Appl Biochem Biotechnol (2012) 167:1–13
DOI 10.1007/s12010-012-9611-1

Advances in Non-snake Venom L-Amino Acid Oxidase

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Received: 8 August 2011 / Accepted: 10 February 2012 /
Published online: 26 February 2012

Abstract L-amino acid oxidase is widely found in diverse organisms and has different properties. It is thought to contribute to antimicrobial activity, amino acid catabolism, and so forth. The purpose of this communication is to summarize the advances in non-snake venom L-amino acid oxidase, including its enzymatic and structural properties, gene cloning and expression, and biological function. In addition, the mechanism of its biological function as well as its application is also discussed.

Keywords L-amino acid oxidase · Non-snake venom · Enzymatic and structural properties · Gene cloning and expression · Biological function and application

Introduction

So far, all the described L-amino acid oxidases (LAAOs; EC 1.4.3.2) are flavoproteins [1], except the lysine oxidase of *Marinomonas mediterranea* [2], with a dimeric structure, and each subunit contains a non-covalently bound flavin adenine dinucleotide (FAD) molecule as cofactor, with the exception that the LAAO of rat kidney has a tetrameric structure for the active enzyme, containing half [3] or one [4] flavin mononucleotide coenzyme per subunit, a highly unusual finding. LAAO is generally classified as *N*-terminal FAD-bound reductase because of the presence of two highly conserved dinucleotide-binding motifs [1]. It is able to catalyze the stereospecific oxidative deamination of L-amino acids to the corresponding α-imino acids which are then hydrolyzed to corresponding α-keto acids with release of NH_4^+ via non-enzymatic oxidation, along with two electrons transferring from the amino acid to the flavin cofactor which subsequently reduces molecular oxygen to H_2O_2. When H_2O_2 is not degraded by catalase, it can cause a decarboxylation of the α-keto acid to the corresponding carboxylic acid. Faust et al. demonstrated that the mechanism of substrate

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dehydrogenation by LAAO is hydride transfer by directing the trajectory of the interacting orbitals, which can proceed efficiently without the involvement of amino acid functional groups, and most groups present at the active site are involved in substrate recognition, binding, and exact orientation [5].

This enzyme is widely distributed in nature including snake venoms, insect drugs [6], sea hare [7], fungi [8], bacteria [9, 10], and algae [11]. The first LAAO described was from bacterium *Proteus vulgaris* [12]. Since then, an increasing number of LAAOs from different organisms have been reported. Before 1980, the studies on LAAO were mainly focused on its reaction mechanism, and enzymatic and physiochemical properties. Between the 1980s and 1990s, the reports mostly dealt with purification and characterization of LAAO from different organisms. From the late 1990s to now, its biological functional properties and mechanism, and structure as well as cloning and expression are mainly attracted. So far, snake venom LAAOs are the best characterized members of this enzyme family with respect not only to toxicology but also biochemistry, physiology, and medicine. An excellent review on snake venom LAAO can be found elsewhere [13]. In contrast, relatively little is known about non-snake venom LAAOs, especially on their structure, biological function, and related mechanisms. The purpose of this communication is to summarize the advances in non-snake venom LAAOs.

Enzymatic Properties

Enzymatic properties of LAAO from a variety of sources show some differences as indicated in Table 1. Generally, the optimum pH of LAAO depends on its substrate and the enzyme activity is relatively stable under 60 °C. Most LAAOs exhibit a broad range of substrate spectra (mainly studied for natural amino acids). The LAAO identified from *Amphiroa crassissima* shows activity toward aliphatic, aromatic, basic, sulfur-containing, γ-hydroxy amino acids, L-Asn, and L-Gln [11]. Several natural L-amino acids, *S*-adenosyl-L-homocysteine, L-homocysteine, and *S*-adenosyl-L-methionine are substrates for the LAAO from rat kidney [14, 15]. Surprisingly, the LAAO isolated from *Rhodococcus opacus* can oxidize 39 L-amino acids, including not only all the 20 L-amino acids but also some derivatives [16]. Among them, basic, aromatic, and aliphatic L-amino acids generally seem to be good substrates. This LAAO was found to have the broadest substrate specificity hitherto. In addition, LAAOs of *Proteus rettgeri* [17], *Mytilus edulis* [18], *P. vulgaris* [12], *Cellulomonas cellulans* [19], and *Corynebacterium* [20] also exhibit broad substrate specificity. Although a few LAAOs accept D-amino acids as substrates, the reaction rates are relatively low compared with those with L-amino acids. The LAAO from *Bacillus carotarum* 2Pfa was found to accept ten L-amino acids and seven D-amino acids as substrates [9]. Among these amino acids, three aromatic L-amino acids (Phe, Tyr, and Trp) are the preferred.

