

Natural Occurrence, Biological Functions, and Analysis of *D*-Amino Acids

Shuang-Xi Gu^{1,2} Hai-Feng Wang^{1,2} Yuan-Yuan Zhu³ Fen-Er Chen^{1,4}

¹Pharmaceutical Research Institute, Wuhan Institute of Technology, Wuhan, People's Republic of China

²Key Laboratory for Green Chemical Process of Ministry of Education, School of Chemical Engineering & Pharmacy, Wuhan Institute of Technology, Wuhan, People's Republic of China

³School of Chemistry & Environmental Engineering, Wuhan Institute of Technology, Wuhan, People's Republic of China

⁴Department of Chemistry, Fudan University, Shanghai, People's Republic of China

Address for correspondence Shuang-Xi Gu, PhD, Pharmaceutical Research Institute, Wuhan Institute of Technology, No. 206 Guanggu 1st Rd, Wuhan 430205, People's Republic of China (e-mail: shuangxigu@163.com). Fen-er Chen, PhD, Engineering Center of Fudan University, 220 Handan Rd, Shanghai 200433, People's Republic of China (e-mail: rfchen@fudan.edu.cn).

Pharmaceut Fronts 2020;2:e79–e87.

Abstract

This review covers the recent development on the natural occurrence, functional elucidations, and analysis of amino acids of the *D* (dextro) configuration. In the pharmaceutical field, amino acids are not only used directly as clinical drugs and nutriment, but also widely applied as starting materials, catalysts, or chiral ligands for the synthesis of active pharmaceutical ingredients. Earlier belief hold that only *L*-amino acids exist in nature and *D*-amino acids were artificial products. However, increasing evidence indicates that *D*-amino acids are naturally occurring in living organisms including human beings, plants, and microorganisms, playing important roles in biological processes. While *D*-amino acids have similar physical and chemical characteristics with their respective *L*-enantiomers in an achiral measurement, the biological functions of *D*-amino acids are remarkably different from those of *L*-ones. With the rapid development of chiral analytical techniques for *D*-amino acids, studies on the existence, formation mechanisms, biological functions as well as relevant physiology and pathology of *D*-amino acids have achieved great progress; however, they are far from being sufficiently explored.

Keywords

- ▶ *D*-amino acids
- ▶ natural occurrence
- ▶ biological functions
- ▶ physiology
- ▶ chiral analysis

Introduction

Amino acids are fundamental components of proteins, enzymes, peptides, peptide hormones, receptors, antibodies, and signaling molecules in living organisms. They are a family of important molecules in nature as well as in food, biotech, chemical, and pharmaceutical industries. In pharmaceutical field, amino acids are not only used directly as nutriment and clinical drugs, but also widely applied as starting materials, catalysts, or chiral ligands for the synthesis of active pharmaceutical ingredients. The first amino acid asparagine was isolated and discovered by French chemists Louis-Nicolas Vauquelin and Pierre-Jean Robiquet in the plant asparagus in 1806.¹ Since then many amino acids including 20 common ones

have been discovered, structurally confirmed, synthesized, and studied.² In 1851, Louis Pasteur revealed the optical activity of asparagine and aspartic acid,³ leading to the realization that the optical activity of most common amino acids arises from their differing orientations around the α -carbon.⁴ With the only exception of glycine, all common amino acids exist in two possible specular structures which are mirror images of each other, called *D*-(dextro) and *L*-(levo) enantiomers (▶ **Fig. 1**).⁵ During evolution, *L*-amino acids were preferred for protein synthesis and main metabolism. The initial discovery and configurational assignment of amino acids led to a view that *L*-amino acids were solely found in nature and *D*-amino acids are artificial products.^{4,6} However, the *D*-amino acids, playing different and specific functions in different organisms,^{7–10}

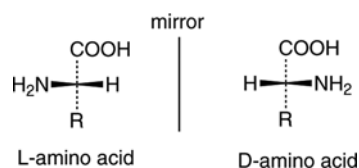


Fig. 1 Fisher projection formulae for *L*- and *D*-amino acids.

were found naturally occurring in a wide variety of living organisms both in their free form and as isomeric residues in many proteins in the past decades.^{11,12} Interestingly, *D*-amino acids are naturally occurring in animals including human beings, plants, and microorganisms, and could be formed during food processing and originate from microbial sources and from aqueous, soil, and other environments.⁵ In this review, the natural occurrence, biological function, and analytical methods of *D*-amino acids are summarized and discussed.

Natural Occurrence and Biological Functions of *D*-Amino Acids

Recognition of the biological importance of *D*-amino acids was enabled by the development of relevant analytical techniques, and has stimulated interdisciplinary collaborations. *D*-Amino acids were previously called “nonnative” or “unnatural” because they are not encoded by RNA, and in most cases they are not used as the building blocks of structural proteins of cellular and noncellular forms of life.¹³ While the driving force for nature’s choice of *L*- over *D*-amino acids for protein transcription from RNA seems to be arbitrary, nevertheless it has been a source of fascination to scientists probing the origin of life. In fact, the existence of *D*-amino acids is not independent from that of *L*-amino acids. *L*-Amino acids are the

overwhelmingly predominant enantiomers of amino acids found in living organism proteins, meanwhile they act as the substrate to generate *D*-amino acids. Conversions from *L*- to *D*-amino acids occur in the presence of the enzyme racemase that changes the stereochemistry of the chiral α -carbon in amino acids.¹⁴ For example, *D*-serine (*D*-Ser) is an important amino acid found abundant in different parts of rat brain including cerebral cortex, hippocampus, anterior olfactory nucleus, and amygdala.¹⁵ In mammals, the enzyme serine racemase (Srr) converts *L*- to *D*-Ser in the presence of pyridoxal 5'-phosphate (PLP; **Fig. 2**)¹⁴ as well as Mg^{2+} and ATP.^{16,17} In **Fig. 2**, the enzyme catalyzes the racemization between *L*-Ser and *D*-Ser (path A) and the α,β -elimination of water from *L*-Ser or *D*-Ser to produce iminopyruvate (path B), which nonenzymatically hydrolyzes to form pyruvate and ammonia.¹⁴ Since Ca^{2+} or Mn^{2+} is necessary for enzyme activity, the presence of chelators such as ethylenediaminetetraacetic acid (EDTA) could completely deactivate the enzyme Srr.¹⁶

Interestingly, free *D*- and *L*-amino acids have been found in both Arctic and the Antarctic aerosol. In 2015, Feltracco's group first determined the free and combined *L*- and *D*-amino acids in Arctic aerosol, which were collected at the Gruvebadet observatory (Svalbard Islands). The mean relative contents of *D*- and *L*-alanine are 10 and 4% in all free amino acid in all samples.¹⁸ Another early investigation reported by Kaplan and Moore's group suggested the indigenous nature of amino acids and hydrocarbons in the Murchison meteorite. The presence of the amino acids such as glycine, alanine, valine, proline, glutamic acid, 2-methylalanine, and sarcosine was unequivocally established. The presence of almost equal amounts of the *D*- and *L*-enantiomers of valine, proline, alanine, and glutamic acid minimized the possibility of terrestrial contamination and suggested a possible extraterrestrial origin.¹⁹

