Review

D-Serine and its important role in the endocrine and central nervous systems

Klaus D. Schnackerz

Physiologische Chemie I, Theodor-Boveri-Institut für Biowissenschaften, Universität Würzburg, Am Hubland, 97072 Wuerzburg, Germany.

ABSTRACT

For a long time D-amino acids were believed to be present only in prokaryotes. More recently, D-serine and D-amino acids were discovered in eukaryotes. In prokaryotes, D-serine is an important component of the cell wall. In some bacteria D-serine competes with β-alanine in the formation of coenzyme A. Thus, D-serine has to be eliminated in prokaryotes by degradation to pyruvate and ammonia using D-serine dehydratase, a fold- type II pyridoxal 5'phosphate-dependent enzyme, or by D-amino acid oxidases. D-Serine and other D-amino acids have been found essential in reactions of the endocrine and central nervous systems. D-Serine has been discovered in the human brain, with the highest concentrations found in those areas enriched in N-methyl-D-aspartate (NMDA) glutamate receptors, such as the forebrain. Robust receptor activation by glutamate occurs only if D-serine is present at the synaptic cleft; such D-serine serves as the coagonist (or the coactivator) to NMDA receptors. D-Serine is synthesised by serine racemases from L-serine. This review describes the purification, reaction mechanism and the three-dimensional structure of serine racemases, D-amino acid oxidases and D-serine dehydratases.

KEYWORDS: pyridoxal 5'-phosphate, D-amino acid oxidase, serine racemase, D-serine dehydratase.

INRODUCTION

For a long time it was believed that D-serine is present only in prokaryotes where they are used as building blocks in cell walls. Improvements in analytical techniques have led to the discovery of D-amino acids in eukaryotes (biological systems as simple as those of baker's yeast or as complex as mammalian systems) [1].

Eukaryotes use these unusual amino acids as chemical messengers allowing for cell communication and regulation of specific functions [2]. D-Aspartate and D-alanine are the most widely studied D-amino acids in humans, exerting their actions in the endocrine and central nervous systems [3]. More recently D-serine has been discovered in the human brain, with the highest concentrations found in those areas enriched in N-methyl-D-asparte (NMDA) glutamate receptors, such as the forebrain. Robust receptor activation by glutamate occurs only if D-serine or glycine is present at the synaptic cleft. Those receptors, localised on the postsynaptic membrane, are involved in excitatory neurotransmission and higher brain functions such as learning and memory [4]. The major enzyme for synthesis of D-serine is the brain pyridoxal 5'-phosphate-dependent serine racemase acting on L-serine [5]. The protein product is a bifunctional enzyme, catalyzing racemization and the α/β-elimination reaction of D,L-serine. SR from *Schizosaccharomyces pombe* (spSR) contains 322 amino acid residues, one pyridoxal 5'-phosphate (PLP) per subunit with a molecular weight of 34,917 daltons [6]. 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. The enzyme is activated by MgATP. Human serine racemase is allosterically modulated by nicotinamide adenine

dinucleotide (NADH) and its reduced nicotinamide derivatives [7]. Ionotropic 2-amino-3(3-hydroxy-5-methyl-isoxazol-4-yl)propionic acid (AMPA) and NMDA glutamate receptors are ligand-gated ion channels that mediate fast synaptic transmission in the brain and play a crucial role in learning and memory. Dysfunction of these receptors is believed to be associated with a number of neuropsychiatric disorders like schizophrenia [8].

Cell death is an obligatory process in development, maintenance, and plasticity of the nervous system, and both anti-death and pro-death modulators are key elements in designing neural architecture [9]. Conversely, the dysregulation of mechanisms controlling cell death machinery activation can cause developmental, neoplastic, and neurodegenerative disorders [10]. Cell death can manifest many forms, which can be distinguished by various histological criteria (apoptosis, necrosis, autophagy), enzymological criteria (with and without the involvement of nucleases or of distinct classes of proteases, such as caspases, calpains, cathepsins, and transglutaminases), functional aspects (programmed or accidental, physiological or pathological) or immunological characteristics. Death signals are spatially and temporally segregated in neurons, for example, at remote synaptic sites [9]. Much of the biochemical machinery involved in apoptosis can be activated in synaptic terminals, where it can remodel synapses or alter synaptic function and promote localized degeneration of synapses and neuritis under both physiological and pathological conditions. For example, caspase-3 activity is crucially involved in monitoring, locally, protein levels in retinal growth cone formation [7], and NMDAR-dependent caspase-3 activity is required for memory storage in long-term depression (LTD) and AMPA receptor internalisation in hippocampal neurons [11, 12]. Unlike other neurotransmitter receptors, the simultaneous binding of two coagonists, glutamate and glycine or D-serine in ion permeation is required to activate NMDAR [12].

A fine balance between cell survival and cell death is required to sculpt the nervous system during development. However, an excess of cell death can occur following trauma, exposure to neurotoxins or alcohol, and some developmental and neurodegenerative diseases such as Alzheimer's disease (AD). N-Methyl-D-aspartate receptors (NMDARs) support synaptic plasticity and survival of neuronal populations whereas inappropriate activation may promote various forms of cell death, apoptosis, and necrosis representing the two extremes of a continuum of cell death processes "*in vitro*" and "*in vivo*" [9].

Altered D-serine levels have been manifested in the spinal sera of patients affected by brain disorders, such as schizophrenia, Alzheimer's disease or amyotrophic lateral sclerosis (ALS) [13]. Hence monitoring D-serine levels in the blood may contribute significantly to the diagnosis of various brain pathologies.

