Journal of Chromatography B, 879 (2011) 3162–3168

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Review

D-Amino acid metabolism in mammals: Biosynthesis, degradation and analytical aspects of the metabolic study☆

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ARTICLE INFO

Article history:
Received 5 December 2010
Accepted 15 June 2011
Available online 22 June 2011

Keywords:
Serine racemase
D-Amino-acid oxidase
Aspartate racemase
D-Aspartate oxidase
D-Serine
D-Aspartate

ABSTRACT

It was believed for long time that D-amino acids are not present in mammals. However, current technological advances and improvements in analytical instruments have enabled studies that now indicate that significant amounts of D-amino acids are present in mammals. The most abundant D-amino acids are D-serine and D-aspartate. D-Serine, which is synthesized by serine racemase and is degraded by D-amino-acid oxidase, is present in the brain and modulates neurotransmission. D-Aspartate, which is synthesized by aspartate racemase and degraded by D-aspartate oxidase, is present in the neuroendocrine and endocrine tissues and testis. It regulates the synthesis and secretion of hormones and spermatogenesis. D-Serine and D-aspartate bind to the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors and function as a coagonist and agonist, respectively. The enzymes that are involved in the synthesis and degradation of these D-amino acids are associated with neural diseases where the NMDA receptors are involved. Knockout mice for serine racemase and D-aspartate oxidase have been generated, and natural mutations in the D-amino-acid oxidase gene are present in mice and rats. These mutant animals display altered behaviors caused by enhanced or decreased NMDA receptor activity. In this article, we review currently available studies on D-amino acid metabolism in mammals and discuss analytical methods used to assay activity of amino acid racemases and D-amino-acid oxidases.

Contents

1. Introduction

D-Amino acids have been historically considered unnatural amino acids. Even after their discovery in bacteria, researchers still believed that D-amino acids were not present in eukaryotes, especially in higher animals. However, recent technological advances and improvements in analytical instruments have enabled studies that have shown that D-amino acids are present in mammals [1–3]. The most abundant D-amino acids in mammals are D-serine and D-aspartate. The enzymes that synthesize and degrade these D-amino acids have also been discovered.

In a landmark study in 1992, Hashimoto et al. [4] found a large quantity of D-serine in rat brains. A second study indicated that D-serine was abundant, specifically in the cerebral cortex, hippocampus, anterior olfactory nucleus, olfactory tubercle, and the amygdala of rats [5]. D-Serine was originally detected in the glial cells, but is also present in the neurons [6,7]. D-Serine, which is synthesized by serine racemase and degraded

☆ This paper is part of the special issue "Analysis and Biological Relevance of D-Amino Acids and Related Compounds", Kenji Hamase (Guest Editor).

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doi:10.1016/j.jchromb.2011.06.028

by D-amino-acid oxidase, binds to the coagonist binding site of the *N*-methyl-D-aspartate (NMDA)-subtype of glutamate receptors and modulates NMDA receptor activity. The NMDA receptors are involved in numerous physiological and pathological processes, including synaptic plasticity, learning, memory, neuronal cell migration, and neural disease [8,9].

In 1986, Dunlop et al. [10] reported that D-aspartate is present in mammals. Since then, a large quantity of D-aspartate has been detected in the brain, pineal gland, pituitary gland, adrenal gland, and testis [11]. D-Aspartate, which is synthesized by aspartate racemase and degraded by D-aspartate oxidase, regulates the synthesis and secretion of hormones and spermatogenesis. Similar to D-serine, D-aspartate also regulates NMDA receptor activity. In this review, we summarize recent findings on the enzymes involved in D-amino acid metabolism and analytical methods utilized in these studies.

2. Analytical methods for the assay of amino acid racemase activity

In mammals, only two amino acid racemases have been reported; serine racemase (EC 5.1.1.18) and aspartate racemase (EC 5.1.1.13). Various assay methods have been reported to measure serine racemase activity. In the first report, luminol reaction was used [12]. Substrate L-serine was converted to D-isomer by serine racemase. The resultant D-serine was converted to 2-oxo acid (α-keto acid), ammonia and hydrogen peroxide by D-amino-acid oxidase. The generated hydrogen peroxide was reacted with luminol in the peroxidase reaction. Emitted luminescence was quantified with a luminometer.