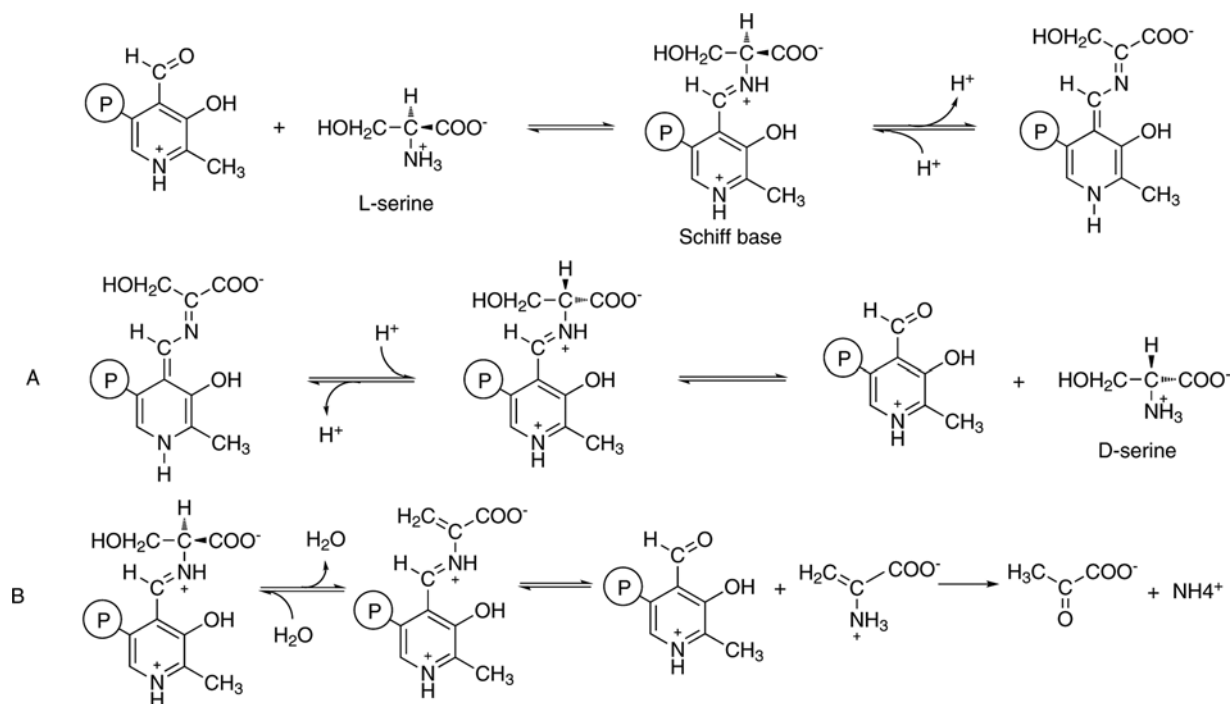


Fig. 2 Serine racemase pyridoxal-5'-phosphate-mediated conversion.

In an achiral environment, the physical and chemical characteristics of *D*-amino acids is similar to those of *L*-amino acids. However, the physiological functions of *D*-amino acids are remarkably different from those of *L*-amino acids. Albeit in much smaller proportions, *D*-amino acids exist widely in animals (including human beings), plants, microorganisms, and other circumstances in natural world, in free form, or as a part of other substances, playing various roles in different biological systems. In particular, free *D*-Ser and *D*-aspartate (*D*-Asp) have been identified in a wide variety of mammalian tissues and cells at relatively high concentrations.

In Animals (Including Human Beings)

With the development of sensitive analytical techniques, such as chromatography and mass spectrometry, various *D*-amino acids were successively discovered. An increasing number of evidence show that free *D*-amino acids as novel bioactive substances play important roles in physiological functions and involvement in human pathophysiology.²⁰ Among various *D*-amino acids, *D*-Ser is an important *D*-amino acid with multiple biological functions relevant to brain development and hence is among the most well studied.²¹ Early around 1970, *D*-amino acids was found to exist in some classes of bacteria and some insects and worms.^{6,22} In the following two decades, the presence of *D*-amino acids in significant quantities was discovered in various classes of marine and terrestrial animals.²³ In 1992 and 1993, Hashimoto et al adopted gas chromatography (GC) and mass spectrometry to find that natural *D*-Ser was present in rodents and human brains at significantly higher concentrations than other *D*-amino acids, such as *D*-Asp and *D*-alanine (*D*-Ala).^{24,25} Their findings pointed that *D*-Ser as a potential endogenous co-ligand for the *N*-methyl-*D*-Asp (NMDA) receptor. Moreover, *D*-amino acid oxidase (DAAO), the enzyme that degrades *D*-Ser, had been discovered in mammals before the demonstration of endogenous *D*-Ser. The structures and functions of DAAO have received intense attention in recent years due to its versatility.^{26–30} In 1995, Schell et al discovered that *D*-Ser was localized principally within glial cells. Specifically, they found that type-2 astrocytes, which were cultured from cerebral cortex, expressed particularly high levels of *D*-Ser.³¹ The key enzyme *Srr*, which converts *L*-Ser to *D*-Ser, was thought to be responsible for the synthesis of *D*-Ser. *Srr* was initially found in astrocytes and microglia in the mammalian brain. However, later research showed that *Srr* was also identified in neurons, exemplifying that *D*-Ser was not generated solely by astrocytes.²¹ Further research indicated that neurons were not the sole source of *D*-Ser.³²

The mammalian brain contains unusually high levels of *D*-Ser. Several studies demonstrated that *D*-Ser was a physio-

logical co-agonist of the NMDA type of glutamate receptor—a key excitatory neurotransmitter receptor in the brain. *D*-Ser binds with high affinity to a co-agonist site at the NMDA receptors, along with glutamate, and mediates several important physiological and pathological processes, including NMDA receptor transmission, synaptic plasticity, and neurotoxicity.³³ Scientists have revealed the mechanisms of *D*-Ser mediated pain induction.³⁴ Some presentative research studies showed that DAAO-mediated antinociceptive actions occur along with a significant decrease of *D*-Ser levels in the brain^{35,36} and the spinal cord.³⁶ Moreover, *D*-Ser-induced nephrotoxicity is believed to be associated with oxidative stress caused by hydrogen peroxide, a byproduct of DAAO-mediated metabolism of *D*-Ser³⁷ (►Fig. 3).¹⁴

D-Asp is another amino acid existing extensively in animals. Dunlop et al first reported the existence of free *D*-Asp in mammals including human beings in 1986.³⁸ Since then, substantial amounts of *D*-Asp have been found in various mammalian tissues, particularly the central nervous, neuroendocrine, and endocrine systems. Alterations in *D*-Asp levels during development and localization of *D*-Asp in these tissues have been investigated in detail. In several regions of the brain, concentrations of *D*-Asp are elevated during early development.³⁹ *D*-Asp levels in the rat cerebrum (approx. 140 nmol/g wet weight) and rat cerebellum (approx. 70 nmol/g wet weight) are both relatively high after birth. The former rapidly decreases to trace levels by 3 weeks of age, and the latter gradually decreases thereafter.⁴⁰ In human prefrontal cortex, the concentrations of *D*-Asp reach as high as approx. 0.36 μmol/g wet weight at 14 weeks of gestation.²⁵ Its content exceeds that of its enantiomer *L*-Asp, and then decreases rapidly to trace levels at birth and remains low.⁷ Immunohistochemical analysis of the rat embryonic brain with a specific anti-*D*-Asp antibody revealed that *D*-Asp is initially expressed in the hindbrain, after which it spreads into the forebrain and then throughout the entire brain.⁴¹

