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Review

D-Amino acids in mammals and their diagnostic value

Kenji Hamase, Akiko Morikawa, Kiyoshi Zaitsu*

Graduate School of Pharmaceutical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

Abstract

Substantial amounts of D-amino acids are present in mammalian tissues; their function, origin and relationship between pathophysiological processes have been of great interest over the last two decades. In the present article, analytical methods including chromatographic, electrophoretic and enzymatic methods to determine D-amino acids in mammalian tissues are reviewed, and the distribution of these D-amino acids in mammals is discussed. An overview of the function, origin and relationship between the amino acids and pathophysiological processes is also given.

Keywords: Reviews; Enantiomer separation; D-Amino acids

Contents

*Corresponding author. Tel.: +81-92-642-6596; fax: +81-92-642-6601.
E-mail address: zaitsu@phar.kyushu-u.ac.jp (K. Zaitsu).

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1. Introduction

Amino acids are one of the most important molecules for living beings, and most of the amino acids have stereoisomers (L-form and D-form) caused by the chiral center on the α carbon. It was long believed that only L-amino acids are present in higher animals and that the enantiomers, D-forms, are utilized only in the lower species such as microorganisms and bacteria. However, recent progress in analytical chemistry has enabled the sensitive and selective determination of D-amino acids in mammalian tissues and revealed that substantial amounts of D-amino acids are present in mammals. These D-amino acids are present as their free form or in proteins as D-amino acid residues. In the present article, we focused on the free D-amino acids in mammals. For the determination of D-amino acids in mammals, various chromatographic methods, electrophoretic methods and enzymatic methods are used. These methods are reviewed in Section 2. They are widely applied to determine the intrinsic amounts of various D-amino acids; especially, D-Ser and D-Asp are well investigated. The tissue distribution of these D-amino acids is shown in Section 3. Much attention has been paid to the physiological functions and origins of D-amino acids in mammals in the last decade. D-Ser is now thought to have an important function in the central nervous system of mammals to modulate the *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptor. D-Asp is reported to regulate the hormonal release in the endocrine glands such as testis, pituitary gland and pineal gland. The findings are discussed in Sections 4 and 5. In addition to the functions and origins, the relationship between pathophysiological processes (Alzheimer's disease, Parkinson's disease, schizophrenia and renal disease) and the amounts of D-amino acids is also described here.

2. Analytical methods

Chiral separation is one of the most important themes in analytical chemistry, and a number of methods have been developed [1,2]. Especially, analyses of D-amino acids in biological samples are challenging, because a large amount of L-amino acids and a large number of biological substances such as peptides and amines interfere with the analyses. Therefore, a sensitive and selective method should be needed for the determination of D-amino acids. Until now, various methods listed in Table 1, including gas chromatographic methods, high-performance liquid chromatographic methods, high-performance capillary electrophoretic methods and enzymatic methods have been reported [3,4], which are discussed below.

2.1. Gas chromatography

Gas chromatography (GC) is widely used to separate and determine enantiomers [5]. For the determination of L- and D-amino acids in biological samples, diastereomer formation by the reaction with an optically pure reagent or separation on a chiral stationary phase has been adapted. As a chiral derivatizing reagent, D(+)-2-butanol has been used to form diastereomeric esters with amino acids [6]. An amino acid derivative, *N*-trifluoroacetyl-L-prolyl chloride (TPC) is also used to obtain diastereomeric dipeptides with D- and L-amino acids, and the resultant diastereomers could be separated on a silica-based packing column [7,8]. This TPC reagent has been widely used to determine the enantiomers of various amino acid derivatives; however, Payan et al. reported that TPC is racemized by triethylamine added to maintain basic pH during the derivatizing reaction [8], and more accurate determination could be performed by using the *N*-trifluoroacetyl-D/L-

Table 1
Analytical methods used for the determination of D-amino acids

Methods		Examples	Ref.
GC	DIA	2-Butanol	[6]
		TPC	[7,8]
		MTPA-Cl	[9]
	CSP	Chirasil-L-Val	[10–13]
LC	DIA	OPA+chiral thiols	[16–23]
		FDAA	[24–27]
		FLEC	[28,29]
		Chiral isothiocyanates	[30–32]
	CSP	Pirkie-type CSP	[33–40]
		CD-bonded CSP	[41–44]
		Crown ether-bonded CSP	[42,45]
		FDAA-bonded CSP	[46]
		Protein-bonded CSP	[47,48]
	CMP	Metal ion complexes	[49,50]
		CDs	[51]
		Amino acid derivatives	[52]
	CS-system	NP or RP + CSP or CMP	[38–40,42, 50,51,53, 54]
CE		CD-CZE	[58]
		DIA+MEKC	[59]
		CD-MEKC	[60–62]
Enz		DAO	[63–66]
		DAspO	[67–69]
		DAT	[70]

GC, gas chromatography; LC, liquid chromatography; CE, capillary electrophoresis; Enz, enzymatic method; DIA, diastereomer formation; CSP, chiral stationary phase; CMP, chiral mobile phase; CS, column-switching. Other abbreviations are described in the text.

amino acid isopropyl esters, which can be separated into their enantiomers on a chiral column. Recently, Hasegawa et al. reported a method for the simultaneous enantiomer determination of leucine and [$^2H_7$]leucine in plasma samples using a chiral derivatizing reagent, (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA-Cl) [9]. The procedure involves the derivatization of amino acids with hydrochloric acid in methanol to form methyl esters, followed by the subsequent chiral derivatization with MTPA-Cl to form the diastereomeric amide. This method is successfully applied to the pharmacokinetic studies of leucine enantiomers without the interference of naturally occurring leucine.

Chiral stationary phases are also used to determine D-amino acids in biological samples. The most widely used one is Chirasil-L-Val, a stationary phase in which L-Val is coupled to a fused-silica glass. This chiral stationary phase enables the enantioseparation of most protein amino acids as their *N*,*O*-pentafluoropropionyl isopropyl esters [10–13]. With this method, Hashimoto et al. showed for the first time that substantial quantities of free D-serine are present in mammalian brain tissue (Fig. 1). The amount of D-Ser in the rat frontal brain is 270 nmol/g wet tissue (23% of the amount of total serine) [10]. Bruckner et al. determined the enantiomers of 14 amino acids in human urine and blood serum using Chirasil-L-Val and demonstrated that D-Ala and D-Ser are most abundant both in urine and blood sera [11].

2.2. High-performance liquid chromatography

High-performance liquid chromatography (HPLC) is the most widely used technique for the determination of D-amino acids in mammalian tissues, and a variety of methods have been reported [14,15]. Most of the amino acids have low absorptivity of light and do not fluoresce; therefore, precolumn or postcolumn derivatization should be needed for the sensitive determination of D-amino acids in biological samples. Precolumn diastereomer formation using chiral derivatizing reagents followed by the separation on a non-chiral stationary phase, or enantiomer separation using a chiral stationary phase or a chiral mobile phase following the derivatization with non-chiral derivatizing reagents is usually performed. The details are described below.