There exist some LAAOs with relatively narrower substrates specificity, such as LAAOs from fresh water cyanobacteria *Synechococcus elongatus* PCC 6301, *S. elongatus* PCC 7942, *Synechococcus cedrorum* PCC 6908 [21], and *Anacystis nidulans* [22], with high preference only to basic L-amino acids.

In contrast, there are several LAAOs with a very strict preference for a specific substrate. Therefore, they are named after their favored substrate, such as the L-glycine oxidase from *Bacillus subtilis* [23], the L-cysteine oxidase from *Neisseria meningitidis* [24], the L-glutamate oxidases from *Streptomyces endus* [25] and *Streptomyces* sp. [26], the L-lysine oxidases from *Trichoderma viride* [27], *Pseudoalteromonas tunicate* [28], *M. mediterranea* [29], and

Table 1 Enzymatic properties of LAAO from various sources

Organism	Optimum pH	Storage stability	Temperature sensitivity
B. carotarum 2Pfa [9]	8.0–8.5 with L-Phe as substrate; showing broad pH range of 4.5–10.5	It was stable when stored at 4 °C and −20 °C for at least 4 months, even at concentration < 0.1 mg/ml.	The activity increased with rising temperature up to 50 °C and then fell rapidly.
R. opacus [15]	8.0–9.0 with L-Phe as substrate having broad pH range of 4–11	It was stable for several months at 4 °C in 50 mM pH 8.6 glycine/NaOH buffer or at −18 °C with 50% glycerol. Freezing at −18 °C without addition of glycerol-inactivated the enzyme.	It was strongly influenced by the chosen buffer; an improved stability at elevated temperatures could be obtained when using glycine/NaOH buffer.
Morganella morganii [32]	6.35 and 7.4 with L-Leu and L-Phe as substrate, respectively	It was stable at 4 °C, but lost 90% activity at 37 °C for 24 h.	The enzyme exhibited a wide optimum temperature range of 35–43 °C.
Gymnogongrus flabelliformis [33]	8.0–9.0 with L-Arg as substrate; being inactive at pH 5.0	When stored at −20 °C for 2 weeks, the activity was almost completely decomposed; when stored at −25 °C for 2 months, the activity became faint.	The activity was stable below 30 °C, but decreased sharply above 30 °C.
C. cellulans AM8 [19]	6.5–7.5 with L-Phe as substrate	It was stable for several months and some days when stored at −30 °C and 35 °C, respectively.	The activity increased linearly with increasing temperature with a doubling of the activity from 25 °C to 40 °C.
N. crassa [8]	9.5 with L-Phe as substrate; having broad pH range of 3.5–12	Purified enzyme was stable at 4 °C for several weeks.	
Corynebacterium [20]	8.0–9.0 with L-Ala as subtract	It lost 5% to 10% activity when stored at 4 °C for 1 month in the presence of 66 mM KCl and 10 mM 2-mercaptoethanol.	
A. crassissima [21]	8.0–9.0 with L-Arg as substrate		The optimum temperature was about 50 °C. It is stable up to 60 °C for 10 min, but lost all activity at 70 °C for 10 min.
Rat [14]	8.8–9.2 with *S*-adenosyl-L-homocysteine as substrate		It was stable after 5 min at 45 °C, but lost above 51% activity after 5 min at 51 °C and was completely inactivated after 5 min at 71 °C.
Aplysia punctata [34]			It was stable at 0~50 °C. Incubation at 60 °C reduced the activity by 80% and at 70 °C destroyed the enzyme.