Among the free *D*-amino acids that have been identified in mammals, *D*-Asp plays a crucial role in the neuroendocrine and endocrine systems as well as in the central nervous system.⁷ It works as a hormone-like substance in the human body.⁴² For example, it promotes testosterone synthesis in the testicle,⁴³ and regulates synthesis of oxytocin, vasopressin, and prolactin in the posterior pituitary gland.^{44–46} The role of *D*-Asp as a neurotransmitter has been demonstrated in recent years.⁴⁷ The generation of *D*-Asp might be catalyzed by *Srr* in mice⁴⁸; however, some mechanisms are unclear regarding its synthesis in mammals.⁴⁶ In addition to the synthesized *D*-Ser and *D*-Asp catalyzed by various enzymes in cells, some *D*-amino acids from various foods and enteric bacteria are also ingested and metabolized in the human body. D'aniello et al

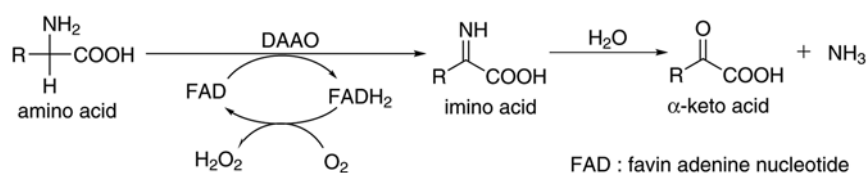


Fig. 3 DAAO-mediated metabolism of *D*-Ser.

investigated the ingestion of *D*-amino acids by feeding adult animals of different species, including man, rat, mouse, rabbit, chicks, with a given quantity of *D*-amino acids followed by measuring the levels of *D*-amino acids and their oxidative products in the blood and solid tissues after a time interval. The results showed that no intestinal barrier existed for absorption of *D*-amino acids, which were absorbed by the intestine and transferred through the blood to solid tissues. Only 10 to 20% of the total *D*-amino acid ingested was excreted in the feces and urine. The other 80 to 90% was absorbed by the intestine and metabolized in the liver and kidney, which were the richest sources of DAAO and *D*-Asp oxidase.²³ Another interesting discovery is that *D*-amino acids in proteins can be interpreted as molecular markers of aging.⁴⁹

Functions of *D*-Amino Acid Residues

D-Amino acid residues in various sequences often play key roles in biological functions. The venom of the North American funnel-web spider *Agelenopsis aperta* contains a variety of proteinaceous toxins which are able to block calcium channels. These peptides called agatoxins differ only by the presence of *D*-Ser in the amino acid sequence. The toxin that contains *D*-amino acid is more effective than its *L*-analogue.^{50,51} *D*-methionine residue is found present in the defensin-like peptide (DLP-2) isolated from the platypus venom. Male platypus (*Ornithorhynchus anatinus*) could produce a poisonous secretion, which is quite capable of killing a small animal like dingo. The poisonousness of DLP-2 has been found to be associated with the presence of *D*-methionine.^{52–54} *D*-Asp residues have also been discovered in proteins associated with age-related human disorders, such as cataract and Alzheimer's disease. In 2017, Ha et al investigated *D*-amino acid-containing peptides in adult human serum by a qualitative analytical method based on diastereomer and liquid chromatography/mass spectrometry (LC-MS/MS) method. Two *D*-Asp-containing peptides were detected in serum, in which one was fibrinopeptide B, preventing fibrinogen from forming polymers of fibrin, and the other was the same peptide with C-terminal arginine missing. The research provides a new direction on the serum proteome and fragmentome.⁵⁵

D-Amino Acids as Nutritious Ingredients of Animal Fodders

Amino acids are important nutritious ingredients of animal fodders. Farm animals remain the basis of the global food supply. Almost all functions of a living organism are related to their protein components to some extent. Proteins perform various functions such as catalytic, regulatory, structural, receptor, protective, molecular transport, and respiratory ones. The proteins are built exclusively from the proteins and *L*-amino acids of the food. Some of these amino acids are produced by microbiological synthesis in certain types of autotrophic soil bacteria, while others are produced by chemical synthesis in the form of chlorinated racemic mixtures requiring subsequent separation procedures. In most cases this procedure is not used because of a significant increase in the cost of the final fodder product, thus for farm animals, *D,L*-racemates are frequently given. The removal of *D*-isomers of

Table 1 Nutritional value of *D*-isomers of amino acids as a percentage of that of the *L*-isomers¹³

Amino acid	Chick	Dog	Pig
<i>D</i> -lysine	0	–	–
<i>D</i> -threonine	0	–	–
<i>D</i> -tryptophan	20	35	80
<i>D</i> -methionine	90	100	100
<i>D</i> -arginine	0	–	–
<i>D</i> -histidine	10	–	–
<i>D</i> -leucine	100	–	–
<i>D</i> -valine	70	–	–
<i>D</i> -isoleucine	0	–	–
<i>D</i> -phenylalanine	75	–	–
<i>D</i> -tyrosine	100	–	–

amino acids from fodder is necessary for several reasons: they have low metabolic and nutritional value (► **Table 1**), reduce the availability of *L*-amino acids, and require the involvement of at least two energy-consuming pathways for their utilization in animal cells. Several previous studies indicate that several *D*-isomers of amino acids have been shown to have toxic effects for some mammals and poultry.¹³ Undoubtedly, *D*-amino acids are closely related to the health of animals including human beings.

In Plants

Some common *D*-amino acids including *D*-Asp, *D*-asparagine (*D*-Asn), *D*-glutamic acid (Glu), *D*-glutamine (Gln), *D*-Ser, and *D*-Ala could be detected in most of the plants, and *D*-proline (Pro), *D*-valine (Val), *D*-leucine (Leu), and *D*-lysine (Lys) in certain plants.⁵⁶ Amino acids are also abundant in various fruits and vegetables. For example, *D*-Ala, *D*-Asp, *D*-Arg, and *D*-Glu are present not only in some fruits such as apples, grapes, oranges, but also in vegetables such as carrots, tomatoes, cabbages.⁵⁷

