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Chapter 1

Therapeutic Uses of Amino Acids

Amraibure Odia and Oaikhena Zekeri Esezobor

Additional information is available at the end of the chapter

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Abstract

Amino acids, which are the building blocks of peptides and proteins, are indispensable chemicals needed by the body for optimal metabolism and proper body functioning. Classified as essential, nonessential and conditionally essential, amino acids play vital roles in the body such as in protein synthesis and as precursors in the production of secondary metabolism molecules. Amino acid oxygenases also play vital metabolic roles such as in prevention of diseases; as a result, amino acids and their oxygenases isolated from various organisms are potent candidates in treatment of diseases which include cancers, inflammations, as well as antibacterial agents.

Keywords: amino acids, oxygenase, therapeutic, bacteria, flavoprotein, enzyme

1. Introduction

The use of amino acids in medicine today continues to be explored using clinical research and applications. Amino acids play several roles in the body [1]; they are essential in the synthesis of proteins and precursors in the formation of secondary metabolism molecules [2], and as a result, amino acids are found in all parts of the body [1].

Amino acids are mainly found as L-enantiomers in all forms of life. However, significant amounts of D-amino acids are produced by bacteria, which are the major producers of D-amino acids [3]. In bacteria, D-amino acids are involved in the synthesis and cross-linking of peptidoglycan [4].

In humans, amino acids participate in various physiological processes, such as skeletal muscle function, atrophic conditions, sarcopenia, and cancer. They play key roles in cell signalling, homeostasis, gene expression, synthesis of hormones, phosphorylation of proteins and

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also possess antioxidant abilities [2, 5]. Amino acids are also key precursors in the synthesis of low molecular weight nitrogenous compounds, which have numerous biological importance. The existence of amino acids and their metabolites, such as glutathione, polyamines, taurine, serotonin and thyroid hormones, in physiological amounts is important for proper body functions [5].

Traditionally, amino acids were classified as essential and nonessential amino acids [5]. However, another class known as conditionally essential amino acids now exists. These classifications are based on whether the body is able to synthesise the amount that it needs for metabolic maintenance [1]. Essential amino acids are those that cannot be synthesised or those that are synthesised inadequately by the body relative to needs and hence must be obtained from diets to meet physiological requirements. Amino acids which the body can synthesise in sufficient amounts to meet the body's maximum requirements are known as nonessential amino acids. Conditionally essential amino acids are those which the body can synthesise in adequate amounts, but under situations of higher utilisation rate, the body obtains them from diets in order to meet optimal requirements [5].

Inadequate intake of amino acids from diets and below optimal synthesis by the body may expose an individual to amino acid deficiency symptoms, such as weight loss, poor growth and development. Because amino acids are not stored in the body for long periods of time and in sufficient amount, meeting maximum daily requirements from diets and/or amino acid supplements is necessary for healthy living [1].

The therapeutic use of amino acids presents a viable and important option for natural medicine. Some of the most prominent areas of therapeutic applications of amino acids are for treatment of brain metabolism and neurotransmission imbalances. Other areas in which amino acids also find key applications are immune function, cardiovascular and gastrointestinal (GI) health [1], treatment of liver diseases, fatigue, skeletal muscle damage, cancer prevention, burn, trauma and sepsis, maple urine disease and diabetes [6].

2. D-Amino acids

Bardaweel [2] investigated the antibacterial activities of some D-amino acids, which include D-alanine, D-lysine, D-serine and D-proline (**Figure 1**), against *Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Pseudomonas aeruginosa* and *Xanthomonas vesicatoria* and reported that the amino acids exhibited relatively low inhibitory effectiveness against the pathogens. However, D-lysine, followed by D-alanine, was more potent than the other amino acids examined, even though their minimum inhibitory concentration (MIC) values were in the millimolar ranges.