Serine racemase activity was also determined colorimetrically or fluorimetrically. D-Serine produced from L-serine was treated with D-amino-acid oxidase as described above, and the resultant hydrogen peroxide was quantified using horseradish peroxidase and *o*-phenylenediamine. Absorbance at 411 nm was measured [13]. In another method, hydrogen peroxide was quantified using horseradish peroxidase and 10-acetyl-3,7-dihydroxyphenoxazine. Fluorescence emission was measured [14].

Serine racemase activity was also measured using HPLC. In this method, the generated D-serine and unreacted L-serine were separated and quantitated using reversed-phase HPLC after derivatization with chiral reagents, *N*-*tert*-butyloxycabonyl-L-cysteine and *o*-phthalaldehyde (OPA) [15] or 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide [13,16]. The use of a selective chromatographic system enables the precise assay of serine racemase.

Concerning the aspartate racemase, the enzymatic activity is measured by a colorimetric method using a specific enzyme catalyzing D-aspartic acid [17], or by HPLC to determine D-aspartic acid produced by the racemase reaction [18]. In the former colorimetric method, L-aspartate is converted to D-aspartate by aspartate racemase. The D-aspartate is converted to oxalacetate by D-aspartate oxidase. The oxalacetate is reacted with dinitrophenyl hydrazine and the resultant hydrazone is measured using a photometer. In the latter method, produced D-aspartate is quantitated using reversed-phase HPLC after derivatization with *N*-*tert*-butyloxycabonyl-L-cysteine and OPA.

3. Analytical methods for the assay of oxidase activity toward D-amino acids

As the oxidation enzyme against D-amino acids, D-amino-acid oxidase (EC 1.4.3.3) and D-aspartate oxidase (EC 1.4.3.1) have been reported in mammals. D-Amino-acid oxidase catalyzes the oxidation of neutral and basic D-amino acids, and D-aspartate oxidase catalyzes the oxidation of acidic amino acids.

A number of assay methods have been developed to measure D-amino-acid oxidase activity. In the past, it was measured by the consumption of oxygen dissolved in the reaction mixture using a manometer. However, the reduction of oxygen is now measured using an oxygen electrode [19,20]. These days, D-amino-acid oxidase activity is determined colorimetrically or chromatographically. In the colorimetric assay, 2-oxo acid produced from D-amino acid is reacted with 2,4-dinitrophenylhydrazine, and the resultant hydrazone is measured at 445 nm [21]. Another reaction product, hydrogen peroxide, is reacted with methanol to produce formaldehyde. The formaldehyde is reacted with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole and the absorbance at 550 nm is measured [22]. In other method, benzoyl formic acid produced from the substrate D-α-phenylglycine is measured at 243 nm [20].

D-Amino-acid oxidase activity is also determined using HPLC. Fluorescent 5-fluoroindole-3-acetic acid produced from the substrate 5-fluoro-D-tryptophan is separated and quantified using a reversed-phase HPLC system [23].

D-Aspartate oxidase activity is determined colorimetrically or chromatographically. In the colorimetric method, oxalacetate produced from D-aspartate is reacted with 2,4-dinitrophenylhydrazine, and the resultant hydrazone is measured at 445 nm [21]. D-Aspartate oxidase activity is also determined using HPLC. Produced oxalacetate is treated with 3-methyl-2-benzothiazoline hydrochloride. The resultant azine derivative is separated and quantified by HPLC [24]. By using these methods, the biosynthesis and degradation of D-amino acids in mammals have been studied. Results from these studies are summarized in Sections 4–7.

4. Serine racemase (EC 5.1.1.18)

It was historically believed that amino acid racemases were only present in prokaryotes and lower eukaryotes. Strikingly, in 1999, the mammalian serine racemase was purified from rat brains [12]. Shortly thereafter, cDNAs encoding mouse and human serine racemase were cloned [25,26].