Serine racemase (SR)

D-Serine is synthesized from L-serine by the glial and neuronal enzyme serine racemase (SR, EC 5.1.1.18) [14] and is selectively degraded by both SR and the peroxisomal D-amino acid oxidase (DAAO, EC 1.4.3.3). Thus, it is not surprising that D-serine and the enzymes involved in its metabolism are crucially involved in several physiological and pathological processes related to NMDAR function and dysfunction. Serine racemase was isolated for X-ray structural work from *Schizosaccharomyces pombe*. The first crystal structure of the *pombe* enzyme was reported by Goto *et al*. [14] in 2009 (Fig. 1). Lys56 of SR binds to the aldehyde group of PLP, forming an internal aldimine, then SR reacts with D-serine to yield an external aldimine. α-Proton abstraction from this intermediate results in a resonance-stabilized carbanion. Details of the mechanism are shown in a graph of the active site of SR (Fig. 2). Previous immunohistochemical analysis of SR expression in rat brain did not detect its presence in neurons [14]. However, the previously employed antibody was suitable only for fresh frozen sections fixed with methanol [15]. In agreement with a previous study [14], it was observed that glial cells were immunoreactive for serine racemase [15]. Competitive inhibitors of L-serine racemization by recombinant mSR (mouse) and hSR (human) are malonate ($K_{i,hSR}$ 33 μM) L-erythro-3-hydroxyaspartate ($K_{i,hSR}$ 11 μM) and glycine ($K_{i,hSR}$ 366 μM) [16]. The reaction mechanism of SR as proposed by Goto *et al*. [14] is depicted in Fig. 3. The structures of the following three forms of the mammalian enzyme homolog from
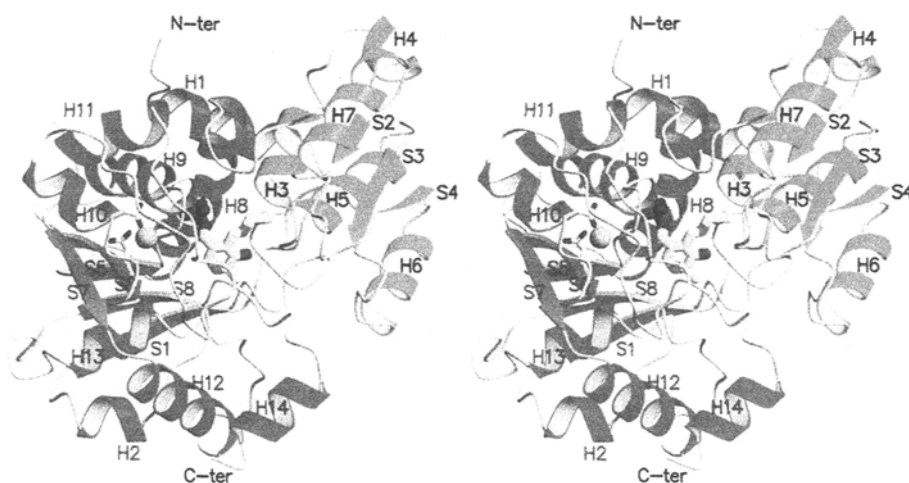
Fig. 1. Stereo view of the monomeric structure of serine racemase from *Schizosaccharomyces pombe* (Reproduced from Goto, M., Yamauchi, T., Kamiya, N, Miyahara, I., Yoshimura, T., Mihara Kurihara, T., Hirotsu, K. and Esaki, N. 2009, J. Biol. Chem., 284, 25944-25952 with permission from The American Society for Biochemistry and Molecular Biology).
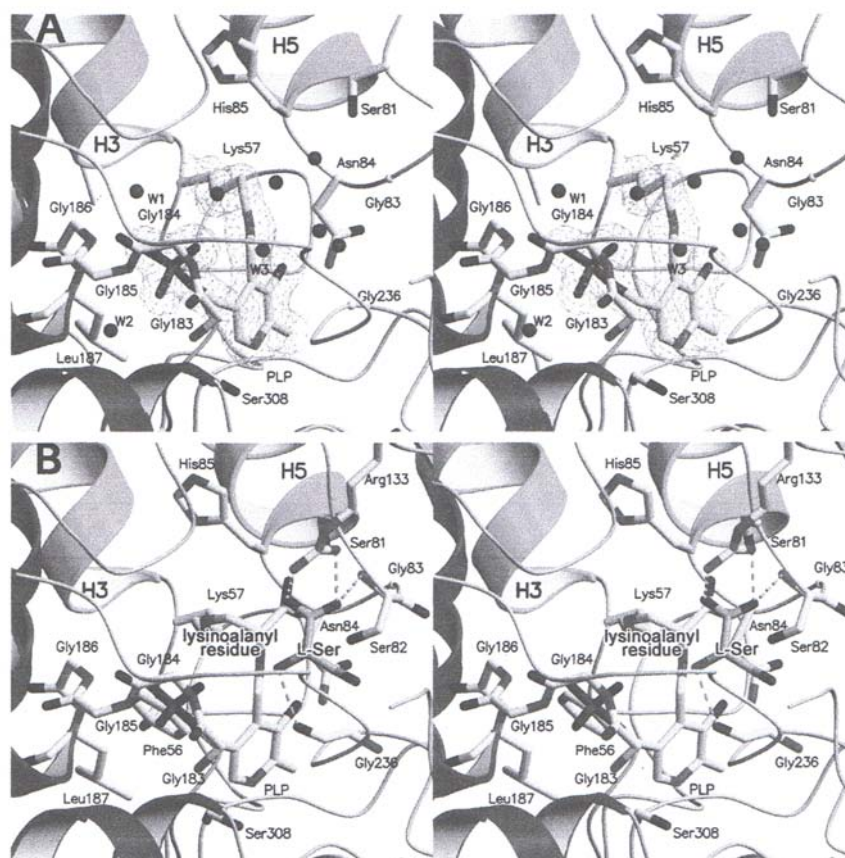