2.2.1. Chiral derivatizing reagents

The structures of chiral derivatizing reagents discussed here are presented in Fig. 2. *o*-Phthalaldehyde (OPA), in combination with chiral thiols, is widely used to determine amino acid enantiomers. All primary amino acid enantiomers are derivatized with OPA and homochiral thiol compounds to form highly fluorescent diastereomeric isoindole derivatives within a few minutes and could then be separated on a reversed-phase column. As a chiral thiol, *N*-acetyl-L-cysteine (NAC) was reported by Aswad [16], and simultaneous analysis of most common protein amino acid enantiomers was

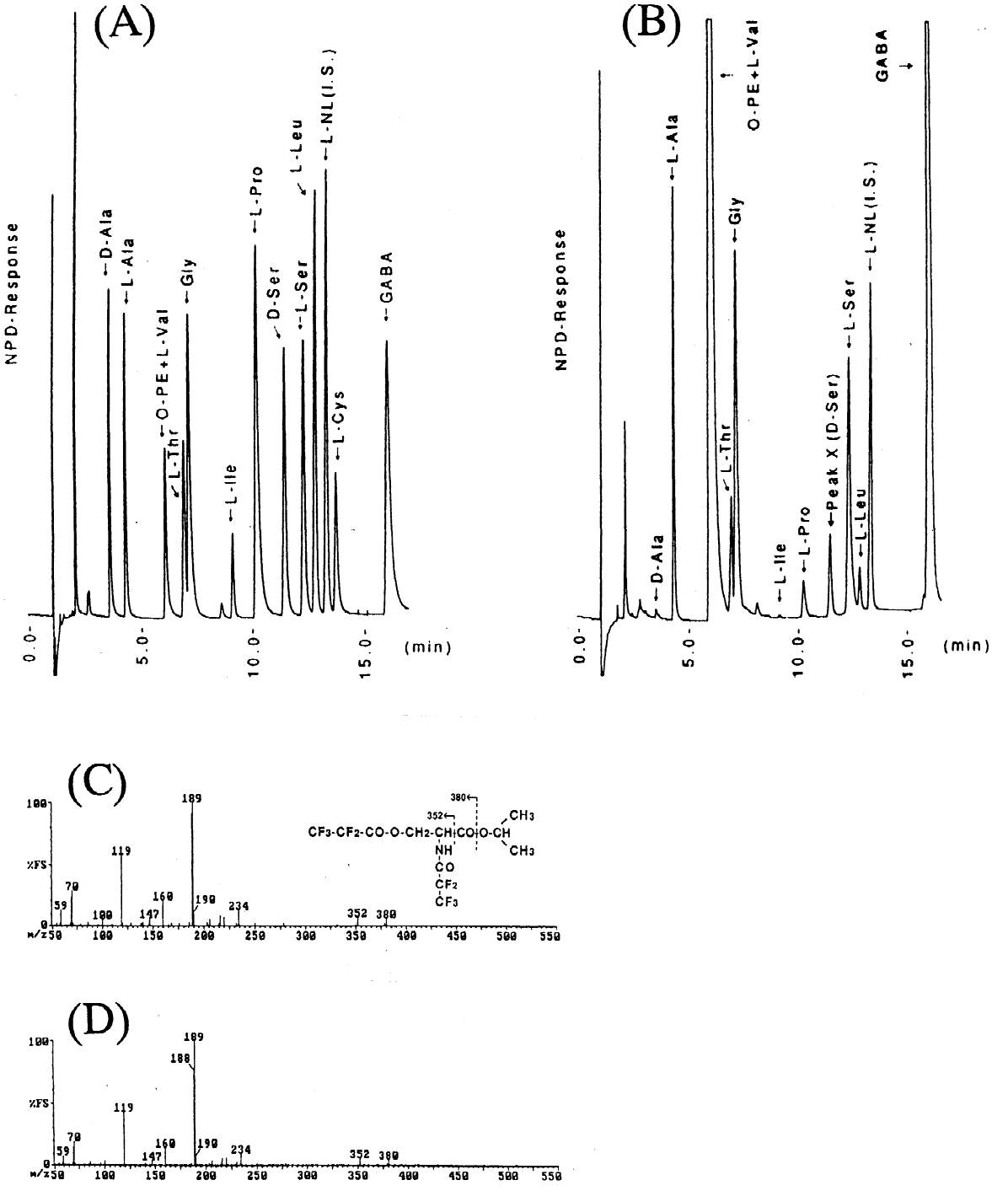

Fig. 1. Gas chromatograms of *N,O*-pentafluoropropionyl (PFP)-isopropyl derivatives of standard amino acids (A) and free amino acids in the rat brain (B) obtained using Chirasil-L-Val as a stationary phase. Electron impact mass spectra of *N,O*-PFP-isopropyl derivative of authentic D-Ser and Peak X observed in the chromatogram (B) are shown in (C) and (D). Reproduced from Ref. [10] with permission.

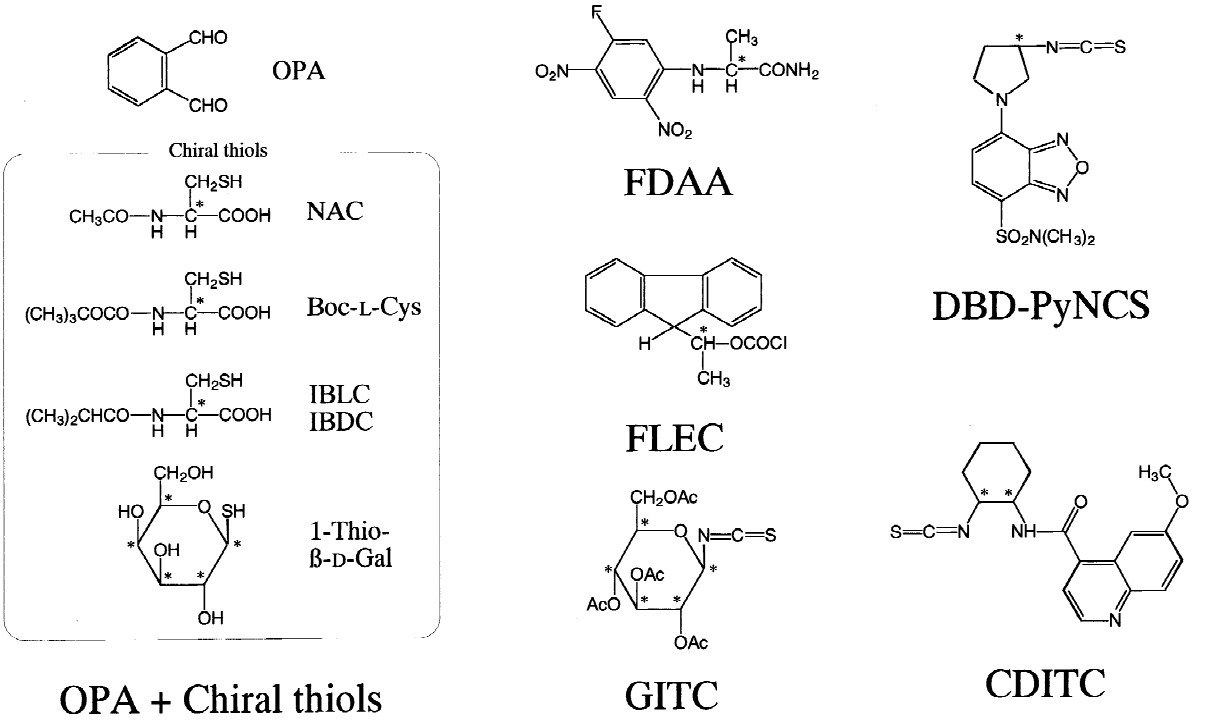

Fig. 2. Chiral derivatizing reagents used for the separation of amino acid enantiomers by HPLC.

achieved [17]. With the reagent, Kera et al. demonstrated the existence of D-Glu and D-Asp in the rat kidney and liver [18]. Large amounts of D-Glu and D-Asp are observed in the liver of male rats (133 nmol/g wet tissue, 60 nmol/g wet tissue, respectively), which are significantly higher than those in female rats. A cysteine analogue, *N-tert.*-butyloxycarbonyl-L-cysteine (Boc-L-Cys), is also used as a chiral thiol in combination with OPA [19–21]. Simultaneous determination of 18 amino acid enantiomers and non-chiral amino acids commonly observed in the rat brain and serum has been reported by Hashimoto et al. and successfully applied to the determination of D-Ser and D-Asp in the rat brain [20]. This method revealed the presence of a large amount of free D-Ser in the rat frontal brain area (220 nmol/g wet tissue, 25% of total Ser). Recently, Morikawa et al. reported a simultaneous determination method for free D-Asp, D-Ser and D-Ala in the mouse brain using OPA and Boc-L-Cys [21]. These three D-amino acids were successfully determined in seven brain areas and serum, showing that D-Ser is localized to frontal brain areas such as the cerebrum and hippocampus. Meanwhile, large amounts of D-Asp and D-Ala are observed in the pineal gland and pituitary gland, respectively. Bruckner et al. utilized *N*-isobutyryl-L-cysteine (IBLC) and *N*-isobutyryl-D-cysteine (IBDC) for the enantiomer separation of amino acids in human serum and urine [22]. Replacement of OPA-IBLC with OPA-IBDC leads to a reversal in the elution order of the derivatives of D- and L-amino acids on an ODS column, enabling a simple confirmation of D-amino acid amounts in biological samples (Fig. 3). Monosaccharide derivatives, 1-thio-β-D-glucose and 1-thio-β-D-galactose, are also used in combination with OPA for the enantiomer separation of primary amino acids [23].