Similar to other racemases, serine racemase has pyridoxal 5′-phosphate (PLP) as a prosthetic group and catalyzes the conversion between L-serine and D-serine (Fig. 1). It was originally thought that serine racemase produced D-serine in the brain, but it was soon shown that serine racemase also catalyzed the α,β-elimination of water from L-serine or D-serine to form pyruvate and ammonia [15,27] (Fig. 1). Interestingly, the eliminase (dehydratase) activity of serine racemase was much higher than the racemase activity, casting doubt on the synthesis of D-serine by serine racemase. However, mice that were genetically deficient in the serine racemase gene had decreased levels of D-serine in the brain, indicating that serine racemase indeed produces D-serine in wild-type mice [28,29]. Various factors that are described below may influence the balance between the racemase/eliminase activities of the enzyme.

Originally detected in protoplasmic astrocytes [26], serine racemase is present in the cerebral cortex, hippocampus, striatum, and olfactory bulb [7,26]. However, Kartvelishvily et al. [6] showed that serine racemase protein is also present in neurons, and a second study by Yoshikawa et al. [30] showed that serine racemase mRNA is expressed in neurons. Immunohistochemistry with the serine racemase knockout (KO) mice as a control confirmed that this enzyme is present predominantly in the neurons [29].

Human, rat, and mouse serine racemase is a 36–37 kDa protein that comprised 340, 333, and 339 amino acids, respectively. Compared to the human enzyme, there is one amino acid deletion in the rodent serine racemases. Furthermore, rat serine racemase is

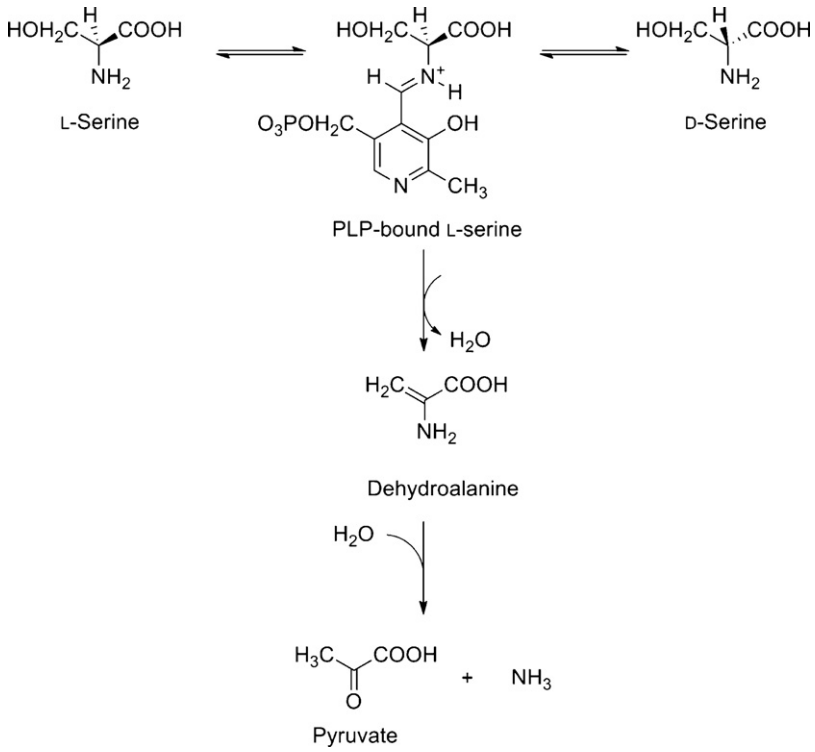

Fig. 1. The serine racemase-catalyzed reaction. Serine racemase catalyzes the interconversion between L-serine and D-serine (horizontal reaction) and the α,β-elimination of water from serine to produce dehydroalanine, which hydrolyzes to form pyruvate and ammonia (vertical reaction). PLP, pyridoxal-5′-phosphate.

truncated at the carboxy terminus [31]. Although there are some minor differences between the species, there is high homology.