Fig. 2. Stereo view of the active site of spSR (Reproduced from Goto, M., Yamauchi, T., Kamiya, N, Miyahara, I., Yoshimura, T., Mihara Kurihara, T., Hirotsu, K. and Esaki, N. 2009, J. Biol. Chem., 284, 25944-25952 with permission from The American Society for Biochemistry and Molecular Biology).
A. Close-up view of the active site of spSRw in the open form. B. Close-up view of the spSRm complex with the substrate serine.
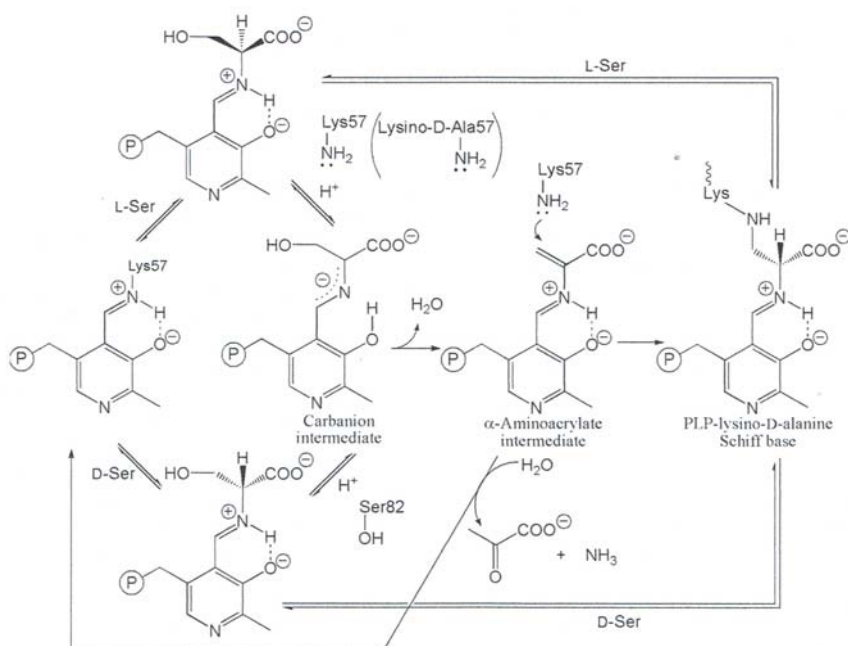
Fig. 3. Proposed reaction mechanisms of the substrates L-serine or D-serine of spSRw and spSRm (Reproduced from Goto, M., Yamauchi, T., Kamiya, N, Miyahara, I., Yoshimura, T., Mihara, Kurihara, T., Hirotsu, K. and Esaki, N. 2009, J. Biol. Chem., 284, 25944-25952 with permission from The American Society for Biochemistry and Molecular Biology).

Schizosaccharomyces pombe were used by Goto *et al*. [14]: the wild-type enzyme, the wild-type enzyme in complex with an ATP analog and the modified enzyme in complex with serine at 1.7, 1.9 and 2.2 Å resolution. On binding of the substrate, the small domain rotates toward the large domain to close the active site. The ATP-binding site is 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 mechanism of both reactions. Overall, it can be concluded that the inhibition determinant of NADH is the N-substituted 1,4-dihydronicotinic ring. The redox state of the 1,4-dihydronicotinic ring is crucial; neither the oxidized nor the fully reduced piperidinic form showed any inhibitory activity (Fig. 4) [7]. Lys-57 located on the protein and solvent sides, respectively, with respect to the cofactor plane, are acid-base catalysts that shuttle protons to the substrate (see also Fig. 3). A 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 a catalytic base in the same manner as inherent Lys-57 of the wild-type enzyme.

Mammalian PLP-dependent serine racemase 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 MgATP *in vivo* [7, 7a]. The interaction of its non-hydrolisable counterpart AMP-PCP (β,γ-methylene adenosine triphosphate) is shown in [14]. The superposition of the external aldimine models between spSRw-D-serine and spSRw-L-serine is shown in [14].

The NMDA receptors (NMDAR) are voltage- and glutamate-gated heteromeric ion channels. The GluN1A and GluN1 subunits bind glutamate and glycine/D-serine, respectively. In the agonist-free state, these domains form homomeric interactions which are disrupted by binding of the respective agonists (Fig. 5). This is direct evidence that the agonism is an essential step in forming functional ion channel in NMDARs [17].

D-Amino acid oxidase (DAO)

