MINI-REVIEW

Antimicrobial properties of L-amino acid oxidase: biochemical features and biomedical applications

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Abstract

Mucus layer that covers the body surface of various animal functions as a defense barrier against microbes, environmental xenobiotics, and predators. Previous studies have reported that L-amino acid oxidase (LAAO), present in several animal fluids, has potent properties against pathogenic bacteria, viruses, and parasites. LAAO catalyzes the oxidative deamination of specific L-amino acids with the generation of hydrogen peroxide and L-amino acid metabolites. Further, the generated hydrogen peroxide is involved in oxidation (direct effect) while the metabolites activate immune responses (indirect effect). Therefore, LAAO exhibits two different mechanisms of bioactivation. Previously, we described the selective, specific, and local oxidative and potent antibacterial actions of various LAAOs as potential therapeutic strategies. In this review, we focus on their biochemical features, enzymatic regulations, and biomedical applications with a view of describing their probable role as biochemical agents and biomarkers for microbial infections, cancer, and autoimmune-mediated diseases. We consider that LAAOs hold implications in biomedicine owing to their antimicrobial activity wherein they can be used in treatment of infectious diseases and as diagnostic biomarkers in the above-mentioned diseased conditions.

Key points
•*Focus on biochemical features, enzymatic regulation, and biomedical applications of LAAOs.*
•*Mechanisms of antimicrobial activity, inflammatory regulation, and immune responses of LAAOs.*
•*Potential biomedical application as an antimicrobial and anti-infection agent, and disease biomarker.*

Keywords Antimicrobial protein · L-amino acid oxidase · Interleukin (IL)-4-induced gene 1 · Hydrogen peroxide · Metabolites · Biomarker

Introduction

L-amino acid oxidase (LAAO; EC: 1.4.3.2) is a flavoenzyme that catalyzes the oxidative deamination of an L-amino acid to an α-keto acid with the generation of ammonia and hydrogen peroxide (Wellner and Meister 1961). Previously, we described the bioactivities of LAAOs, including their antibacterial activity (Kasai et al. 2015b) that can be inhibited by catalases, suggesting that their antibacterial potential is due to the hydrogen peroxide produced during the reaction.

Hydrogen peroxide is a powerful oxidizing agent that acts as an intracellular signaling molecule and is involved in the oxidative bursts of phagocytes, thereby leading to the elimination of invading microorganisms (Clifford and Repine 1982). Briefly, hydrogen peroxide is an antimicrobial agent that induces DNA damage and cell death and is attributed to direct cytotoxicity and generation of reactive oxygen species (ROS) (Ueda and Shah 1992). This molecule regulates cell proliferation, growth arrest, apoptosis, and necrosis in various organisms (Vatansever et al. 2013). Owing to their diverse properties, LAAOs hold potential applications in detection and treatment of several diseased conditions, such as microbial infections, cancer, and autoimmune diseases. In this review,

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we have elaborated the biochemistry of LAAOs and focused on their potential applications in the field of biomedicine. This comprehensive overview of the current state of the LAAOs studies may facilitate the development of novel strategies for the diseased conditions.

Biochemical features of LAAOs and their mechanism of action

LAAOs are found in various animal fluids, including serum, snake venom, sea hare ink, body surface mucus of the snail and fish, mammary milk, and tissue extracts (mammary gland, intestine, liver, and kidney). Reportedly, several LAAOs have characteristic functions and excellent bioactivity in various species (Table 1). Further, Fig. 1 illustrates the molecular phylogenetic tree of LAAOs from various organisms. LAAOs are involved in innate immunity with selective and specific antibacterial properties (Kasai et al. 2015a (Table 1 V1), Kasai et al. 2015b (Table 1 V1), Teixeira et al. 2016 (Table 1 M2), Lazo et al. 2017 (Table 1 G), Rey-Suárez et al. 2018 (Table 1 N), Salama et al. 2018 (Table 1 L), Elsheimer-Matulova et al. 2020 (Table 1 D), Soares et al. 2020 (Table 1 E)), antiparasites (Carone et al. 2017 (Table 1 H2), Wiezel et al. 2019 (Table 1 M1), Soares et al. 2020 (Table 1 E), Barbosa et al. 2021) and induce DNA fragmentation (Mukherjee et al. 2015, Burin et al. 2016a, 2016b, Machado et al. 2018 (Table 1 H2), Machado et al. 2019a, 2019b (Table 1 H2)), apoptosis (Mukherjee et al. 2015, Fung et al. 2015 (Table 1 O), Burin et al. 2016a, 2016b, Ribeiro et al. 2016, Tan et al. 2017, Carone et al. 2017 (Table 1 H2), Machado et al. 2018 (Table 1 H2), Mukherjee et al. 2018, Rey-Suárez et al. 2018 (Table 1 N), Bedoya-Medina et al. 2019 (Table 1 N)), regulation of immune responses (Cousin et al. 2015, Scarlata et al. 2015, Romagnani 2016, Bod et al. 2018, Ramspott et al. 2018 (Table 1 A), Aubatin et al. 2018 (Table 1 A), Elsheimer-Matulova et al. 2020 (Table 1 D), Sadik et al. 2020), and cell cycle arrest (de Melo Alves Paiva et al. 2011 (Table 1 F), Machado et al. 2019a, 2019b (Table 1 H2)).