Serine racemase activity is regulated by several physiological pathways. The enzyme is activated by divalent cations and nucleotides [15,25]. Yeast two-hybrid studies have shown that the enzyme binds to the glutamate receptor interacting protein (GRIP) [32], protein interacting with C kinase 1 (PICK1) [33], and Golgi-localized protein (Golga 3) [34]. The carboxy terminus of both the mouse and human enzyme contains an amino acid domain that binds to PSD-95/DlgA/zo-1 (PDZ)-containing proteins, such as GRIP and PICK1, which subsequently activates the racemase. The PDZ domain is an important protein–protein interaction motif. However, because rat serine racemase lacks the C-terminal PDZ-recognition sequence, it is unknown whether GRIP and PICK1 bind to and activate rat racemase. The N-terminal of serine racemase contains residues that bind to Golga 3, which results in inhibition of ubiqitin-proteosomal serine racemase degradation. In addition to the binding to above proteins, serine racemase can also be activated by phosphorylation [35], whereas *S*-nitrosylation [36] or membrane binding [37,38] inhibits racemase activity. More detailed information on the regulation of serine racemase can be found in a review article by Baumgart and Rodriguez-Crespo [39].

D-Serine binds to the coagonist site of the NMDA receptors and enhances neurotransmission. Thus, serine racemase, which synthesizes D-serine, has been associated with a number of diseases where the NMDA receptors play a role. For example, NMDA receptor dysfunction is associated with schizophrenia [40], and serine racemase has been implicated in this disease [33,41,42]. Although one study has shown that the serine racemase protein level was decreased in schizophrenic patients [43], another study did not support this finding [44].

Three strains of serine racemase KO mice have been reported [7,28,41], and none of these mice have any obvious defects. However, as expected, neurotransmission and behavior mediated by the NMDA receptors are altered in these mice. Serine racemase KO mice exhibit a schizophrenia-like phenotype and have impaired spatial memory [28,41], reduced prepulse inhibition [41], decreased sociability [41], and elevated anxiety [28]. Injections of NMDA and β-amyloid peptide into the forebrain [29] produced less brain damage in the KO mice compared to wild-type mice. Cerebral ischemia also produced less brain damage in the KO mice [45]. These injections and ischemia result in overactivation of the NMDA receptors, and the KO mice had a decreased level of D-serine, which protected against this overstimulation. Mori and Inoue [8] have summarized the similarities and differences between these three strains of KO mice in their recent review. They note that some behavioral differences may be caused by differences in the genetic background of the mice.

5. D-Amino-acid oxidase (EC 1.4.3.3)

Among the enzymes that are involved in D-amino acid metabolism, D-amino-acid oxidase (DAO or DAAO) was the first enzyme discovered in mammals [46]. D-Amino-acid oxidase contains flavin adenine dinucleotide (FAD) as the prosthetic group and catalyzes the oxidative deamination of D-amino acids. This enzyme has a wide range of substrate specificity and metabolizes a number of neutral and basic D-amino acids. During the initial step of catalysis, the D-amino acid is oxidized to an imino acid and FAD is concomitantly reduced. The reduced FAD is subsequently oxidized by oxygen to release hydrogen peroxide, and the imino acid is non-enzymatically hydrolyzed to produce the corresponding 2-oxo acid and ammonia (Fig. 2). In mammals, D-amino-acid oxidase is mainly present in the kidneys, liver, and brain, although this enzyme is not present in the mouse liver [47]. The physiological function of D-amino-acid oxidase in mammals was unknown for a long time, because D-amino acids were historically not considered to be present in higher animals. However, the physiological significance of this enzyme was partially revealed when a landmark study showed that a large amount of D-serine is present in mammalian