A study by Hochbaum et al. [7] reported that D-amino acids were effective in preventing biofilm (communities of cells held together by a self-produced extracellular matrix typically consisting of protein, exopolysaccharide and often DNA) development in *S. aureus*, which is a leading cause of hospital-acquired infections. The D-isomers that were found to be active in inhibiting biofilm formation were D-phenylalanine (**Figure 2a**) and D-proline and D-tyrosine (**Figure 2b**). Mixture of D-tyrosine, D-proline and D-phenylalanine was more effective in preventing biofilm formation

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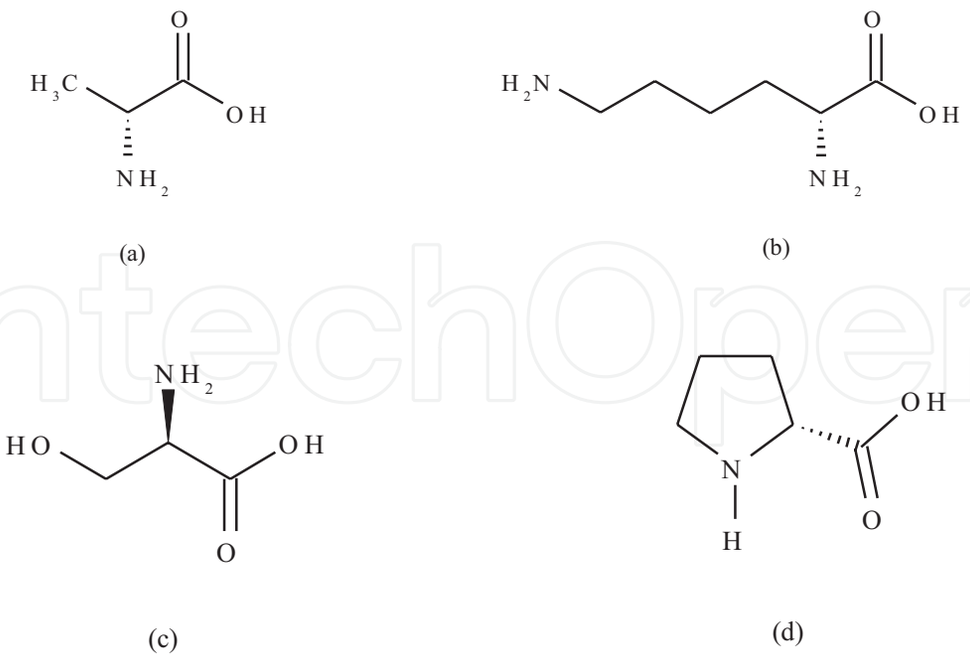


Figure 1. Chemical structures of (a) D-alanine, (b) D-lysine, (c) D-serine and (d) D-proline.