1-Fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA, Marfey's reagent) is a powerful tool for determining D-amino acids [24], and a variety of analogues has been reported [25]. Nagata et al. reported a method of determining 15 D-amino acids in biological samples using FDAA [26]. The tissue extracts were derivatized with FDAA, and each amino acid was separated by a preparative two-

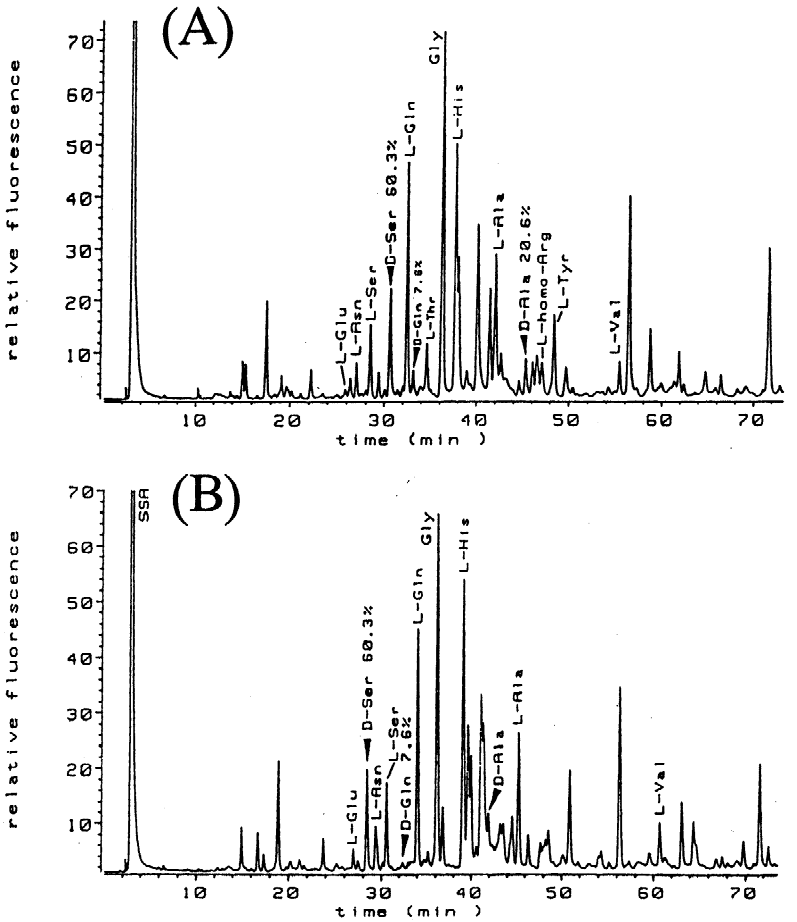

Fig. 3. Elution profile of amino acids from the urine of human male; derivatization with (A) OPA-IBLC and (B) OPA-IBDC. Reproduced from Ref. [22] with permission.

dimensional thin-layer chromatography, followed by an RP-HPLC determination of each D-amino acid. With this method, D-Ser, D-Ala and D-Pro in human plasma and mouse kidney were determined [26,27]. A fluorescence chiral derivatizing reagent (+)-1-(9-fluorenyl)ethyl chloroformate (FLEC) is used to separate optically active amines. With this reagent, highly fluorescent diastereomers of amino acids could be obtained without racemization within 4 min at room temperature. The FLEC reagent enabled the reversed-phase separation of the enantiomers of 17 primary amino acids in a single HPLC run [28] and demonstrated that large amounts of free D-Ala and D-Arg are present in crustaceans [29]. Chiral isothiocyanate reagents, *O*-tetraacetyl-β-D-glucopyranosyl isothiocyanate (GITC) [30], *R*(−)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(*N*,*N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazole (*R*(−)-DBD-PyNCS) [31] and (1*R*,2*R*)-*N*-[(2-isothiocyanato)-cyclohexyl]-6-methoxy-4-quinolinylamide ((*R*,*R*)-CDITC) [32], are also used to determine enantiomers of amino acids. The enantiomers of 14, 17 and 18 amino acids could be separated using GITC, *R*(−)-DBD-PyNCS and (*R*,*R*)-CDITC, respectively.

2.2.2. Chiral stationary phases

Pirkle-type chiral stationary phases (CSPs) are widely used for the separation of enantiomers, and dozens of CSPs have been synthesized and evaluated [33–35]. Fukushima et al. reported a sensitive enantiomeric determination of DL-amino acid using

Pirkle-type CSPs following the precolumn derivatization with highly fluorescent derivatizing reagents, 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), 4-(*N*,*N*-dimethylaminosulphonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) and 4-aminosulphonyl-7-fluoro-2,1,3-benzoxadiazole (ABD-F) [36]. With their method, the enantiomers of 11 amino acids were separated and successfully applied to the determination of D-Ala in human serum and D-Lys in myeloma patient serum. Hamase et al. determined 17 D-amino acids in rat brain using a Pirkle-type CSP [37]. The method includes fluorescence labeling of amino acids with NBD-F, isolation of each amino acid by reversed-phase HPLC, followed by enantiomeric separation with a Pirkle-type CSP. A large amount of D-Ser was found in the cerebrum, hippocampus and hypothalamus (210, 231 and 210 nmol/g wet tissue, respectively), while D-Asp was localized in the pineal gland (3524 nmol/g wet tissue). Small amounts of D-Ala (pituitary gland, 25.9 nmol/g wet tissue) and D-Leu (hippocampus and pineal gland, 1.6 and 3.4 nmol/g wet tissue, respectively) were also observed. Sensitive and specific methods to determine minute amounts of D-Leu [38], D-Pro [39] and D-Asp [40] in mammalian tissues have also been developed.

Cyclodextrin (CD)-bonded CSPs are useful for separating amino acid enantiomers [41]. Armstrong et al. demonstrated the presence of D-Pro in the serum and urine of laboratory rodents using a β-CD bonded column following the derivatization of D-Pro with 9-fluorenylmethyl chloroformate (FMOC-Cl) [42]. Using the modified β-CD bonded CSP, enantiomeric separation of amino acids derivatized with DBD-NCS and PITC was reported by Matsunaga et al. and Iida et al. for the purpose of amino acid sequencing with D/L-configuration determination [43,44]. Chiral crown ethers are also used for the CSP to separate amino acid enantiomers [42,45]. Armstrong et al. determined D-Phe, D-Tyr, D-Trp and D-Leu using a chiral crown ether column in combination with an ODS column [42]. Fluorescence detection of amino acids was carried out by postcolumn derivatization with OPA and mercaptoethanol, and a large amount of these D-amino acids was determined in rat and mouse urine. A CSP synthesized by covalent bonding of aminopropylsilica to FDAA (Marfey's reagent) was reported by Bruckner et al. and utilized to separate 2,4-dinitrophenyl and 3,5-dinitrobenzoyl-DL-amino acid esters [46]. Protein-bonded CSPs (α-chymotrypsin [47], bovine serum albumin [48]) have also been used to separate amino acid derivatives.