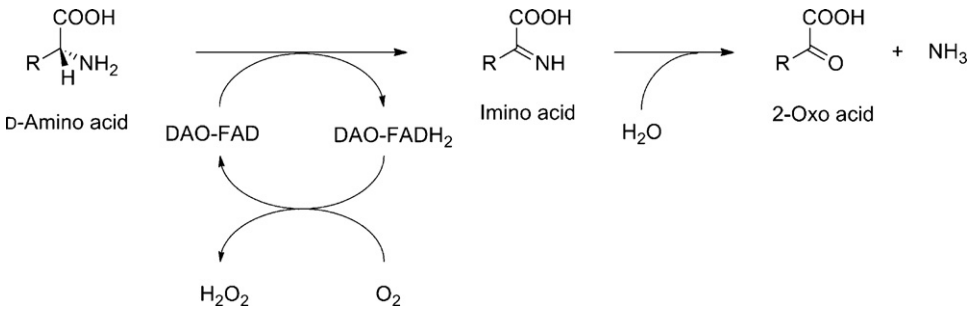

Fig. 2. The D-amino-acid oxidase-catalyzed reaction. The D-amino acid is oxidized to an imino acid, and FAD is reduced to $FADH_2$ which is subsequently oxidized whereas molecular oxygen is reduced to hydrogen peroxide. The imino acid is then non-enzymatically hydrolyzed to the corresponding 2-oxo acid (α-keto acid) and ammonia.

brains [4]. This enzyme attracted much attention when Chumakov et al. [48] reported in 2002 that D-amino-acid oxidase is associated with schizophrenia.

Mammalian D-amino-acid oxidases are approximately 39 kDa proteins and consist of 347 amino acid residues, although the rat and mouse orthologs consist of 346 and 345 amino acid residues, respectively. It is because deletions of amino acid residues have occurred during the evolution of these rodents. Despite these minor differences, the amino acid homology is high among these species. D-Amino-acid oxidase is present in the epithelial cells of the proximal tubules in the kidneys, as well as the liver hepatocytes. The D-amino-acid oxidase protein contains a peroxisomal targeting signal at the carboxyl terminus [49] that localizes the enzyme to the peroxisomes of these cells [50]. Histochemical staining studies have shown that this enzyme is present in the glial cells of the cerebellum and the brainstem [51]. However, D-amino-acid oxidase mRNA and immunoreactivity were also detected in the forebrain [52–55]. Currently, there is no explanation for this difference between D-amino-acid oxidase activity and expression [44].

Because D-serine enhances the activity of the NMDA receptors, D-amino-acid oxidase, which degrades D-serine, has been associated with diseases that involve NMDA receptor malfunction. Chumakov et al. [48] found that a new human gene (*G72*), which encodes a product that activates D-amino-acid oxidase, is associated with schizophrenia. Whereas D-amino-acid oxidase itself has a weak association with schizophrenia, the combination of D-amino-acid oxidase and the G72 activator has a synergistic effect. Chumakov et al. [48] hypothesized that this combination decreases the level of D-serine that is bound to the NMDA receptors, resulting in decreased NMDA receptor function, which is hypothesized to play a role in schizophrenia. Other studies have shown that D-amino-acid oxidase is genetically associated with schizophrenia [52,56]. However, a conflicting second study indicated that the *G72* product inactivates D-amino-acid oxidase [54].

Additional studies have shown that the concentration of D-serine is decreased in the serum [57] and cerebrospinal fluid [43,58] of schizophrenic patients compared to healthy controls. Moreover, D-amino-acid oxidase expression was increased in post mortem brains of schizophrenic patients [55,59–61]. However, there are a number of studies that conflict with these results. Several reports have found no genetic association between D-amino-acid oxidase and schizophrenia [44]. Additionally, another study detected no difference in the concentration of D-serine in the serum or cerebrospinal fluid between schizophrenic patients and normal subjects [62]. Because schizophrenia is a complex disease, many factors could play a role in disease manifestation; D-amino-acid oxidase may simply be a risk factor, rather than the etiology. However, the mechanism of how D-amino-acid oxidase function influences in schizophrenia is not known. Additional information regarding brain D-amino-acid oxidase is present in the following review [44].