The mechanism of LAAOs' antibacterial action has been investigated extensively and the most prevalent mechanism is via generation of hydrogen peroxide, as described above. An early report showed that LAAO isolated from *Crotalus adamanteus* venom to be effective against bacteria (Skarnes 1970). Many previous reports have highlighted that the bactericidal activity of LAAOs is generally inhibited by catalases (Stiles et al. 1991, Stábeli et al. 2007, Nagashima et al. 2009 (Table 1 U), Kasai et al. 2015a (Table 1 V1), Soares et al. 2020 (Table 1 E)), thereby the antibacterial action of LAAOs is due to the generated hydrogen peroxide.

Mammalian LAAO, which is capable of generating ROS upon exposure to an aromatic and hydrophobic amino acid, such as L-Trp or L-Phe in the presence of oxygen, has been found in milk (cow, donkey, and mouse), expressed immune cells (human, mouse, and chicken). Mammalian LAAO, which has an immunoregulatory function, was first detected in murine B cells (Chu and Paul 1997) and has been known to inhibit T cell proliferation (Boulland et al. 2007), cytotoxicity, and IFN-γ production by tumor-infiltrating CD8$^+$ T cells, which favors tumor escape. Human LAAO, encoded by interleukin (IL)-4-induced gene 1 (*IL4I1*), is secreted and expressed by macrophages (Mφ) and dendritic cells (DCs) stimulated by microbial-derived products or interferons, which are endowed with immunoregulatory properties, and block the growth of gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* (Puiffe et al. 2013).

Interestingly, Il4I1 is found in the mammalian spermatozoa head (human, equine, and bovine), particularly in the acrosomal. The aromatic amino acids were substrates for equine sperm LAAO, eliciting the dose- and time-dependent generation of ROS via mechanisms that were enhanced by cell death (Aitken et al. 2015). The combination of aromatic amino acids and nonviable cells was also found to enhance the levels of lipid peroxidation in live spermatozoa. Houston et al. (Table 1 A) reported that the stimulation of LAAO activity results in the induction of several hallmarks of capacitation, including tyrosine phosphorylation of the sperm flagellum and concomitant activation of phospho-SRC expression, that are important in the regulation of growth and differentiation of eukaryotic cells (2015). In addition, stimulation of IL4I1 resulted in an increase in the levels of acrosomal exocytosis in both the presence and absence of progesterone stimulation via a mechanism that could be significantly reversed by the presence of catalase. Their results suggested that the existence of human spermatozoan LAAO has a potential role in driving the redox regulation of sperm capacitation and acrosomal exocytosis (Houston et al. 2015 (Table 1 A)).

On the contrary, mice have two isoforms of LAAO encoded by *Lao1* or *Il4i1* that are localized and expressed in lactating mammary glands during the lactation period and immune cells by microbial-derived products or interferons, respectively, similar to humans. LAO1 present in milk protects against bacterial infections by producing hydrogen peroxide (Sun et al. 2002 (Table 1 C), Nagaoka et al. 2009 (Table 1 C)). Recently, gut microbiota profiles were compared between wild type and *Lao1*-knockout mouse pups to determine whether LAO1 affects infant gut microbiota development via mother's milk consumption (Shigeno et al. 2019). In particular, the gut microbiota of lactating *Lao1*-knockout pups was highly diverse, whereas those of the wild type were composed of only a few dominant bacteria, such as *Lactobacillus* sps. Furthermore, cross-fostering indicated that milk of wild type mothers has the ability to suppress this diversity in *Lao1*-knockout pups. Moreover, the stomach contents of pups fed milk containing LAO1 could produce hydrogen peroxide. They suggested that hydrogen peroxide is

