L-Amino Acid Oxidases-Microbial and Snake Venom

Susmita Singh*
Department of Molecular Biology and Biotechnology, Tezpur University, India

Abstract

L-amino acid oxidases (EC 1.4.3.2, L-aao) are flavoenzymes that catalyse the stereospecific deamination of an L-amino acid to their corresponding α-keto acid with the production of hydrogen peroxide and ammonia. These enzymes are widely distributed across diverse phyla from bacteria, fungi to mammals and many venomous snakes. Although they are mainly involved in cellular amino acid catabolism, many other physiological functions are attributed to L-aao including their antibacterial property and ability to protect from infection. L-aao has also been correlated with penicillin production, violacein synthesis and biofilm development and cell dispersal. Snake venom L-aaos are studied extensively for their ability to induce apoptosis, aggregate platelets, induce haemorrhage, edema and many other toxic effects. L-aaos have been characterized biochemically and found to differ in terms of biochemical parameters not only among different species but also among members of the same species. L-aao act on L-amino acids, preferentially basic, aromatic and aliphatic L-amino acids. The snake venom enzyme shows broad oxidizing activity towards aromatic and hydrophobic L-amino acids such as leucine, phenylalanine and isoleucine.

L-aao have practical value in biochemical and chemical investigations as they have been used to destroy L-isomer of a racemic DL-amino acid and thus yield an optically pure preparation of the D-isomer. As such, L-aao finds numerous applications as catalysts in biotransformation and for production of keto acids. L-aao has also been used for the determination of L-amino acids as part of biosensors. L-amino acids are reported to be found in physiological fluids of patients with certain diseases and disorders. In addition, the content of certain amino acids essentially controls the nutritional quality of the food. L-aao is useful in this aspect by development of biosensors to detect the L-amino acids.

Snake venom L-aao are known to induce apoptosis and antibacterial effects mediated by the hydrogen peroxide produced during the L-aao reaction. The hydrogen peroxide induces oxidative stress which in turn activates the heat shock proteins and initiates an array of functions ultimately leading to apoptosis and cell death. In this aspect, L-aao can be greatly useful for the development of efficient therapeutics and drugs to control tumor cells, bacterial, leishmanial, viral and protozoal infections. L-aao is also reported to display dose dependent inhibition on HIV-1 infection and replication and as such can be studied for development of anti HIV medicine.

Keywords: L-amino acid oxidases; Function; Characterization; Substrate specificity; Structure; Applications

Introduction

Enzymes that catalyse the oxidation of amino acids have been known for many years. L-amino acid oxidases (EC 1.4.3.2) (L-aaos) are flavoenzymes that catalyse the stereospecific deamination of an L-amino acid substrate to their corresponding α-keto acid with the production of hydrogen peroxide and ammonia via an imino acid intermediate. These enzymes are widely distributed across diverse phyla from bacteria to mammals and many venomous snakes. L-aao in microorganisms are involved in the utilization of nitrogen sources and those in animals have been characterized as having distinct biological and physiological functions.

Much attention has been given on the snake venom L-aao which have become an interesting subject for pharmacological as well as structural and molecular biology studies. Snake venom L-aao has been characterized extensively in terms of their molecular mass, substrate preference, apoptosis, cytotoxicity, bacterial activities etc.

In the present review emphasis will be given on the relatively recent advances in my knowledge of the flavoprotein L-aao of organisms other than snakes and also to some extent on the snake venom L-aao.

Physiological role of L-aao

L-aao are reported to be extracellular enzymes in Aplysia californica [1], and Myxoxecphalus polyacanthocephalus [2] while the enzyme was found to be localized in the cell envelope of Proteus mirabilis [3] and cytoplasmic membrane of Proteus sps [4]. In Myxoxecphalus polyacanthocephalus, the skin mucus isoyme MPLAO3 contains a signal peptide, comprising residues Met1-Ala26. Synecchoccus elongatus L-aao was found in the membrane where it helps in photosynthesis as part of photosystem II particles [5]. Different localization of L-aao was reported in Meleagris gallopavo [6] in mitochondria, while Chlamydomonas reinhardti [7] and Synecchoccus elongatus L-aao were found in the periplasm [8]. Soluble L-aao was also reported from Corynebacterium sp [9] and Neurospera crassa [10].