A recent study reported that D-amino-acid oxidase is associated with the pathogenesis of amyotrophic lateral sclerosis (ALS) [63]. Specifically, a missense mutation in the D-amino-acid oxidase gene that decreased D-amino-acid oxidase activity was found in a family with ALS. This oxidase mutation increased the D-serine concentration in the spinal cord, which overactivated the NMDA receptors and ultimately led to the destruction of the motor neurons. Correspondingly, in an ALS mouse model, the D-serine concentration in the spinal cord was elevated compared to the wild-type mice, and the concentration of D-serine increased as the disease symptoms progressed [64].

A mutant mouse strain that lacks D-amino-acid oxidase activity due to a missense mutation has been established fairly earlier [65]. Although these mice did not show any obvious abnormalities, large quantities of D-amino acids were present in the organs and body fluids of these mice [66,67]. These results confirm that the physiological function of D-amino-acid oxidase is to metabolize D-amino acids. Detailed characteristics of the mutant mice are presented in the following review [67]. Interestingly, the D-serine concentration was significantly elevated in the cerebellum whereas it was almost unchanged in the cerebrum of the mutant mice (Fig. 3). These results are consistent with the distribution of D-amino-acid oxidase activity in the brain; D-amino-acid oxidase activity is present in the cerebellum and brainstem, but not in the cerebrum [51].

Additionally, NMDA receptor activity was enhanced in the mutant mice, because the excitatory post synaptic currents mediated by the NMDA receptors were augmented [68]. The mutant mice performed better in the Morris water maze [69,70] and had enhanced learning in association with fear-based tasks [69]. These results are not surprising in that learning and memory are known to be dependent upon NMDA receptor function within the hippocampus. Enhanced long-term potentiation (LTP) was observed in hippocampal slices from the mutant mice [70]. Moreover, the mutant mice were resistant to non-competitive antagonists of the NMDA receptors, which typically induce schizophrenia-like behavior [71,72]; the female mutant mice also exhibited increased anxiety [69]. These results were most likely caused by the elevated concentration of D-serine, which bound to the NMDA receptors. An additional study showed that there was increased occupancy of the coagonist sites of NMDA receptors [71]. However, because D-amino-acid oxidase activity is not present in the cerebrum, and because the D-serine concentration was not elevated in the cerebrum of these mutant mice (Fig. 3), the precise mechanism underlying these phenomena is not known. There may be cryptic D-amino-acid oxidase which controls the local D-serine concentration.

There was no compensatory effect for the absence of D-amino-acid oxidase activity in the mutant mice [71]. These results indicate that D-amino-acid oxidase inhibitors, which increase the level of

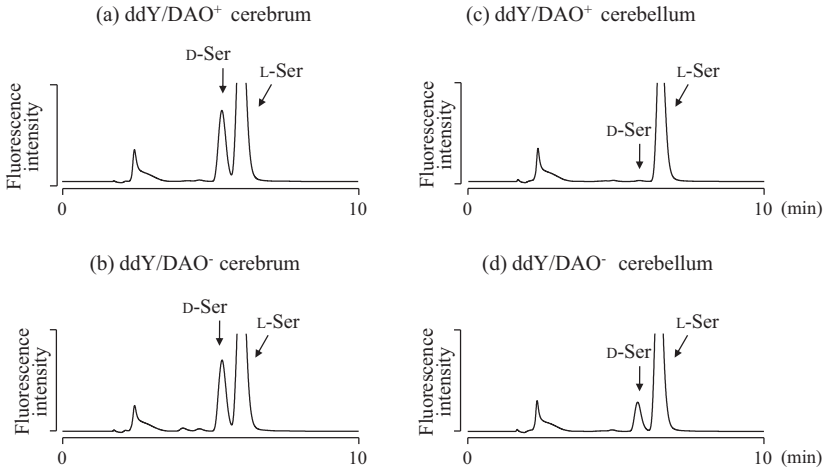

Fig. 3. Chromatograms from the HPLC analysis of D-serine and L-serine in mouse cerebrum (a and b) and mouse cerebellum (c and d). Mouse tissues were homogenized and deproteinized. Amino acids were derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole. D- and L-Serine were separated in a two-dimensional micro-HPLC system (NANOSPACE SI-II series, Shiseido, Tokyo, Japan) combining a microbore-monolithic-ODS column and a narrowbore-Pirkle-type (chiral stationary phase) column. (a) The cerebrum of a normal ddY/DAO$^+$ mouse, (b) the cerebrum of a mutant ddY/DAO$^-$ mouse lacking D-amino-acid oxidase activity, (c) the cerebellum of a normal ddY/DAO$^+$ mouse, and (d) the cerebellum of a mutant ddY/DAO$^-$ mouse.

