Properties, biosynthesis, and catalytic mechanisms of hydroxy-amino-acids

D Y Sun[1,2,3], X T Cheng[3], D K Gao[3], P P Xu[3], Q Q Guo[3], Z L Zhu[3], M L Zhu[3], X Y Wang[3], H M Qin [1,2,3,5] and F P Lu[1,2,3,4,5]

[1]Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education, Tianjin 300457, People's Republic of China
[2]Tianjin Key Laboratory of Industrial Microbiology, Tianjin 300457, People's Republic of China
[3]College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, People's Republic of China
[4]National Engineering Laboratory for Industrial Enzymes, Tianjin 300457, People's Republic of China

E-mail: huiminqin@tust.edu.cn/lfp@tust.edu.cn

Abstract. The hydroxy amino acids have its unique effects in the biotechnology and molecular biology, and all sorts of synthetic hydroxy amino acids have also been developed. The hydroxy amino acids have been proved to be valuable for antifungal, antibacterial, antiviral and anticancer properties and known as a constituent of pharmaceutical intermediates. In addition to these fundamental researches, the hydroxy amino acids are applied widely to the synthesis of chiral drugs, such as (2*S*, 3*R*, 4*S*)-4-hydroxyisoleucine (4-HIL) is proved to be worthy in medical treatment, *cis*-4-hydroxy-L-proline (C4LHyp) and trans-4-hydroxy-L-proline (T4LHyp) are applied to build the chiral intermediates in pharmaceutical synthesis. Through comparisons chemical synthesis with biological catalysis to form hydroxy amino acids, enantioselective biocatalysts will undoubtedly be used in the synthesis of chiral pharmaceuticals. Mononuclear non-heme Fe(II)/α-ketoglutarate-dependent dioxygenases (Fe/αKGDs) that use an Fe(IV) complex intermediate to active diverse oxidative transformations with key biological roles. Studies identifying the important intermediates in catalysis and the proposed mechanisms are explained. In summary, we describe the physiological properties and synthesis regarding hydroxy amino acids, particularly 4-HIL and hydroxyproline. And the proposed catalysis mechanisms of Fe/αKGDs are also explained, while we also discussed the applications of hydroxy amino acids in fundamental research and industrial.

1. Introduction

Amino acids are pervasive chemical compounds of significant importance in the origin of life stem from their multiple critical functions in organisms. The hydroxylation of L-amino acids are an interesting and growing field of biotechnology and molecular biology research which have many physiological activities, for instance, that are the components of glycopeptide antibiotics, cyclodepsipeptides and collagen. These peptides contain hydroxy amino acid residues and are proved have properties of antifungal, antibacterial, antiviral and anticancer. Besides, some hydroxy amino

acids could be utilized as blocks in the asymmetric synthesis of chiral drug [1].

There has been an increasing focus on the great potential of microorganisms and enzymes leading to the transformation of synthetic chemicals with high enatio-selectivities [2]. The enzyme-catalyzed has advantages over chemical synthesis with highly enantioselective and regioselective. Biocatalysis is a new developing environmentally-friendly technologies to obtain specific compounds, which could be carried out at room temperature and atmospheric pressure avoiding the utilize of more extreme conditions. Microbial cells and enzymes catalysis can be immobilized and reused. Meanwhile, the enzymes can be obtained from inexhaustible biological resources, thus making biocatalytic processes economically efficient [3]. For example, sitagliptin, an antidiabetic drug is synthesized by the biocatalytic approach [4]. Compared with the chemical synthesis requiring heavy atoms (rhodium), organic solvent under high pressure and resulting in a yield of 97% of stereoselective compounds, biocatalytic approach only requires pyridoxal phosphate (PLP), transaminases under normal pressure and results in a yield of 99.95% of enantioselective compounds. In future, more enantioselective biocatalysts will undoubtedly be used in the synthesis of chiral pharmaceuticals. Moreover, enzymatic catalysis will greatly benefit in pharmaceutical manufacture.

Ferrous iron and α-ketoglutarate dependent dioxygenases (Fe/αKGDs) were first identified in studies on collagen biosynthesis, where the Fe/αKGDs were found could catalyze the hydroxylation of prolyl and lysyl residues [5]. Following the pioneering work, dioxygenases are a very widely distributed family when it emerged. The dioxygenases catalyze a number of oxidation reactions, possibly the widest of any identified enzyme family. The dioxygenases were applied in business started with plant growth retardants and now extend to a clinical drug compound used for heart protection. Several dioxygenases are now explored to finish the target for therapeutic intervention for diseases including anemia, inflammation and cancer [6].

In this review, we describe the recent discoveries regarding the synthesis of physiological characteristics and microbial production of hydroxy-L-amino acids, particularly (2*S*, 3*R*, 4*S*)-4-hydroxyisoleucine and hydroxyproline. In addition to its properties the catalytic mechanisms of hydroxylase and applications in fundamental research and business are also discussed.