Plants are readily able to uptake *D*-amino acids from the soil. The proportion of *D*-amino acids in the total amino acid pool of different plant parts (including seeds, fruits, leaves, etc.) can reach around 1.5%.⁵⁸ The natural amounts of *D*-amino acids in fruits and vegetables are usually lower than 3.4 and 0.7%, respectively. The highest amount of individual *D*-amino acids found was 3.4% *D*-Asn and 1.9% *D*-Asp in grapefruit, 2.7% *D*-Ala and 1.7% *D*-Ser in apples, and 1.3% *D*-Glu in clementines.⁵ For a long time, plant growth inhibition by certain *D*-amino acids and slow degradation of *D*-amino acids by plants were neglected, as well as the possibility that *D*-amino acids could be serving as a nitrogen source or play a role as important regulatory molecules.⁵⁹ *D*-Ser, *D*-Ala, and *D*-Arg have been shown to strongly inhibit the growth of *Arabidopsis*.⁵⁸ Contrarily, *D*-isoleucine (Ile) and *D*-Val can promote growth of *Arabidopsis*,⁶⁰ and *D*-Lys can promote growth of both *Arabidopsis* and tobacco.⁶¹

D-Amino acid derivatives are widely present in algal thalli and seedlings of higher plants, mainly in the form of

dipeptides and malonic acid esters.^{13,56} The growth of higher plants such as soybeans and tomatoes is likely to be relevant to the presence of a *D*-tryptophan (Trp) derivative as precursors of plant hormones auxins.⁶²

Increasing evidence showed that *D*-amino acids can be both produced and metabolized by plants, since *D*-amino-acid-related enzymes, such as racemases, *D*-amino acid aminotransferases, or DAAOs, have been discovered in different plants.⁵⁹ Moreover, *D*-Ala can be taken up and assimilated by wheat from the solution of mixed nitrogen sources, where *D*-Ala uptake was fivefold faster than NO_3^- .⁶³ Michard et al brought yet another argument in 2011 for the role of *D*-amino acids as important modulators of plant development. Their study has shown that *D*-Ser influences pollen tube development in *Arabidopsis* and tobacco, and *D*-Ser racemase is important for *D*-Ser-mediated signal transduction.^{59,64}

In Microorganisms

Microorganisms produce, use, and metabolize *D*-amino acids, and could potentially serve as a supply source for them. In all bacteria, *D*-Ala and *D*-Asp were found in high concentrations; besides, the dipeptide *D*-Ala-*D*-Ala contributes to the antibiotic resistance.⁶⁵ The bacterial cell wall maintains the integrity and morphology of bacteria and comprises membrane layers and a rigid peptide-glycan scaffold known as peptidoglycan (PG).⁶⁶ In the 1970s, *D*-Ala, *D*-glutamate, and *D*-Asp were found in bacterial cell walls as constituents, and *D*-amino acids were observed to exist widely in nature. The initial findings led to the conclusion that *D*-amino acids were only rarely present in bacterial cell walls, but with the development of chiral analytical techniques, various kinds of *D*-amino acids were identified in free forms in diverse organisms.⁴⁶ In 2013, Mutaguchi et al reported that lactic fermentation is responsible for the *D*-amino acid production. And obvious increases in *D*-amino acids were seen during lactic fermentation, but not during alcoholic or acetic fermentation. This suggests that lactic acid bacteria have a greater ability to produce *D*-amino acids than yeast or acetic acid bacteria.⁶⁷ In 2018, Matsumoto et al performed simultaneous analysis of chiral amino acids using the highly sensitive LC-MS/MS technique and 12 free *D*-amino acids (*D*-Ala, *D*-Arg, *D*-Asp, *D*-Gln, *D*-Glu, *D*-allo-Ile, *D*-Leu, *D*-Lys, *D*-methionine (Met), *D*-phenylalanine (Phe), *D*-Ser, and *D*-Trp) produced by intestinal were identified, which belong to *Firmicutes* as the relevant bacterial candidates.⁶⁸

In *Vibrio cholerae*, the production of *D*-amino acids in its stationary phase and their incorporation into the PG polymer control the strength and amount of this structure, thereby providing fitness against low osmolarity and stationary phase stresses such as starvation, growth arrest, or accumulation of secondary metabolites.⁵⁹ The presence of *D*-amino acids in the peptide moieties of the PG of bacteria makes the cell wall invulnerable to most proteases designed to cleave between *L*-amino acids. Additionally, the presence of alternative *D*-amino acids like *D*-Asp or *D*-Ser at the terminal position of the stem peptide provides tolerance to certain bactericidal agents such as vancomycin.

Based on different mechanisms, some *D*-amino acids serve to prevent the formation of the biofilms of various

kinds of bacteria and disassemble formed biofilms.^{69–71} Their research results showed that *D*-Leu, *D*-Met, *D*-tyrosine (Tyr), *D*-Trp and their mixture can inhibit the biofilm formation of *Bacillus subtilis*⁶⁹ and *Staphylococcus aureus*.⁷⁰ Due to their antibiofilm and bactericidal effects, application of *D*-amino acids is an attractive antimicrobial strategy both alone or in synergy with existing antibiotics.⁵⁹

Biologically active peptides containing *D*-amino acids are also found in the cells of higher eukaryotic organisms; the only difference is that they are primarily formed as a result of posttranslational modification of a precursor consisting of *L*-amino acids.¹³ Some natural and synthetic peptides, containing *D*-amino acids, have strong antimicrobial properties. Synthetic peptides, containing glycyl-*D*-Ala, myristoyl-*D*-Asp, and sorbyl-*D*-Trp, can inactivate *Clostridium botulinum*.⁷² In natural antibiotics, *D*-Asp, *D*-Glu, *D*-Phe, and *D*-ornithine (Orn) are present in bacitracin; *D*-Val in penicillin G; *D*-Ala, *D*-Leu, and *D*-Val in actinomycin, gramicidin, and valinomycin; *D*-Asp and *D*-Glu in mycobactin; *D*-Phe and *D*-Trp in fungisporin, tyrocidine A, B, C, and D.¹⁴

Analysis of *D*-Amino Acids

Because of their important roles implicated in the biological system, there has been a ceaseless pursuit for ever more sensitive analysis of *D*-amino acids in various biological samples. Chiral analysis of amino acids is rather challenging especially in the case of complex biological systems.⁷³ Chiral analyses of amino acids are encumbered by the following difficulties: (1) while a high level of *L*-amino acids can be found in the tissues, their *D*-enantiomers are usually present in one or two orders of magnitude lower concentration, which requires extremely high sensitivity of instruments. (2) In many cases, derivatization is often required for their optical detection, because most amino acids lack either a chromophore or a fluorophore moiety. However, derivatization is not only time consuming but also the source of several analytical errors. And also, racemization of amino acid enantiomers might occur at a high temperature or under acidic conditions. (3) For many biological samples containing *D*-amino acids at low concentrations, derivatization is not reliable due to complex competing reactions.