D-Amino oxidase (EC 1.4.3.3, DAO) was first identified by Hans Krebs in 1935 [18] and was later recognized to be the first enzyme to require flavin adenine dinucleotide (FAD) as a cofactor.
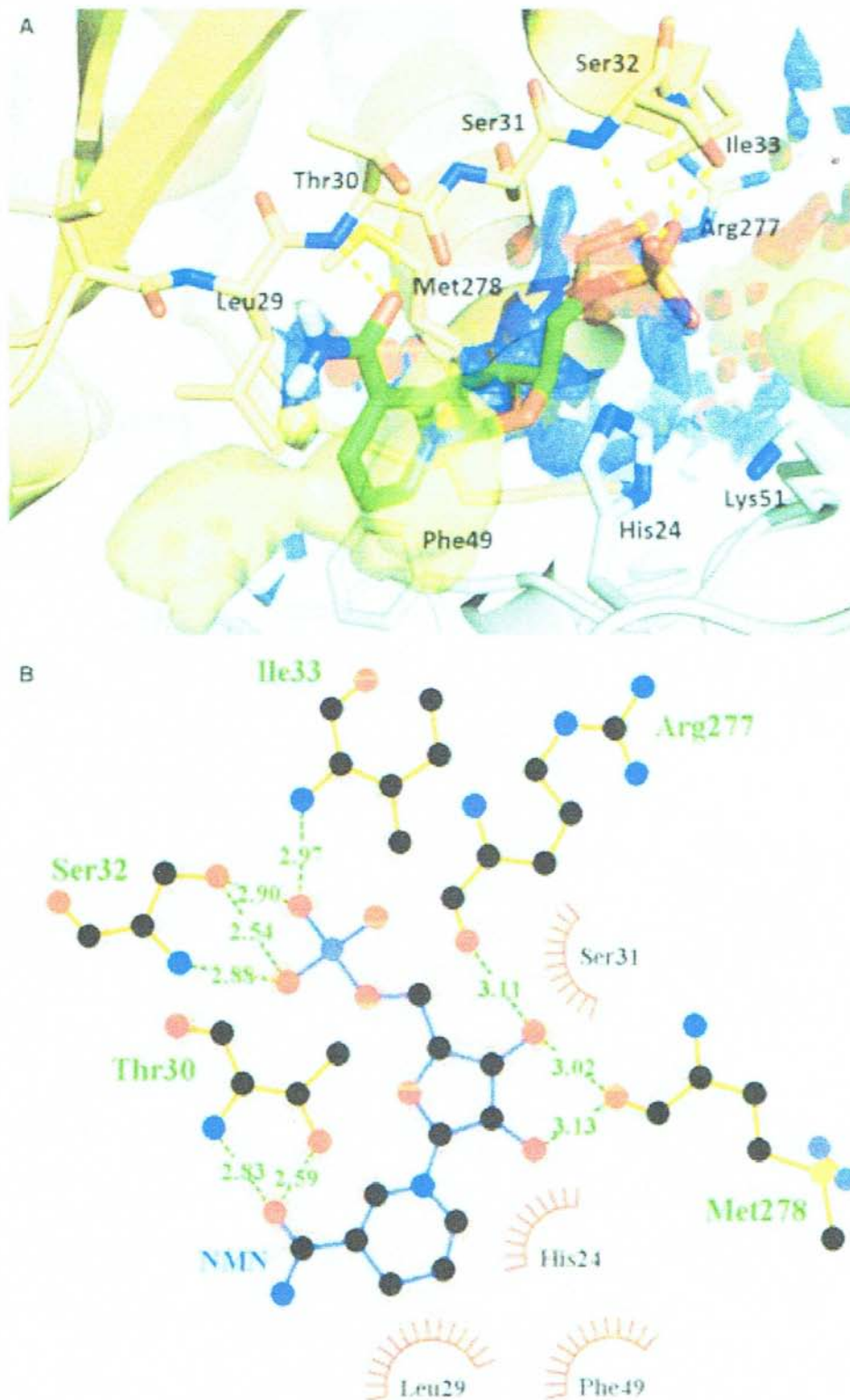
Fig. 4. Close-up of the 1,4-dihydronicotinamide mononucleotide fragment binding to hSR (A) and corresponding 2D schematic representation (B) (Originally published in Bruno, S., Marchesani, F., Dellafiora, L., Margiotta, M., Faggiano. S., Campanini, B. and Mozarelli, A. 2016, Biochem. J., 473, 3505-3516).
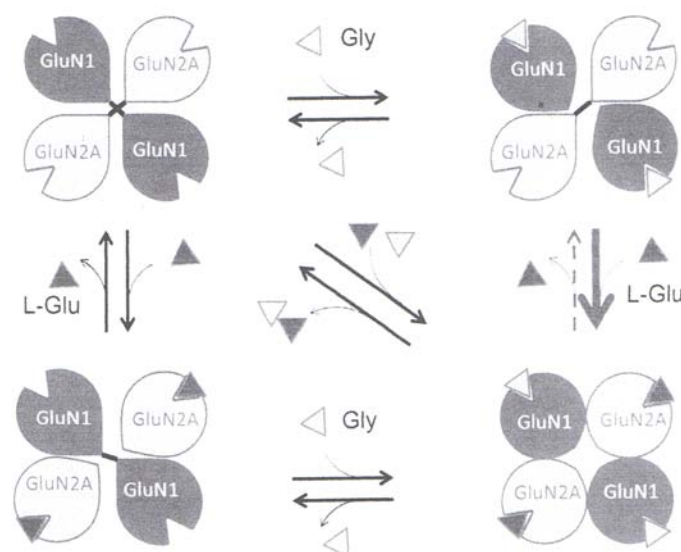
Fig. 5. Proposed mechanism of co-agonist regulation in NMDAR activation (Reproduced from Cheriyan, J., Mezes, C., Zhou, N., Balsara, R. D. and Castellino, F. J. 2015, Biochemistry, 54, 787-794). The subunit GluN2A binds glutamate. The regulatory subunit binds either D-serine or glycine as a co-agonist.

DAO noncovalently binds to FAD as a prosthetic group and catalyzes the oxidative deamination of D-amino acid to their corresponding imino acids with concomitant reduction of FAD [19]. The reduced flavin is subsequently reoxidized by molecular oxygen generating H_2O_2, and the imino acid is released into the solvent where it nonenzymatically hydrolyzes, yielding the corresponding α-keto acid and ammonia [20].

The loop is thought to switch between the "closed" conformation observed in the crystal structures and an "open" state required for substrate binding and product release (Fig. 6). Loop closure is likely to have a role in catalysis by increasing the hydrophobicity of the active site.

In a pioneer study, Mothet and Synder [19] depleted endogenous D-serine from neural cultures by applying D-amino acid oxidase which specifically degrades D-amino acids, but not L-amino acids. Careful characterization of a naturally occurring DAO knockout mouse strain suggests the primary purpose of DAO is to degrade D-amino acids [19]. DAO has a selective distribution, being particularly abundant in cranial motor nuclei and occurs in the facial nerve nucleus, magnocellular cells of the reticular formation, cellular nuclei and is present in cerebellum in both Purkinje cells and Bermann glial cells [21, 22].

In human spinal cord, DAO and D-serine immunoreactivity are prominent in motor neurons [22]. DAO immunoreactivity is highly concentrated in spinal cord motor neurons, fibers and glial cells and at the cellular level, the distinct peroxisomal localization of DAO is evident [23]. D-Serine appears punctuate and in vesicular structures throughout the cytosol of motor neurons and glial cells. SR is also present in both motor neurons and glial cells in control spinal cord. In familial amyotrophic lateral sclerosis (ALS), levels of D-serine, DAO, and Serin spinal cord are all severely depleted reflecting the substantial loss of motor neurons with disease. Whilst SR-positive motor neuron loss occurs in ALS, the number of SR-positive glial cells is substantially increased in ALS.