2. Synthesis and properties of hydroxy amino acids

2.1. The synthesis and properties of the 4-Hydroxyisoleucine (4-HIL)

2.1.1. The properties of 4-HIL. The 4-HIL, which was detected in the seeds of fenugreek firstly and that own 80 percent of total content free amino acid, the annual herb Trigonella foenumgraceum, has insulinotropic and anti-obesity effects and knowned as a promising drug for diabetes [7,8]. The 4-HIL has been used for the treatment of type II diabetes (e.g. sulfonylureas) as the insulin response mediated by 4-HIL that is directly based on the glucose concentration when compared with several types of pharmacological drugs. It is this unique property of 4-HIL that prevents adverse side effects such as hypoglycemia in the treatment of type II diabetes [9,10].

Besides, 4-HIL has more effect in regulating blood lipid and lowering cholesterol levels, on the one hand, the 4-HIL had efficacy on anti-hyperglycaemic and anti-dyslipidemic for the first time in a well-characterised model of type II diabetes in mice [11,12], on the other hand, it also could significantly decrease the levels of plasma triglyceride, total cholesterolby, and free fatty acids in the dyslipidemic hamster model [13]. In summary, the 4-HIL should be a very promising treatment for prevention of chronic disease and its therapeutic benefits includes anti-infective, antioxidant, anti-infammatory, cardioprotective and digestive stimulant activities [14-22]. Thus, the research of 4-HIL synthesis of receives more and more attention, urgent requirements for 4-HIL were also put forward by more and more people in modern times. The paragraph text follows on from the subsubsection heading but should not be in italic.

2.1.2. The synthesis of 4-HIL. Although several kinds of chemical and biological synthesis methods for

4-HIL have been reported, all in all, there are mainly three synthesis methods in industries including extraction and separation processes, chemical synthesis and biological enzyme synthesis (figure 1(a)) for the moment. Fenugreek (Trigonella foenum-graecum) is a promising medicinal herb. It has been used for many years and also applied to raw material for extraction of 4-HIL. Briefly, the amino acids were extracted by absolute ethanol, then the washed fractions were further purified by ion exchange resin, and the eluted fractions contain amount of the right 4-HIL, pure 4-HIL was obtain after further elution at last [23,24].