Fungal L-aao is involved in the utilization of amino acids as nitrogen sources. Neurospera crassa expresses an L-aao whose synthesis is induced in nitrogen starved cultures by amino acid addition [11]. Depending on the amino acid used as nitrogen source, the catabolic pathway using a broad range L-aao can coexist with alternative pathways in Aspergillus nidulans [12]. An L-aao having antitumour

*Corresponding author: Dr. Susmita Singh, UGC Dr. D. S. Kothari Post-Doctoral Fellow, Department of Molecular Biology and Biotechnology, Tezpur University, Napaam-784028, Assam, India, Tel: +91-9957722523; E-mail: susmitasingh123@gmail.com

Received February 27, 2014; Accepted February 27, 2014; Published March 07, 2014


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activity has been identified in the fungus Trichoderma viride. This enzyme is highly specific and is an L-lysine oxidase which also has promising antibacterial and cytotoxic properties [13].

Lysyl oxidase is a different type of L-aao found in mammalian tissues which catalyse the E-oxidative deamination of lysyl residues in mammalian sclerotic proteins, especially collagen and elastin to yield alanyl residues that rapidly crosslink those proteins during the formation of the extracellular matrix and therefore play an important role in the development, elasticity and extensibility of the connective tissue [14].

Mouse milk is enriched in various nutrients like proteins, carbohydrates, lipids, minerals and vitamins together with many bioactive substances and hence bears a great risk for bacterial infection and proliferation. But milk also contains antibacterial factors that protect the mother and the offspring. This antibacterial capacity of milk can be attributed to some extent on the presence of L-aao [15]. It displays antibacterial effect through the production of hydrogen peroxide from the oxidative deamination of free amino acids [16].

An interesting observation was made by Knight [17], on the correlation between the penicillin producing ability and the L-aao content of the penicillin producing molds. The highest penicillin producers (P. chrysogenum, P. notatum) are more active and have more L-aao content as compared to the low penicillin producers like P. expansum, P. sanguineum, Aspergillus niger etc.

Hebeloma sp and Laccaria bicolor contain L-aao that is involved in cellular amino acid catabolism. They are potential candidates for causing nitrogen mineralization from amino acids at the ecosystem level [18].

Since the first finding of antibacterial activity in an L-aao from snake (Crotalus adamanteus) venom [19], antibacterial L-aao have been reported from various snake venom of Pseudochis australis [20], Trimeresurus jerdoni [21] and Bothrops alternatus [22], the body surface of the giant African snail, Achatina fulica Ferussac (term as echinac) [23], the albumen gland of the sea hare, Aplysia kurodai (term as aplysianin A) [24] and the ink of the sea hare Aplysia californica (term as echapin) [1]. Antibacterial LAO isozymes may be involved in the innate immunity which was demonstrated in the rockfish (Sebastes schlegelii) and the sculpin Myoxocephalus polyacanthocephalus [25]. The skin secretion of the rockfish Sebastes schlegeli and the sculpin Myoxocephalus polyacanthocephalus contains an L-aao which is a potent antibacterial protein with strict selectivity against Gram negative bacteria like Aeromonas hydrophila, A. salmonicida, Photobacterium damselae sp piscicida and Vibrio parahaemolyticus, but not against enteric bacteria such as Escherichia coli and Salmonella typhimurium suggesting the importance of the antibacterial protein as a primary innate immunity strategy in the rockfish skin. The antibacterial action is elicited by hydrogen peroxide generated from the enzyme reaction [25]. The skin mucus of Myxoxecephalus polyacanthocephalus contains an L-aao which has antibacterial activity against Gram positive and Gram negative bacteria and it is most active against Aeromonas salmonicida. This enzyme containing the antibacterial activity help in the innate immunity of the sculpin skin [2].

