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Journal of Molecular Catalysis B: Enzymatic 12 (2001) 37–41

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

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Distribution of D-amino-acid oxidase and D-serine in vertebrate brains

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Received 2 August 1999; received in revised form 18 October 1999; accepted 18 October 1999

Abstract

In mammalian brains, D-amino-acid oxidase activity is absent or scarce in the forebrain, is confined to the brain stem and cerebellum, and its localization is extended to the spinal cord. The oxidase-containing cells are astrocytes including Bergmann glial cells. Neither neurons, endothelial cells, oligodendrocytes nor ependymal cells show the oxidase activity. Free D-serine, a potent activator of the *N*-methyl-D-aspartate (NMDA) receptor, is in high levels in the forebrain (ca. 0.4 μmol/g wet weight), and in low levels in the hindbrain. Thus, the localization of the oxidase activity is inversely correlated with the distribution of D-serine in mammalian brains. This inverse correlation is generally found in vertebrate brains. These results indicate that D-amino-acid oxidase decomposes D-amino acids including D-serine in vertebrate brains, and that the magnitude of its activity is important in determining the regional concentrations of D-amino acids in the steady states.

Keywords: D-Amino-acid oxidase; D-Amino acid; D-Serine; Astrocytes; Vertebrate; Brain; NMDA receptor

1. Introduction

In animals, there are two D-amino-acid-degrading enzymes, viz., D-amino-acid oxidase (EC 1.4.3.3) and D-aspartate oxidase (EC 1.4.3.1). Both are FAD-dependent oxidases, and localized in peroxisomes [1–3]. D-Amino acids having the negative charge on their side chains such as D-aspartate and D-glutamate are not the substrates of D-amino-acid oxidase, but of D-aspartate oxidase.

D-Amino-acid oxidase was first found in mammalian kidneys and livers by Krebs in 1935 [4,5]. It has been studied extensively since then. The enzyme first oxidizes its D-amino acid substrate to the corresponding 2-imino acid, forming a reduced enzyme–imino acid complex [6,7]. This then transfers its electrons to dioxygen, producing hydrogen peroxide and an oxidized enzyme–imino acid complex. The 2-imino acid finally dissociates from the enzyme. With cyclic amino acids such as D-proline, the 2-imino acid is the final product; with noncyclic amino acids the 2-imino acid is non-enzymatically hydrolyzed to the corresponding 2-oxo acid and ammonia. The high-resolution three-dimensional structure of the oxidase has been recently solved by X-ray crystallography, and the structure-reaction linkage in the oxidase is investigated in atomic detail [8–10].

Since the discovery of the oxidase [4], a number of reports on physiological functions of D-amino-acid oxidase have been published, with the belief that free

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1381-1177/01/$ – see front matter
PII: S1381-1177(00)00201-0

D-amino acids in animals are of microbial origin and not physiological substrates, e.g. [1,3,11,12]. However, the recent development of analytical methods of amino acid enantiomers has shown the occurrence of substantial amounts of D-amino acids in animals, e.g. [11–13]. It is further shown that D-amino-acid oxidase catabolizes neutral free D-amino acids in vivo [14–18]. In this paper, we review the distribution of both free D-serine and D-amino-acid oxidase in vertebrate brains, and discuss the physiological role of the oxidase in the brain.

2. Free D-serine content in vertebrate brains

The L-serine levels are similar (1–2 μmol/g wet weight) among the brains of fish (carp), amphibian (frog), bird (chick), and mammal (mouse, rat, and bull) [19–23]. The L-serine contents are high in all vertebrate brains examined, but D-serine is not detectable in the brains of carp and frog [22]. In the brains of chick, D-serine is present at low levels of 10–20 nmol/g wet weight (the D/L ratio of ca. 0.01). In the mammals (mouse, rat, and bull), the D-serine contents are high in the forebrain; 0.4–0.5 μmol/g wet weight and the D/L ratio is about 0.4 (Table 1) [22].

No significant difference in the free L-serine level is found among the various regions of mammalian brains. Substantial regional differences are found in the distribution of D-serine in the mammalian brains (Table 1): the D-serine levels in the cerebella and brain stems are considerably lower than those in the cerebra (D/L ratios for mammalian cerebella < 0.03).