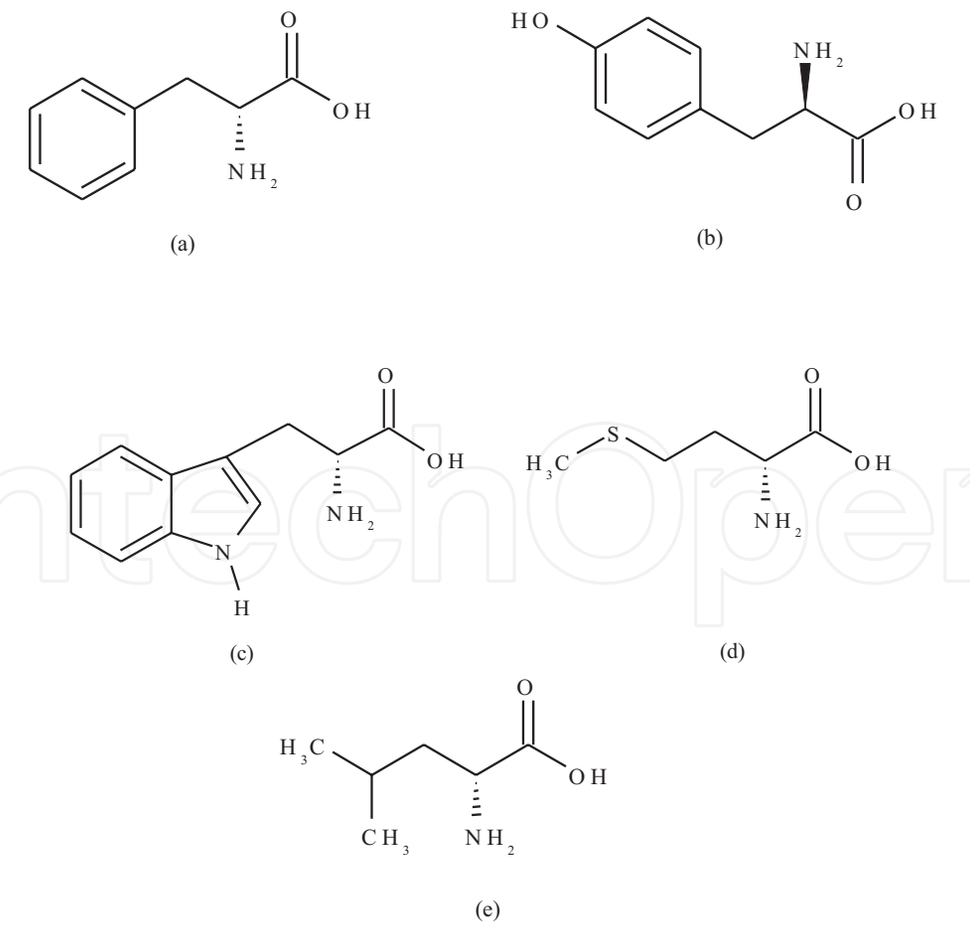


Figure 2. Chemical structures of (a) D-phenylalanine, (b) D-tyrosine, (c) D-tryptophan, (d) D-methionine and (e) D-leucine.

than the mixture of D-tryptophan, D-methionine, D-leucine (**Figure 2c–e**) and D-tyrosine. Earlier, the study by Kolodkin-Gal et al. [8] reported that D-tyrosine, D-leucine, D-tryptophan and D-methionine were active in inhibiting biofilm formation by *B. subtilis*, whereas D-isomers of other amino acids, such as D-phenylalanine, were inert in inhibiting biofilm formation.

The therapeutic potential of D-amino acid oxidase (DAAO) inhibitors, a flavoenzyme that degrades D-amino acids through the process of oxidative deamination, in schizophrenia patients has also been studied [9]. DAAO catalyses the metabolism of D-serine, a known full agonist at the allosteric glycine binding site of the *N*-methyl- D-aspartic acid (NMDA) (**Figure 3**) receptor, which has been reported to improve negative and cognitive symptoms of schizophrenia [10]. As a result, several studies have focused on the design and development of selective DAAO inhibitors, which when administered to schizophrenia patients have been shown to increase the concentrations of D-serine in the blood and the brain [9].

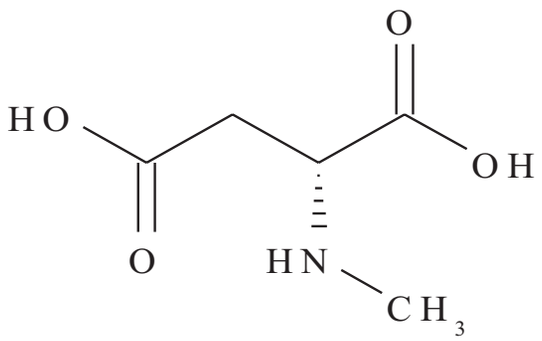


Figure 3. Chemical structure of *N*-methyl-D-aspartic acid.

3. Branched-chain amino acids

Branched-chain amino acids (BCAAs) are essential amino acids required for synthesis of body proteins. BCAAs play vital roles in regulation of protein synthesis and maintenance of glutamate-glutamine levels in the body. BCAAs are oxidised during high-energy-demanding and stressful conditions, and as a result, limit their accessibility in body tissues, which in the long run upsets mechanisms controlling the synthesis of proteins and body glutamate-glutamine pool [6].

The use of BCAA supplements in treatment of diseases is a developing nutritional strategy in disease management. Several studies have reported that when BCAA supplements are administered, patients experience improvements in health, although there are some disease conditions where BCAAs showed no effects. However, increased levels of BCAAs in the body have been observed to be involved in disease pathology [6].