Rheinheimera sp [30], and the L-phenylalanine oxidase from *Pseudomonas* sp. P-501 [31]. This "strictness" property makes it an interesting catalyst for enzymatic syntheses.

Structural Properties

As shown in Table 2, LAAOs have a wide range of isoelectric points from 4.0 to 9.4 and molecular mass from around 80 to 300 kDa. In general, LAAO is first synthesized as precursor carrying a signal peptide and then forms a mature protein by post-translational modification through limited proteolysis. This could be due to their potential toxicity [6]. It was speculated that the LAAO activity that catalyzes the stereospecific oxidation of L-amino acids along with the release of ammonia and hydrogen peroxide is toxic for or has negative effect on the growth of host cells. Therefore, LAAO exists in cells as a precursor with low

Table 2 Comparison of LAAOs from different organisms

Organisms	N-terminal sequence	Molecular weight	pI
Cyanobacterium *Synechococcus* PCC6301 [41]	MPYVLSYIRHFKLPLLT	39.2 kDa according to DNA sequence analysis	9.81
M. polyacanthocephalus [42]	VSPKEHLAMCLKDDDYQVLL	56 kDa in SDS-PAGE and 140 kDa by gel filtration	4.96
A. punctata [34]	DGICRNRRQC	60 kDa in SDS-PAGE	4.6
R. opacus DSM43250 [16]	AGDLIGKVKGSHSVVVLGGG PAGLCSAFEL	A dimmer; 99 kDa by gel filtration and 108 kDa by MALDI-TOF analysis	4.8
Mouse milk [43]	LYENLVKXFQDPDYEAFLLI	A dimmer; 60 kDa in SDS-PAGE and 113 kDa by gel filtration	
S. schlegeli [36]	ISLRDNLAD	A homodimer; 0.53 kDa in SDS-PAGE and 120 kDa by gel filtration HPLC	
A. nidulans [22]		49 kDa in SDS-PAGE and 98 kDa by gel exclusion chromatography	
S. oligofermentans [44]		43 kDa in SDS-PAGE	
T. viride [27]		116 kDa comprising two subunits identical in molecular weight about 56 kDa	
C. cellulans AM8 [19]		55 kDa in SDS-PAGE and 300 kDa in native PAGE	
Pseudoalteromonas luteoviolacea [45]		110 kDa	
S. endus [24]		90 kDa by gel chromatography and 50 kDa by SDS-PAGE	6.2
B. carotarum 2Pfa [9]		A dimmer; 102 ~ 115 kDa comprising two identical subunits of 54 kDa.	4.8
P. flavipulchra [46]		60 kDa in native PAGE	9.4
Cyanobacterium *S. cedrorum* PCC 6908 [21]			8.52
Mouse B lymphocytes [47]			4.0

activity first and then is secreted with high activity from cells after digestion by an endopeptidase. For example, the LAAO from *Neurospora crassa* consists of 695 amino acids as a precursor, 129 amino acids larger than the mature enzyme [35]. Similarly, the *Sebastes schlegeli* mature LAAO includes 496 residues that are 58 amino acids fewer than the precursor [36]. The *R. opacus* LAAO gene shows an open reading frame (ORF) coding for 534 amino acids including a signal peptide of 45 amino acids [16].

The structural knowledge about non-snake venom LAAO is fairly limited. This situation has been changed to a certain extent in recent years. In 2006, the crystallization and preliminary X-ray analysis of the first bacterial LAAO from *R. opacus* was reported [37]. So far, the structures of the LAAO from *R. opacus* [5], the L-glutamate oxidase (LGOX) from *Streptomyces* sp. X-119-6 [38], and the L-aspartate oxidase (Laspo) from R386L mutant of *Escherichia coli* [39] have been described. It was found that each LAAO subunit consists of three domains according to the topology as described in snake venom LAAO by Pawelek et al. [40]: an FAD-binding domain, a substrate-binding domain, and a helical domain. The structure of Laspo is very similar with LAAO which includes three domains: the FAD-binding domain with the classic dinucleotide binding fold observed in many flavoproteins, the capping domain with an irregular $\alpha+\beta$ topology, and the helical C-terminal domain. The structure of LGOX also resembles that of LAAO to a certain extent. As shown in Table 3, there are several differences between LGOX and LAAO. Like LAAO [37], D-amino acid oxidase (DAAO) [48], monoamine oxidase (MAO) [38], and polyamine oxidase (PAO) [49] are all flavoproteins as well. The structures of these four enzymes are functionally similar with highly conserved sequence motif for the cofactor binding, showing a quite similar overall topology within the FAD-binding domain. Their substrate binding