D-Serine dehydratase (DSD)

Another enzyme which degrades D-serine is D-serine dehydratase (EC 4.2.1.18, DSD) from *Escherichia coli*. This enzyme was first discovered in 1960s in Esmond Snell's laboratory. It catalyses the pyridoxal 5'-phosphate-dependent conversion of D-serine to pyruvate and ammonia [23]. To our knowledge, it is the only monomeric PLP-dependent enzyme of amino acid metabolism. More recently, D-serine dehydratase was discovered in *Saccharomyces cerevisae* as a zinc- and PLP-dependent enzyme [24]. Even more recently, D-serine dehydratase
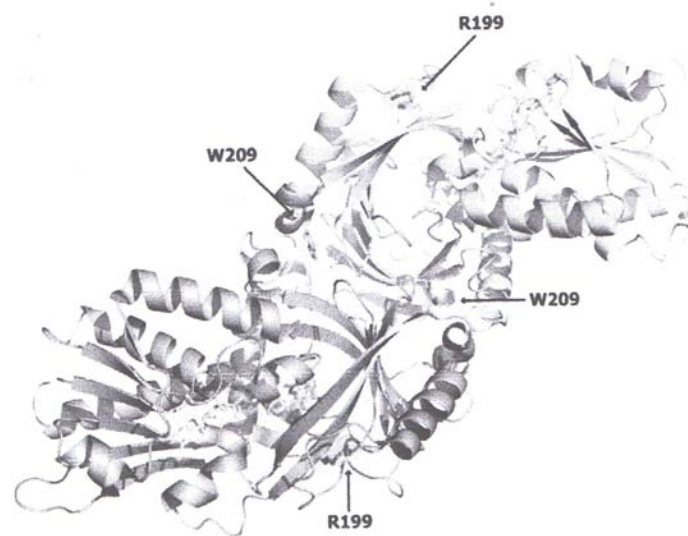
Fig. 6. Three dimensional structure of D-amino acid oxidase (Reproduced from Cappelletti, M. Piubelli, L., Murtas, G., Caldinelli, L., Valentino, M., Molla, G., Pollegioni, L. and Sacchi, S. 2015, Biochim. Biophys. Acta, 1854, 1150-1159 with permission from Elsevier).
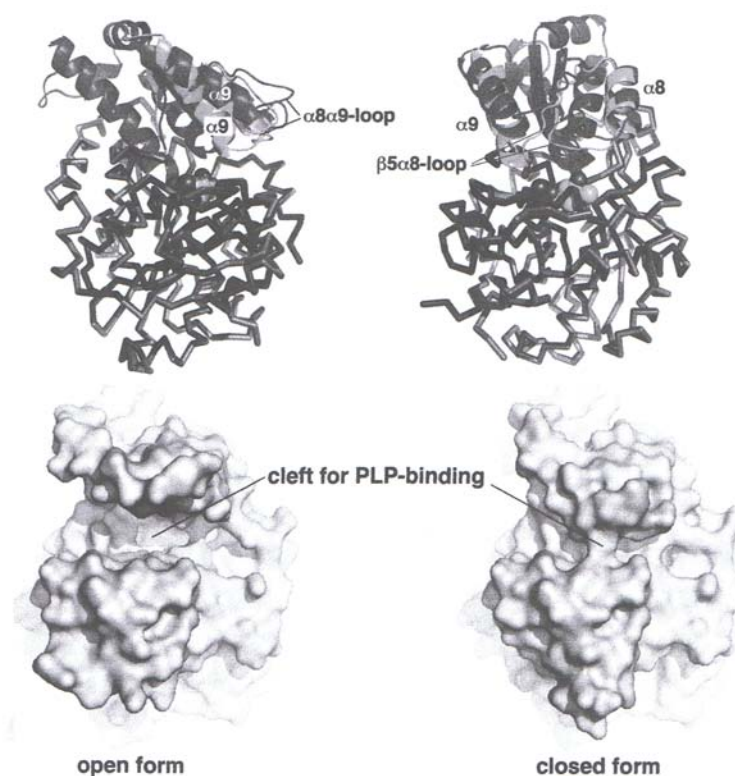

Fig. 7. Open and closed conformation of DSD (Reproduced from Urusova, D. V., Isupov, M. N., Antonyuk, S., Kachalova, G. S., Obmolova, G., Vagin, A. A., Lebedev, A. A., Burenkov, G. P., Dauter, Z., Bartunik, H. D., Lamzin, V. S., Melik-Adamyan, W. R., Mueller, T. D. and Schnackerz, K. D. 2012, Biochemica et Biophysica Acta, 1824, 422-432 with permission from Elsevier). The small domain is shown in light dark, whereas the large one appears in dark. At the interface of the two domains the PLP-binding site is located.

was isolated from chicken kidney [25]. Again the enzyme is zinc-dependent like the dehydratase from *Saccharomyces cerevisiae*. A crystal structure of the zinc-dependent D-serine dehydratase from chicken kidney has just been published [26].

D-Serine dehydratase from *Escherichia coli* was crystallized in two forms, orthorhombic (1.97 Å) and monoclinic (1.55 Å). The monomer reveals a fold-type II, characteristic of β-family PLP-dependent enzymes and is divided into two domains, the larger PLP-binding domain and a smaller domain. The orthorhombic and monoclinic structures of DSD show large differences in the arrangement of the small domain with respect to the large domain. In the closed conformation (monoclinic form) the movement of the small domain blocks the entrance to the active site of DSD [27]. The domain movement plays an important role in the formation of the substrate recognition site and the catalysis of the enzyme (Fig. 7).

Even though the sequence identity or sequence similarity of DSD when compared to the subunits of tryptophan synthase, O-acetylserine sulfhydrylase, allosteric threonine deaminase and L-serine dehydratases is less than 20 per cent, all enzymes mentioned above have the same fold-type II, i.e., have similar active sites [28]. Interestingly enough, serine racemases, the main protein source to produce D-serine from L-serine in the mammalian brain, is also a fold-type II protein [14].

The PLP-binding site of DSD is depicted in Fig. 8. The phosphate group of PLP is surrounded by the so-called tetraglycine loop (G279, G281, G282, and G283) [29, 30]. The aldehyde residue of the pyridoxal moiety exists in a Schiff base linkage to K118. The 3-hydroxyl group is hydrogen-bonded to the side chain of N170. The pyridinium nitrogen is hydrogen-bonded to the side chain of T424. The so-called asparagine loop (S167, T168, G1669, and N170) resides on the O3'side chain of PLP and acts as the recognition site for the substrate carboxylate in the closed form. A similar arrangement of the active site residues has been found in serine racemase from rat liver and *Schizosaccharomyces pombe* [15]. In early publications of DSD, Snell's group reported that monovalent cations greatly influence the enzymatic