Yang et al. [1] described a monomeric antibacterial protein from the purple ink of sea hare Aplysia californica which was named as echapin because of its potential role in sea hare defence. The echapin is released when the sea hare is attacked by predators and it has cytotoxic effects against a predatory sea anemone. This protein which is an L-aao has a wide spectrum of antimicrobial activities including that against bacteria, yeasts and fungi but with variable efficacies. The hydrogen peroxide generated from the enzyme reaction plays a prominent role in the bacteriostatic effect and a weak role in bactericidal effect.

The physiological role of L-aao in bacteria is greatly unknown. A tryptophan oxidase from Chromobacterium violaceum is involved in violacein synthesis [26]. In Marinomonas mediterranea Lod A shows lysine oxidase activity and it is described as a novel antimicrobial protein [27].

Pseudoalteromonas tunicata also expresses a protein similar to Lod A that has important role in biofilm development and cell dispersal [28,29].

The L-aao produced from Pseudoalteromonas luteoviolacea is also a broad range L-aao which has antimicrobial activity [30].

Snake venom is useful sources of bioactive substances showing a wide range of pharmacological activities. This complex cocktail of both toxic and nontoxic components includes several peptide and enzymes such as L-aao which may represent 1-9 % of the total venom proteins. Although the exact biological function of snake venom L-aao is still unknown, these enzymes are postulated to be toxins that may be involved in the allergic inflammatory response and specifically associated with mammalian endothelial cell damage [31,32], cytotoxic activities [33], induction of apoptosis, platelet aggregation, hemorrhage, edema and other toxic effects [34-36].

### Enzymatic Properties of L-aaos

L-aao differ in the parameters of enzymatic properties greatly, not only among different species but also among the same species. A comparison of various L-aaos and their properties is given in Table 1.

In terms of substrate preference, L-aao show great variations. The Pseudoalteromonas L-aao has broad substrate specificity, oxidizing several amino acids but it shows some preference for L-glutamine [30].

Mouse milk L-aao accepts a broad substrate range i.e., phenylalanine, methionine, tyrosine, leucine, lysine and histidine but they do not oxidize isoleucine [15].

L-aao produced from Marinomonas mediterranea has high affinity for L-lys. α-N-acetyl-L-lys is a good substrate indicating that the amino group in position α is not oxidized by this enzyme. On the contrary, ε-N-acetyl-L-lys is not a substrate which points out that the modification of the ε-group abolished the enzyme activity. Other substrates with lower affinity are L-orn, D-lys and 5-hydroxy-L-lys. This shows the importance of the appropriate distance between the amino and the carboxyl groups in L-lys, the stereospecificity and the negative effects of the introduction of a hydroxyl group adjacent to the ε-amino group on the side chain of lysine. Other compounds with structural similarity to L-lysine, such as the tetrapeptide LSKL, amino acids such as arginine and p-amino-L-phenylalanine are not substrates of this enzyme [37].

The mold Penicillium chrysogenum enzyme deaminates simple short chain amino acids like L-alanine, L-methionine, L-cysteine more rapidly than longer, branched or substituted amino acids [17] while the substrate specificity of N. crassa enzyme is broad with the best utilized including L-histidine, L-a amino butyrate, L-canavaine and L-tyrosine [38].

Basic, aromatic and aliphatic L-amino acids are generally good substrates for Rhodococcus opacus L-aao. Threonine, proline and
glycine are the only proteogenic amino acids that are not accepted by this broad range enzyme. Among the aliphatic amino acids, alanine, valine and isoleucine. Phenylalanine, tyrosine and tryptophan showed similar activity among the aromatic amino acids. Among the basic amino acids, asparagine showed the highest activity followed by leucine, valine and isoleucine. Glycine are the only proteogenic amino acids that are not accepted by this enzyme [43].