Table 3 Structure comparison between LAAO and LGOX

	LAAO from *R. opacus*	LGOX from *Streptomyces* sp. X-119-6
Primary structure	Per monomer consisting of 489 amino acids and a non-covalently bound FAD molecule	Per monomer consisting of 701 amino acids and a FAD
Secondary structure	Being a homodimer with each protomer containing 12 α-helices, 26 β-strands and five 3_{10}-helices that fold into three defined domains	Being an oligomeric dimer with each protomer containing three fragments (αβγ), which interacts in a head-to-tail orientation with the substrate-binding site facing away from the dimmer interface; the chain of each fragment is substantially entangled with the other chains
Entrance to the active site	Missing funnel and the substrate entry to the active site by diffusion	Having two funnel-shaped narrower entrances extending from the surface of the protein into the active site
Active site	The active site of the binary complex is filled with a tight network of water molecules and an aspartate residue (Asp227) is involved in transfer of a proton to the solvent.	Asp227 of LAAO is replaced by Gly in LGOX (Gly316).
Substrate-binding site	The arrangements of many residues composing the substrate-binding sites of both LAAO and LGOX are similar. Differences in terms of the properties of their side chains are apparent in several residues.	
Others	LGOX has three regions (Asp150–Asn192, Ser246–Trp262, and Thr450–Ala480) on the surface that were not found in the LAAO structure.	

domains are also very similar. However, comparison of their structures also displays great differences. First, the structural identity of LAAO to DAAO is around 25%, to MAO 45% and to PAO 39%; second, remarkably, in contrast to the DAAO from yeast with a shallow funnel and the PAO from *Zea mays* with a U-shaped funnel, there is no funnel orientating and directing the substrates to the active site in the LAAO from *R. opacus*. The substrate specificity is probably associated with the funnel conformation and structure of the enzyme. Therefore, the missing funnel may explain the broader substrate specificity of the LAAO from *R. opacus* where the substrate entry relies on diffusion [37]; third, the helical domain of LAAO contains three α-helices and a short two-pleated antiparallel β-sheet. PAO, MAO, and DAAO do not have helical domains; fourth, the LAAO from *R. opacus* lacks glycosylation whereas PAO shows glycosylation as found in the LAAO from *Calloselasma rhodostoma* [40]; fifth, in FAD-binding domain, LAAO, PAO, and MAO all have a conserved region, R-x-G-G-R-x-x-T/S. However, DAAO does not contain such region. In addition, both LAAO and PAO harbor either amino acid residue or side chain amino group in FAD-binding region which can interact with water molecule whereas DAAO lacks a similar residue at the identical position. Thus, if hydrolysis of the imino acid was to be mediated by the protein, this might mean that, in LAAO or PAO and DAAO, it happens through different mechanisms. Indeed, hydrolysis in DAAO is thought to be non-enzymatic.

Gene Cloning and Expression

So far, a great variety of LAAO coding sequences have been published, revealing that LAAO family members have common flavin as coenzyme, except the lysine oxidase of *M. mediterranea* [2] and two characteristic sequence motifs. One is a dinucleotide-binding motif comprising β-strand/α-helix/β-strand of the secondary structure; the other is a GG motif (R-x-G-G-R-x-x-T/S) shortly after the dinucleotide-binding motif [1]. In 1990, the *N. crassa* LAAO gene that is devoid of introns was cloned and sequenced [35]. According to the *Synechococcus* PCC6301 LAAO nucleotide sequence [41], it can be deduced that this protein consists of 355 amino acid residues which show no homologies with the LAAO of *N. crassa* [35]. The cDNA cloning of mouse milk LAAO indicated that the protein consists of 497 amino acids and a signal peptide of 26 amino acids [43]. In 2007, the LAAO gene in the skin mucus of rockfish *S. schlegeli* was cloned, showing a full-length cDNA of 2,037 bp including an ORF of 1,662 bp that encodes 554 amino-acid residues as a precursor [36]. Similarly, cDNA cloning of the LAAO from the skin mucus of *Myoxocephalus polyacanthocephalus* revealed a full-length cDNA with 2,659 bp encoding the signal peptide (Met1–Ala26) and the mature protein (Val28–Phe520), which has 74% identity with the antibacterial LAAO from the skin mucus of the rockfish *S. schlegeli* [42].