The BCAAs—leucine, isoleucine (**Figure 4**) and valine—are metabolically very active. In the peripheral tissues, they may be oxidised to produce energy or act as anticatabolic factors (particularly leucine) by stimulating the synthesis and lowering the rate of degradation of muscle protein [11]. They are three of nine essential amino acids that are not synthesised by

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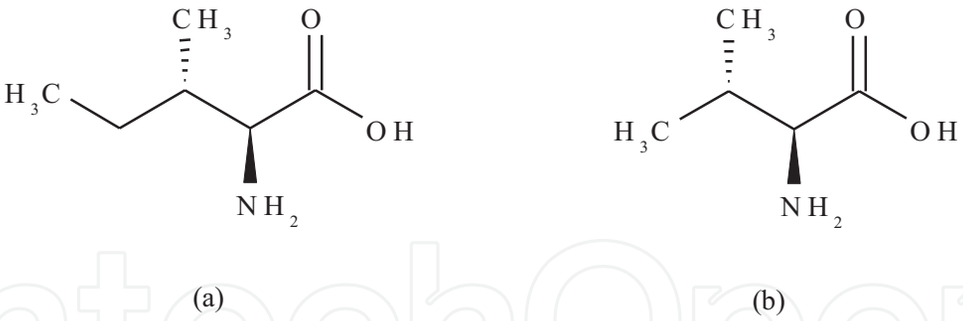


Figure 4. Chemical structures of (a) isoleucine and (b) valine.

the human body and therefore must be obtained from diet. Approximately 35% of indispensable muscle proteins and 40% of total amino acids required by mammals are composed of these BCAAs [6]. The three BCAAs either together or with leucine alone can stimulate protein synthesis and can also inhibit protein degradation depending on the context [12].

Although most of the amino acids are degraded in the liver, BCAAs are primarily broken down in the extrahepatic tissues (muscle, adipose, kidney and brain). Catabolism of these amino acids is initiated by transamination reaction with α-ketoglutarate to form glutamate and branched-chain keto acids (BCKAs). Then, the glutamate is converted to glutamine by the action of the glutamine synthetase enzyme [6]. Glutamine (**Figure 5**), which is derived mainly from skeletal muscles [13], is one of the most abundant amino acids in the body [14]. It is utilised readily by the liver, kidneys, GI tract and the immune system. Glutamine transports nitrogen and carbon inside the organs and plays a vital role in proper immune system function and GI integrity, as well as maintenance of overall amino-acid balance in the body [13, 14].

BCAAs have been considered as potential intervention for repair of damaged muscle tissues and some studies have suggested that BCAA supplementation may improve the repair of re-induced damaged muscle [15]. BCAAs, particularly leucine, have been reported to possess anabolic potential. They stimulate the metabolic pathways that initiate protein synthesis [16] and are involved in the control of protein breakdown (proteolysis) in impaired muscles [17].

The transamination product of BCAAs, α-ketoisocaproate (α-KIC) (**Figure 6**), is known to prevent the enzymatic action of branched-chain α-keto dehydrogenase complex (BCKDH), which increases the oxidation of BCAAs [18]. These anabolic potentials of BCAAs have led

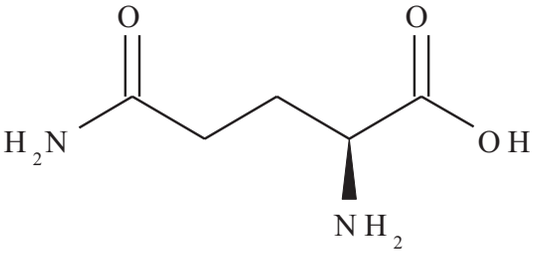


Figure 5: Chemical structure of glutamine.