activity, K^+ ions enhance, whereas Na^+ ions inhibit the enzymatic activity [31]. A similar arrangement of the potassium ion binding site was discovered for DSD and human serine racemase. Residues C311 (S200), G279 (G198), E395 (E194), S309 (A198), L340 (L233) and V342 (V225) form the binding site of the catalytically important potassium ion (the amino acids in parentheses are those found for rat L-serine dehydratase (LSD)). It is specially noteworthy that G279 is part of the tetraglycine loop as well as the monovalent ion binding site, i.e., the monovalent cation binding site is actually not part of the active site, but located adjacent to it (Fig. 9). ^{31}P NMR (nuclear magnetic resonance spectroscopy) experiments of DSD revealed information about the properties of the DSD-bound cofactor phosphate. The pH-dependence of the ^{31}P chemical shift of PLP bound to DSD suggests that the 5'-phosphate group of the enzyme-bound cofactor is exposed to the solvent, i.e., is in the open conformation. The ^{31}P
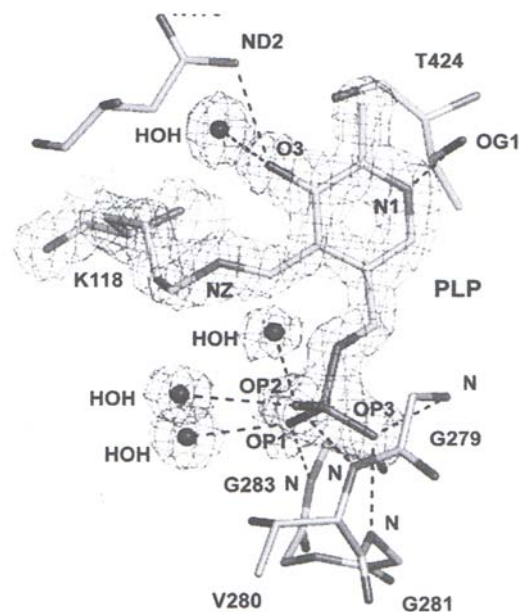

Fig. 8. View of the PLP-binding site showing the electron density peaks of PLP aldimine with K118 (Reproduced from Urusova, D. V., Isupov, M. N., Antonyuk, S., Kachalova, G. S., Obmolova, G., Vagin, A. A., Lebedev, A. A., Burenkov, G. P., Dauter, Z., Bartunik, H. D., Lamzin, V. S., Melik-Adamyan, W. R., Mueller, T. D. and Schnackerz, K. D. 2012, Biochemica et Biophysica Acta, 1824, 422-432 with permission from Elsevier).

line width of 13 Hz indicates that the cofactor is tightly bound to the protein and tumbles with protein [32]. In the presence of a potent competitive substrate analogue isoserine (D,L-3-amino-2-hydroxy-propionate), a new external Schiff base linkage is formed. The ^{31}P NMR signal is now pH-independent, and the phosphate group is locked in its dianionic form in the closed conformation [32].

Modeling of D-serine into the active site of DSD suggests that the hydroxyl group of D-serine is coordinated to the carboxyl group of D238. The carboxyl oxygens of D-serine are coordinated to the hydroxyl group of S167 and the amide group of L171 (O1 oxygen) and the hydroxyl group of S167 and the amide group of T168 (O2 oxygen) via hydrogen bonding (Fig. 10).

The putative mechanism of the reaction of DSD is summarized in Fig. 10. The catalytic mechanism of DSD is very similar to that proposed for LSD [30]. The amino group of the substrate, D-serine, forms a hydrogen bond with an oxygen of the cofactor phosphate (Fig. 10, 1). The phosphate becomes protonated and the amino group changes into a nucleophile attacking the nitrogen of the internal Schiff base by releasing the neutral K118 (Fig. 10, 2). The O1 of the carboxyl group of D-serine is coordinated to the hydroxyl of S167 (3.3 and 2.8 Å) and the amide nitrogen of L171 (2.8 Å) via hydrogen bonds. The hydroxyl group of S167 (3.3 Å) and amide nitrogen of T168 are hydrogen-bonded to the carboxyl group of D238 (3.4 Å). The hydroxyl group of D-serine is hydrogen-bonded to the carboxyl of D238 (2.7 Å) and to the phosphate group of PLP via a water molecule (Fig. 10). The pyridine ring of PLP cannot form a quinonoid structure since N1 of PLP is not protonated, and therefore the abstraction of the α-hydrogen from $C_α$ of D-serine by K118 would not occur. It is, however, very likely, that the phosphate group of PLP can act as a general acid to donate a proton to the oxygen OG of D-serine and abstraction of a hydrogen from $C_α$ by K118 occurs in a concerted fashion. Water and the PLP-α-aminoacrylate Schiff base are released (Fig. 10, 4). K118 attacks C4' of the Schiff base of the PLP-α-aminoacrylate intermediate to form the PLP-K118 (internal) Schiff base (Fig. 10, 5) and releases α-aminoacrylate (Fig. 10, 6) which in a non-enzymatic hydrolysis is deaminated to pyruvate and ammonia.
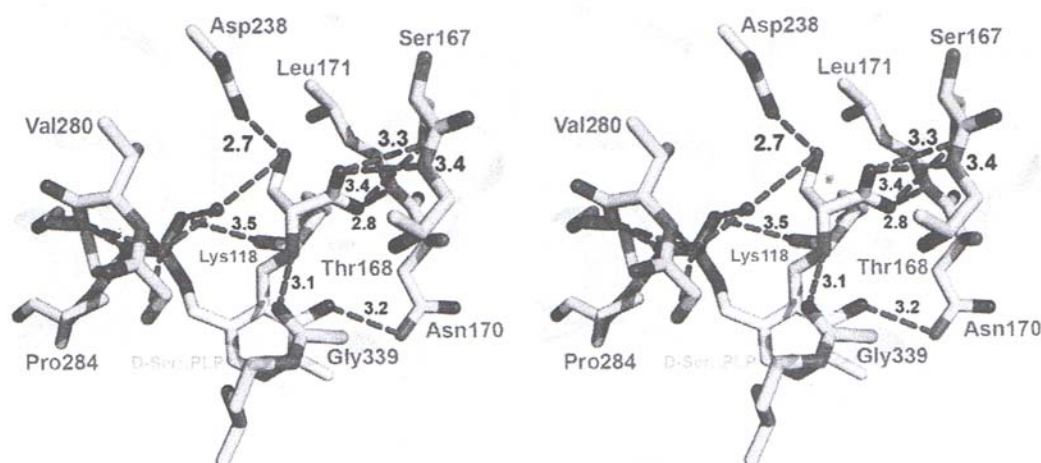