Table 1 Bioactive properties of LAAOs from several sources

Source of LAAO: Serial symbol: species	Protein name	Chemical features, function, and bioactivity: Chemical characterization (location)	Optimal condition (pH, temp.) and regulation of enzymatic activity	Function and bioactivity	Substrate specificity	UniProtKB/Swiss-Prot, GeneID, Refseq, PDB	Reference
A: *Homo sapiens*	IL4I1	MW = 63 kDa, N-glycosylated secreted flavoprotein (sperm, immune cells)		Bactericidal activity, tumor escape, inhibits T-lymphocyte proliferation, redox regulation of sperm	Phe > Trp > Asp > leu > others	Q96RQ9, 259307, NP_690863.1, 2I8H	Ramspott et al. (2018), Aubatin et al. (2018), Houston et al. (2015), others
B: *Mus musculus*	Fig. 1 (Il4i1)	MW = 63 kDa, N-linked glycosylated flavoprotein (B lymphocytes)	pH4.0, Inhibited by aromatic competitors (benzoic acid and para-aminobenzoic acid)	Immune response	Phe > Trp > others	O09046, 100328588, NP_001164495.1, 2I8W	Mason et al. (2004)
C: *Mus musculus*	Lao1	MW = 58 kDa, flavoprotein (milk, mammary gland > small intestine > others: thymus, spleen, salivary gland, testis)		Bactericidal activity, innate immunity of mammary glands	Leu, Phe, Met, and Tyr	B1ARV3, 100470, NP_598653.3	Sun et al. (2002), Nagaoka et al. (2009), Fujii et al. (2015)
D: *Gallus gallus*	IL4I1	MW = 59 kDa, flavoprotein (macrophages, granulocytes, CD4$^+$ and $\gamma\delta$ T-lymphocytes)		Bactericidal activity, control of inflammatory response, not directly bactericidal activity of phagocytes		F1NM87, 417039, NP_001092821.2	Elsheimer-Matulova et al. (2020)
E: *Micrurus lemniscatus*	ML-LAAO	MW = 69 kDa, monomer, glycoprotein (venom)	Optimal activity at pH 8.5	Bactericidal activity, parasiticidal activity	Try > Trp > Leu > Phe > Met > others		Soares et al. (2020)
F: *Bothrops atrox*	BatroxLAAO	MW = 57 kDa, glycosylated flavoprotein (venom)	Stabilized by Zn^{2+}	Bactericidal activity, parasiticidal activity, caspase-mediated apoptosis, cell cycle arrest evidence	Met > Leu > Phe > Trp > Tyr > Ile > others	P0CC17, 5TS5	de Melo Alves Paiva et al. (2011), Feliciano et al. (2017)
G: *Bothrops pictus*	Bpic-LAAO	MW < 65 kDa, homodimeric glycosylated flavoprotein (venom)	pH 8.5 (7.0–10), 35–45 °C, stabled until 55 °C. Inhibit by Zn^{2+}	Bactericidal activity	Leu	X2L4E2	Lazo et al. (2017)
H1: *Bothrops jararacussu*	BjussuLAAO-I (BjsuLAAO)	MW = 54 kDa, pI5.7, flavoprotein (venom)		Antiprotozoal effects	Met > Leu > Phe > Ile > Tyr > Trp > Cys > others	Q6TGQ9, 4E0V	França et al. (2007), Ullah et al. (2012), França et al. (2007), Ullah et al. (2012)
H2: *Bothrops jararacussu*	BjussuLAAO-II	MW = 60 kDa, pI3.9, glycosylated flavoprotein (venom)	pH 7.0, 65 °C, inhibited by Zn^{2+}, Al^{2+}, Cu^{2+}, Ni^{2+}	Antiprotozoal effects, tumor resistance, cytotoxicity effects, cytotoxicity and apoptosis in MCF-7, but not MCF10A. DNA	Met > Leu > Ile > Phe >		Costa et al. (2018), Carone et al. (2017), Machado

Table 1 (continued)

Source of LAAO: Serial symbol: species	Protein name	Chemical features, function, and bioactivity			Substrate specificity	UniProtKB/ Swiss-Prot, GeneID, Refseq, PDB	Reference
		Chemical characterization (location)	Optimal condition (pH, temp.) and regulation of enzymatic activity	Function and bioactivity			
				methylation of cell-cycle genes in HepG2, upregulates the inflammatory genes, downregulates the apoptotic-related genes	Tyr > others		et al. (2018), Machado et al. (2019a, 2019b)
I: Bothrops moojeni	BmooLAAO-I	MW = 65 kDa, homodimeric glycoslyated flavoprotein (venom)	pH 7.0–8.0, 60 °C, inhibited by Zn^{2+}, Al^{2+}, Cu^{2+}, Ni^{2+}	Bactericidal activity, trypanocidal, antitumor effects	Met > Leu > Ile > Phe > Tyr > others	Q6TGQ8	Stábeli et al. (2007), Costa et al. (2018)
J: Bungarus multicinctus	BM-Apotxin	MW = 65 kDa, homodimeric glycoslyated flavoprotein (venom)	pH 8.4, 45 °C, deglycoslyation can significantly reduce the oxidation and antitumor activity	Antitumor effects, selectively kill tumor, with less cytotoxicity to the normal cells	Met > Leu > Phe > Ile > Tyr > Pro > others	A8QL51, ABN72539.1	Lu et al. (2018)
K1: Cerastes cerastes	CC-LAAO	MW = 58 kDa, homodimeric (115 kDa), glycosylated flavoprotein (venom, venom gland)	pH 7.8, 50 °C	Thermoactive	Met > Leu > Phe > Trp > Ile > Tyr > Val > His > others	X2JCV5, AH-N53388.1,	Abdelkafi-Koubaa et al. (2016)
K2: Cerastes cerastes	Cc-LAAOI/II	MW = 60 kDa, 56 kDa/60 kDa, 53 kDa, heterodimeric (115 kDa) flavoprotein (venom)	pH 7.8, 50 °C/pH 7.0, 60 °C, increased by Mn, inhibited by Zn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, Al^{2+}	Thermally stable up to 70 °C	Arg > Met > Phe > Leu > Tyr > Ile > Trp		El Hakim et al. (2015)
L: Cerastes vipera	Cv-LAAO	MW = 60 kDa, homodimeric (120 kDa) flavoprotein	Inhibited by Co^{2+}, Ni^{2+}, Hg^{2+} and Cu^{2+}	Bactericidal activity, breast cancer cell MCF-7 (IC50:2.75 ± 0.38 μg/mL)	Met > Leu > Phe > Try > Arg > Ile > Trp > others		Salama et al. (2018)
M1: Crotalus durissus terrificus	bordonein-L	MW = 59 kDa, homodimeric (101 kDa) glycosylated flavoprotein (venom)	pH 7.0		Met > Leu > Trp > Phe > Ile > others	C0HJE7	Wiezel et al. (2019)
M2: Crotalus durissus terrificus	LAAOcdt	MW = 113 kDa, pI9.0 (venom)	pH 7.4, 44 °C	Bactericidal activity, hemotoxicity, antiproliferative effect, cytotoxic activity on human cancer cell lines did not induce apoptosis, upregulated p-H2AX, decreased P42/P44 (ERK) activation	Phe > Tyr > Cys > Leu > others		Teixeira et al. (2016)
N: Micrurus mipartitus	MipLAAO	MW = 59 kDa, monomeric flavoprotein (venom)	pH 8.0	Bactericidal activity, specifically induces caspase3-mediated apoptosis in ALL, but not in PBL	Trp > Leu > Tyr > others	A0A2U8QPE6, AW-M11661.1	Bedoya-Medina et al. (2019), Rey-Suárez et al. (2018)