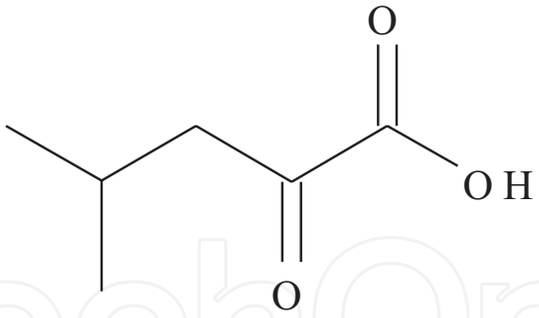


Figure 6. Chemical structure of α-ketoisocaproate.

to suggestions that BCAA supplementation could stimulate repair of impaired muscles by reducing oxidation of proteins, promoting the formation and development of muscle components and improving muscle functioning ability [15].

In a study by Soomro et al. [11], BCAAs were reported to be effective in the management of hepatic encephalopathy. While comparing the recovery and recurrence of hepatic encephalopathy of patients who were on BCAAs given initially intravenously and then orally with those of group without BCAAs, the results showed that those on BCAAs showed early improvements and recovery and subsequently on follow-up visits at 4 months. Improvements were observed in ammonia levels which were initially raised, but however decreased subsequently at 6 days and on 4 months of follow-up. In comparison to patients who did not receive BCAAs, the albumin levels of patients administered with BCAAs also increased from initial reading noticed at 4 months of follow-up.

BCAAs act as energy substrates, substrates for gluconeogenesis and modulators of muscle protein metabolism. These properties make their use in amino acid-enriched solutions theoretically appropriate for the management of the metabolic alterations that occur in sepsis. Forty-five percent branched-chain amino acid-enriched solutions have been suggested to intensify synthesis of proteins in the liver [19] and proteins whose plasma levels are elevated or reduced (acute-phase proteins) during severe illness [20]. Because acute-phase proteins might play vital roles in a septic patient's defence mechanisms against infections, the administration of solutions containing high amounts of BCAAs may increase the likelihood of quick recovery and survival for such patients [19].

4. Amino acid oxidases

Amino acid oxidases (AAOs) are flavoenzymes that catalyse the oxidative deamination of amino acids to α-keto acids with the generation of ammonia and hydrogen peroxide [21], as shown in (**Figure 7**) [22]. Depending on the amino acid isomer used as a substrate, it is possible to differentiate between L-amino acid oxidases and D-amino acid oxidases [23]. However, of particular interest in AAOs are the L-amino acid oxidases (LAAOs) because AAOs are highly specific for L-amino acids, and generally hydrophobic amino acids (such as phenylalanine,

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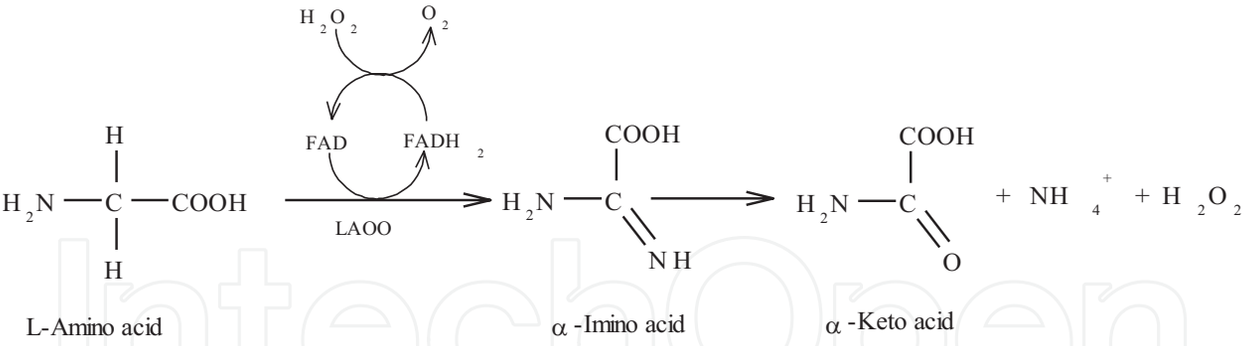


Figure 7. Mechanism of chemical reaction catalysed by L-amino acid oxidases (LAAOs) [22].

tryptophan, tyrosine and leucine) [24] are the best substrates [25]. These flavoenzymes are found in diverse organisms, such as bacteria, fungi, algae, fish, snails as well as venoms of snake families [26].