D-serine and stimulate NMDA receptors, may not cause serious side effects. Smith et al. have summarized the various D-amino-acid oxidase inhibitors [73].

Recently, a rat mutant strain that lacks D-amino-acid oxidase activity was described [74,75]. In contrast to the mutant mice above, the mutant rats do not have D-amino-acid oxidase protein. Because of their increased body size, rat models would facilitate detailed studies regarding D-amino-acid oxidase and D-serine function in the brain.

6. Aspartate racemase (EC 5.1.1.13)

Although it was known that a large amount of D-aspartate is present in the tissues and organs of mammals, it was unknown for a long period of time how this D-amino acid is produced. Several studies have shown that the enzyme aspartate racemase is present in bacteria, mollusks, and amphibians. Wolosker et al. [76] showed that embryonic neuronal primary culture cells from rats can produce [^{14}C]D-aspartate from the [^{14}C]L-isomer. Topo et al. [17] developed a method to measure aspartate racemase activity in rats and mice. The assay showed that aspartate racemase activity is present in numerous tissue types in these animals, with the pituitary gland having the highest activity, followed by the testis, the thyroid, and the brain. The liver and kidneys had low aspartate racemase activity. As is expected, the highest aspartate racemase activity is observed in tissues where D-aspartate is present at high concentrations.

Kim et al. [18] used a novel procedure to clone the aspartate racemase gene. They initially attempted to identify a candidate gene via homology to mammalian serine racemase or bacterial aspartate racemase but were unsuccessful. However, several reports have stated that glutamate-oxalacetate transaminase can generate a small amount of D-aspartate during the transamination of L-aspartate to L-glutamate and that D-aspartate formation was augmented with a mutant form of this enzyme. Kim et al. [18] capitalized on this information and discovered aspartate racemase based on homology to glutamate-oxalacetate transaminase. The candidate gene was subsequently cloned and characterized.

Aspartate racemase is a 45.5-kDa protein that is a PLP-dependent enzyme, similar to serine racemase. Although the enzymatic mechanism is not known, the reaction probably proceeds similarly as shown in Fig. 1. Aspartate racemase is highly expressed in the brain, heart and testis, and is moderately expressed in the adrenal gland, which are all tissues where D-aspartate is present. Specifically within the brain, the enzyme is present in the paraventricular and supraoptic nuclei, the cerebral cortex, the hippocampus, the dentate gyrus, the pineal gland, and the posterior pituitary gland.

Retrovirus-mediated expression of short-hairpin RNA complementary to aspartate racemase in newborn neurons of the hippocampus resulted in a defect in dendritic development and impaired survival of the newborn neurons [18]. These results seem to be consistent with previous results where D-aspartate is abundant in the embryonic brain, but drastically decreases during postnatal development. D-Aspartate, therefore, could modulate neurogenesis.

Because aspartate racemase was just recently discovered, there is little information regarding its function. Aspartate racemase KO mice production seems underway, which will facilitate additional studies of this enzyme.

7. D-Aspartate oxidase (EC 1.4.3.1)

D-Aspartate oxidase, which is an enzyme that is similar to D-amino-acid oxidase, was discovered a number of years ago [77]. D-Aspartate oxidase (DDO or DAspO) has FAD as a prosthetic group, and catalyzes the oxidative deamination of acidic D-amino acids, such as D-aspartate and D-glutamate, to produce the corresponding 2-oxo acid, hydrogen peroxide and ammonia. The reaction mechanism is similar to the mechanism that is shown in Fig. 1.