Table 1 (continued)

Source of LAAO Serial symbol: species	Protein name	Chemical features, function, and bioactivity		Function and bioactivity	Substrate specificity	UniProtKB/Swiss-Prot, GeneID, Refseq, PDB	Reference
		Chemical characterization (location)	Optimal condition (pH, temp.) and regulation of enzymatic activity				
O: Ophiophagus hannah	Oh-LAAO	MW = 70 kDa, homodimeric (150 kDa), pI4.5, glycosylated flavoprotein (venom)		Bactericidal activity, antitumor effects, cytotoxicity and specifically induces caspase 8/9-mediated apoptosis in human tumor, but not normal, thermal stability	Lys, Leu, Arg, and Met	P81383, ABN72538.1	Ahn et al. (1997), Lee et al. (2011), Fung et al. (2015), others
P: Achatina fulica	Achacin	MW = 56 kDa, glycosylated flavoprotein (mucus)		Bactericidal activity, tumoricidal activity	Met > Leu > Trp > Lys > Arg > Phe > Cys > Asn > others	P35903, CAA45871.1	Ehara et al. (2002)
Q: Aplysia californica	Escapin	MW = 60 kDa flavoprotein (ink gland, opaline)		Bactericidal activity	Arg > Lys > Tyr > others	Q6IWZ0, AAT12273.1, NP_001191570.1	Yang et al. (2005)
R: Aplysia punctata	APIT	MW = 60 kDa, glycosylated flavoprotein (purple ink)		Tumoricidal activity, apoptosis-inducing activity	Lys > Arg > others	Q6T277, AAR14185.1	Butzke et al. (2005)
S: Epinephelus akaara	LAAO	MW = 67 kDa, 124 kDa, 256 kDa, 450 kDa, secreted flavoprotein (serum)		Bactericidal activity, upregulated by blood less in the kidney	Trp > Met > Phe > Ala > Leu > others		Osaka and Kitani (2021)
T: Gadus morhua	GmLao	MW = 56.2 kDa (monomer), pI5.85 (skin, gill, spleen, head kidney, liver)		Induce by bacterial infection	Lys	A0A0C6EYL8, BAQ36070.1	Kitani et al. (2015)
U: Myoxocephalus polyacanthocephalus	MPLAO3			Bactericidal activity	Lys	B5U982, BAG72078.1	Nagashima et al. (2009)
V1: Platichthys stellatus	psLAAO1	MW = 52 kDa, pI5.3, olygomeric non-glycosylated secreted flavoprotein, (skin mucus, gill, serum)	Stabilized by Mg^{2+}, may be inhibited by Zn^{2+}	Bactericidal activity	Lys > Leu > Tyr > others (date not published)	D2KWB6, BAI66016.1	Kasai et al. (2010), Kasai et al. (2015a), Kasai et al. (2020)
V2: Platichthys stellatus	psLAAO2	Flavoprotein (skin tissues)				D2KWB7, BAI66017.1	Kasai et al. (2010)
V3: Platichthys stellatus	psLAAO3	Secreted flavoprotein (liver)		Bactericidal activity (date not published)	Lys > others (date not published)	D2KWB8, BAI66018.1	Kasai et al. (2010)
W: Scomber japonicus	AIP		pH 7.4, 70 °C, inhibited by Co^{2+},	Apoptosis-inducing activity, dependent on infection of the *C. mackerel* with the larval	Lys > others	Q9I8A7, CAC00499.1	Jung et al. (2000), Tani et al. (2015)

Table 1 (continued)

Source of LAAO		Chemical features, function, and bioactivity				UniProtKB/Swiss-Prot, GeneID, Refseq, PDB	Reference
Serial symbol: species	Protein name	Chemical characterization (location)	Optimal condition (pH, temp.) and regulation of enzymatic activity	Function and bioactivity	Substrate specificity		
		MW > 50 kDa, homodimer (133.9 kDa) flavoprotein (visceral extracts)	stable below 60 °C and at pH range of 5.5–7.5	nematode, Anisakis simplex			
X: *Sebastes schlegeli*	SSAP	MW = 53 kDa, dimeric (120 kDa) flavoprotein (skin, gill, stomach, intestine, spleen, kidney)		Bactericidal activity	Lys > others	A1IGW6, BAF43314.1	Kitani et al. (2007a), Kitani et al. (2007b), Kitani et al. (2008)