The *Saccharomyces cerevisiae* has a specific lysine oxidase activity; however it also accepts L-arginine, L-asparagine and other L-amino acids like alanine, leucine, glutamic acid and tryptophan as substrates [40].

*Streptomyces endus* is a specific L-glutamate oxidase which oxidizes only L-glutamate. L-aspartate, even in high concentrations is not converted to any extent. However the L-glutamic acid 4-benzyl ester is oxidized by the enzyme but the diester is not. The 4-substituted L-glutamic acid derivatives, L-glutamine and glutathione as well as peptide derivatives are also not substrates for this enzyme [41].

Proteus rettgeri contains two separable L-aaos that differ in their substrate specificities. One of them catalyses the oxidative deamination of aromatic, monoaminomonocarboxylic, sulphur containing, imino and β-hydroxy L-amino acids with no affinity for the basic amino acid, L-citrulline. The other oxidase catalyses the oxidative deamination of L-arginine, histidine, ornithine, citrulline and lysine only [42] while the *Proteus vulgaris* enzyme oxidizes the unsubstituted monocarboxylicaminoacid, primary amino acids like nor-leucine, phenylalanine, leucine, tryptophan, methionine with the exceptions of alanine and valine [43].

### Table 1: Comparison between different L-aao in terms of some enzymatic properties.

<table>
<thead>
<tr>
<th>Species</th>
<th>Metal and ions</th>
<th>Inhibitors</th>
<th>pl value</th>
<th>pH optima</th>
<th>Temp. optima</th>
<th>Mol.wt. and subunits</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Anacystis nidulans</em></td>
<td>Ni</td>
<td>2-naphthol, Ba²⁺, Ca²⁺, Cd²⁺, Co²⁺, chloropromazine, Cu²⁺, La³⁺, Mg²⁺, Mn²⁺, Na⁺, Ni⁺, o-phenanthroline, NaNO₂, Sr²⁺, Zn²⁺</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>98 kDa (dimer of 49 kDa)</td>
<td>[5]</td>
</tr>
<tr>
<td><em>Rhodococcus opacus</em></td>
<td>Ni</td>
<td>Glycine (competitive inhibition)</td>
<td>4.8</td>
<td>8</td>
<td>30°C</td>
<td>99-104 kDa (dimer)</td>
<td>[39]</td>
</tr>
<tr>
<td><em>Proteus sp.</em></td>
<td>Ni</td>
<td>Atebrine, HgCl, KCN, quinine sulphate, NaNO₂</td>
<td>NI</td>
<td>7.0-7.6</td>
<td>NI</td>
<td>NI NI</td>
<td>[4]</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>Ni</td>
<td>Caprylic alcohol, Ag, Cu, Hg, 0.01 M HCN (98% inhibition) under aerobic condition</td>
<td>NI</td>
<td>8.8</td>
<td>50°C</td>
<td>NI</td>
<td>[43]</td>
</tr>
<tr>
<td><em>Proteus rettgeri</em></td>
<td>Ni</td>
<td>KCN, o,o’-diprydil, salicylaldehyde, 1, 10-phenanthroline, 8-hydroxyquinoline</td>
<td>NI</td>
<td>7.4-7.8</td>
<td>NI</td>
<td>NI NI</td>
<td>[42]</td>
</tr>
<tr>
<td><em>Meleagris gallopavo</em></td>
<td>Mni²⁺ (activator)</td>
<td>Mg, Fe and molydbden</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>NI NI</td>
<td>[6]</td>
</tr>
<tr>
<td><em>Chlamydomonas reinhardtii</em></td>
<td>CaCl₂ (2 mM, 50% activation)</td>
<td>EDTA (10 mM, 90% inhibition), hydroxylamine (5mM,75% inhibition) KCN (10mM, complete inhibition) NaF (10mM,85%inhibition)</td>
<td>NI</td>
<td>9</td>
<td>NI</td>
<td>900-1300 kDa (oligomer)</td>