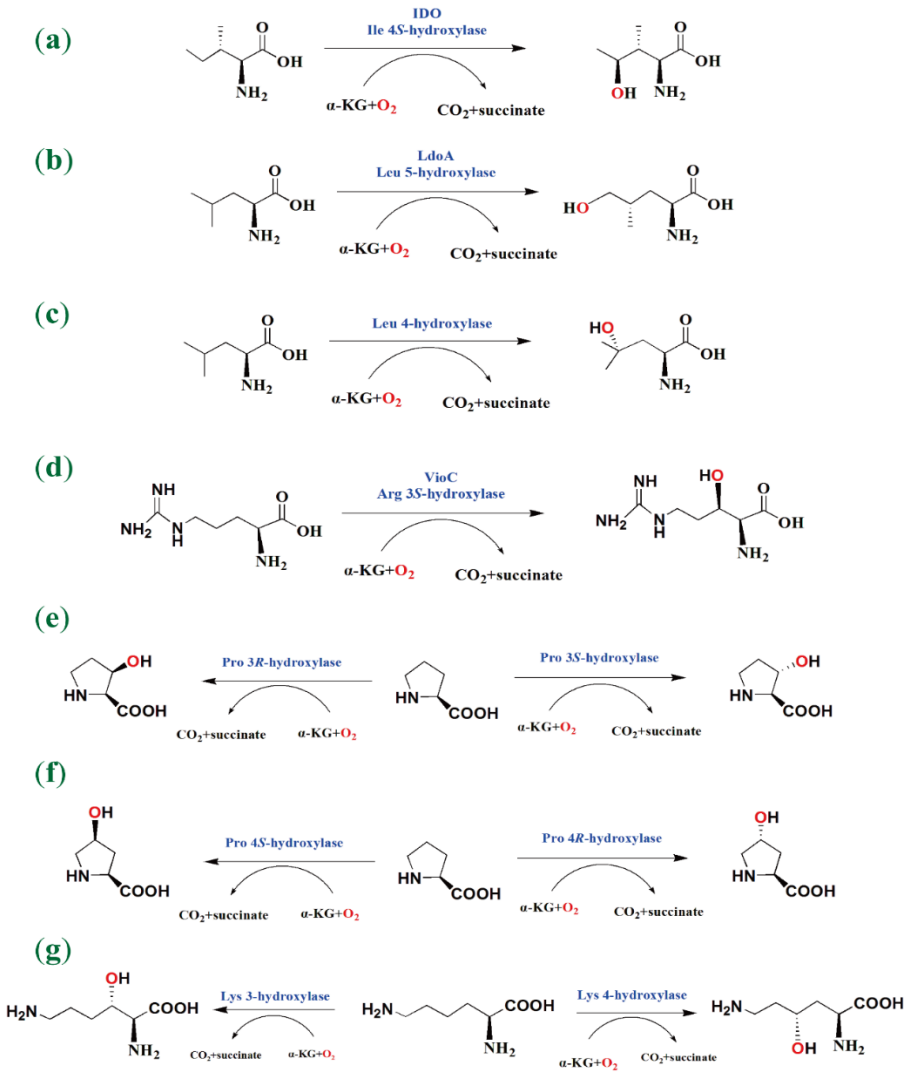

Figure 1. Typical biocatalytic synthesis of hydroxylated L-amino acids by Fe/αKG-DOs. (a) The C-4 hydroxylation of L-isoleucine forms L-4-hydroxyisoleucine (4-HIL). (b) The C-5 hydroxylation of L-leucine forms L-5-hydroxyisoleucine. (c) The C-4 hydroxylation of L-leucine generates L-4-hydroxyleucine. (d) The C-3 hydroxylation of L-arginine. (e) The C-4 hydroxylation of L-peoline forms 4*S* and 4*R*-hydroxyproline, respectively. (f) The C-3 hydroxylation of L-peoline forms 3*S* and 3*R*-hydroxyproline, respectively. (g) The C-3 and c-4 hydroxylation of L-lysine form L-3-hydroxylysine and L-4-hydroxylysine, respectively.

The chemical synthesis processes are often employed to chemical products and drug synthesis including eathyl-2-methyl acetoacetate, tert-Butyl bromoacetate, Ethyl 3-piperidinecarboxylate and so on. Wang et al provided an eight-step synthesis method as described for 4-HIL which has been achieved a 39% overall yield, one crucial step is the biotransformation of ethyl-2-methylacetoacetate to ethyl (2*S*,3*S*)-2-methyl-3-hydroxybutanoate by Geotrichum candidum [25]. There are another six-step chemical synthesis of 4-HIL with total control of stereochemistry, the final step being the bio-catalytical preparation by hydrolysis of a N-phenylacetyl lactone derivative using the commercial penicillin acylase G, and the yield was up to 40% [26]. Arthrobacter simplex AKU 626 was found that could produce 4-HIL from acetaldehyde, α-ketobutyric acid, and L-glutamate by the function of the branched chain amino acid transaminase gene with yield of 3.2 mM 4-HIL [27].

The fully enzymatic synthesis of 4-HIL is to clone and highly express the bifunctional L-isoleucine-4-dioxygenase to provide active protein as a target for new drugs which seems to be getting faster, more efficient, therefore, this enzymatic synthesis strategy has been researched further and applied widely abroad as well as popular at home. Haefelé et al research results showed that isoleucine could be catalyzed forming 4-HIL by the dioxygenase on the presence of ferrous iron, 2-oxoglutarate (α-KG), ascorbate and oxygen, and it also indicated 2-oxoacid dependent dioxygenase has important roles in this biosynthetic pathway [28]. One bacillus thuringiensis strain 2e2 AKU 0251 that could hydroxylate the L-isoleucine to produce 4-HIL was screened, then the purified hydroxylase was measured, the results indicated it was a α-KG dependent L-isoleucine dioxygenase (IDO) which had high stereoselectivity to produce only (2*S*, 3*R*, 4*S*)-4-HIL [7]. This new study also provides a direction for developing microbial fermentations and biological catalysis to produce 4-HIL.

Based on the mechanism of the IDO hydroxylation process could couple the 2Δ strain destroyed TCA (figure 2), Smirnov et al cloned the IDO gene into *Escherichia coli* 2Δ strain which the α-KG dehydrogenase, isocitrate liase, and isocitrate dehydrogenase kinase/ phosphatase were knocked out leading to a highly biotransformation of L-isoleucine into 4-HIL [24]. Shi *et al* modified the key enzyme 4-HIL dehydrogenase (HILDH) using protein modification strategies to enhance one-step carbonyl reduction to only produce (2*S*, 3*R*, 4*S*)-4-HIL, and this strategy achieved commercial-scale production of 4-HIL [29].