Fig. 1 Phylogenetic tree of *LAAO* from different vertebrates. The tree is constructed by neighbor-joining (NJ) method using *LAAO* sequences with the bootstrap values indicated (1000 replicates). Bootstrap values (≥ 50) are shown on the branch points. Scale bar represents 0.1 nucleotide substitutions per site
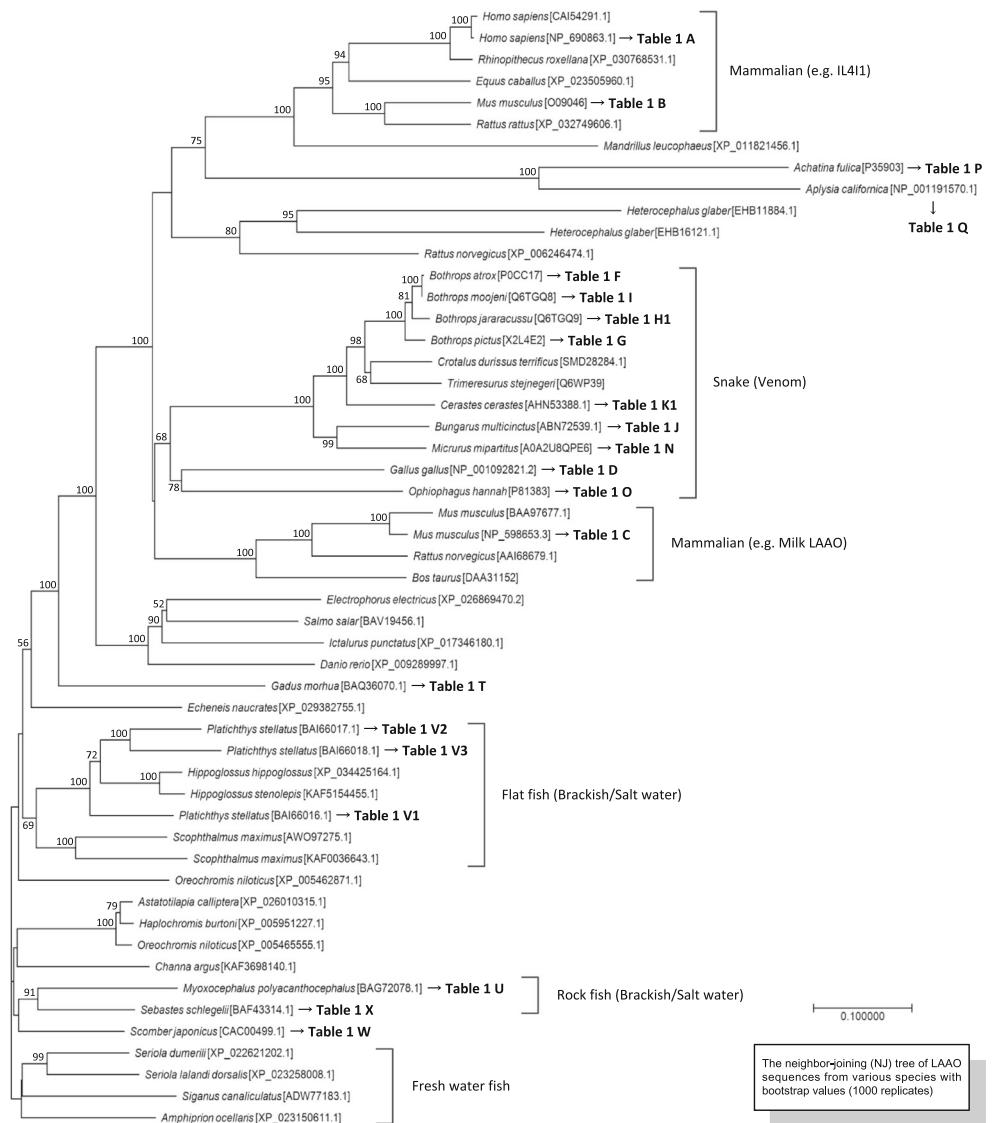
generated from the process of free amino acid metabolism in the mother's milk and may play a key role in regulating initial acquisition and development of the gut microbiota. Conclusively, LAO1-containing milk protects organs from bacterial infection via innate immunity.

Snake LAAOs are a major component of their venom and exhibit bactericidal (Lazo et al. 2017 (Table 1 G), Salama et al. 2018 (Table 1 L), Rey-Suárez et al. 2018 (Table 1 N)), parasiticidal (Carone et al. 2017 (Table 1 H2), Wiezel et al. 2019 (Table 1 M1), Barbosa et al. 2021), and anticancer effects through caspase activation (Fung et al. 2015 (Table 1 O), Mukherjee et al. 2015, Burin et al. 2016a, 2016b, Carone et al. 2017 (Table 1 H2), Tan et al. 2017, Mukherjee et al. 2018, Machado et al. 2018 (Table 1 H2)). CR-LAAO, isolated from *Calloselasma rhodostoma* venom, has antitumor potential and induces acute inflammatory responses similar to mammalian LAAO in vivo, with recruitment of neutrophils and release of IL-6, IL-1β, leukotriene

B4 (LTB$_4$), and prostaglandin E2 (PGE$_2$). In vitro, IL-6 and IL-1β production by peritoneal macrophages stimulated by CR-LAAO was dependent on the activation of the Toll-like receptors, TLR2 and TLR4 (Costa et al. 2017). CR-LAAO and several other LAAOs promote apoptosis of tumor cells mediated by the release of hydrogen peroxide and activation of immune cells, resulting in oxidative stress and production of cytokines, such as IL-6 and IL-1β, which trigger a series of events, such as activation of caspase (-8, -9, and -3) cascade, and modulate cell cycle delay or arrest in the G0/G1 and S phases of tumor cells (Costa et al. 2017). Moreover, CR-LAAO alters regulation of apoptosis by modulating expression of miRNAs related to anti-apoptotic genes (*Bid*, *Bim*, *Bcl-2*, *Cip-2*, *c-Flip*, and *Mcl-1*) in BCR-ABL$^+$ cells (Burin et al. 2016a). They suggested that CR-LAAO is a potential tool for enhancing apoptosis-related miRNA expression in chronic myeloid leukemia therapy (Burin et al. 2016a; Burin et al. 2016b).