<td>[7]</td>
</tr>
<tr>
<td><em>Aplysia californica</em></td>
<td>Ni</td>
<td>Ni</td>
<td>NI</td>
<td>7</td>
<td>37°C</td>
<td>60 kDa (monomer)</td>
<td>[1]</td>
</tr>
<tr>
<td><em>Neurospora Crassa</em></td>
<td>Ni</td>
<td>Ni</td>
<td>9.5</td>
<td>49°C</td>
<td>300 kDa</td>
<td>NI</td>
<td>[10]</td>
</tr>
<tr>
<td><em>P. luteoviolacea</em></td>
<td>Ni</td>
<td>Ni</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>110 kDa, oligomeric</td>
<td>[30]</td>
</tr>
<tr>
<td><em>Lechevaleria aerocolonigenes</em></td>
<td>Ni</td>
<td>Ni</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>101 kDa (dimer of 55 kDa)</td>
<td>[68]</td>
</tr>
<tr>
<td><em>Marinomonas mediterranea</em></td>
<td>Ni</td>
<td>B-APN, cadaverine, 5-aminoacaproic acid (strong inhibitors) 5-aminovaleric acid, amiloride aminoguandine, (weak inhibitors)</td>
<td>NI</td>
<td>NI</td>
<td>NI</td>
<td>140 kDa</td>
<td>[27]</td>
</tr>
<tr>
<td><em>Penicillium chrysogenum</em></td>
<td>Ni</td>
<td>Capryl alcohol, CuSO₄, (NH₄)₂SO₄, 2,4-dinitrophenol, benzolic acid, iodoacetic acid</td>
<td>NI</td>
<td>8-8.5</td>
<td>50-55°C</td>
<td>NI</td>
<td>[17]</td>
</tr>
<tr>
<td><em>Streptomyces endus</em></td>
<td>Ni</td>
<td>Ag⁺ and Hg²⁺ ions</td>
<td>6.2-6.3</td>
<td>6.5-8.0</td>
<td>30-45°C</td>
<td>90 kDa, dimeric</td>
<td>[41]</td>
</tr>
<tr>
<td><em>Bombys mori</em></td>
<td>Ni</td>
<td>CuSO₄, HgCl, (10⁻² M), KCN, EDTA (10⁻³ M), riboflavin, isoriboflavine.</td>
<td>NI</td>
<td>7.2</td>
<td>55°C</td>
<td>NI</td>
<td>[61]</td>
</tr>
<tr>
<td><em>Calloselasma rhodostoma</em></td>
<td>Ni</td>
<td>Anthranilate (competitive)</td>
<td>NI</td>
<td>9</td>
<td>NI</td>
<td>132 kDa (dimer of 66 kDa)</td>
<td>[58,32]</td>
</tr>
<tr>
<td><em>Crotilus durissus cascadavella</em></td>
<td>Ni</td>
<td>Aspirin, indomethacin</td>
<td>5.4</td>
<td>6.5</td>
<td>NI</td>
<td>68 kDa (dimer of 49 kDa)</td>
<td>[82]</td>
</tr>
<tr>
<td><em>Trimeresurus macropsopus</em></td>
<td>Ni</td>
<td>Benzoic acid, CdCl₂, HgCl, Iodoacetamide, KCN, MnCl₂, o-amino-benzoic acid, ZnCl₂, 2-chloromercuribenzoate</td>
<td>5.6</td>
<td>9 (L-trp), 8(L-his), 7(L-leu,phe,tyr)</td>
<td>140 kDa (dimer of 70 kDa)</td>
<td>[59]</td>
<td></td>
</tr>
<tr>
<td><em>Crotilus adamanteus</em></td>
<td>Cl (activator)</td>
<td>Aromatic carboxylates, benzoate, iodoacetic acid, orthanalic acid, vinylglycine, mandelate</td>
<td>Ni</td>
<td>7.5</td>
<td>NI</td>
<td>Dimeric with 58.7 kDa subunit</td>
<td>[63,84]</td>
</tr>
<tr>
<td><em>Agkistrodon piscivorus piscivorus</em></td>
<td>Ni</td>
<td>Benzoic acid, iodoacetic acid, NH₄⁺</td>
<td>7.2-7.5</td>
<td>7.5</td>
<td>150 kDa</td>
<td>NI</td>
<td>[56]</td>
</tr>
<tr>
<td><em>Rattus norvegicus</em></td>
<td>Ni</td>
<td>Benzenearsonic acid, Benzoic acid, CuSO₄, iodoacetic acid, p-chloromercuribenzoate, NH₄⁺</td>
<td>NI</td>
<td>8.8-9.2</td>
<td>NI</td>
<td>138 kDa</td>
<td>[56]</td>
</tr>
<tr>
<td><em>Bothrops pirajai</em></td>
<td>Ni</td>
<td>Ni</td>
<td>4.9</td>
<td>6.0-7.4</td>
<td>37°C</td>
<td>130 kDa (dimer of 66 kDa)</td>
<td>[50]</td>
</tr>
</tbody>
</table>