2.2.3. Chiral mobile phases

Enantiomer separation could be achieved by a non-chiral stationary phase with a mobile phase having a chiral selector. Copper (II) ion complexes, which perform chiral discrimination by a ligand-exchange mechanism, are used in combination with chiral amino acid derivatives. Copper (II) and *N*-(*p*-toluenesulfonyl)-L-phenylalanine are added to the mobile phase to separate 14 amino acid enantiomers [49]. *N*-Methyl-L-phenylalanineamide and *N*,*N*-dimethyl-L-phenylalanineamide are also used with copper (II) ion to separate the enantiomers of amino and imino acids [50]. Cyclodextrin, a useful chiral selector, is added to the mobile phase to determine D-amino acids. With this system, an assay for monitoring traces of D-Ala and D-Glu in protein and peptide hydrolysate is presented by Rizzi et al. [51]. Dunlop et al. used L-aspartyl-L-phenylalanine methyl ester as a chiral selector added to the mobile phase to determine D-Asp [52]. Enantiomers of Asp are derivatized with naphthyl isocyanate, isolated by an ODS column, and subsequently determined using an ODS column saturated with L-aspartyl-L-phenylalanine methyl ester. With this system, D-Asp was determined in the brain and peripheral tissues of rat and human blood. Especially, a large amount of D-Asp was observed in the brain of neonatal rat.

2.2.4. Column-switching system

A highly sensitive and selective analysis could be performed using a column-switching HPLC system, which is applied to the precise determination of target D-amino acids in biological samples. For the determination of D- and L-amino acids using a column-switching procedure, D- and L-amino acids are first isolated as the racemic mixtures, following the derivatization with non-chiral reagents or in their native forms. The isolated fractions are introduced to the second column (a CSP or a non-chiral column with a chiral selector added in the mobile phase) in order to separate the enantiomers. Chiral derivatization reagents are not used because of the difficulty of

isolation of racemic mixtures in the first step. Combination of a reversed-phase column and a Pirkle-type CSP has been used following the pre-column derivatization with NBD-F [38–40]. Long et al. reported a system in which NBD-Asp was separated on an octyl silica column, and subsequently, the enantiomers were separated and determined by a Pirkle-type CSP and applied to the highly selective determination of D-Asp in human serum [40]. Inoue et al. [38] and Hamase et al. [39] used a micro ODS column to isolate the racemic NBD-amino acids, followed by the enantiomeric separation on a Pirkle-type CSP to determine D-Leu and D-Pro (Fig. 4). Using a micro ODS column, the volume of the isolated amino acid fraction could be reduced to 125 μl. Therefore, almost all of the fraction could be introduced to the second column without the loss of sensitivity, and minute amounts of D-Leu and D-Pro could be determined in eight regions of the mouse brain. CD-bonded stationary phases and chiral crown ether-bonded stationary phases are also used as the CSP of a column switching system. Khan et al. demonstrated a system for the determination of OPA precolumn-labeled D-Phe using a β-CD-bonded CSP [53]. Armstrong et al. reported a system for D-Pro determination using a combination of an ODS column and a β-CD-bonded CSP, following the derivatization with FMOC-Cl, and utilized for the

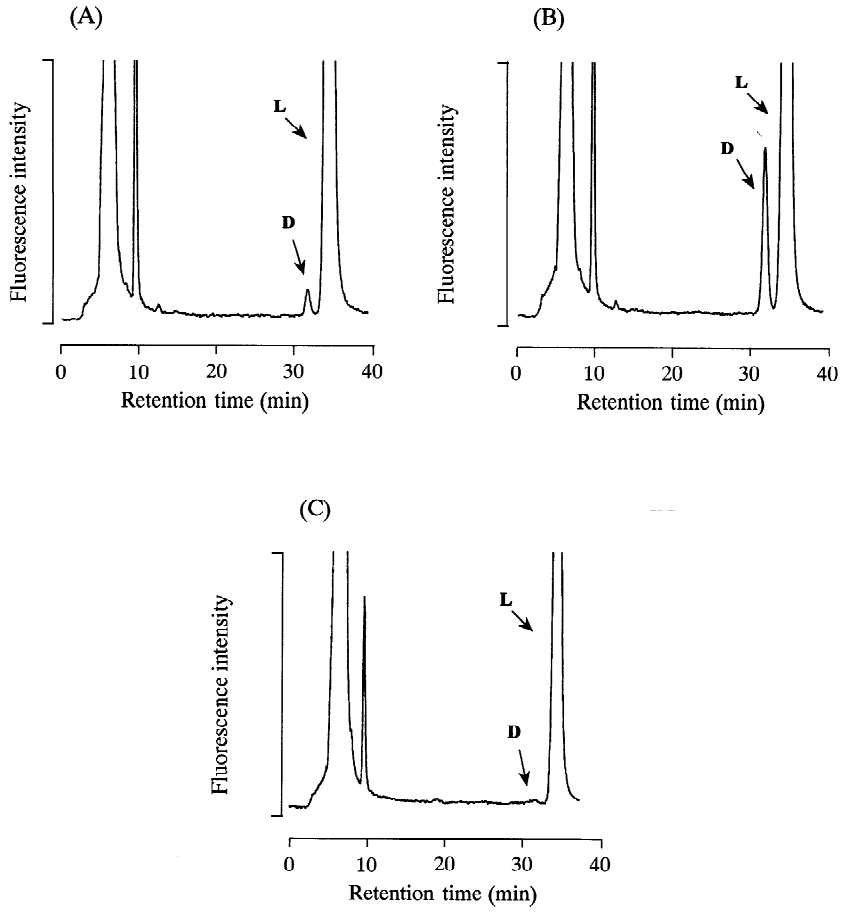

Fig. 4. Chromatograms of NBD-D- and NBD-L-Leu in the cerebrum of a ddY/DAO$^+$ mouse (A) and a ddY/DAO$^-$ mouse (B). To confirm the presence of D-form, D-Leu in the cerebrum of ddY/DAO$^-$ mouse were determined after enzymatic degradation with D-amino acid oxidase (DAO) (C). Peaks: D, NBD-D-Leu; L, NBD-L-Leu. Reproduced from Ref. [39] with permission.

determination of Pro enantiomers in the urine of rodents [42]. In the same article, Armstrong et al. reported a system using a chiral crown ether as a CSP and successfully determined the enantiomers of Phe, Tyr, Trp and Leu [42]. Another column switching system using a chiral crown ether for the determination of Asp, Ser, Glu and Ala has also been reported by Vandemerbel et al. [54]. An ODS-column could also be used as the second column to separate enantiomers with the addition of chiral selectors to the mobile phase. Rizzi et al. reported a system for determining dansyl-amino acid enantiomers using β-CD as a chiral selector [51], and Dossena et al. reported a system using copper (II) complexes as a chiral selector for unlabeled amino acids [50].