Previously, we reported that 2.5 μg/mL of psLAAO1 isolated from the flounder *Platichthys stellatus*, which favors L-Lys as a substrate, produced approximately 2.7 mM of hydrogen peroxide within 1 h in the bacterial culture media (Kasai et al. 2015a (Table 1 V1)). This result suggests that sufficient levels of hydrogen peroxide can be produced by the action of psLAAO1 during the early bacterial growth phase to kill the bacteria under liquid culture conditions. Interestingly, psLAAO1 and other LAAOs (Achacin, OH-LAAO, SSAP, and CC-LAAO) bind to the bacterial membrane, which leads to localized high concentrations of hydrogen peroxide and antibacterial action in or near the bacterial surface (Ehara et al. 2002 (Table 1 P), Lee et al. 2011, Kitani et al. 2008 (Table 1 X), Kasai et al. 2015a (Table 1 V1), Abdelkafi-Koubaa et al. 2016). In summary, LAAOs selectively and specifically bind to sensitive bacteria and exhibit their antibacterial effect by exposing the cells to high, localized concentrations of hydrogen peroxide near the bacterial surface.

Characterization, structural stability, and regulation by metal ions

Recently, it has been reported that snake LAAOs have unique characteristics, namely, wide range of optimal pH, resistance to high temperatures, and structural conformation for enzymatic stability via regulation by metal ions. LAAOs from the venom of *Bothrops jararacussu* (BjussuLAAO-II) and *Bothrops moojeni* (BmooLAAO-I) showed high enzymatic activity in a wide temperature range of 25 to 75 °C with broad range pH and high optimum temperature (pH 6.0 to 9.0 at 65 °C and pH 5.5 to 9.5 at 60 °C).

Metal ions (Zn^{2+}, Al^{3+}, Cu^{2+}, and Ni^{2+}) negatively modulate the enzymatic activity of the aforementioned LAAOs (Costa et al. 2018 (Table 1 I)). A homodimeric Cv-LAAOI from the venom of *Cerastes vipera* has antibacterial, antiproliferative, and cytotoxic effects via extensive hydrogen peroxide production and optimum enzymatic activity on L-Leu at 50 °C. Moreover, the enzymatic activity is enhanced by Mn^{2+}, while Cu^{2+}, Hg^{2+}, Ni^{2+}, and Co^{2+} have suppressive effects on the oxidative activity (Salama et al. 2018 (Table 1 L)). LAAO from Peruvian *Bothrops pictus* (Bpic-LAAO), which is a homodimeric glycoprotein, exhibited optimal activity at pH 8.5 and remained stable up to 55 °C (Lazo et al. 201 7 (Table 1 G)). The kinetic activity of Bpic-LAAO was inhibited by Zn^{2+}, while Ca^{2+}, Mg^{2+}, and Mn^{2+} ions induced no significant change. Furthermore, enzymatic activity of Cc-LAAOI and Cc-LAAOII, purified from *Cerastes Tomato* venom, was increased by Mn^{2+}, while Zn^{2+}, Ni^{2+}, Co^{2+}, Cu^{2+}, and Al^{3+} ions markedly inhibited the activities of both isoforms. Notably, the optimal temperature of both enzymes remains stable up to 70 °C (El Hakim et al. 2015). They suggested that the thermoactivity of Cc-LAAO is related to the hydrogen bond

between Ser73 and Gly70, which is an external loop consisting of residues 64–73 for contact with the FAD cofactor and might act as an energy barrier to adopt an optimal conformation for the catalytic activity. Therefore, the optimal temperature for Cc-LAAO can be surpassed at elevated temperatures. Moreover, the crystal structure and molecular dynamics simulations of LAAO from the venom of *Bothrops atrox* (BatroxLAAO) presented a dimeric arrangement that can be stabilized by Zn^{2+} (Feliciano et al. 2017 (Table 1 F)). These results suggest that metal coordination is critical for dimerization and eventually rendering effective catalytic activity.

We recently reported that psLAAO1 pre-treated with a chelating agent showed transiently increased antibacterial activity that was either decreased or diminished after one or two freeze–thaw cycles. Moreover, the mutant constructs of His348, whose position is predicted to be a metal coordination site by homology modeling simulation, did not show antibacterial activity or Mg^{2+} coordination (Kasai et al. 2020 (Table 1 V1)), indicating that Mg^{2+} coordination is essential for the enzymatic activity and structural stability of psLAAO1 at His348. Metal detection assay revealed that pre-chelated psLAAO1 did not detect Zn^{2+}, whereas 1 mole of the wild type or mutant constructs (His348Ala and His209Ala) detected approximately 0.5 mole of Zn^{2+}. These results suggest that Zn^{2+} does not coordinate with monomeric psLAAO1 and that the metal ion probably inhibits its enzymatic activity. Therefore, metal removal can potentially increase the enzymatic activity or vulnerability of LAAOs via changes in structural complex and conformation.