The LAAO gene is expressed throughout lactation in the mouse mammary glands, not in the brain, heart, lung, muscle, and placenta. Glucocorticoid is essential for expression [43]. Kitani et al. revealed the dominant expression of the *S. schlegeli* LAAO mRNA in skin and gill, and the weak expression in ovary or kidney [50]. In the case of *N. crassa* [51–54], the expression of LAAO can be induced by addition of L-amino acids to nitrogen-starved cultures as well as upon addition of protein synthesis inhibitors or D-amino acids. Its gene expression is controlled by NIT2 and *nmr* gene product, and LAAO gene is regulated at the transcriptional level.

Probably due to the requirement of post-translational modification of LAAO, only some heterologous expression systems have been reported hitherto. In 2003, the first bacterial

heterologous expression system for an LAAO of *R. opacus* was reported [55]. The *lao*-gene coding LAAO was cloned into different *E. coli* and *Streptomyces lividans* expression vectors. Expression in *E. coli* results in the accumulation of insoluble protein, but *S. lividans* is a relatively suitable host for the heterologous production of LAAO. Comparison of native and recombinant LAAOs showed the same specific activities, similar spectral properties, and the same molecular mass. The LAAO isolated from sea hare was first functionally expressed in *E. coli*, but expression level for soluble recombinant LAAO is relatively low, ca. 0.2 mg/L culture medium [7]. The following reasons can account for this phenomenon. First, this level is for soluble LAAO, while much of the LAAO is present in insoluble inclusion bodies. Second, LAAO inhibits the growth of *E. coli* and other bacteria at doses below 1 mg/L, and this would likely inhibit the level of bacterial expression. It was found that the recombinant LAAO from sea hare in *E. coli* is three to four times less effective against bacteria than the wild-type one. The authors deduced that it might be because the recombinant form contains inactive LAAO. In 2008, the LAAO gene of *Streptococcus oligofermentans* was successfully cloned and overexpressed in the *E. coli* BL21(DE3)pLysS strain [44]. To date, the heterologous expression of LAAO is still a big challenge.

Biological Functional Properties

One of the main biological functions is antimicrobial activity. Some gastropods have LAAO on their surfaces, for example, the albumen gland packaging the egg masses, to prevent colonization by microorganisms [56], or to prevent infections of the skin after an attack [57]. The LAAO found in *S. schlegeli* only has antibacterial activity against specific Gram-negative bacteria, including fish pathogens and a marine bacterium, but not against enteric Gram-negative bacteria [36]. While the *M. polyacanthocephalus* LAAO showed a broad spectrum of antibacterial activity against both Gram-positive and Gram-negative bacteria [42]. The sea hare *Aplysia californica* LAAO inhibited not only the growth of Gram-positive and Gram-negative bacteria, but also the growth of yeast and fungi, with different efficiencies [7]. The *Pseudoalteromonas flavipulchra* LAAO with broad substrate specificity is against methicillin-resistant *Staphylococcus aureus*, which occurs during the stationary phase of the strain growth curve [46]. It is believed that antimicrobial activity of LAAO is probably associated with H_2O_2 formation generated by the enzyme activity because its antimicrobial effect is significantly decreased in the presence of H_2O_2 scavengers such as catalase and peroxidase.