2.3. High-performance capillary electrophoresis

Recent progress in high-performance capillary electrophoresis (HPCE) enables rapid and sensitive enantiomer separation of many biological compounds and pharmaceuticals including amino acids with high efficiency [55–57]. However, there have been few reports on determining D-amino acids in mammalian tissues and biological fluids by HPCE. Recently, Tsunoda et al. determined the amount of D-Asp in the rat pineal gland [58]. The amino acids are derivatized with NBD-F and separated using heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CD) as a chiral selector. Thorsen et al. reported the micellar electrokinetic separation of 14 D- and L-amino acids [59]. In the paper, (+)-1-(9-anthryl)-2-propyl chloroformate ((+)-APOC) is used as a chiral derivatizing reagent, and the amino acids are separated as their diastereomers using a mixed micellar system of sodium dodecyl sulfate (SDS) and sodium deoxycholate. Using this system, D-Ala and D-Asp were determined in human cerebrospinal fluid, and D-Ala and D-Glu were determined in human urine. Zhao et al. also reported the CD-micellar electrokinetic chromatography (MEKC) system to determine various D-amino acids in biological samples [60–62]. The amino acids are derivatized with naphthalene-2,3-dialdehyde and determined using laser-induced fluorescence detection. Using this method, postnatal changes in the D-Asp amounts in the rat cerebrum and cerebellum have been demonstrated. In both tissues, the amounts of D-Asp decrease rapidly after birth within a week [60]. The enantiomers of Trp in human urine, and the enantiomers of Asp, Phe and Leu in neurons are also reported [61,62].

2.4. Enzymatic methods

Enzymatic methods are valuable because of their specificity and simplicity. As the enzymes, D-amino acid oxidase (DAO, EC 1.4.3.3) and D-aspartic acid oxidase (DAspO, EC 1.4.3.1) are commonly used. DAO catalyzes the oxidation of most of the neutral D-amino acids to form α-keto acids and hydrogen peroxide. The α-keto acids react with hydrazine to form hydrazones; therefore, D-amino acids could be determined by the absorbance of the hydrazones at 445 nm. Using this method, Nagata et al. determined the total amount of D-amino acids in human blood samples [63,64]. However, the amount of individual D-amino acids could not be determined by the procedure. D'Aniello et al. determined D-Ala using DAO and lactate dehydrogenase (LDH) [65]. Pyruvate derived from D-Ala and NADH are converted to L-lactate and NAD^+ by LDH. The amount of NADH oxidized in the reaction is stoichiometric with the amount of D-Ala and is determined by the absorbance at 340 nm. An immobilized enzyme reactor containing DAO has also been used following the separation of each amino acid to determine the D-enantiomer of each amino acid and has been successfully applied to the determination of D-amino acids spiked in human serum [66].

DAspO catalyzes the oxidation of D-Asp and D-Glu to form oxaloacetate and α-ketoglutarate. These α-keto acids are converted to L-malate and L-Glu in combination with NADH by malate dehydrogenase and glutamate dehydrogenase, respectively, and the amount of each D-amino acid is determined by the alteration of NADH [67,68]. D'Aniello et al. determined D-Asp in the rat brain and endocrine glands using DAspO in combination with HPLC [69]. In this procedure, D- and L-Asp were derivatized with OPA and *N*-acetyl-L-Cys to form diastereomeric fluorescence derivatives and are separated by an RP-HPLC. The same sample was analyzed after treatment with DAspO, and D-Asp was determined by the reduction of the HPLC peak area observed at the retention time of D-Asp. By this procedure, the

amounts of D-Asp in six brain areas and three endocrine glands of rats were determined. D-Amino acid transaminase (DAT) has also been used to determine D-amino acids [70]. This system employs DAT coupled with 2-oxohexanoate, which accepts the amino group from D-amino acids to form D-norleucine, and the amounts of amino acids are determined by amino acid analysis. The amounts of D-enantiomers are determined by the decrease in the corresponding amino acid amount after treatment with DAT.

3. Anatomical distribution of free D-amino acids in mammals

A variety of studies have been performed from the 1980s to demonstrate the presence of free D-amino acids in mammalian tissues. Especially, D-Ser and D-Asp have been well investigated. The amounts of other D-amino acids in mammalian tissues are small in many cases and are determined using sensitive and selective methods such as column switching HPLC. The amounts and distribution of D-amino acids reported before the middle of the 1990s are clearly summarized in sophisticated reviews [3,71] and are not discussed here in detail.

3.1. Anatomical distribution of D-serine

A large amount of naturally occurring D-Ser in mammalian tissue was first demonstrated by Hashimoto et al. in 1992 using GC–MS [10] and HPLC [20]. The amount of D-Ser in the rat frontal brain area is more than 200 nmol/g wet tissue, which is more than 20% of the amount of total Ser (D-enantiomer plus L-enantiomer). Because D-amino acids had been believed to be scarce in mammalian tissues, many researchers have been interested in the presence of D-Ser in the mammalian brain and a variety of investigations have been performed. Naturally occurring D-Ser was widely found in the brain [10,20,21,37,72–80], peripheral tissues [72,74,77,81] and physiological fluids [11,20–22,27,73,77,82–84]. The amounts are summarized in Table 2. In the brain of various species of mammals, D-Ser is localized to the frontal brain areas such as the cerebrum, hippocampus, hypothalamus and striatum [72,74]. While, the amounts of D-Ser are small in the cerebellum, medulla oblongata [21,72,74] and spinal cord [74]. Small amounts of D-Ser are also observed in several peripheral tissues including the endocrine glands [21,37,72,74,81]. Postnatal changes were also investigated [72,78]. In the cerebellum of neonatal rats and mice, a substantial amount of D-Ser is present, which decreases with age and could hardly be detected in mature animals. Meanwhile, a large amount of D-Ser is present in the frontal brain areas throughout the life span.

3.2. Anatomical distribution of D-aspartic acid

D-Asp is widely observed in the brain and peripheral tissues in adult animals, especially in the endocrine glands [18,21,22,37,40,52,58,60,69,72,73,75,81,85–94]. The amounts are summarized in Table 3. In the adult rat, an especially high amount of D-Asp is observed in the pineal gland (more than 1000 nmol/g wet tissue), followed by the testis, adrenal gland and pituitary gland. The amounts of D-Asp in these glands increase after birth and reach their maximum values in cooperation with the tissue maturation. In the brain, a large amount of D-Asp is observed in the neonatal rat, which decreases rapidly after birth, and only a small amount of D-Asp is observed in the cerebrum of an adult rat.

3.3. Other D-amino acids

There are some reports demonstrating the presence of D-amino acids other than D-Ser and D-Asp in mammals. Hoeprich et al. found D-Ala in the blood of guinea pigs and mice in 1965 [95], which was the first time the presence of D-amino acids in mammals was demonstrated [96]. Until now, the amounts of D-Ala have been determined in various brain regions, peripheral tissues and physiological fluids [11,21,22,27,36,37,73,77,81,83,87,95–98], showing that this D-amino acid is localized in the pituitary gland. Urinary excretion of a large amount of D-Ala is also reported. A small amount of D-amino acids such as D-Pro [11,27,39,42,77] and D-Leu [11,37–39,81,94] could be determined using sensitive and selective methods. Hamase et al. reported the amounts of D-Pro and D-Leu in seven brain regions of mice, showing that the amounts of these D-amino