The increasing discoveries involving *D*-amino acids as well as their derivatives are ascribed to the development of analytical methods, especially chiral chromatography. A large number of documents are based on LC as well as its relevant combination techniques. For example, in 1995, Fukushima et al used chiral high-performance LC (HPLC) with fluorometric detection (HPLC-FD) to analyze human serum and found *D*-Ala at a concentration of 0.48 to 3.10 $\mu\text{mol/L}$.⁷⁴ In 2006, Song et al adopted a sensitive chiral HPLC-MS/MS method to determine amino acid enantiomers in biological samples of 3-day and 90-day rat brain, and found 12 amino acids, and among them, the content of *D*-Ser is 82.3 $\mu\text{g/g}$ (3-day-old) and 241.3 $\mu\text{g/g}$ (90-day-old).⁷⁵ In 2020, Kimura et al developed a cognitive function marker based on *D*-amino acid proportions using new chiral tandem LC-MS/MS systems, which was used to analyze the

enantiomeric proportions of amino acids in blood samples.⁷⁶ More methods based on chiral LC such as HPLC-UV, 2D-HPLC-FD, and UPLC-MS/MS are not listed herein; please refer to the review.⁷³ Chiral GC is also a very common method for enantiomeric amino acid analysis. Since free *D*-Ser was first identified in rat brain,²⁴ an increasing number of documents reported GC-based methods such as GC-MS, 2D GC-TOFMS, etc.^{73,77–80} Capillary column Chirasil-*L*-Val and cyclodextrin-based chiral stationary phases both are frequently used chiral solid phases.

In addition to chiral LC and GC techniques, some other analytical methods such as chiral capillary electrophoresis (CE) as well as chiral CE-MS,^{81–84} enzyme-based microbiosensors,⁸⁵ chiral micellar electrokinetic capillary chromatography,^{86,87} and chiral microchip electrophoresis^{88,89} have been applied in the analysis of *D*-amino acids in biological samples. It is noteworthy that CE has been widely used in the enantiomeric analysis of various chiral compounds including amino acids due to their high enantioselectivity, low cost, and chromatography/CE compatibility.^{90–93} Measurements of low-abundance, heterogeneously distributed, and endogenous *D*-amino acids in complex biological samples require the implementation of sensitive and selective analytical approaches. To measure the *D*- and *L*-forms of aspartate and glutamate, in 2017, Patel et al developed and applied a stacking chiral CE with a laser-induced fluorescence detection method, which enabled the relative quantification of *D*-aspartate and *D*-glutamate in individual neurons mechanically isolated from the central nervous system of the sea slug *Aplysia californica*.⁹⁴ In 2018, Zhang et al used optical-gated CE with LIF detection (OGCE-LIF) to quickly and efficiently separate amino acid enantiomers. Under the optimal OGCE-LIF conditions, five pairs of *D/L*-amino acid pairs could be separated in less than 1 minute with the low limit of detection of 1.3 $\mu\text{mol/L}$.⁹⁵

Since the *D*- and *L*-enantiomers of amino acids are naturally occurring, directly used, and also applied as versatile catalysts or synthetic precursors to diverse functional organic compounds and as chirality sources for asymmetric synthesis and catalysis,^{96–99} great attention has been paid to exploring the chiral analysis of amino acids. In recent decades, various analytical techniques have achieved success in determining the yield and enantioselectivity of organic molecules, including LC and GC methods (as mentioned above), nuclear magnetic resonance spectroscopy, mass spectrometry, circular dichroism spectroscopy,¹⁰⁰ and fluorescence measurements.¹⁰¹ Among them, fluorescence measurement for chiral analysis has become a research hotspot due to its multiple advantages such as high sensitivity, multiple detection modes, noninvasive real-time imaging, and potentials in online and high-throughput analysis.^{102–107} Enantioselective fluorescent probes for chiral recognition of protected and free amino acids have sprung up like mushrooms in recent years.^{108–114} These chiral fluorescent probes have great potential for analysis of the optical purity and concentrations of amino acids, as well as high-throughput screening of asymmetric reactions for preparing chiral amino acids.

Conclusions

Amino acids, as components of protein, peptides, receptors as well as antibodies, play important roles in life processes. With the development of chiral analytical methods, scientists have found that *D*-amino acids are widely present in living organisms including animals, plants, and microorganisms. The various biological functions of *D*-amino acids have attracted much attention, and many interesting and significant discoveries have been successively revealed. Accumulating data indicate that *D*-amino acids are closely relevant to human physiology and pathophysiology. Many recent investigations have demonstrated the formation and increasing amount of *D*-amino acid forms in food processing. The roles of *D*-amino acids in the development, pathophysiology, and treatment of cancer are being slowly revealed. Significantly altered levels of specific *D*-amino acids are found in cancer cells compared with nontumorigenic cells. There has been growing recognition that bacteria contribute significantly to the body's *D*-amino acid pool, and overprocessed foods might contain more *D*-amino acids, which is a worrying issue. However, on the other hand, *D*-amino acids also have many useful functions for animals including human beings. In view of these, effects of *D*-amino acids and their metabolic products on cellular and tissue functions, and the relationship between *D*-amino acids in the human body and health/diseases, are far from being sufficiently explored. With their far-reaching impacts in biology, physiology, pathology, and medicine, we believe that with the development of ever more sensitive and selective analytic technologies, the formation mechanisms of *D*-amino acids and their derivatives in living organisms and their biological functions will be elucidated more clearly to better serve the interest of science and humanity.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

The support from the National Natural Science Foundation of China (Nos. 21602164, 21877087) is greatly appreciated.

References

- Vauquelin LN, Robiquet PJ. The discovery of a new plant principle in *Asparagus sativus*. *Ann Chim* 1806;57:88–93
- Vickery HB, Schmidt CLA. The history of the discovery of the amino acids. *Chem Rev* 1931;9(02):169–318
- Gal J. Louis Pasteur, language, and molecular chirality. I. Background and dissymmetry. *Chirality* 2011;23(01):1–16
- Weatherly CA, Du S, Parpia C, Santos PT, Hartman AL, Armstrong DW. *D*-amino acid levels in perfused mouse brain tissue and blood: a comparative study. *ACS Chem Neurosci* 2017;8(06):1251–1261
- Marcone GL, Rosini E, Crespi E, Pollegioni L. *D*-amino acids in foods. *Appl Microbiol Biotechnol* 2020;104(02):555–574
- Corrigan JJ. *D*-amino acids in animals. *Science* 1969;164(3876):142–149
- Katane M, Homma H. *D*-Aspartate—an important bioactive substance in mammals: a review from an analytical and biological