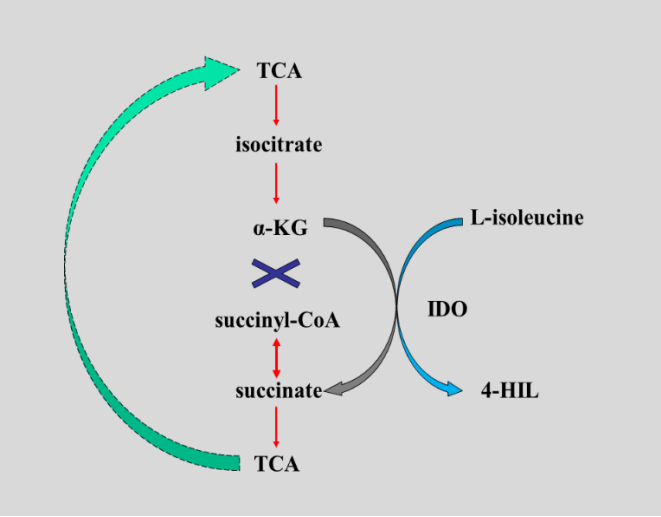

Figure 2. Reconstruction of the TCA cycle in the *E. coli* 2Δ strain producing more α-KG to couple the simultaneous oxidation of L-isoleucine (Smirnov *et al* 2010).

According to a comparison with three methods for 4-HIL (table 1), we could find that the extraction and chemical synthesis required more complex multilevel reactions, due to the complexity

of the products obtained by the two methods mentioned above, it was very difficult to isolate and purify the single 4-HIL, moreover, there is a struggle for control to stereoselectivity for 4-HIL. Thus, developing microbial fermentations and biological catalysis would become new direction to produce 4-HIL for industrialized application.

Table 1. Comparison of 4-HIL industrial production methods.

Methods	Raw material	Procedure feature	Product feature
Extraction and separation processes	Fenugreek	Restrictions of local materials, complex process	Low-purity, difficult of separation
Chemical synthesis	Methyl 2-Ethylacetoacetate, acetaldehyde, α-KG and L-glutamate et al	Complex process, low yield, low efficiency for stereoselectivity of product	Low-safety, low-purity
Biological enzyme	L-isoleucine, L-isoleucine dioxygenase, α-KG et al	Efficient production, simple technique, Environment protecting	High-pure, high security

2.2. The synthesis and properties of hydroxyproline

Hydroxyproline has many isomers, which could produce in cis- or trans-forms, such as *cis*-3-hydroxy-L-proline (C3LHyp) and trans-3-hydroxy-l-proline (T3LHyp) (figure 1(e)), *cis*-4-hydroxy-L-proline (C4LHyp) and trans-4-hydroxy-L-proline (T4LHyp) (figure 1(f)). These hydroxyprolines exist in some proteins such as collagen, peptide antibiotics and plant cell wall, moreover, some isomers are used for the synthesis of pharmaceuticals as chiral building blocks.

2.2.1. The properties of T3LHyp and C3LHyp. T3LHyp is generated by prolyl 3-hydroxylase hydroxylation, which is the most plentiful in collagen. And another stereoisomer, C3LHyp has been realized the value as a drug to treat some diseases, such as cancer and collagen disorders, becoming the important biologically active components for chiral drug synthesis [30].

2.2.2. The synthesis of T3LHyp and C3LHyp. T3LHyp come mainly from the biosynthetic pathway, nevertheless, its biosynthetic pathway is still not clarified up to now as there are few researches to the proline trans-3-hydroxylase at present. However, C3LHyp has more understand as microbial production for pharmaceutical drugs [31]. C3LHyp is produced by a whole-cell biocatalyst, Johnston et al cloned the L-proline cis-3-hydroxylase gene from Streptomyces sp. TH1 into E. coli cells and was overexpress, then L-proline was put into the fermentation system as substrate, after cultivation 60 hours, the yield of C3LHyp could be up to 99% [32].

2.2.3. The properties of T4LHyp and C4LHyp. T4LHyp is formed by posttranslational modification, which is the most common in nature and key for the synthesis of procollagen, and it was provided to the cosmetics industry. C4LHyp is one ingredient of phallotoxin generated by the poisonous mushroom Amanita phalloides [33], which is effect on inhibiting the growth of new cells, meanwhile have also been applied to detect cells antitumor activity in tissue culture and in vivo [34]. In addition, C4LHyp has been used as an anticancer drug. [35,36].

2.2.4. The synthesis of T4LHyp and C4LHyp. The hydroxyproline could be synthesized by some chemical synthesis from D-glucose, D-mannitol, the synthesis method including conversion of D-glucose into N-benzyloxycarbonyl-c-alkenyl amine [37]. However, the hydroxyproline isomers, such as T4LHyp and C4LHyp, were not studied enough until enantioselective L-proline hydroxylases were

found.