Fig. 9. Modeling of the active site of DSD with the substrate D-serine (Reproduced from Urusova, D. V., Isupov, M. N., Antonyuk, S., Kachalova, G. S., Obmolova, G., Vagin, A. A., Lebedev, A. A., Burenkov, G. P., Dauter, Z., Bartunik, H. D., Lamzin, V. S., Melik-Adamyan, W. R., Mueller, T. D. and Schnackerz, K. D. 2012, Biochemica et Biophysica Acta, 1824, 422-432 with permission from Elsevier). Model of the PLP cofactor loaded with D-serine (shown with C-atoms) in the active site of DSD. Surrounding residues of the DSD are shown in sticks, hydrogen bonds between the cofactor::D-serine complex and the dehydratase are shown as stippled lines. Residues participating in the coordination of PLP and/or the D-serine residue are indicated. D238, involved in the dehydration mechanism is coordinated via hydrogen bond with the hydroxyl group of the D-serine. L-serine attached to PLP would lack this hydrogen bond as its side chain is oriented away from the active site.

Comparison of the structures of DSD from *Escherichia coli* and serine racemase from *Schizosaccharomyces pombe*

The X-ray structure of SR was first determined from crystals of a homolog of mammalian serine racemase from *Schizosaccharomyces pombe* in 2009 by Goto *et al*. [14]. Evidence for conformational changes upon inhibitor binding in the structure of mammalian serine racemase was reported by Smith *et al*. [33] in 2010. The alignment of the monomers of DSD and SR are shown in Fig. 11. The sequence similarities are quite apparent.
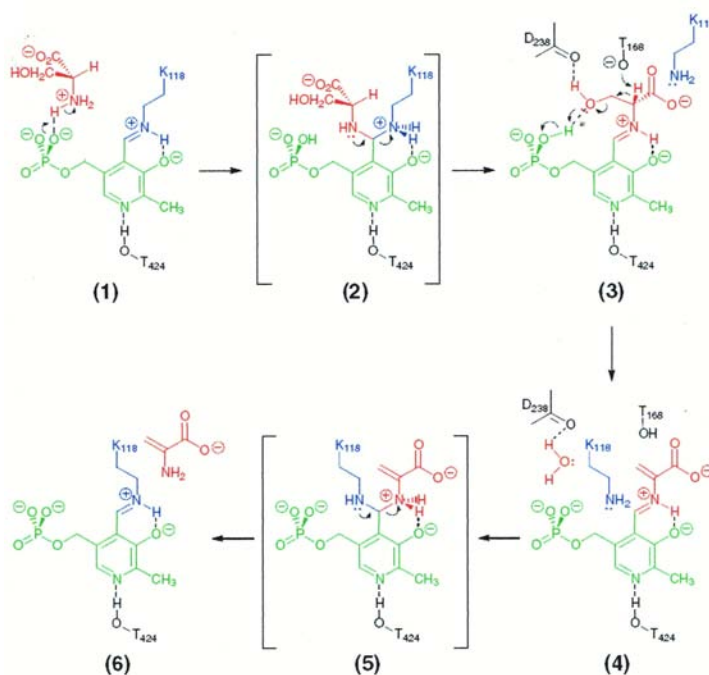

Fig. 10. Putative catalytic mechanism of DSD (Reproduced from Urusova, D. V., Isupov, M. N., Antonyuk, S., Kachalova, G. S., Obmolova, G., Vagin, A. A., Lebedev, A. A., Burenkov, G. P., Dauter, Z., Bartunik, H. D., Lamzin, V. S., Melik-Adamyan, W. R., Mueller, T. D. and Schnackerz, K. D. 2012, Biochemica et Biophysica Acta, 1824, 422-432 with permission from Elsevier).
Curved arrows indicate movements of electrons. Possible hydrogen bonds are represented by dashed lines.
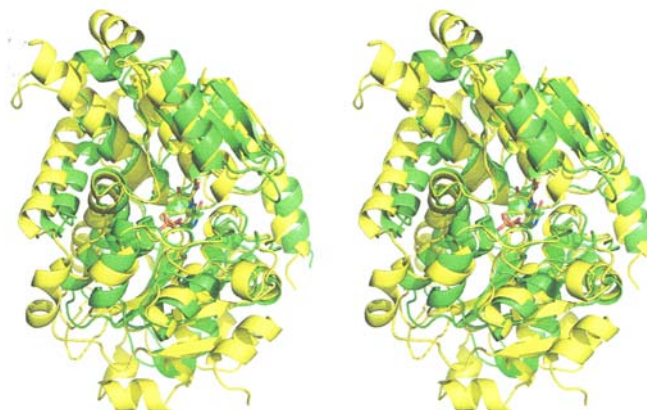

Fig. 11. Stereo view of the superposition of DSD (green) and serine racemase (SR) (yellow).
The distinction of two domains in both proteins is recognisable.

ACKNOWLEDGEMENT

CONFLICT OF INTEREST STATEMENT

The author declares that there are no competing interests associated with this review.

ABBREVIATIONS

DSD, D-serine dehydratase; SR, serine racemase; DAO, D-amino acid oxidase; PLP, pyridoxal 5'-phosphate; NMDA, N-methyl-D-aspartate; NMDAR, N-methyl-D-aspartate receptor; spSR, SR from *Schizosaccharomyces pombe*; spSRw, SR from *Schizosaccharomyces pombe* wild-type; spSRw, SR from *S. pombe* wild-type protein; spSRm, SR from human tissue; ALS, amyotrophic lateral sclerosis.