- point of view. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011;879(29):3108–3121
- 8 Fujii N, Kaji Y, Fujii N. *D*-Amino acids in aged proteins: analysis and biological relevance. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011;879(29):3141–3147
 - 9 Ohide H, Miyoshi Y, Maruyama R, Hamase K, Konno R. *D*-Amino acid metabolism in mammals: biosynthesis, degradation and analytical aspects of the metabolic study. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011;879(29):3162–3168
 - 10 Raucci F, Di Fiore MM. *D*-Asp: a new player in reproductive endocrinology of the amphibian *Rana esculenta*. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011;879(29):3268–3276
 - 11 Konno R, Brückner H, D'Aniello A, Fischer N, Fujii N, Homma H, eds. An improved HPLC method for determination and quantification of *D*- and *L*-Asp. In: *D*-Amino acids: a new frontier in amino acid and protein research—Practical methods and protocols. New York, New York: Nova Science Publishers; 2007: 148–149
 - 12 Hamase K. Analysis and biological relevance of *D*-amino acids and relating compounds. *J Chromatogr B Analyt Technol Biomed Life Sci* 2011;879(29):3077
 - 13 Grishin DV, Zhdanov DD, Pokrovskaya MV, et al. *D*-amino acids in nature, agriculture and biomedicine. *All Life* 2020;13(01):11–22
 - 14 Genchi G. An overview on *D*-amino acids. *Amino Acids* 2017;49(09):1521–1533
 - 15 Zhao J, Taylor CJ, Newcombe EA, et al. EphA4 regulates hippocampal neural precursor proliferation in the adult mouse brain by *D*-serine modulation of *N*-methyl-*D*-aspartate receptor signaling. *Cereb Cortex* 2019;29(10):4381–4397
 - 16 Cook SP, Galve-Roperh I, Martínez del Pozo A, Rodríguez-Crespo I. Direct calcium binding results in activation of brain serine racemase. *J Biol Chem* 2002;277(31):27782–27792
 - 17 De Miranda J, Panizzutti R, Foltyn VN, Wolosker H. Cofactors of serine racemase that physiologically stimulate the synthesis of the *N*-methyl-*D*-aspartate (NMDA) receptor coagonist *D*-serine. *Proc Natl Acad Sci U S A* 2002;99(22):14542–14547
 - 18 Feltracco M, Barbaro E, Kirchgorg T, et al. Free and combined *L*- and *D*-amino acids in Arctic aerosol. *Chemosphere* 2019; 220:412–421
 - 19 Kvenovolden K, Lawless J, Pering K, et al. Evidence for extraterrestrial amino-acids and hydrocarbons in the Murchison meteorite. *Nature* 1970;228(5275):923–926
 - 20 Katane M, Homma H. The Free Form of *D*-amino acids as a novel bioactive substance: physiological functions and involvement in human pathophysiology. *Rinsho Byori* 2017;65(01): 67–80
 - 21 Van Horn MR, Sild M, Ruthazer ES. *D*-serine as a gliotransmitter and its roles in brain development and disease. *Front Cell Neurosci* 2013;7:39
 - 22 Neims AH, Hellerman L. Flavoenzyme catalysis. *Annu Rev Biochem* 1970;39(01):867–888
 - 23 D'Aniello A, D'Onofrio G, Pischetola M, et al. Biological role of *D*-amino acid oxidase and *D*-aspartate oxidase. Effects of *D*-amino acids. *J Biol Chem* 1993;268(36):26941–26949
 - 24 Hashimoto A, Nishikawa T, Hayashi T, et al. The presence of free *D*-serine in rat brain. *FEBS Lett* 1992;296(01):33–36
 - 25 Hashimoto A, Kumashiro S, Nishikawa T, et al. Embryonic development and postnatal changes in free *D*-aspartate and *D*-serine in the human prefrontal cortex. *J Neurochem* 1993;61(01):348–351
 - 26 Pollegioni L, Sasabe J. Editorial: bioscience of *D*-amino acid oxidase from biochemistry to pathophysiology. *Front Mol Biosci* 2018;5:108
 - 27 Sharma J, Kulshrestha R, Singh N, Jaggi AS. Dual role of *D*-amino acid oxidase in experimental pain models. *Eur J Pharmacol* 2019; 855:98–102
 - 28 Padhi AK, Hazra S. Insights into the role of *d*-amino acid oxidase mutations in amyotrophic lateral sclerosis. *J Cell Biochem* 2018; 120(02):2180–2197
 - 29 Lin CH, Yang HT, Chiu CC, Lane HY. Blood levels of *D*-amino acid oxidase vs. *D*-amino acids in reflecting cognitive aging. *Sci Rep* 2017;7(01):14849
 - 30 Pollegioni L, Sacchi S, Murtas G. Human *D*-amino acid oxidase: structure, function, and regulation. *Front Mol Biosci* 2018;5:107
 - 31 Schell MJ, Molliver ME, Snyder SH. *D*-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. *Proc Natl Acad Sci U S A* 1995;92(09): 3948–3952
 - 32 Benneyworth MA, Li Y, Basu AC, Bolshakov VY, Coyle JT. Cell selective conditional null mutations of serine racemase demonstrate a predominate localization in cortical glutamatergic neurons. *Cell Mol Neurobiol* 2012;32(04):613–624
 - 33 Wolosker H, Dumin E, Balan L, Foltyn VN. *D*-amino acids in the brain: *D*-serine in neurotransmission and neurodegeneration. *FEBS J* 2008;275(14):3514–3526
 - 34 Choi SR, Moon JY, Roh DH, et al. Spinal *D*-serine increases PKC-dependent GluN1 phosphorylation contributing to the sigma-1 receptor-induced development of mechanical allodynia in a mouse model of neuropathic pain. *J Pain* 2017;18(04):415–427
 - 35 Hamase K, Konno R, Morikawa A, Zaitso K. Sensitive determination of *D*-amino acids in mammals and the effect of *D*-amino acid oxidase activity on their amounts. *Biol Pharm Bull* 2005;28(09):1578–1584
 - 36 Miyoshi Y, Hamase K, Tojo Y, Mita M, Konno R, Zaitso K. Determination of *D*-serine and *D*-alanine in the tissues and physiological fluids of mice with various *D*-amino-acid oxidase activities using two-dimensional high-performance liquid chromatography with fluorescence detection. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009;877(24):2506–2512
 - 37 Ferraris D, Duvall B, Ko YS, et al. Synthesis and biological evaluation of *D*-amino acid oxidase inhibitors. *J Med Chem* 2008;51(12):3357–3359
 - 38 Dunlop DS, Neidle A, McHale D, Dunlop DM, Lajtha A. The presence of free *D*-aspartic acid in rodents and man. *Biochem Biophys Res Commun* 1986;141(01):27–32
 - 39 Bastings JJA, van Eijk HM, Olde Damink SW, Rensen SS. *D*-amino acids in health and disease: a focus on cancer. *Nutrients* 2019;11(09):2205
 - 40 Hashimoto A, Oka T, Nishikawa T. Anatomical distribution and postnatal changes in endogenous free *D*-aspartate and *D*-serine in rat brain and periphery. *Eur J Neurosci* 1995;7(08):1657–1663
 - 41 Sakai K, Homma H, Lee JA, et al. *D*-aspartic acid localization during postnatal development of rat adrenal gland. *Biochem Biophys Res Commun* 1997;235(02):433–436
 - 42 Li Y, Han H, Yin J, Li T, Yin Y. Role of *D*-aspartate on biosynthesis, racemization, and potential functions: a mini-review. *Anim Nutr* 2018;4(03):311–315
 - 43 Nagata Y, Homma H, Lee JA, Imai K. *D*-Aspartate stimulation of testosterone synthesis in rat Leydig cells. *FEBS Lett* 1999;444(2-3):160–164
 - 44 Wang H, Wolosker H, Pevsner J, Snyder SH, Selkoe DJ. Regulation of rat magnocellular neurosecretory system by *D*-aspartate: evidence for biological role(s) of a naturally occurring free *D*-amino acid in mammals. *J Endocrinol* 2000;167(02):247–252
 - 45 Long Z, Lee JA, Okamoto T, Nimura N, Imai K, Homma H. *d*-Aspartate in a prolactin-secreting clonal strain of rat pituitary tumor cells (GH(3)). *Biochem Biophys Res Commun* 2000;276(03):1143–1147
 - 46 Kobayashi J. *D*-Amino acids and lactic acid bacteria. *Microorganisms* 2019;7(12):690
 - 47 D'Aniello S, Somorjai I, Garcia-Fernández J, Topo E, D'Aniello A. *D*-Aspartic acid is a novel endogenous neurotransmitter. *FASEB J* 2011;25(03):1014–1027