Several kinds of LAAOs have been isolated and their crystal structure presented (**Figure 8**). Most of the LAAOs isolated and characterised structurally to date are flavoproteins, which exist as dimers. The subunits in the structures are joined by noncovalent bonds with flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) [27]. The venoms of many snakes have characteristic yellow colour which has been attributed to the flavin component in LAAOs isolated from the snakes. The flavins have also been reported to contribute to the toxicity of such venoms because of the oxidative stress caused by H_2O_2 production [26].

In a study by Joseph et al. [24], LAAOs isolated from snake venom induced platelet aggregation and cytotoxicity in various cancer cell lines. The enzyme also showed antibacterial activity by inhibiting the growth of Gram-positive *(B. subtilis)* and Gram-negative *(E. coli)* bacteria. Snake venom LAAOs have also been reported to exhibit oedema-inducing, apoptotic-inducing as well as anti-bacterial, anti-coagulant and anti-HIV effects [25].

In a study of the king cobra *(Ophiophagus hannah)* venom L-amino acid oxidase, Lee et al. [32] reported that the heat-stable enzyme exhibited very potent anti-proliferative activity against human breast and lung tumorigenic cells, but not in their non-tumorigenic counterparts. They further reported that after eight weeks of treatment of mice samples with the isolated LAAOs, the enzyme markedly inhibited PC-3 tumours when compared to the control group.

In another study of the heat stable L-amino acid oxidase isolated from the king cobra *(O. hannah)* venom, Phua et al. [33] reported that the LAAO showed antibacterial activity against Gram-positive bacteria, such as *B. subtilis, Bacillus cereus, S. aureus* [including methicillin-resistant *S. aureus* (MRSA)], and *S. epidermidis*. The LAAO also showed antibacterial activity against gram-negative bacteria such as *Salmonella enteridis, P. aeruginosa, Serratia marcescens, Klebsiella pneumoniae, E. coli* and *Enterobacter cloacae*. They further reported that the snake venom showed the highest antibacterial activity against *Staphylococcus* spp. and *E. coli*, even though the inhibition zones increased with increasing concentration of venom in all cases.