Because of having antimicrobial activity, LAAOs from animals may act as defense or attack weapons via H_2O_2. LAAO as a humoral defense factor was purified from the mucus of the giant snail *Achatina fulica* Ferussac. It can bind to the plasma membranes of growth-phase-specific pathogens to inhibit the bacterial growth by increasing the local concentration of H_2O_2 [58]. The LAAO isolated from the defensive purple ink secretion of the sea hare *A. californica* is only released when a sea hare is attacked by predators, and it has a cytotoxic effect against a predatory sea anemone [7]. Thus, LAAO may play a role in sea hare chemical defense. The LAAO extracted from skin mucus of the rockfish *S. schlegeli* was found to be involved in the innate immunity of fish skin [36]. Further study indicated that binding of LAAO to pathogens may induce the production of highly localized concentration of H_2O_2 in or near the binding interfaces, which induced morphological changes in the bacteria to release its antibacterial effect [59]. As a result, LAAO is significantly less than H_2O_2 itself needed to inhibit the bacterial growth, for example, a minimum inhibitory concentration of 0.2 and 0.25 μg/mL for achacin [60]

and escapin [61], respectively. The LAAO of *S. schlegeli* is as potent as an MIC of 0.078 μg/mL for *Aeromonas salomonicida* [36].

Most LAAOs from microorganisms have broad substrate spectra, appointing them as ideal molecular mechanism for the acquisition of nitrogen from diverse amino acid sources. Thus, LAAOs presented in many microorganisms are functionally linked with other pathways for efficient catabolism of amino acids. It was found that the LAAO of *Neurospora crassus* can be induced by L-Arg and L-Phe in the absence of readily metabolizable nitrogenous compounds [62]. The *Chlamydomonas reinhardtii* LAAO is also inducible if no primary nitrogen source is available to produce ammonia [63]. In 2005, Davis et al. found that the LAAO of *Aspergillus nidulans* is the primary route of catabolism for some amino acids, such as L-Cys, L-His, L-Leu, L-Lys, L-Met, L-α-amino butyrate, and L-citrulline, while other amino acids are metabolized through alternative pathways that yield either ammonium or glutamate for growth [64]. LAAOs expressed in *Hebeloma* spp. and *Laccaria bicolor* can catalyze the mineralization of amino acid nitrogen to NH_4^+ [65]. The released inorganic nitrogen might then be re-assimilated and distributed to organic compounds, which complements the previously identified amino acid catabolic machinery, e.g., NADPH-GDH, aspartate aminotransferase, and alanine aminotransferase. It is assumed that, in general, the bacterial LAAO might be necessary in the nitrogen and L-amino acid metabolism.

The biological roles of the algal LAAO have been ascribed both to amino acid catabolism and to cellular defense. A survey has shown that at least three genera *Pleurochrysis*, *Pryrnnesium*, and *Arnphidinium* have cell-surface LAAOs which metabolize a wide spectrum of free amino acids and allow for growth with amino acids as the nitrogen source [66]. The LAAOs isolated from algae are believed to be responsible for controlling amino acid distribution in natural water where these organisms are found. Extensive accumulation of an extracellular LAAO during gametogenesis of *C. reinhardtii* may operate in vivo as an efficient scavenger of ammonium, but high ammonium concentration (≥5 mM) totally represses amino-acid oxidase [67]. There is interplay between the metabolisms of nitrogen and carbon. Reduced carbon and metabolic energy availability seems to be a pre-requisite for induction of amino-acid oxidase. However, Weinberger et al. pointed out that the primary role of the *Chondrus crispus* LAAO is to control the infection by its filamentous endophytic alga *Acrochaete operculata*, not nitrogen sequestration [68].

In addition, the LAAO of *S. oligofermentans* seems to have a role in competition between species [44]. A function of LAAO from cyanobacteria *A. nidulans* is for electron transfer on the thylakoid membrane [69]. The LAAO found in insects is cytotoxic, killing tumors cells by generation of H_2O_2 [6]. Similarly, the L-tryptophan oxidase isolated from *Chromobacterium violaceum* was thought to be involved in the biosynthesis of violacein which is believed to have bactericidal and tumoricidal activities [70]. The L-phenylalanine oxidase as an immunosuppressive enzyme expressed in a series of 315 human malignancies could be a general mechanism affecting the relation of tumor cells with the immune system [71]. It was reported that the mouse milk LAAO can kill bacteria in the mammary gland and is responsible for preventing mastitis [43]. Moreover, α-keto acids, the products of the LAAO reaction, seem to function as siderophores, forming complexes with iron, as in *Proteus mirabilis* [72].