NI = No information
A. fumigatus L-aao also shows certain degree of substrate preference. The enzyme has a greater specificity towards hydrophobic aromatic amino acids namely tyrosine and phenylalanine. D-amino acids are not attacked. The enzyme does not act on basic amino acids [44] and the best substrates for this enzyme are the L-isomers of phenylalanine, tyrosine, leucine and isoleucine.

L-aao from various organisms exhibit different substrate specificity. For e.g. leukocyte L-aao (IL411) prefers aromatic L-amino acids such as phenylalanine [45] while the enzyme from ink of sea hare is most active to positively charged L-amino acids like arginine and lysine [1,46].

The snake venom enzyme shows broad oxidizing activity towards aromatic and hydrophobic L-amino acids such as leucine, phenylalanine and isoleucine [47,48]. In general, the best substrates for this enzyme are the L-isomers of phenylalanine, tyrosine, leucine, isoleucine, methionine and tryptophan [49-51].

Bungarus fasciatus L-aao (BF L-aao) substrate specificity is similar to that of L-aao from Calloselasma rhodostoma, Naja naja kaouthia, Agkistrodon blomhoffi assursensis, Bothrops jararaca, Daboia russelli siamensis, Vipera lebetina in that these enzymes have affinity towards hydrophobic amino acids including phenylalanine, tryptophan, tyrosine and leucine. However, BF L-aao is also active towards acetic L-amino acids aspartic acid and glutamic acid [33,52,53]. The Agkistrodon contortrix laticeps enzyme shows greater activity against hydrophobic L-amino acids but a significant activity was also present for the basic L-amino acids arginine and histidine but not detected for lysine [49].

FAD acts as a cofactor for these enzymes. L-aao family members possess in common, flavin as a coenzyme and two motifs, a dinucleotide binding motif comprising of β-strand/α-helix/β-strand of the secondary structure and a GG motif (R-X-G-x-x-T/S) shortly after the dinucleotide binding motif [54]. In Anacytis nidulans [5], 1 mol of FAD is bound per mol of enzyme while in Bacillus carotarum [55], Rhodococcus opacus [39] and most other organisms, the enzyme is a homodimeric complex containing 2 FAD molecules.

Snake venom L-aao of Agkistrodon piscivorus piscivoros, Crotalus adamanteus [56], Ophiopogon hannahi [57] binds to 2 FAD molecules per enzyme, while Calloselasma rhodostoma [58], Trimeresurus jerdonii [21], and T. mokusquamateus [59] contain 2 mol of FMN per mol of enzyme.