The synthesis of T4LHyp had only been reported using method of biological catalysis [38]. Shibasaki et al used special strains to realize overexpression of L-proline-4-hydroxylase (L4HL) from *Dactylosporangium* sp. RH1 to obtain T4LHyp in proline oxidase-deficient cells [39]. Based on this method, many special strains such as *Clonostachys cylindrospora*, *Gliocladium* sp., and *Nectria gliocladioides* were chosen for producing T4LHyp in in an optimized medium [40,41]. Thus, to obtain more characteristic properties on the enzyme for catalysis T4LHyp, the L-proline-trans-4-hydroxylase (LT4HL) from *S. griseoviridus* P8648 has been studied for purifying and identifying in vitro evaluation. To improve the yield of T4LHyp, Shibasaki *et al* have explored to build commercial scale microbial production for T4LHyp [38].

The fully enzymatic synthesis of C4LHyp was obtained by using L-proline-*cis*-4-hydroxylase (LC4HL) to hydroxylates L-proline, and the LC4HL was discovered from *rhizobia Sinorhizobium meliloti* and *Mesorhizobium loti* [42]. In addition, the N-acetyl C4LHyp had also been produced from L-proline by LC4HL and N-acetyltransferase Mpr1 [43,44]. Bach *et al* designed that the conversion of LC4HL that came from *rhizobia Sinorhizobium meliloti* into C4LHyp from L-proline in vitro, which was evaluated to be approximately 10 % [43]. According to these reports we could find that the biosynthesis of C4LHyp utilized L-proline as initial material.

However, in these cases, it was revealed that whether T4LHyp or C4LHyp synthesis were all relied on direct hydroxylation of L-proline by L4HL as the flexible conformation of hydroxyproline, and the biological catalysis method became worthy of study to produce hydroxyproline.

2.3. The synthesis and properties of L-5-Hydroxytryptophan

L-5-Hydroxytryptophan (5-HTP) is produced by L-tryptophan hydroxylase from l-tryptophan, which is an intermediate to synthesize serotonin. 5-HTP has been used for medical treatment as a precursor of the neurotransmitter serotonin for many years [45]. Thus, the 5-HTP has been used to treat some disease conditions, including in the therapy of depression, insomnia, fibromyalgia, obesity, antidepressant, chronic headaches [46-50].

At present, the main ways to obtain 5-HTP is extraction and separation processes from the seeds of the Griffonia simplicifolia [51], and which is commonly used in industry. The chemical and multienzymatic methods for synthesis of 5-HTP was reported by Winnicka *et al*, and the key intermediate was to obtain DL-alanine [52]. The multienzymatic synthesis of L-tryptophan and 5-HTP both in carboxylic group has been reported [53]. However, these methods of synthesis of 5-HTP have low yield, low-purity and difficulty in separation, therefore, the synthesis of 5-HTP need more research in the future.

2.4. The synthesis and properties of hydroxylysine

Hydroxylysine is naturally produced by non-proteinogenic β-hydroxy amino acids which are important component part of the peptides, protein scaffolds and antibiotics [54,55]. It is reported that hydroxylysine has two isomers, 3-hydroxylysine and 5-hydroxylysine (figure 1(g)), which were produced by enzymes catalysis and chemical synthesis, respectively [56,57]. Furthermore, 3-hydroxylysine and 5-hydroxylycine could be used for synthesis of 3-hydroxypipecolic acid and 5-hydroxypipecolic acid as an intermediary, respectively [58].

2.5. Other hydroxy-amino-acids

Although some other hydroxy-amino-acids were reported, there less researches work on it. 3-hydroxy-L-valine was found as synthesis intermediate of a HIV protease inhibitor, and that was important in the synthesis of HIV protease inhibitor [59]. (2*S*,3*S*)-3-hydroxyasparagine is a promising therapeutic for Gram-positive bacterial infections, and it also composes the calcium-dependent lipopeptide antibiotics [60]. (2*S*,3*S*)-3-hydroxyarginine (figure 1(d)) is used as biosynthesis of viomycin which is necessary for keeping the antimicrobial activity. In addition, this antibiotic is effective and valuable for the bacterial infections treat [61]. (2*S*,3*R*)-3-hydroxyleucine is broad development prospects

medical materials for the production of certain cyclic depsipeptides, and cyclic depsipeptides have function to be used as component of an antibiotic or platelet aggregation inhibitors [59], (2*S*,3*R*)-3-hydroxyphenylalanine is reported that found in the structure of lysobactin. (2*S*,4*S*)-5-hydroxyleucine (figure 1(b)) is refer to the biosynthetic pathyway of (2*S*,4*S*)-4-methylproline, which is known to formation of nostopeptolides [62].