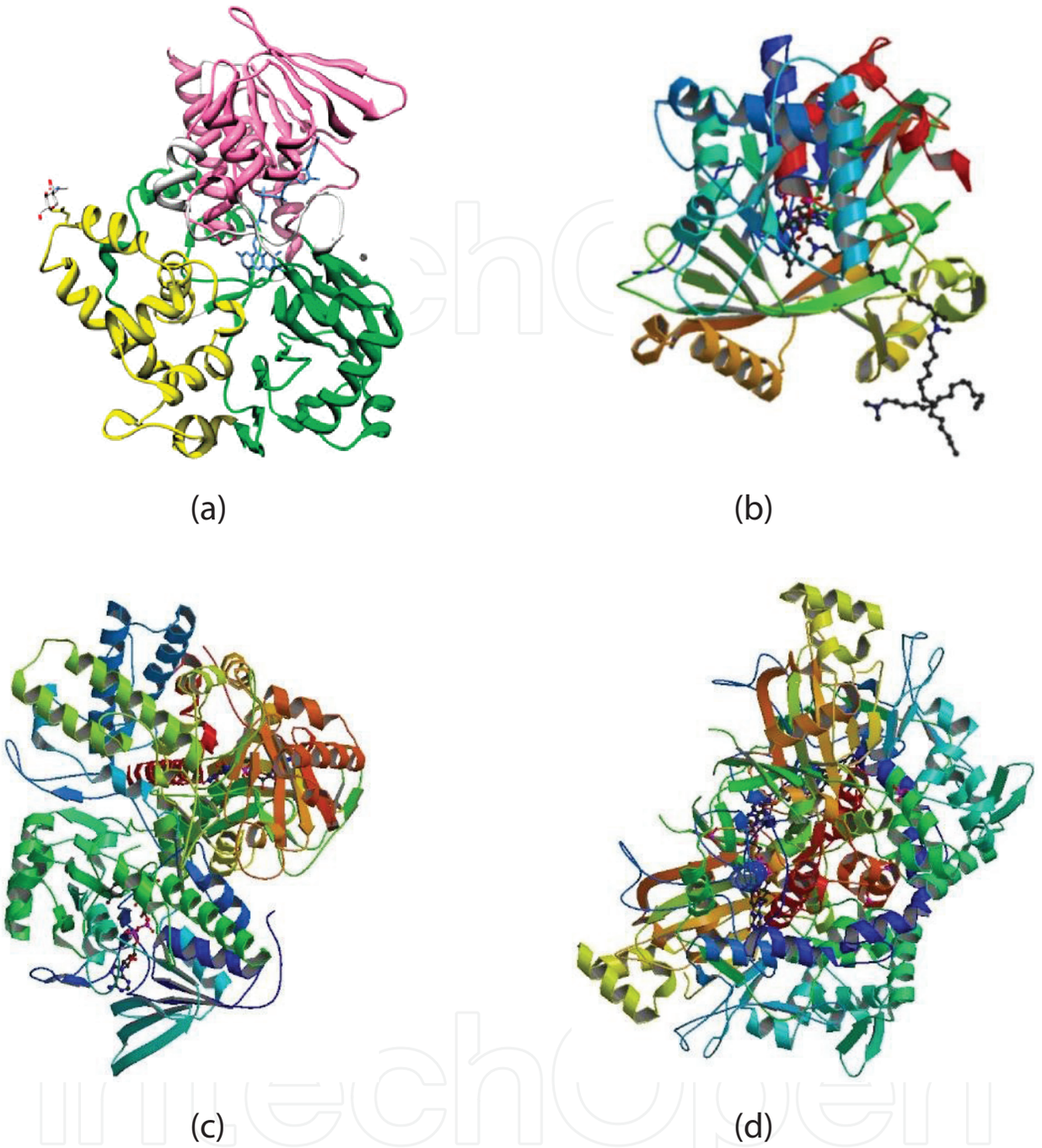


Figure 8. Crystal structures of LAAOs isolated from (a) the venom of *Vipera ammodytes* [28], (b) *Proteus vulgaris* [29], (c) *Rhodococcus opacus* [30] and (d) *Streptomyces* sp. [31].

LAAOs isolated from the venom of *Calloselasma rhodostoma* were also reported by Costa et al. [34] to induce acute inflammatory responses *in vivo*, with recruitment of neutrophils and release of IL-6, IL-1β, LTB4 and PGE2. An *in vitro* study showed IL-6 and IL-1β production by peritoneal macrophages stimulated with LAAOs, which was dependent on the activation of the Toll-like receptors TLR2 and TLR4. They also reported that LAAOs promoted apoptosis of HL-60 and HepG2 tumour cells mediated by the release of hydrogen peroxide and

activation of immune cells, resulting in oxidative stress and production of IL-6 and IL-1β that triggered a series of events, such as activation of caspase 8, 9 and 3, and the expression of the pro-apoptotic gene BAX.

L-Lysine α-oxidase (LysOx) isolated from the extracellular growth medium of *Trichoderma cf. aureoviride* was reported by Pokrovsky et al. [35] to have shown considerable cytotoxicity and anti-tumour effects *in vitro* against a panel of murine and human tumour cell lines and *in vivo* on murine tumours and on animals with human tumour xenografts (breast cancer SKBR3, melanoma Bro, colon cancer HCT116 and ovarian adenocarcinoma SCOV3). L-Amino acid oxidase isolated from *Bothrops marajoensis* has also been reported [36] to cause nephrotoxicity in isolated perfused kidney and cytotoxicity in MDCK renal cells.

5. Conclusion

The therapeutic effects of amino acids and amino acid oxygenases present interesting prospects for the use of these chemicals in management of diseases. The future potential of amino acid-based therapeutics in treatment of diseases and the diverse effects of naturally occurring amino acid oxygenase is far reaching.

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