An exception to this observation is that, the enzyme from Marinomonas mediterranea is an unusual amine oxidase. This melanogenic marine bacterium synthesizes marilnoicin, which is a type of lysine oxidase that has antibacterial activity. It does not depend on flavin as a cofactor, and is copper enzyme, requiring tyrosine derived quinine as a cofactor [37].

Although L-aao are flavoprotein enzymes, glycoprotein L-aao are reported in many organisms like Aplysia californica where the L-aao sequence contains one potential glycosylation site but glycosylation is not essential for its antimicrobial activity [1], Chlamydomonas reinhardtii [7] and Sebastes schlegeli where the protein contains N-linked glycochains [25]. Most snake venom L-aao are reported to be glycoproteins [22,60].

Structural properties of L-aao

Primary structures of snake venom L-aao were determined for Crotalus adamanteus [61], A. contortrix laticeps [49], Crotalus atrox [62], Agkistrodon halys blomhoffi [63], Trimeresurus stejnegeri [64] and Bothrops sp [65]. Phylogeny analysis shows that Bothrops and Crotalus adamanteus L-aao form a cluster and are more closely related to each other.

The complete nucleotide sequence of Rhodococcus opacus L-aao gene was determined and its primary structure was deduced [39]. The nucleotide sequence revealed that the L-aao is synthesized as a precursor carrying a signal peptide of 45 amino acids, which is processed after translation. The proteolytic cleavage site of the precursor protein does not agree with the predicted cleavage site. The nucleotide sequence of Neurospora crassa L-aao gene was obtained from a partial c-DNA and a complete genomic DNA clone [66]. The gene encodes a protein consisting of 695 amino acids and unlike most of the cloned genes of N. crassa, the L-aao gene is devoid of introns. The enzyme is synthesised as a precursor exceeding the mature form (566 amino acids) by 129 amino acids.

Various workers report the cloning of the L-aao gene into vectors for their expression in large quantities. Geuseke and Hummel [39] report the expression of Rhodococcus opacus L-aao in E. coli but, this yields only inclusion bodies, while the expression in a Streptomyces lividans strain yields both soluble and active enzyme, but at low yields. The Calloselasma rhodostoma L-aao (CRL-aao) was cloned in a yeast expression system (Pichia pastoris) after the α-MF signal sequence that promotes secretion. The expression was repressed when glycerol was used as the sole carbon source while switching the carbon source to methanol leads to the secretion of recombinant CRL-aao with good yields [67]. The L-aao of Aplysia californica (escapin) was cloned, sequenced and functionally expressed in E. coli [1]. The bioactive recombinant escapin level was relatively low because much of the escapin is present in the form of insoluble inclusion bodies. Also the escapin inhibits growth of E. coli which likely inhibits the level of bacterial expression. Lechevalieria aerocolonigenes produces an L-aao (rebeccamycin) (RebO) which was overexpressed in E. coli using an expression vector (pDHS5514). A KLAAALEHHHHHHH amino acid sequence was engineered onto the C-terminus of RebO to facilitate purification by a Ni-nitrilotriacetic acid strategy. The co-expressed plasmid was used to facilitate protein folding and to prevent aggregation and degradation of RebO in E. coli. Thus, the recombinant RebO protein was found to be >95% pure [68]. Nagashima et al. [2] report the c-DNA cloning of Myxocephalus polyactecephalus L-aao (MPL-aao) which show that the full length of c-DNA was 2659 bp and it encodes the signal peptide (Met1-Ala26) and the mature protein (Val28-Phe520). MPL-aao shares 74% sequence identity with the antibacterial L-aao from skin mucus of the rockfish Sebastes schlegeli.