3. Catalytic mechanisms of ferrous iron [Fe(II)] and α-ketoglutarate (αKG)-dependent hydroxylase

It is the asymmetric hydroxylation that is still a significant reaction to synthetize valuable chiral compounds in the industry [63]. The Fe(II)/α-ketoglutarate-dependent dioxygenases (Fe/αKGDs) are applied urgently to design as amino acids hydroxylation biocatalysts for its highly regio- and stereoselectivity of the reactions [64]. And some hydroxylases belong to Fe/αKGDs which play an important role to specifically catalyze L-amino-acids producing hydroxylation of amino acids including 4-HIL, 5-Hydroxyleucine, (2*S*,3*R*)-3-hydroxyleucine, N-succinyl-(2*S*,3*R*)-3-hydroxyleucine, *cis*-4-hydroxy-L-proline, (2*S*)-4-hydroxyvaline [65], and so on. Fe/αKGDs are used to act on free amino acids with different types of regioselective hydroxylations. Thus, amino acids have been put to use as typical substrates of Fe/αKGDs to be converted into hydroxy amino acids.

In some cases, Fe/αKGDs are parts of multidomain proteins which is in accordance with catalysis mechanism. The substrates hydroxylation is typical reactivity of Fe/αKGDs and is also researched most frequently and deeply. The functions of Fe/αKGDs contain to repair of DNA/RNA alkylation damage, demethylation of 5-methylcytosine and degrade environmental substances [5,66-68]. The hydroxylation process needs Fe(II) and α-KG as cofactors. Oxygen is required in the hydroxylation to oxidatively break down α-KG into succinate and CO_2 while another oxygen atoms attacks the substrate to form hydroxy amino acid. The Fe/αKGDs have common structural features, including a typical protein fold known as double-stranded b-helix (DSBH) fold, an HXD/EXnH iron binding motif that is the core of the protein structure [68-70]. The α-KG binds to the iron in a bidentate manner via its 1-carboxylate and 2-oxo groups and its binding sites are relatively conserved. However, it is variation of the substrate-binding sites, leading to the hydroxylation has highly stereoselectivity and substrate specificity.

In all reported structures of the enzyme Fe/αKG-DOs complex, Hanauske-Abel *et al* [71] first put forward a mechanism involving the special relations and coordination of three metal-amino acid side chains in the active metallocentre and generating a valent iron: oxygen atom intermediate. After that, many other Fe/αKGDs structures were found which permitted redefinition of this structure model and producing a basic common mechanism as shown in figure 3. The catalysis starts with Fe(II) attached to the 2-His-1-carboxylate facial triad [72,73], and the other three coordination sites occupied by water molecules (figure 3(a)). Then the α-KG binds to the Fe(II) active metallocentre in a bidentate configuration, with its keto group and carboxylate opposite the structure of 2-His-1-carboxylate facial triad, which results in replacing two metal-bound water molecules (figure 3(b)). Following α-KG binding, it is the binding of the remaining water to the metal was weakened by substrate-triggered leading to substrate can be easy to bind the active coordination site displacing the third metal-bound water molecule (figure 3(c)). The substrate binding process triggered an oxygen molecule to bind Fe(II) active center and occur oxidation reactions with generating an Fe(III)-superoxo intermediate [74] (figure 3(d)). Then 2-ketogroup of α-KG are attacked by the distal oxygen atom of the Fe(III)-superoxo species leading to generating a Fe(IV)=O species (figure 3(e)). Soon to come is oxidative decarboxylation of α-KG triggered by Fe(IV)=O species, and then the CO_2 is released [75], the ferryl intermediate and succinate are generated (figure 3(f)). The last second step is that a substrate radical and Fe(III)-OH species are formed after Fe(IV)-oxo species acquired a hydrogen atom from the substrate (figure 3(g)). Lastly, the hydroxyl radical rebound (or more complex chemistry) completes substrate hydroxylation and recycles the enzyme to the initial Fe(II) state [76].