Table 2
D/L Ratios of free serine in the gray and white matter[a]

	Gray matter	White matter
Rat cerebrum		
Right	0.43	0.29
Left	0.42	0.25
Bovine cerebrum	0.27	0.62
Bovine cerebellum	0.01–0.03	0.01–0.03

[a] Data taken from Ref. [22].

In the rat forebrain, there is no significant difference in the D- and L-serine levels or in the D/L ratio among the various regions of the cerebral cortex [22]; D-serine is evenly distributed in the rat cerebral cortex. The D/L ratios in the hippocampus and hypothalamus are comparable to the cerebral cortex ($D/L = 0.4$), while that in the olfactory bulb is lower ($D/L = 0.12$).

In the rat cerebral cortex, D-serine content in the gray matter is higher than that in the white matter; the D/L ratio for the gray matter is higher than that for the white matter (Table 2) [22]. In the bull, the D/L ratio for cerebral gray matter ($D/L = 0.27$) is lower than that for the cerebral white matter ($D/L = 0.62$),

Table 1
D-Serine contents and D-amino-acid oxidase activities in mammalian brains

Species	Brain region	Free serine (nmol/g wet weight)[a]			D-Amino-acid oxidase activity[b]
		L	D	(D/L)	
Mouse, 8 w	Cerebrum	1290	520	0.425	−
	Brain stem	1110	127	0.115	+
	Cerebellum	989	28	0.029	++
Rat	Cerebrum	928	395	0.425	−
	Cerebellum[c]	∼600	n.d.[c]		++
Bovine	Cerebrum	917	355	0.405[d]	−
		1020	430		
	Cerebellum	835	20	0.027[d]	++
		801	24		

[a] Data taken from Ref. [22].
[b] Data taken from Ref. [24–28], and [32]. −, the oxidase activity undetected histochemically and/or biochemically; +, the oxidase activity detected histochemically and/or biochemically; ++, the intense activity detected histochemically and/or biochemically.
[c] Data taken from Ref. [20], n. d., not detectable.
[d] Values are calculated by using the mean values for two animals.

whereas the ratios for the cerebellar gray and white matter are similar (D/L = 0.01–0.03).

In developing mice, the free L-serine contents in the various brain regions increases with age, and reach the level of the adult mouse (8-weeks old). There is no difference in the L-serine level among the brain regions at each developing stage. The free D-serine contents in the cerebrum increases with age and attain the adult level (D/L = 0.40) 8 weeks after birth. In the cerebellum and brain stem, by contrast, D-serine contents increases with age until 2 weeks (D/L ratio of 0.18 for the brain stem, and of 0.08 for the cerebellum), followed by a decrease to the adult levels (D/L = 0.12 for the brain stem and D/L = 0.03 for the cerebellum) [22].

3. Localization of D-amino-acid oxidase in rat whole brain

Horiike et al. devised a sensitive peroxidase-coupled procedure for histochemical detection of D-amino-acid oxidase activity [24]. The method is based on the intensifying effect of nickel ions on diaminobenzidine-based product formation with peroxidase [24–26]. Using this method, the localization and the identification of D-amino-acid oxidase containing cells in rat whole brain was systematically investigated [25,26].

The oxidase activity is absent in the telencephalon, scarce in the diencephalon, and is almost completely confined to the brain stem (the midbrain, pons, and medulla oblongata) and cerebellum, and its localization is extended to the spinal cord. The hypophysis and the pineal body show no detectable activity (Table 1 and [26]).

In the brain stem, the oxidase activity is not observed in the tectum, but is almost exclusively restricted to the tegmentum, characteristically in the reticular formation. The intense oxidase activities are present in the red nucleus, oculomotor nucleus, trochlear nucleus, ventral nucleus of the lateral lemniscus, dorsal and ventral cochlear nuclei, vestibular nuclei, nuclei of posterior funiculus, nucleus of the spinal tract of the trigeminal nerve, lateral reticular nucleus, inferior olivary nucleus, and hypoglossal nucleus [26]. In the cerebellum the oxidase activity in the cortex is much more intense than that in the medulla [25].