Applications of L-aao

L-amino acid oxidases have practical value in biochemical and chemical investigations. The usefulness of these enzymes arises from the fact that they exhibit absolute antipodal specificity. It is therefore possible to detect as little as one part of a susceptible amino acid isomer in the presence of ten thousand times the concentration of its enantiomorph. The kinetic resolution of racemic and other isomers is highly successful strategy for the synthesis of enantiomerically pure chiral compounds and has found widespread usage in industry. The amino acid oxidases have been used to destroy one isomer of a racemic amino acid and thus yield an optically pure preparation of the other isomer [69]. We demonstrated the racemic resolution of DL-
amino acids to yield optically pure D-amino acids. DL-tyrosine, DL-phenylalanine and DL-alanine were successfully resolved with the help of *Aspergillus fumigatus* L-aaos to yield D-tyrosine, D-phenylalanine and D-alanine respectively [70]. Further applications of this enzyme were demonstrated as catalysts in biotransformation [71] and could also be used for production of keto acids, which can function as siderophores [72].

The determination of amino acids is important for several purposes. Determination of certain amino acids in food essentially controls the nutritional quality of the food. L-aaos can be used in this aspect by development of amperometric biosensors to detect the amino acids. Amperometric biosensors based on screen printed electrodes have been developed by Sarkar et al. [73]. An amperometric microbial biosensor based on *Saccharomyces cerevisiae* cells was developed for selective and rapid determination of L-lysine [40]. In addition, L-aaos was physically immobilized on diamond paste to construct an amperometric biosensor that detects L-leucine by measuring the hydrogen peroxide formed when L-aaos catalyses the conversion of L-leucine to their keto acids and H$_2$O$_2$ [74]. L-aaos was immobilized on a preactivated nylon membrane by using glutaraldehyde to develop an enzyme sensor for L-amino acids that can detect ammonia [75].

Snake venom L-aaos are known to induce apoptosis and antibacterial effects mediated by the hydrogen peroxide produced during the L-aaos reaction. The hydrogen peroxide is a strong inducer of apoptosis in promastigote forms of *Leishmania* spp. The hydrogen peroxide induces oxidative stress which in turn activates the heat shock proteins and initiates cell membrane/ cytoplasmic disorganization, DNA fragmentation, apoptosis and therefore cell death [76]. In this aspect, L-aaos can be greatly useful for the development of efficient therapeutics and drugs to control tumor cells, bacterial and leishmanicidal infections. Also the *Trimeresurus stejnegeri* L-aaos displays dose dependent inhibition on HIV-1 infection and replication [64].

Antiviral (against Dengue virus) and antiprotozoal (trypanocidal and leishmanicidal) activities have been reported from *Bothrops jararaca* L-aaos (LAAO-I) [77]. The *B. jararaca* L-aaos was found to significantly inhibit Ehrlich ascites tumour growth and induce an influx of polymorphonuclear cells, as well as spontaneous liberation of H$_2$O$_2$ from peritoneal macrophages. Later, LAAO-I induce mononuclear influx and peritoneal macrophage spreading. Animals treated with L-aaos show a marked increase in survival time and thus, the application of these enzymes in tumour inhibition was implicated.

In *Deinagkistrodon acutus*, L-aaos (ACTX-6) demonstrates cytotoxicity in vitro and inhibits tumour growth in vivo and can markedly increase accumulation of sub-G1 phase, which suggests that this enzyme can induce apoptosis. ACTX-6 is a potential substance to develop into an antitumor drug [79] since it induces apoptosis in Hela cervical cancer cells in a concentration- and time-dependent manner. Caspase activation and PARP cleavage are involved in ACTX-8-induced apoptosis. ACTX-8 activates a mitochondrial pathway of apoptosis, which is regulated by Bcl-2 family members. Reactive oxygen species generated by ACTX-8 are involved in apoptosis [80].

In *Vipridivipera stejnegeri* the L-aaos enzyme displays dose dependent inhibition on HIV-1 infection and replication [64] and as such can be studied for development of anti HIV medicine. Thus L-aaos are useful enzymes due to their biotechnological potential as model of therapeutic drugs and medicine.

**Acknowledgement**

The author would like to acknowledge UGC for providing Dr. D. S. Kothari Post-Doctoral Fellowship.

**References**