REFERENCES

1. Gogami, Y., Ito, K., Katitani, Y., Matsushima, Y. and Oikawa, T. 2009, Phytochemistry, 70, 380-387.
2. Mattineau, M., Baux, G. and Mothet, J. P. 2006, Trends Neurosci., 29, 481-491.
3. Wolosker, H., Dumin, E., Balan, L. and Foltyn, V. N. 2008, FEBS J., 275, 3514-3426.
4. Mothet, J. P., Parent, A. T., Wolosker, H., Brady, R. O. Jr., Linden, D. J., Ferris, C. D., Rogawski, M. A. and Snyder, S. H. 2000, Proc. Natl. Acad. Sci. USA, 97, 4926-4931.
5. Synder, S. H. and Kim, P. M. 2000, Neurochem. Res., 25, 553-560.
6. Campbell, D. S. and Holt, C. E. 2003, Neuron, 37, 939-952.
7. Bruno, S., Marchesani, F., Dellafiora, L., Margiotta, M., Faggiano, S., Campanini, B. and Mozarelli, A. 2016, Biochem. J., 473, 3505-3516. (a) Bruno, S., Margiotta, M., Marchesani, F., Paredi, G., Orlando, V., Faggiano, S., Ronda, L., Pampanini, B. and Mozzarella, A. 2017, Biochim. Biophys. Acta, 1865, 381-387.
8. Li, Z., Jo, J., Jia, J. M., Lo, S. C., Whitcomb, D. J., Jiao, S., Cho, K. and Sheng, M. 2010, Cell, 141, 850-871.
9. Kroemer, G., Galluzzi, L., Vandenabeele, P., Adams, J., Alnemri, E. S., Baenrecke, E. H., Blagosklonny, M. V., El-Deiry, W. S., Golstein, P., Green, D. R., Hengartner, M., Knight, R. A., Kumar, S., Lipton, S. A., Malori, W., Nunez, G., Peter, M. E., Tschopp, J., Yuan, J., Piacentini, M., Zhivotovsky, B. and Melino, G. 2009, Cell Death Differ., 16, 3-11.
10. Canu, N., Tufi, R., Serafino, A. I., Amadori, G., Ciotti, M. T. and Calissano, P. 2005, J. Neurochem., 92, 1228-1242.
11. Johnson, J. W. and Ascher, P. 1990, Biophys. J., 57, 1085-1090.
12. Canu, N., Ciotti, M. T. and Pollegioni, L. 2014, Front. Synaptic Neurosci., 6, 9. doi10.3389/frisyn.2014.00009.
13. Wolosker, H., Sheth, K. N., Takahashi, M., Mothet, J. P., Brady, R. O. Jr., Ferris, C. D. and Snyder, S. H. 1999, Proc. Natl. Acad. USA, 96, 721-725.
14. Goto, M., Yamauchi, T., Kamiya, N, Miyahara, I., Yoshimura, T., Mihara Kurihara, T., Hirotsu, K. and Esaki, N. 2009, J. Biol. Chem., 284, 25944-25952.
15. Yang, C. R. and Svensson, K. A. 2008, Pharmacol. Therapy, 120, 317-332.
16. Suzuki, M., Sasabe, J., Miyoshi, Y., Kuwasako, K., Muto, Y., Harnase, K., Matsuoka, M., Imanishi, N. and Aiso, S. 2015, Proc. Natl. Acad. Sci. USA, 112, E2217-E2224.
17. Cheriyan, J., Mezes, C., Zhou, N., Balsara, R. D. and Castellino, F. J. 2015, Biochemistry, 54, 787-794.
18. Krebs, H. A. 1935, Biochem. J., 29, 1620-1644.
19. Mothet, J. P. and Synder, S. H. 2012, Amino Acids, 43, 1809-1810
20. Kawazoe, T., Tsuge, H., Pilone, M. S. and Fukui, K. 2006, Prot. Sci., 15, 2708-2717.
21. Mitchell, J., Paul, P., Chen, H. J., Morris, A., Payling, M., Falchi, M., Habgood, J., Panoutson, S., Winkler, S., Tisalo, V., Hajitou, A., Smith, B., Vance, C., Shaw, C. Mazarakis, N. D. and deBelle, J. 2010, Proc. Natl. Acad. Sci. USA, 107, 7556-7561.
22. Paul, P., Murphy, T., Oseni, Z., Sivalokanathan, S. and de Belleroche, J. S. 2014, Neurobiol. Aging, 35, 876-885.
23. Dowhan, Jr. W. and Snell, E. E. 1970, J. Biol. Chem., 245, 4618-462
24. Ito, T., Hemmi, H., Kataoka, K., Mukai, Y. and Yoshimura, T. 2008, Biochem J., 409, 399-406.
25. Tanaka, H., Yamamoto, A., Ishida, T. and Horiike, K. 2008, Biochem. J., 143, 49-57.

26. Tanaka, H., Senda, M., Venugopalan, N., Yamamoto, A., Senda, T., Ishida, T. and Horiike, K. 2011, J. Biol. Chem., 286, 27548-27558.

27. Urusova, D. V., Isupov, M. N., Antonyuk, S., Kachalova, G. S., Obmolova, G., Vagin, A. A., Lebedev, A. A., Burenkov, G. P., Dauter, Z., Bartunik, H. D., Lamzin, V. S., Melik-Adamyan, W. R., Mueller, T. D. and Schnackerz, K. D. 2012, Biochemica et Biophysica Acta, 1824, 422-432.

28. Schnackerz, K. D., Keller, J. W., Phillips, R. S. and Toney, M. D. 2006, Biochim. Biophys. Acta 1764, 230-238.

29. Schiltz, E. and Schmitt, W. 1981, FEBS Lett., 134, 57-62.

30. Obmolova, G., Tepliakov, A., Haruntyunyan, E., Wahler, G. G. and Schnackerz, K. D. 1990, J. Mol. Biol. 214, 641-642

31. Dupourque, D., Newton, A. W. and Snell, E. E. 1966, J. Biol. Chem., 241, 1233-1238.

32. Schnackerz, K. D., Feldmann, K. and Hull, W. E. 1979, Biochemistry, 18, 1536-1539.

33. Smith, M. A., Mack, V., Ebneth. A., Moraes, I., Fellcetti, B., Wood, M., Schonfeld, D., Mather, D. and Barker, J. 2010, J .Biol. Chem., 285, 12873-12881.

34. Cappelletti, M. Piubelli, L., Murtas, G., Caldinelli, L., Valentino, M., Molla, G., Pollegioni, L. and Sacchi, S. 2015, Biochim. Biophys. Acta, 1854, 1150-1159.