Table 2
Anatomical distribution of D-Ser in mammals

Species	Age	Sex	Brain and peripheral tissues (nmol/g wet tissue)							Physiological fluids (nmol/ml)		Method	Ref.
			Cerebrum	Hippocampus	Hypothalamus	Cerebellum	Pituitary gland	Liver	Kidney	Blood	Urine		
Human	Fetus	–	280	–	–	–	–	–	–	–	–	HPLC-DIA	[75]
	0–101 years	–	130	–	–	–	–	–	–	–	–	HPLC-DIA	[75]
	20–75 years	F	–	–	–	–	–	–	–	0.4–1.8[p]	–	HPLC-DIA	[27]
	23–41 years	F	–	–	–	–	–	–	–	2.9[s]	60–197	GC-CSP	[11]
	27–49 years	M	–	–	–	–	–	–	–	–	30.1–379.8	HPLC-DIA	[83]
	27–51 years	M	–	–	–	–	–	–	–	1.5[s]	26–221	GC-CSP	[11]
	34 years	M	–	–	–	–	–	–	–	–	224	HPLC-DIA	[22]
	50–71 years	M	66	–	–	–	–	–	–	–	–	HPLC-DIA	[76]
	76 years	M	–	–	–	–	–	–	–	0.7[p]	–	HPLC-DIA	[27]
Rat	1 week	M	143	–	–	267	41	15	38	–	–	HPLC-DIA	[72]
	6 weeks	M	210	231	210	0	12	–	–	–	–	HPLC-CSP	[37]
	7 weeks	M	276	–	–	0	24	0	18	–	–	HPLC-DIA	[72]
	7 weeks	M	275	235	–	0	–	–	–	–	–	HPLC-DIA	[74]
	7 weeks	M	220[a]	–	–	–	–	–	–	3.0[s]	–	HPLC-DIA	[20]
	7 weeks	M	270[a]	–	–	–	–	–	–	–	–	GC-CSP	[10]
	9 weeks	M	395	208	250	–	–	–	–	–	–	HPLC-DIA	[78]
Mouse	1 week	F	93	–	–	48	–	–	–	–	–	HPLC-DIA	[78]
	7 weeks	M	310[a]	–	–	0	–	–	–	2.1[s]	–	HPLC-DIA	[73]
	8 weeks	F	520	–	–	28	–	–	–	–	–	HPLC-DIA	[78]
	8 weeks	M	353	–	–	32	–	1.1	3.4	2.9[s]	–	HPLC-DIA	[77]
	9 weeks	M	423	342	105	12	3	–	–	2.1[s]	–	HPLC-DIA	[21]
Bovine	Adult	M	393	–	–	22	–	–	–	–	–	HPLC-DIA	[78]
Dog	6 years	M	–	–	–	–	–	–	–	4.2[s]	–	HPLC-DIA	[22]

DIA, diastereomer formation using chiral derivatizing reagents; CSP, chiral stationary phases.
[a] Values obtained for frontal brain.
[s] Values obtained for serum.
[p] Values obtained for plasma.

acids are relatively high in the pineal gland and pituitary gland [39]. Urinary excretion of D-Phe is also reported [11,42,83]. The amounts of D-Ala, D-Pro, D-Leu and D-Phe are summarized in Table 4. Other D-amino acids such as D-Glu, D-Gln, D-Tyr, D-Trp, D-Lys, D-Thr and D-Val are also reported [11,18,22,42,83].

4. Function of D-amino acids in mammals

4.1. Function of D-Ser as a neuromodulator

Many in vivo and in vitro studies have suggested that D-amino acids, especially D-Ser, have a crucial role in NMDA receptor-mediated neurotransmission. The NMDA receptor is one of the subtypes of glutamate receptors and has been considered to play very important functions in physiological and pathophysiological processes, such as learning [99], nociception [100,101], schizophrenia [102,103] and epilepsy [104,105]. The NMDA receptor has several modulation sites, including a glycine-binding site [71]. D-Ser binds to the glycine-binding site of the NMDA receptor with high affinity as does glycine and potentiates the L-glutamate-mediated responses of cloned NMDA receptors expressed in Xenopus oocytes [106–108]. However, glycine was initially considered to be a naturally occurring modulator, because D-Ser had never been considered to exist in

Table 3
Anatomical distribution of D-Asp in mammals

Species	Age	Sex	Brain and peripheral tissues (nmol/g wet tissue)									Blood (nmol/ml)	Method	Ref.
			Cerebrum	Cerebellum	Pituitary gland	Pineal gland	Liver	Kidney	Adrenal	Testis	Thymus			
Human	Fetus	–	360[a]	–	–	–	–	–	–	–	–	–	HPLC-DIA	[75]
	0–101 years	–	8[a]	–	–	–	–	–	–	–	–	–	HPLC-DIA	[75]
	76 years	–	26	12	–	–	–	–	–	–	–	–	HPLC-DIA	[86]
Rat	0 days	–	100	–	–	–	–	–	–	–	–	–	HPLC-DIA	[85]
	0 days	M	–	–	40	–	–	–	–	20	–	–	HPLC-DIA	[89]
	1 day	–	64	48	–	–	–	–	–	–	–	–	CD-MEKC	[60]
	1 day	–	100	28	19	–	28	49	–	–	–	8.9[w]	HPLC-CMP	[52]
	1 week	M	32	47	51	–	26	32	157	53	40	–	HPLC-DIA	[72]
	3 weeks	M	–	–	–	–	–	–	–	75	–	–	HPLC-CSP	[81]
	6 weeks	M	–	–	137[c]	–	–	–	–	–	–	–	HPLC-CSP	[93]
	6 weeks	M	–	–	–	2810[d]	–	–	–	–	–	–	HPLC-CSP	[94]
	6 weeks	M	0	0	81	3524	–	–	–	–	–	–	HPLC-CSP	[37]
	6 weeks	M	–	–	–	1030[d]	–	–	–	–	–	–	HPLC-CSP	[92]
	7 weeks	M	–	–	–	1290[d]	–	–	–	–	–	–	CE-CD-CZE	[58]
	7 weeks	M	21[b]	–	–	–	60	39	–	–	–	–	HPLC-DIA	[18]
	7 weeks	F	20[b]	–	–	–	25	26	–	–	–	–	HPLC-DIA	[18]
	7 weeks	M	0	7	94	–	8	0	201	177	60	–	HPLC-DIA	[72]
	7 weeks	–	40[b]	–	–	–	50	70	–	–	–	–	Enz	[87]
	7 weeks	–	13	22	127	–	11	12	–	–	–	2.2[w]	HPLC-CMP	[52]
	8 weeks	–	–	–	–	–	–	–	–	–	–	1.7[w]	HPLC-CSP/CS	[40]
	10 weeks	M	–	–	–	–	–	–	–	220	–	–	HPLC-CPS	[81]
	11 weeks	M	–	–	190	–	–	–	–	152	–	–	HPLC-DIA	[89]
	12 weeks	M	15–21	18	114[c]	–	7	8	78	90	–	2[w]	HPLC-DIA/Enz	[69]
	12 weeks	–	6.7	10	–	–	–	–	–	–	–	–	CD-MEKC	[60]
Mouse	7 weeks	M	50[a]	0	–	–	–	–	–	–	–	0[s]	HPLC-DIA	[73]
	9 weeks	M	43	20	15	43[d]	–	–	–	–	–	1.0[s]	HPLC-DIA	[21]
Dog	6 years	M	–	–	–	–	–	–	–	–	–	1.0[s]	HPLC-DIA	[22]

Enz, enzymatic methods; CS, column-switching system.
[a] Values obtained for frontal brain.
[b] Values obtained for whole brain.
[c] Values obtained for anterior pituitary gland.
[d] Values represent pmol/whole pineal gland.
[s] Values obtained for serum.
[w] Values obtained for whole blood.

mammalian tissues. In 1992, Hashimoto et al. first demonstrated the presence of a large amount of intrinsic D-Ser in the rat frontal brain [10]. The naturally occurring D-Ser is localized in close vicinity to the NMDA receptor, while glycine is distributed differently from D-Ser and the NMDA receptor [75,109]. In addition, Mothet et al. showed that depletion of D-Ser by D-amino acid oxidase (DAO) treatment attenuates NMDA receptor-mediated neurotransmission [110]. Schell et al. demonstrated that D-Ser is localized in type 2 astrocytes expressing a high level of non-NMDA glutamate receptor, from which D-Ser is released by the stimulation of the glutamate receptor [111]. Based on these reports, D-Ser is considered to be released from astrocytes and activates the NMDA receptor as a naturally