Current and Potential Applications

Bleaching agents incorporated into detergent compositions are most often precursors of hydrogen peroxide. A novel LAAO of *Trichoderma harzianum* in conjunction with its

substrate has be applied for in situ generation of hydrogen peroxide and may be advantageously incorporated, together with a substrate, into detergent compositions which comprise also a peroxidase system for inhibiting the transfer of dye from dyed fabric to other fabrics during washing [73]. It also can be mixed with toothpaste or used for cosmetic preservation. Moreover, enzyme has been mentioned in applications for wastewater treatment, pulp bleaching in the paper industry, and water treatment in the pulp production and lignin improvement.

Another field of application of LAAOs is as biosensors. In such applications, the LAAOs are usually coupled with a detector of the oxygen consumed [74] or the hydrogen peroxide produced [75]. LAAO has been used as biosensors in food industry, in order to determine the quality of many products by their L-amino acid content [76, 77], especially determination of L-Lys in maize, rice and wheat. The LAAO of the black rockfish *S. schlegeli* has been proposed for sensing L-Lys [78]. In food processing, LAAO can be applied in the enzymatic treatment for dough to reduce its viscosity and provide a better workability [79].

The LAAO is also well-known used for biotransformations including the industrial production of α-keto acids and the separation of enantiomers from racemic mixtures of amino acids [80, 81]. The LAAO of *Rhodococcus* sp. is successful in the bioconversion to synthesize aminoadipic derivatives that are precursors for β-lactam antibiotics [82]. The *Providencia alcalifaciens* LAAO has been found to be applied as catalysts in biotransformation [83].

The fungal L-lysine oxidase has been tested in vivo on several tumors with good results [84]. The studies proved the efficacy of sequential pretreatment with LAAO and LAAO antiserum in the modulation of melphalan activity against intracranial glioma [85]. In addition, a LAAO from an ocean fish, *Chub mackerel*, infected with *Anisakis simplex* had dose-dependent apoptotic activity on a variety of mammalian tumor cells, further displaying its potential for therapeutic application as an antitumor compound [86]. Mastitis is caused by an intramammary bacterial infection. A LAAO found in mouse milk together with free amino acids was speculated to be responsible for killing bacteria in the mammary gland, indicating the potentials of therapeutic agent [43]. Investigators proposed an application of the red seaweed *C. crispus* produced LAAO for controlling the pathogen, the green algal endophyte *A. operculata* Correa and Nielsen [68]. LAAOs that show not only antibacterial and antipathogen activity but also the ability to release nitrogen from amino acids [65] are present in many soil microorganisms that exhibit different lifestyles. Therefore, LAAO-producing microorganisms are potential candidates for biological control at the ecosystem level.

Conclusion

LAAOs form a family of proteins with various enzymatic properties, similar overall structure, different biological functions, and wide applications. Unlike snake venom LAAOs which have been widely and deeply investigated to show broad bioactivities such as apoptosis, cytotoxicity, edema, hemolysis, hemorrhage, platelet aggregation, parasite-killing activity, and antimicrobial activity, non-snake venom LAAOs need to be further study and their functional roles and applications remain to be revealed. The pharmacological mechanisms of these proteins are not well understood, and their products have not secured a source for further development as a drug. Therefore, further studies are required to arrive at a better understanding of non-snake venom LAAO and to explore the proper formulation in applications in order to address the commercialization of LAAO.

Acknowledgment We thank Professor J. Tze-Fei Wong at Hong Kong University of Science and Technology for critical reading of this manuscript and anonymous reviewers for their comments on the manuscript. This work was supported by Natural Science Foundation of Zhejiang Province, China (Y5100153), Science and Technology Planning Project of Zhejiang Province, China (Welfare Technology Applied Research Project of Zhejiang Province, grant no.: 2011C23007), Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry and Natural Science Foundation of ZJUT to Z Yu.

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