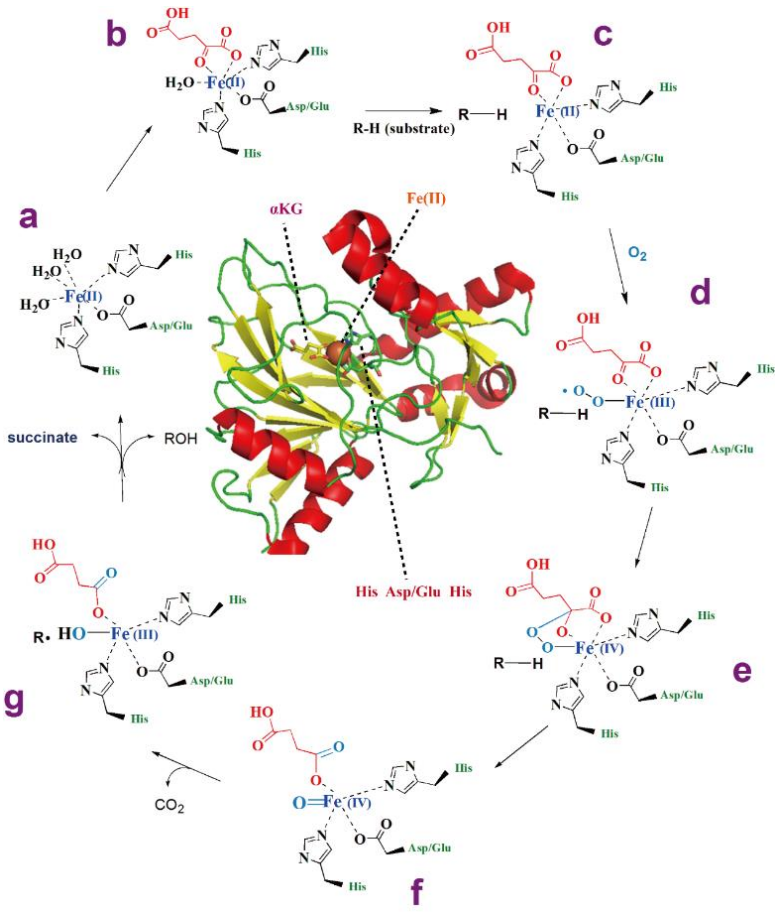

Figure 3. Proposed mechanism of reaction by Fe/αKG-DOs. Middle, Structure of a representative Fe/αKG-DOs (PDB access code 1GY9). TauD is constructed with red α helices, yellow β strands, green unstructured regions, α-KG (sticks with yellow carbons), Fe (orange sphere) and side chains are integrated in stick mode, furthermore the position of the metallocentre relative to the major group (double-stranded β helix core). The (a)-(g) is the simplified hydroxylation mechanism.

The above proposed hydroxylation mechanism was supported by extensive structural studies of Fe/αKGDs that provides experimental data [68,77-79]. And some crystal structures of Fe/αKGDs revealed the consensus facial triads and Fe(II) active center coordination environments as shown in the figure 3. The decarboxylation of α-KG almost is not accompanied with the substrate oxidation that has been studied in many Fe/αKGDs [79]. This substrate 'uncoupled' turnover with α-KG could be observed in many cases, such as AlkB and TauD, consequently leading to aromatic residues auto-oxidation in hydroxylation process which may also be mediated by the reactive Fe(IV)=O species [66]. Tuderman *et al* found [80] that the ascorbate has function of decreasing the oxidation of Fe(II) regenerating the catalytically active Fe(II) species in resting state.

4. Conclusions and perspectives

Hydroxy amino acids are essential and important kind amino acids in the case of its biological functions and biotechnological cosmetic applications. It has physiological activities and function of antifungal, antibacterial, antiviral and anticancer properties, but can be used as precursors belong to

important components of chiral compounds in the asymmetric synthesis of pharmaceuticals. A unique conformation of hydroxy amino acids contributes to the formation of chemical isomers, result in the generation of various hydroxy amino acid analogues. As described previously, 4-HIL has effect on for prevention of chronic disease; and hydroxy-L-proline are also used as medical intermediates for pharmaceutical. Due to the emergency hydroxyproline isomers, it has different effect on biological functions, such as C3LHyp, T3LHyp, C4LHyp and T4LHyp. Nowadays, chemical synthesis has been developed for many hydroxy amino acids synthesis including unnatural compounds, such as 4-HIL and some hydroxyproline. And some other hydroxy amino acids found in biological resources could be obtained by extracted process. However, biological catalysis with existence of related hydroxylase could produce 4-HIL, peptide-like compounds containing hydroxyproline and so on. The biocatalysis of some L-amino acids is poorly studied and needs more comprehensive exploration. The Fe/αKGDs has a prominent ability to trigger the oxygen molecular for realize the important oxidative transformations and the oxidative decarboxylation of the α-KG moiety of the substrate to generate the key ferryl intermediate [80]. Furthermore, new members of the Fe/αKG-DOs superfamily is certain to be discovered in the biosynthesis of hydroxy-L-amino acid, and the research and application of hydroxy-amino acid would be more and more extensive in the future.

Acknowledgments
This research was financially supported by Natural Science Foundation of Tianjin.