Table 4
Anatomical distribution of D-Ala, D-Pro, D-Leu and D-Phe

Species	Age	Sex	Brain and peripheral tissues (nmol/g wet tissue)										Physiological fluids (nmol/ml)		Method	Ref.
			Cerebrum	Hippocampus	Hypothalamus	Cerebellum	Medulla oblongata	Pituitary gland	Pineal gland	Liver	Kidney	Testis	Blood	Urine		
D-Ala																
Human	20–75 years	F	–	–	–	–	–	–	–	–	–	–	0–1.8[p]	–	HPLC-DIA	[27]
	23–41 years	F	–	–	–	–	–	–	–	–	–	–	2.3[s]	16–51	GC-CSP	[11]
	23–49 years	–	–	–	–	–	–	–	–	–	–	–	0.48–3.1[s]	–	HPLC-CSP	[36]
	27–41 years	M	–	–	–	–	–	–	–	–	–	–	–	7.6–53.8	HPLC-DIA	[83]
	27–51 years	M	–	–	–	–	–	–	–	–	–	–	3.6[s]	17–82	GC-CSP	[11]
	34 years	M	–	–	–	–	–	–	–	–	–	–	–	82	HPLC-DIA	[22]
	76 years	M	–	–	–	–	–	–	–	–	–	–	1.1[p]	–	HPLC-DIA	[27]
Rat	3 weeks	M	–	–	–	–	–	–	–	–	–	18.5	–	–	HPLC-CSP	[81]
	6 weeks	M	0	0	0	0	0	26	0	–	–	–	–	–	HPLC-CSP	[37]
	10 weeks	M	–	–	–	–	–	–	–	–	–	2.8	–	–	HPLC-CSP	[81]
Mouse	7 weeks	M	0	–	–	0	–	–	–	–	–	–	6[s]	–	HPLC-DIA	[73]
	8 weeks	M	3.5	–	–	2.2	–	–	–	9.5	4.6	–	2.7[s]	–	HPLC-DIA	[77]
	9 weeks	M	12	11	9	11	5	29	37[a]	–	–	–	9[s]	–	HPLC-DIA	[21]
D-Pro																
Human	27–51 years	M	–	–	–	–	–	–	–	–	–	–	0.9[s]	0–0.3	GC-CSP	[11]
	23–41 years	F	–	–	–	–	–	–	–	–	–	–	0.9[s]	0–0.5	GC-CSP	[11]
	76 years	M	–	–	–	–	–	–	–	–	–	–	0.69[p]	–	HPLC-DIA	[27]
	20–75 years	F	–	–	–	–	–	–	–	–	–	–	0–0.62[p]	–	HPLC-DIA	[27]
Rat	Adult	–	–	–	–	–	–	–	–	–	–	–	0.1[p]	4	HPLC-CSP/CS	[42]
Mouse	9–10 weeks	M	0.31	0.11	0.25	0.29	0.16	0.59	1.11[a]	–	–	–	0.45[s]	–	HPLC-CSP/CS	[39]
	8 weeks	M	–	–	–	–	–	–	–	1.9	4.6	–	0.14[s]	–	HPLC-DIA	[77]
	Adult	–	–	–	–	–	–	–	–	–	–	–	3.7[p]	14	HPLC-CSP/CS	[42]
D-Leu																
Human	27–51 years	M	–	–	–	–	–	–	–	–	–	–	–	0.2–3.2	GC-CSP	[11]
Rat	3 weeks	M	–	–	–	–	–	–	–	–	–	10.5	–	–	HPLC-CSP	[81]
	6 weeks	M	–	–	–	–	–	–	4.2[a]	–	–	–	–	–	HPLC-CSP	[94]
	6 weeks	M	0	1.6	0	0	0	0	3.4	–	–	–	–	–	HPLC-CSP	[37]
Mouse	9–10 weeks	M	0.41	0.39	0.55	0.24	0.27	0.76	1.94[a]	–	–	–	0.39[s]	–	HPLC-CSP/CS	[39]
D-Phe																
Human	23–41 years	F	–	–	–	–	–	–	–	–	–	–	0.3[s]	2.0–2.3	GC-CSP	[11]
	27–49 years	M	–	–	–	–	–	–	–	–	–	–	–	0.35–3.5	HPLC-DIA	[83]
	27–51 years	M	–	–	–	–	–	–	–	–	–	–	–	0.4–2.8	GC-CSP	[11]
Rat	Adult	–	–	–	–	–	–	–	–	–	–	–	–	56	HPLC-CSP/CS	[42]
Mouse	Adult	–	–	–	–	–	–	–	–	–	–	–	0.03[p]	73	HPLC-CSP/CS	[42]

[a] Values represent pmol/whole pineal gland.
[s] Values obtained for serum.
[p] Values obtained for plasma.

occurring modulator. The relationship of D-Ser and the NMDA receptor has been reviewed in excellent articles in detail [71,112–114].

The NMDA receptor is related to some important physiological and pathophysiological processes; therefore, the utility of D-Ser as a therapeutic agent, especially for the treatment of schizophrenia, has been a matter of interest. Schizophrenia is a mental disease characterized by positive symptoms (e.g. hallucinations, delusions), negative symptoms (e.g.

loss of motivation, blunted affect) and cognitive symptoms (e.g. distractibility, concentration difficulties) [115]. Antipsychotic agents targeting dopamine D2 receptors are generally used for the treatment of schizophrenia; however, these agents have poor efficacy on the negative and cognitive symptoms. Therefore, development of a new antipsychotic agent is required, and potentiation of the NMDA receptor is proposed as a new strategy [116]. Administration of D-Ser to laboratory rodents inhibits abnormal behavior similar to schizophrenia induced by a psychotomimeric agent, phencyclidine [117]. In a clinical trial, Tsai et al. reported that D-Ser treatment of schizophrenic patients, as an add-on agent to other anitipsychotics, improved positive, negative and cognitive symptoms without side effects [118].

4.2. Function of D-Asp as a regulator of hormonal secretion

D'Aniello et al. revealed that intrinsic D-Asp is localized in Leydig and Sertoli cells in the testes by an immunocytochemical technique and that intraperitoneal administration of D-Asp results in an increase in D-Asp in the testes and serum testosterone [89]. Nagata et al. also showed that D-Asp stimulates testosterone synthesis in isolated rat Leydig cells via stimulating the steroidogenic acute protein (StAR) gene expression [119,120]. Wang et al. showed that intrinsic D-Asp is subcellularly localized to the heterochromatin and nucleoli of the cells synthesizing oxytocin in the rat hypothalamus [121] and that exogenous D-Asp stimulates the expression of oxytocin mRNA [122]. These reports suggest that D-Asp modulates hormonal secretion by stimulating gene expression directly. D-Asp is also related to the secretion of adenohypophysical hormones such as prolactin [123,124], luteinizing hormone [69] and growth hormone [69], in addition to the pineal hormone, melatonin [125–127].

4.3. Involvement with ontogeny and differentiation

A significant amount of D-Asp has been observed in the human [75], rat [88] and chick [88] embryonic brain and rapidly decreases after birth [75,88]. Sakai et al. demonstrated that, during embryonic development, D-Asp first emerges around the hindbrain, spreads into the forebrain, and then extends over the whole brain [128]. Transient emergence of D-Asp has also been reported in some endocrine organs, such as the testes [89], retina [88], pineal gland [37] and adrenal gland [72,129]. Because D-Asp increases in each tissue corresponding to the period of morphological and functional maturation of the tissue, the possible role of this D-amino acid in ontogeny and differentiation is considered.

5. Origin of D-amino acids in mammals

5.1. Biosynthesis

Though D-amino acids had been considered to be synthesized only by microorganisms [130], plants [131] and invertebrates [132–134], recent studies suggest that naturally occurring D-Ser and D-Asp are synthesized in mammalian tissues. D-Ser is highly concentrated in the mammalian frontal brain, and the origin of D-Ser in the brain is a matter of interest. Dunlop et al. reported that radiolabeled L-Ser is converted to D-Ser in the rat and mouse brain, suggesting that D-Ser is synthesized in the mammalian brain [135]. In 1999, Wolosker et al. first purified and cloned mammalian serine racemase from the rat brain [136]. This enzyme catalyzes direct racemization of L-Ser to D-Ser and is closely associated with D-Ser in the rat brain [137]. Miranda et al. cloned human serine racemase and demonstrated that D-Ser is synthesized by the enzyme in living cells [138].