D-Aspartate oxidase is present in the kidneys, liver, and the brain [11]. Specifically, it is present in the epithelial cells in the proximal tubules of the kidney, as well as the liver hepatocytes. The enzyme contains a peroxisomal targeting signal at its N-terminus and is thus localized within the peroxisomes of these cells. Within the brain, D-aspartate oxidase is abundant in the cerebral cortex, the choroid plexus, the ependyma, the hippocampus, the dentate gyrus, the olfactory bulb, the pituitary gland, the hypothalamus, and the cerebellum [78]. cDNA that encodes for D-aspartate oxidase has been cloned from kidneys of cows, mice, and pigs, as well as from the human brain. The D-aspartate oxidase enzyme is approximately 37–38 kDa and consists of 341 amino acids; all of these proteins are well conserved among species.

Oral and intraperitoneal administration of D-aspartate to rats increased D-aspartate oxidase activity in the liver. When D-aspartate was administered to pregnant or gestating rats, the D-aspartate oxidase activity was increased in the kidneys and livers of the newborn rats. Thus, D-aspartate oxidase activity is induced by excess concentrations of D-aspartate [11], unlike D-amino-acid oxidase, which cannot be induced.

The structure of D-aspartate is similar to NMDA, and this molecule is an NMDA receptor agonist [79] that enhances neurotransmission. D-Aspartate oxidase, which degrades D-aspartate, is associated with diseases that are caused by NMDA receptor malfunction.

Two strains of D-aspartate oxidase KO mice have been generated [80,81]. The D-aspartate concentration was markedly increased in these mice, confirming that D-aspartate oxidase degrades D-aspartate. Because D-aspartate regulates the synthesis and excretion of several neuropeptides and hormones, D-aspartate oxidase KO mice have defects in these signaling pathways. The KO mice had an increase in body mass, impaired sexual performance, and decreased autogrooming [81]. These behaviors are known to depend on the α-melanocyte-stimulating hormone (α-MSH). Correspondingly, the expression of proopiomelanocortin (POMC), which is an α-MSH precursor, was decreased in the intermediate lobe of the pituitary gland in the D-aspartate oxidase KO mice; it has been hypothesized that this decreased expression of POMC may be caused by increased amounts of D-aspartate in the intermediate lobe of the pituitary gland of the KO mice. These results suggest that D-aspartate suppresses POMC synthesis in wild-type mice.

D-Aspartate oxidase KO mice show enhanced NMDA neurotransmission and have changes in behaviors that are mediated by these receptors. The mutant mice perform better in the water maze and contextual fear conditioning, which may be related to the elevated level of D-aspartate in the hippocampus. Correspondingly, enhanced LTP was observed in hippocampus slices from the KO mice [82]. Because the D-aspartate oxidase KO mice have a reduced immobility time in the forced swim test, which assesses animal depression [83], D-aspartate oxidase inhibitors might function as an antidepressant. D-Aspartate oxidase inhibitors that increase the D-aspartate concentration in the brain thus have the potential to become antipsychotics as well. Further information on D-aspartate oxidase can be found in Ref. [11].

8. Future directions

In addition to D-serine and D-aspartate, D-alanine is also present in mammals at moderate levels [75,84]. Although most D-alanine derives from food and intestinal bacteria, it may also be synthesized in the body. Alanine racemase has been purified from crayfish [85], and a mammalian ortholog may be soon discovered in a manner similar to the discovery of aspartate racemase.

Currently, four enzymes that metabolize D-amino acids have been discovered in mammals, and the literature regarding these enzymes continues to grow. The regulation of enzymes that play a role in D-amino acid synthesis/degradation is complicated. Researchers currently do not have enough information to fully illustrate the interplay among the D-amino acids and the various enzymes that are involved in their metabolism. Although there are a number of published studies that describe these interactions, results from several studies are contradictory, indicating that the entire network is not well understood. However, the rapid progress in this field in the past decade indicates that additional studies in the near future will allow us to understand the function of D-amino acids and their metabolic enzymes in physiology and pathology. The creation of genetically deficient animals that lack these enzymes and advances in analytical methods for D-amino acids will definitely improve our knowledge of this important and complex network.

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