Immunoregulatory responses mediated by LAAOs

Human Mφ and DCs are major producers of L-Phe-catabolized IL4I1 under inflammatory conditions; B cells also express IL4I1 in response to NF-κB-activating stimuli (Marquet et al. 2010). IL4I1 inhibits the proliferation of CD3-stimulated T cells with a similar effect on $CD4^+$ and $CD8^+$ T cells via mature DCs. Notably, IL4I1 exerts inhibitory effects via its oxidative activity; these effects are associated with a transient downregulation of T cell receptor zeta (TCRζ) expression (Boulland et al. 2007). According to another study, the expression of IL4I1 has been reported in Th17 cells and a subset of $FOXP3^+$ regulatory T (Treg) to Th17 transdifferentiating cells under inflammatory conditions (Scarlata et al. 2015). Moreover, IL4I1 expression is restricted to cells that do not express Helios, a transcription factor that characterizes natural Treg (nTreg) cells, but Aiolos, which is involved in the differentiation of Th17 and induced Treg (iTreg) cells. Supposedly, the expression of IL4I1 in Th17 and iTreg cells may provide insights into approaches that

aim to modulate the balance of these populations in different pathological conditions involving inflammation-mediated immunosuppression, such as rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis. In fact, previous reports have suggested that the human IL4I1 ortholog is located on chromosome 19q13.3–19q13.4 (Chavan et al. 2002) in a region that is a hot spot for autoimmune disease susceptibility in general, including rheumatoid arthritis, multiple sclerosis, insulin-dependent diabetes mellitus, and systemic lupus erythematosus (Mason et al. 2004 (Table 1 B)). Therefore, an alteration in expression of IL4I1 could contribute to autoimmune disease, and its expression screening is a potential biomarker of inflammation-mediated immunosuppression.

Recently, IL4I1, which uses L-Phe as well as L-Trp as a substrate, activates the aryl hydrocarbon receptor (AHR) through the generation of indole metabolites and kynurenic acid (KynA) (Sadik et al. 2020) where the latter constitutes an important endogenous AHR ligand (Platten et al. 2019). In humans, L-Trp catabolism is initiated by indole-amine-2,3-dioxygenase 1/2 (IDO1/2) or tryptophan-2,3-dioxgenase (TDO2). Cancers express high levels of IDO1 and TDO2, taking advantage of L-Trp catabolite-mediated AHR activation. The AHR–KynA axis enhances the malignant phenotype of cancer cells, particularly cancer cell motility. Moreover, AHR–KynA signaling suppresses T cell proliferation and function by inducing differentiation of Treg cells, expression of programmed cell death protein 1 (PD1) on CD8$^+$ T cells, and recruitment of immunosuppressive tumor-associated macrophages. These results suggest that IL4I1 promotes tumor progression as a metabolic consequence of immune checkpoints.

Recently, Elsheimer-Matulova et al. (Table 1 D) reported that IL4I1 from ISA brown chicken (*Gallus gallus domesticus*) is expressed in the cecum of newly hatched chickens 4 days post-infection with *Salmonella enteritidis* serovar Enteritidis. Moreover, chicken IL4I1 was expressed in Mφ, granulocytes, phagocytes, and CD4$^+$ and γδ T cells, while they were not detected in CD8$^+$ T or B cells (2020). Notably, mutation of the gene encoding chicken IL4I1 in Mφ HD11 did not affect the enzyme's bactericidal capacity against *S. enteritidis* ser. Enteritidis but negatively affected its oxidative burst after phorbol 12-myristate 13-acetate (PMA) stimulation. Their findings suggest that IL4I1 is not directly involved in the bactericidal activity of phagocytes but instead it is likely involved in the control of inflammatory responses and signaling to T and B cells.

In a recent study, an antibacterial LAAO from the serum of red-spotted grouper (*Epinephelus akaara*) showed a broad range of substrate specificity with aromatic and hydrophobic amino acids, similar to mammalian and snake LAAOs (Osaka and Kitani 2021 (Table 1 S)). Interestingly, the encoding gene was expressed at a low level in the kidney under normal conditions, while it was significantly upregulated by blood loss.

On the contrary, *Il4i1*-knockout mice been exhibit an accelerated B cell egress from the bone marrow, resulting in the accumulation of peripheral follicular B cells (Bod et al. 2018) and present a higher serum level of natural immunoglobulins and self-reactive antibodies. Collectively, several LAAOs including IL4I1 may function as extreme responses and vicious circles of inflammation due to bacterial and viral infection in the peripheral or tissue environments, such as bacillemia, ischemic disease, and sepsis. These LAAOs prefer aromatic and hydrophobic amino acids for substrate specificity and are conserved in vertebrates through the process of evolution (Fig. 1).

Perspectives for possible biomedical applications

Previously, we reported that LAAOs have bioactive potential; they can produce hydrogen peroxide and can be used for developing drugs against community and health care-associated bacterial infections. Briefly, LAAOs show significant antibacterial activity against various pathogenic bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Vibrio cholera*, *Yersinia enterocolitica*, *S. aureus*, *S. epidermidis*, *Enterococcus faecalis*, and *Streptococcus pyogenes* that cause infections such as that of the urinary tract, bloodstream, and abdominal cavity, diarrhea, vomiting, intestinal inflammation, endocarditis, tympanitis, and conjunctivitis (Kasai et al. 2015b (Table 1 V1)).

