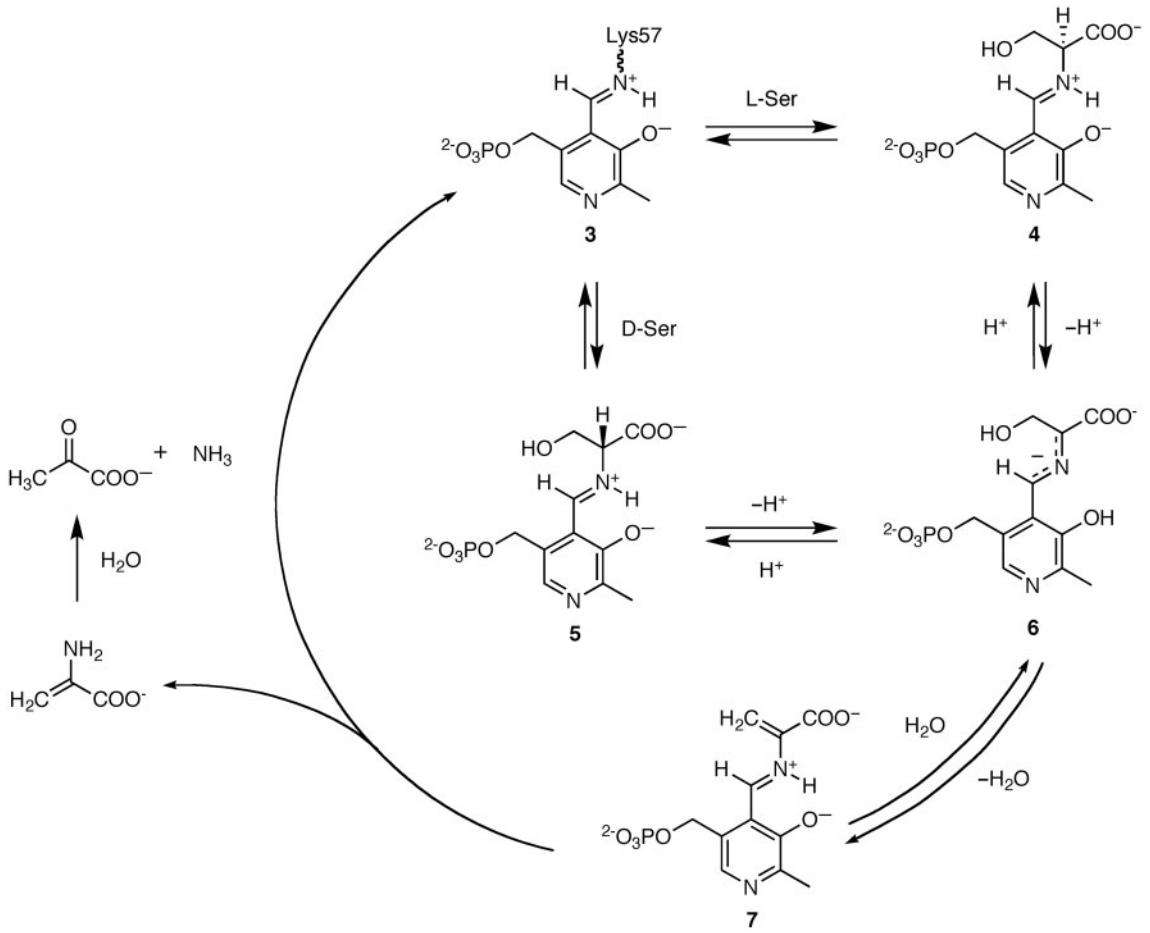

Scheme 2. **Proposed reaction mechanism of spSR with serine.**

that spSR uses PLP-binding lysine (Lys57) to abstract the α-proton from the *S*-substrate because spSR is similar in 3D structure to *O*-acetylserine sulfhydrylase (*11*) and threonine synthase (*12*), both of which act specifically on 2*S* isomers as substrates. Therefore, Lys57 probably occurs on the *si* face side of the planar α-aminoacrylate intermediate to cause Michael addition to the Cβ carbon of α-aminoacrylate. In contrast, PLP-binding lysine of alanine racemase acts as a catalytic base specifically acting on (namely, abstracting α-proton from) *R*-substrate, while tyrosine acts as a counterpart base specific toward the *S*-substrate (*13*).

This is the first report showing that a lysinoalanyl residue substitutes for the original lysine with an important catalytic function. Our results are reminiscent of the previous observation by Panizzutti *et al*. (*14*) that the reaction specificity of mouse serine racemase is also modified on incubation with L-serine *O*-sulphate. It appears likely that mammalian serine racemase is also modified with the substrate to form the lysinoalanyl residue in the same manner as spSR. In fact, we recently found that incubation of mouse serine racemase with L-serine resulted in the formation of an enzyme with a higher mass than the native enzyme (Yamauchi, T. and Esaki, N., unpublished data). Modified spSR is being characterized in detail by means of NMR and other spectroscopies to shed light on its catalytic mechanism.

SUPPLEMENTARY DATA

Supplementary data are available at *JB* online.

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CONFLICT OF INTEREST

None declared.

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