In the midbrain and pons, the oxidase-containing cells are sparse or few in the central gray, cerebral peduncle, the raphe nuclei, and the longitudinal fasciculus of the pons (pyramidal tract). In the medulla oblongata, no oxidase activity is observed in the area postrema, nucleus of the solitary tract, and dorsal motor nucleus of vagus. No activity is also observed in the substantia gelatinosa.

The oxidase-containing cells are exclusively astrocytes including Bergmann glial cells (Golgi epithelial cells) [24–26], which are the particularly modified astrocytes in the cerebellum. Neither neuronal components, endothelial cells, oligodendrocytes nor ependymal cells show the oxidase activity. Most of the oxidase activity is present in the astroglial spaces, i.e., the extraneuronal space, around various kinds of synapses, but not specifically around a single kind of neuron or synapses at which a particular transmitter is released.

The astrocytes are distributed throughout the entire brain, and have morphologically similar characteristics. However, the astrocytes existing in the brain stem, cerebellum and spinal cord express D-amino-acid oxidase, but the astrocytes in the telencephalon and diencephalon do not. These results clearly show that there are two biochemically different types of astrocytes. Viewing astrocytes through D-amino-acid oxidase activity, we conclude that the astrocytes regionally differentiate into two distinct types in the central nervous system.

4. Characteristics of the distribution and possible physiological role of D-amino-acid oxidase

The telencephalon and the diencephalon, which show no activity of D-amino-acid oxidase, come from the alar plate of the neural tube, and embryologically lack the basal plate. The distributing region of the oxidase from the midbrain toward the upper region is limited to the part developed ontogenetically from the basal plate. In the brains of mammals and birds, the oxidase is strongly concentrated in the hindbrain, particularly in the cerebellum, whereas in the lower vertebrates it is distributed fairly evenly over the whole brain [24–29]. These findings indicate that the oxidase comes not to be expressed in the forebrain with the phylogenetic development. The oxidase gene expression is regulated phylogenetically and ontogenetically. In this connection, it is interesting that the

regulatory mechanisms underlying the oxidase gene expression in porcine brain are different from those in kidney and liver [30].

The oxidase activity in rat cerebellum and brain stem is biochemically undetected until 14 days after birth. The adult level is attained by 4–5 weeks [31]. In the cerebrum, the activity is undetectable until day 29, and the maximal activity reaches less than 2% of the cerebellar activity in adult rat [31]. In adult mice, the oxidase activity in the cerebrum is also remarkably lower than that in the cerebellum [27,32]. As described in Section 2, the changes in the D/L ratio among different brain regions of developing mice [22] suggest that D-serine is oxidatively deaminated in situ by D-amino-acid oxidase.

The mammalian brains contain free D-amino acids, in particular D-serine, at a high level corresponding to the order of 10^{-5}–10^{-4} M (Table 1 and [19–23]). The regional D-serine content in rat brain is closely correlated with the distribution of *N*-methyl-D-aspartate (NMDA) receptor [20,33–35]. In contrast, the distribution of the oxidase in rat whole brain [26] is inversely correlated with those of free D-serine and NMDA receptor. This circumstantial evidence suggests that D-amino-acid oxidase decomposes free D-amino acids including D-serine in the brain, and that the magnitude of its activity is important in determining the regional concentrations of D-amino acids in the steady states.

A supplying pathway of D-serine has been recently revealed: serine racemase occurs in eukaryotes such as silkworm [36] and rat brain [37]. The purified racemase depends on pyridoxal 5′-phosphate and acts with high specificity towards serine. In mammalian cerebrum, D-amino-acid oxidase is hardly expressed [26], and D-serine content is very high [22]. Therefore, it is important to elucidate the distribution of serine racemase in mammalian whole brain, and whether the specific degrading system for D-serine exists in brains, especially in cerebrum, because D-serine is a potent activator of the NMDA receptor complex ([11,12] and references cited therein).

Acknowledgements

This work was partly supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (No. 10309005 to KH).

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