Long et al. demonstrated that D-Asp is synthesized in cultured cells derived from adrenal medulla [139] and pituitary tumor [124]. Wolosker et al. showed that D-Asp is synthesized from L-Asp in primary neuronal culture obtained from rat embryos [85]. These reports suggest that D-Asp is synthesized in some mammalian tissues; investigations on the molecular mechanism of D-Asp synthesis are now in progress.

5.2. Exogenous D-amino acids

Some D-amino acids are reported to be derived from nutrition and bacteria. Konno et al. reported that the urine of mutant mice lacking D-amino acid

oxidase (ddY/DAO$^-$ mice) [140] contains high levels of D-methionine (Met) [141,142] and D-Ala [142,143]. The amount of urinary D-Met significantly decreases when the mice are starved or raised on diets containing low levels of D-Met [141,142], suggesting that urinary D-Met mainly comes from the diet. While, the D-Ala amount in the urine of a ddY/DAO$^-$ mouse does not decrease due to starvation [142]. D-Ala is known to be abundant in the cell wall of gastrointestinal bacteria. Therefore, Konno et al. made ddY/DAO$^-$ mice germ-free and demonstrated that the urinary secretion of D-Ala in ddY/DAO$^-$ mice significantly decreased in these mice, suggesting that the urinary D-Ala is mainly derived from intestinal bacteria [143].

As the transport system for D-amino acids, it has been reported that glutamate (Glu) transporter has high affinity for D-Asp as well as for L-Asp and L-Glu [144]. Lee et al. reported that in the rat adrenal cortex, the expression of the Glu transporter transiently increases in parallel with the increase in D-Asp at 3 weeks of age [145]. Therefore, D-Asp is considered to be taken up from the vascular system via a specific transporter in addition to the biosyntheses in some tissues. Nagata et al. reported that oral administration of D-Ala to mice increases the amount of D-Ala in the serum, liver, kidney and brain [77]. Oguri et al. showed that D-arginine, D-Ala and D-Asp are taken up by a sodium ion-dependent transporter on the rat intestinal epithelial cells and that the degree of intestinal absorption of these D-amino acids is highest at 8 weeks of age but decreased thereafter [146]. This developmental profile is similar to the developmental changes in the levels of D-Ala and D-Asp in some rat tissues reported by Hamase et al. [37]. Takigawa et al. reported, using cultured rat pinealocytes, that D-Asp accumulates in the pinealocytes when D-Asp is added to the medium [126]. These reports suggest that exogenous D-amino acids, derived from food and bacterial cell walls, could be distributed to mammalian tissues.

6. Relationship between the D-amino acids and pathophysiological processes, and the diagnostic value of the D-amino acids

D-amino acids are present in free form and in protein-bound form in mammalian tissues, both of which have been implicated in some pathophysiological processes. In this article, we mainly discuss the free D-amino acids as described in Table 5. The relationship between pathophysiological processes and D-amino acids in the proteins is extensively discussed in excellent articles [147–149].

Fisher et al. first reported that free D-Asp levels in the white matter of Alzheimer brains are found in less than 50% of normal subjects; 10.5 ± 6.6 nmol/g for Alzheimer brains and 22.4 ± 4.6 nmol/g for normal brains [150]. D'Aniello et al. reported that the amount of D-Asp regionally decreases in an Alzheimer brain, such as in the frontal and hippocampus, but does not decrease in a region without the neuropathological changes of Alzheimer's disease [86]. In an Alzheimer brain, a reduction in the NMDA receptor, which is involved in learning and memory processes, is also reported [151]. Because D-Asp binds to the glutamate-binding site of the NMDA receptor [71], D'Aniello et al. proposed the possibility that a decrease in the D-Asp level contributes to the memory deficits in Alzheimer's disease [86]. Unlike D-Asp, the free D-Ala levels increase in the gray matter of Alzheimer brains; 20.8 ± 5.3 nmol/g for Alzheimer brains and 9.5 ± 2.9 nmol/g for normal brains [150]. D'Aniello et al. also demonstrated that the presence of protein-bound D-Ala and higher level of D-Ala is observed in the protein in an Alzheimer brain [65]. Fisher et al. showed that free D-Ser, D-Asp and total D-amino acids in Alzheimer ventricular cerebrospinal fluid (CSF) are significantly higher than those in normal subjects [152]. Though further studies are needed to clarify whether the alterations in D-amino acids in an Alzheimer brain and CSF is a factor or a result, the elucidation of the relationship of D-amino acids and Alzheimer's disease might offer new insights to overcome this devastating disease.

The alteration of the amount of D-amino acids is also reported with aging and renal disease. Nagata et al. showed that D-amino acid levels in the plasma of elderly people (age 76 ± 6 years, $n=41$) are significantly higher than those in younger people (age 42 ± 4 years, $n=26$); 6.9 ± 4.8 nmol/ml (mean$\pm$SD) for an elderly group and 2.5 ± 1.8 nmol/ml for a younger group [64]. The amounts of these plasma D-amino acids have a positive correlation with the markers evaluating renal disease such as creatinine, β2-microglobulin and glomerular filtration rate,

Table 5
Relationship between the D-amino acids and pathophysiological processes

Disease	D-Amino acid	Region	Alteration	Ref.
Alzheimer's disease	D-Ala	Brain		
		White matter	→	[150]
		Gray matter	↑	[150]
		CSF	→	[152]
	D-Arg	CSF	→	[152]
	D-Asp	Brain		
		White matter	↓	[150]
		Gray matter	→	[150]
		Frontal brain	↓	[86]
		Hippocampus	↓	[86]
		Parietal	↓	[86]
		Temporal	↓	[86]
		Amygdala	↓	[86]
		Cerebellum	→	[86]
		CSF	↑	[152]
	D-Ser	Brain		
		Temporal cortex	→	[79]
		Frontal cortex	→	[76]
		White matter	→	[153]
		Gray matter	→	[153]
		CSF	↑	[152]
	Total D-AAs	CSF	↑	[152]
Parkinson's disease	D-Ser	Brain		
		Temporal cortex	→	[79]
Schizophrenia	D-Ser	Brain		
		White matter	→	[153]
		Gray matter	→	[153]
Renal disease	D-Ala	Plasma	↑	[27]
		Serum	→	[84]
		Serum	↑	[82]
	D-Asx	Serum	↑	[84]
	D-Pro	Serum	↑	[27]
	D-Ser	Plasma	↑	[27]
		Serum	↑	[84]
	Total D-AAs	Serum	↑	[64]
		Plasma	↑	[27]

↑, Increase from control; ↓, decrease from control; →, comparable to normal subjects. CSF, cerebrospinal fluids.

though individual D-amino acids were not distinguished [64]. Subsequently, it has been reported that the serum levels of D-Asx (D-Asx=D-Asp+D-Asn), D-Ser and D-Ala are positively correlated with the advance of renal failure [27,82,84].

7. Conclusions

Recent progress in analytical chemistry reveals that substantial amounts of D-amino acids are present in mammals including humans. Large amounts of D-Ser are present in the frontal brain areas, and D-Asp is mainly localized to the endocrine glands. These D-amino acids play important roles and are synthesized in mammalian tissues. The relationship between pathophysiological processes and the amounts of D-amino acids is also increasingly realized. These lines of evidence suggest that D-amino acids are essential for mammals and that the alteration of their amounts would indicate some diagnostic value. However, the knowledge of D-amino acids is

still limited and most of the D-amino acids are not well investigated yet; further advances in this area of research are actually expected in the near future.

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