Several studies have reported procedures for the production of recombinant LAAOs. For instance, fungal LAAO from *Rhizoctonia solani* (RsLAAO1) was expressed in *E. coli* BL21 Codon Plus (DE3) as a fusion protein with maltose-binding protein to induce solubility (Hahn et al. 2017a; Hahn et al. 2017b). Further, hcLAAO4, one of the fungal LAAOs from *Hebeloma cylindrosporum*, was expressed in *E. coli* or yeast (*Pichia pastors*) systems with His-Tag (Bloess et al. 2019; Heß et al. 2020). Moreover, L-Lys-specific LAAO isolated from the *Siganus pramin* (serum) or *Platichthys stellatus* (gill), which is an innate immune protein with antibacterial activity, was codon optimized for expression in insect cells (Sf9) or *P. pastoris* expression systems (Han et al. 2020, Kasai et al. 2020 (Table 1 V1)). These recombinant LAAOs can be easily high mass-produced by scale-up and display strong bioactivity via bactericidal, parasiticidal, and apoptotic effects, and bind to the surface of bacteria, similar to native LAAOs, suggesting potential use of LAAOs against clinical pathogens. Moreover, L-Lys α-oxidase (LysOX; EC: 1.4.3.14), a homodimeric flavoenzyme that has substrate specificity for L-Lys as the name suggests, was identified from *Scomber japonicus* as an apoptosis-inducing protein. This enzyme was used for L-pipecolic acid synthesis

using an *E. coli* expression system that expressed both LysOX and Δ^1-piperideine-2-carboxylate reductase (Tani et al. 2015 (Table 1 W)). L-pipecolic acid produced by the action of LysOX is a precursor of numerous natural and synthetic bioactive compounds that function as immunosuppressants, anticancer agents, and local anesthetics. Based on these findings, LysOX is being investigated as a new approach for the production of chemical intermediates or precursors.

L-Trp is not only important in protein synthesis but also as a precursor of various biologically active compounds via the kynurenine pathway, generating metabolites with crucial functions in neurotransmission and regulation of immune responses. Notably, the depletion of L-Trp facilitates tumor immune escape by inducing Treg cells and expression of inhibitory receptors IL3 and IL4 on DCs and downregulating T cell receptor ζ-chain (TCRζ) in CD8$^+$ T cells (Brenk et al. 2009). L-Trp catabolic products downstream of IDO1/2 and TDO2 regulate immune cell function and promote cancer progression by activating AHR (Gutiérrez-Vázquez and Quintana 2018). As mentioned above, IL4I1 is expressed in Th17 cells and a subset of Treg to Th17 transdifferentiating cells under inflammatory conditions. Therefore, profiling IL4I1 expression in immune cells is a probable biomarker of the inflammatory grade and stage.

Hydrogen peroxide is a widely used antimicrobial agent; its efficacy has been demonstrated in several human viruses, among which coronaviruses have been found to be sensitive (Dev Kumar et al. 2020). Recently, several chemical agents used for viral decontamination could effectively inactivate human coronaviruses by surface disinfection procedures using 62–71% ethanol, 0.1% sodium hypochlorite, or 0.5% hydrogen peroxide for 60 s (Kampf et al. 2020). Previously, we described the antiviral activity of LAAOs demonstrated by various studies (Kasai et al. 2015b (Table 1 V1)). For instance, LysOX from the fungus *Trichoderma viride* inhibited the reproduction of the type I herpes simplex virus (Lukasheva and Berezov 2002). Additionally, LysOX was heterologously expressed by *Streptomyces lividans* TK24 (Amano et al. 2015). The enzymatic properties of the purified recombinant LysOX, such as substrate specificity and thermal stability, are same as those of native LysOX. LAAO from venom of the Chinese green tree viper *Trimeresurus stejnegeri* (TSV-LAO) also displayed dose-dependent inhibition of HIV-1 infection and replication. Notably, in the presence of catalase, TSV-LAO inhibited syncytium formation in a dose-dependent manner, whereas anti-HIV-1 activity was not observed with the exogenous addition of hydrogen peroxide (Zhang et al. 2003). These results suggest that the antiviral activity of TSV-LAO does not affect infected cells via generation of hydrogen peroxide but rather by intermediate product or substrate depletion. L-Lys excess is characterized by a significant increase in HIV-1 RNA copies in enriched peripheral blood mononuclear cell fractions of HIV-infected patients and the

copy number did not increase after adding L-Lys with LysOX in the plasma samples (Butorov 2015). Therefore, we consider that LysOX is a suitable antiviral as well as antibacterial agent. However, optimization of high production and yield of bioactive recombinant LAAOs and regulation of their cytotoxicity need further research.

Conclusions

LAAOs show high potency and efficacy as treatment strategy for various diseases and as biomarkers of autoimmune diseases, tumor grade, and immune signaling responses. Selective, specific, and localized activities of LAAOs in the early response are the underlying reasons for their potential biomedical implications. Although we now know that the direct or indirect bioactivities of LAAOs are associated with the catabolism of specific substrates and the production of hydrogen peroxide, several aspects such as the regulation of their enzymatic activities and conformation, selective usage per substrate specificity, production of intermediate products, and immune regulatory mechanisms remain poorly understood. It is, therefore, imperative to channelize future research with a clinical approach rendering each LAAO as a therapeutic agent. Moreover, LAAOs for clinical therapy must not only fulfil improved in vivo production but also pH resistance, thermal stability, and regulated substrate specificity and immune responses. Elucidating the mechanisms of LAAO selectivity against bacterial infections and evaluating the enzyme's effects on resistance, cytotoxicity, and genotoxicity must also be characterized to fully realize the therapeutic potential of these enzymes for biomedical applications.

Declarations

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