- 48 Ito T, Hayashida M, Kobayashi S, et al. Serine racemase is involved in d-aspartate biosynthesis. *J Biochem* 2016;160(06):345–353
- 49 Fujii N. D-amino acid in elderly tissues. *Biol Pharm Bull* 2005;28(09):1585–1589
- 50 Heck SD, Siok CJ, Krapcho KJ, et al. Functional consequences of posttranslational isomerization of Ser46 in a calcium channel toxin. *Science* 1994;266(5187):1065–1068
- 51 Murkin AS, Tanner ME. Dehydroalanine-based inhibition of a peptide epimerase from spider venom. *J Org Chem* 2002;67(24):8389–8394
- 52 Whittington CM, Koh JM, Warren WC, et al. Understanding and utilising mammalian venom via a platypus venom transcriptome. *J Proteomics* 2009;72(02):155–164
- 53 Torres AM, Tsampazi M, Kennett EC, et al. Characterization and isolation of L-to-D-amino-acid-residue isomerase from platypus venom. *Amino Acids* 2007;32(01):63–68
- 54 Torres AM, Menz I, Alewood PF, et al. D-Amino acid residue in the C-type natriuretic peptide from the venom of the mammal, *Ornithorhynchus anatinus*, the Australian platypus. *FEBS Lett* 2002;524(1–3):172–176
- 55 Ha S, Kim I, Takata T, et al. Identification of D-amino acid-containing peptides in human serum. *PLoS One* 2017;12(12):e0189972
- 56 Brückner H, Westhauser T. Chromatographic determination of L- and D-amino acids in plants. *Amino Acids* 2003;24(1–2):43–55
- 57 Gandolfi I, Palla G, Marchelli R, et al. D-alanine in fruit juices: a molecular marker of bacterial activity, heat treatments and shelf-life. *J Food Sci* 1994;59(01):152–154
- 58 Forsum O, Svennerstam H, Ganeteg U, Näsholm T. Capacities and constraints of amino acid utilization in *Arabidopsis*. *New Phytol* 2008;179(04):1058–1069
- 59 Aliashkevich A, Alvarez L, Cava F. New insights into the mechanisms and biological roles of D-amino acids in complex ecosystems. *Front Microbiol* 2018;9:683
- 60 Erikson O, Hertzberg M, Näsholm T. A conditional marker gene allowing both positive and negative selection in plants. *Nat Biotechnol* 2004;22(04):455–458
- 61 Chen IC, Thiruvengadam V, Lin WD, Chang HH, Hsu WH. Lysine racemase: a novel non-antibiotic selectable marker for plant transformation. *Plant Mol Biol* 2010;72(1–2):153–169
- 62 Rekoslavskaya NI. Possible role of N-malonyl-D-tryptophan as an auxin precursor. *Biol Plant* 1986;28(01):62–67
- 63 Hill PW, Quilliam RS, DeLuca TH, et al. Acquisition and assimilation of nitrogen as peptide-bound and D-enantiomers of amino acids by wheat. *PLoS One* 2011;6(04):e19220
- 64 Michard E, Lima PT, Borges F, et al. Glutamate receptor-like genes form Ca²⁺ channels in pollen tubes and are regulated by pistil D-serine. *Science* 2011;332(6028):434–437
- 65 Reynolds PE. Control of peptidoglycan synthesis in vancomycin-resistant enterococci: D,D-peptidases and D,D-carboxypeptidases. *Cell Mol Life Sci* 1998;54(04):325–331
- 66 Hsu YP, Booher G, Egan A, Vollmer W, VanNieuwenhze MS. D-Amino acid derivatives as in situ probes for visualizing bacterial peptidoglycan biosynthesis. *Acc Chem Res* 2019;52(09):2713–2722
- 67 Mutaguchi Y, Ohmori T, Akano H, Doi K, Ohshima T. Distribution of D-amino acids in vinegars and involvement of lactic acid bacteria in the production of D-amino acids. *Springerplus* 2013;2(01):691
- 68 Matsumoto M, Kunisawa A, Hattori T, et al. Free D-amino acids produced by commensal bacteria in the colonic lumen. *Sci Rep* 2018;8(01):17915
- 69 Kolodkin-Gal I, Romero D, Cao S, Clardy J, Kolter R, Losick R. D-amino acids trigger biofilm disassembly. *Science* 2010;328(5978):627–629
- 70 Hochbaum AI, Kolodkin-Gal I, Foulston L, Kolter R, Aizenberg J, Losick R. Inhibitory effects of D-amino acids on *Staphylococcus aureus* biofilm development. *J Bacteriol* 2011;193(20):5616–5622
- 71 Ramón-Peréz ML, Diaz-Cedillo F, Ibarra JA, et al. D-Amino acids inhibit biofilm formation in *Staphylococcus epidermidis* strains from ocular infections. *J Med Microbiol* 2014;63(Pt 10):1369–1376
- 72 Paquet A, Rayman K. Some N-acyl-D-amino acid derivatives having antibiotoxic properties. *Can J Microbiol* 1987;33(07):577–582
- 73 Szökő É, Vincze I, Tábi T. Chiral separations for d-amino acid analysis in biological samples. *J Pharm Biomed Anal* 2016;130:100–109
- 74 Fukushima T, Kato M, Santa T, Imai K. Enantiomeric separation and sensitive determination of D,L-amino acids derivatized with fluorogenic benzofurazan reagents on Pirkle type stationary phases. *Biomed Chromatogr* 1995;9(01):10–17
- 75 Song Y, Feng Y, LeBlanc MH, Zhao S, Liu YM. Assay of trace D-amino acids in neural tissue samples by capillary liquid chromatography/tandem mass spectrometry. *Anal Chem* 2006;78(23):8121–8128
- 76 Kimura R, Tsujimura H, Tsuchiya M, et al. Development of a cognitive function marker based on D-amino acid proportions using new chiral tandem LC-MS/MS systems. *Sci Rep* 2020;10(01):804
- 77 Simek P, Hušek P, Zahradníčková H. Heptafluorobutyl chloroformate-based sample preparation protocol for chiral and nonchiral amino acid analysis by gas chromatography. *Methods Mol Biol* 2012;828:137–152
- 78 Hasegawa H, Masuda N, Natori H, Shinohara Y, Ichida K. Pharmacokinetics and toxicokinetics of d-serine in rats. *J Pharm Biomed Anal* 2019;162:264–271
- 79 Waldhauer MC, Almstetter MF, Nürnberger N, Gruber MA, Dettmer K, Oefner PJ. Improved enantiomer resolution and quantification of free D-amino acids in serum and urine by comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry. *J Chromatogr A* 2011;1218(28):4537–4544
- 80 Majhi KC, Karfa P, Madhuri R. Chromatographic separation of amino acids. In: Inamuddin, ed. *Applications of Ion Exchange Materials in Biomedical Industries*. Basel: Springer; 2019:71–118
- 81 Jakó T, Szabó E, Tábi T, Zachar G, Csillag A, Szökő E. Chiral analysis of amino acid neurotransmitters and neuromodulators in mouse brain by CE-LIF. *Electrophoresis* 2014;35(19):2870–2876
- 82 Wagner Z, Tábi T, Jakó T, Zachar G, Csillag A, Szökő E. Chiral separation and determination of excitatory amino acids in brain samples by CE-LIF using dual cyclodextrin system. *Anal Bioanal Chem* 2012;404(08):2363–2368
- 83 Fanali S. Chiral separations by CE employing CDs. *Electrophoresis* 2009;30(Suppl 1):S203–S210
- 84 Tanaka Y, Otsuka K, Terabe S. Separation of enantiomers by capillary electrophoresis-mass spectrometry employing a partial filling technique with a chiral crown ether. *J Chromatogr A* 2000;875(1–2):323–330
- 85 Pernot P, Mothet JP, Schuvalov O, et al. Characterization of a yeast D-amino acid oxidase microbiosensor for D-serine detection in the central nervous system. *Anal Chem* 2008;80(05):1589–1597
- 86 Carlavilla D, Moreno-Arribas MV, Fanali S, Cifuentes A. Chiral MEKC-LIF of amino acids in foods: analysis of vinegars. *Electrophoresis* 2006;27(13):2551–2557
- 87 Swartz ME, Mazzeo JR, Grover ER, Brown PR. Separation of amino acid enantiomers by micellar electrokinetic capillary chromatography using synthetic chiral surfactants. *Anal Biochem* 1995;231(01):65–71
- 88 Rodríguez I, Jin LJ, Li SF. High-speed chiral separations on microchip electrophoresis devices. *Electrophoresis* 2000;21(01):211–219
- 89 Harrison DJ, Fluri K, Seiler K, Fan Z, Effenhauser CS, Manz A. Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip. *Science* 1993;261(5123):895–897
- 90 Zhang Q, Ren S, Xue S. Investigation of fusidic acid as a chiral selector in capillary electrophoresis. *Separ Purif Tech* 2020;242:116768

- 91 Bernardo-Bermejo S, Sanchez-Lopez E, Castro-Puyana M, et al. Chiral capillary electrophoresis. *Trends Analyt Chem* 2020; 124:115807
- 92 Xue S, Ren S, Wang L, Zhang Q. Evaluation of tetraalkylammonium amino acid ionic liquids as chiral ligands in ligand-exchange capillary electrophoresis. *J Chromatogr A* 2020;1611:460579
- 93 Sun Y, Zhang S. Application of capillary electrophoresis for ephedrine and pseudoephedrine detection: a review. *Int J Electrochem Sci* 2020;15(03):2614–2623
- 94 Patel AV, Kawai T, Wang L, Rubakhin SS, Sweedler JV. Chiral measurement of aspartate and glutamate in single neurons by large-volume sample stacking capillary electrophoresis. *Anal Chem* 2017;89(22):12375–12382
- 95 Zhang N, Tian M, Liu X, Yang L. Enzyme assay for d-amino acid oxidase using optically gated capillary electrophoresis-laser induced fluorescence detection. *J Chromatogr A* 2018;1548:83–91
- 96 Ma D, Cai Q. N,N-dimethyl glycine-promoted Ullmann coupling reaction of phenols and aryl halides. *Org Lett* 2003;5(21): 3799–3802
- 97 Sardina FJ, Rapoport H. Enantiospecific synthesis of heterocycles from alpha-amino acids. *Chem Rev* 1996;96(06):1825–1872
- 98 MacMillan DW. The advent and development of organocatalysis. *Nature* 2008;455(7211):304–308
- 99 Xiao Q, Tang Y, Xie P, et al. Asymmetric amination of α,α -dialkyl substituted aldehydes catalyzed by a simple chiral primary amino acid and its application to the preparation of a S1P 1 agonist. *RSC Advances* 2019;9(57):33497–33505
- 100 Shahriar I, Islam MKB, Iqfath M, et al. Solvent effect on vibrational circular dichroism of chiral amino acids. *Theor Chem Acc* 2019;138:32
- 101 Herrera BT, Pilicer SL, Anslyn EV, Joyce LA, Wolf C. Optical analysis of reaction yield and enantiomeric excess: a new paradigm ready for prime time. *J Am Chem Soc* 2018;140(33): 10385–10401
- 102 Wu X, Shi W, Li X, Ma H. Recognition moieties of small molecular fluorescent probes for bioimaging of enzymes. *Acc Chem Res* 2019;52(07):1892–1904
- 103 Zhang X, Yin J, Yoon J. Recent advances in development of chiral fluorescent and colorimetric sensors. *Chem Rev* 2014;114(09): 4918–4959
- 104 Zhou Y, Yoon J. Recent progress in fluorescent and colorimetric chemosensors for detection of amino acids. *Chem Soc Rev* 2012; 41(01):52–67
- 105 Sun Q, Yang SH, Wu L, Dong QJ, Yang WC, Yang GF. Detection of intracellular selenol-containing molecules using a fluorescent probe with near-zero background signal. *Anal Chem* 2016;88 (11):6084–6091
- 106 Xu J, Yuan H, Zeng L, et al. Recent progress in Michael addition-based fluorescent probes for sulfur dioxide and its derivatives. *Chin Chem Lett* 2018;29(10):1456–1464
- 107 Han J, Yue X, Wang J, et al. A ratiometric merocyanine-based fluorescent probe for detecting hydrazine in living cells and zebra fish. *Chin Chem Lett* 2020 (e-pub ahead of print) . Doi: 10.1016/j.ccl.2020.01.029
- 108 Pu L. Simultaneous determination of concentration and enantiomeric composition in fluorescent Sensing. *Acc Chem Res* 2017;50(04):1032–1040
- 109 Pu L. Enantioselective fluorescent sensors: a tale of BINOL. *Acc Chem Res* 2012;45(02):150–163
- 110 Pu L. Fluorescence of organic molecules in chiral recognition. *Chem Rev* 2004;104(03):1687–1716
- 111 Zhu YY, Wu XD, Abed M, Gu SX, Pu L. Biphasic enantioselective fluorescent recognition of amino acids by a fluorophilic probe. *Chem Eur J* 2019;25(33):7866–7873
- 112 Zhu YY, Wu XD, Gu SX, Pu L. Free amino acid recognition: a bisbinaphthyl-based fluorescent probe with high enantioselectivity. *J Am Chem Soc* 2019;141(01):175–181
- 113 Zeng C, Zhang X, Pu L. Enantioselective fluorescent imaging of free amino acids in living cells. *Chem Eur J* 2017;23(10): 2432–2438
- 114 Wen K, Yu S, Huang Z, et al. Rational design of a fluorescent sensor to simultaneously determine both the enantiomeric composition and the concentration of chiral functional amines. *J Am Chem Soc* 2015;137(13):4517–4524