References

[1] Hibi M, Kawashima T, Kodera T, *et al* 2011 Characterization of *Bacillus thuringiensis* L-isoleucine dioxygenase for production of useful amino acids *Appl Environ Microb* **77** 6926-30
[2] Patel N R 2008 Synthesis of chiral pharmaceutical intermediates by biocatalysis *Coordin Chem Rev* **252** 659-701
[3] Patel N R 2004 Biocatalytic synthesis of chiral pharmaceutical intermediates *Food Technol Biotechnol* **24** 305-25
[4] Savile K C, Janey M J, Mundorff C E, *et al* 2010 Biocatalytic asymmetric synthesis of chiral amines from ketones applied to sitagliptin manufacture *Sci.* **329** 305-9
[5] McDonough M A, Loenarz C, Chowdhury R, *et al* 2010 Structural studies on human 2-oxoglutarate dependent oxygenases *Curr Opin Struct Biol* **20** 659-72
[6] Rose N R, McDonough M A, King O N, *et al* 2011 Inhibition of 2-oxoglutarate dependent oxygenases *Chem Soc Rev* **40** 4364-97
[7] Kodera T, Smirnov S V, Samsonova N N, *et al* 2009 A novel L-isoleucine hydroxylating enzyme, L-isoleucine dioxygenase from *Bacillus thuringiensis*, produces (2*S*,3*R*,4*S*)-4-hydroxyisoleucine *Biochem Biophys Res Commun* **390** 506-10
[8] Moorthy R, Prabhu K M and Murthy P S 2010 Anti-hyperglycemic compound (GII) from fenugreek (Trigonella foenum-graecum Linn.) seeds, its purifcation and efect in diabetes mellitus *Indian J Exp Biol* **48** 1111-8
[9] Jackson J E and Bressler R 1981 Clinical pharmacology of sulfonylurea hypoglycemic agents II *Drugs* **22** 295-320
[10] Jennings A M, Wilson R M and Ward J D 1989 Symptomatic hypoglycemia in NIDDM patients treated with oral hypoglycemic agents *Diab Care* **12** 203-8
[11] Singh A B, Tamarkar A K, Narender T, *et al* 2010 Antihyperglycaemic effect of an unusual amino acid (4-hydroxyisoleucine) in C57BL/KsJ-db/db mice *Nat Prod Res* **24** 258-65
[12] Eidi A, Eidi M and Sokhteh M 2007 Effect of fenugreek (Trigonella foenum-graecum L) seeds on serum parameters in normal and streptozotocin-induced diabetic rats *Nutr Res* **27** 728-33
[13] Narender T, Puri A, Khaliq T, *et al* 2006 4-Hydroxyisoleucine an unusual amino acid as antidyslipidemic and antihyperglycemic agent *Bioorganic & Medicinal Chemistry Letters* **16** 293-6

[14] Kandhare A D, Bodhankar S L, Mohan V, *et al* 2015 Effect of glycosides based standardized fenugreek seed extract in bleomycin-induced pulmonary fbrosis in rats:Decisive role of Bax, Nrf2, NF-κB, Muc5ac, TNF-α and IL-1β *Chem Biol Interact* **237** 151-65
[15] Aboubakr H A, Nauertz A, Luong N T, *et al* 2016 In vitro antiviral activity of clove and ginger aqueous extracts against feline calicivirus, a surrogate for human norovirus *J Food Prot* **79** 1001-12
[16] Sakr S A, Mahran H A and Abo-El-Yazid S M 2012 Effect of fenugreek seeds extract on cyclophosphamide-induced histomorphometrical, ultrastructural and biochemical changes in testes of albino mice *Toxicol Ind Health* **28** 276-88
[17] Panda S, Tahiliani P and Kar A 1999 Inhibition of triiodothyronine production by fenugreek seed extract in mice and rats *Pharmacol. Res.* **40** 405-9
[18] Lamfon H A 2012 Effect of fenugreek seeds extract on carbendazim-inhibited spermatogenesis in albino rats *J App Pharmaceu Sci* **2** 9-13
[19] Becker P M, Widjaja-Greefkes H C and Wikselaar P G 2010 Inhibition of binding of the AB5-type enterotoxins LT-I and cholera toxin to ganglioside GM1 by galactose-rich dietary components *Foodborne Pathog Dis* **7** 225-33
[20] Bin-Hafeez B, Haque R, Parvez S, *et al* 2003 Immunomodulatory efects of fenugreek (Trigonella foenum graecum L.) extract in mice *Int Immunopharmacol* **3** 257-65
[21] Mukthamba P and Srinivasan K 2015 Hypolipidemic infuence of dietary fenugreek (Trigonella foenum-graecum) seeds and garlic (Alliumsativum) in experimental myocardial infarction *Food Funct* **6** 3117-25
[22] Petit P, Sauvaire Y, Ponsin G, *et al* 1993 Effects of a fenugreek seed extract on feeding behaviour in the rat:metabolic-endocrine correlates *Pharmacol Biochem Behav* **45** 369-74
[23] Fowden L, Pratt H M and Smith A 1973 4-Hydroxyisoleucine from seed of Trigonella foenum-graecum *Phytochemistry* **12** 1707-11
[24] Smirnov S V, Kodera T, Samsonova N N, *et al* 2010 Metabolic engineering of *Escherichia coli* to produce (2*S*,3*R*,4*S*)-4-hydroxyisoleucine *Appl Microbiol Biotechnol* **88** 719-26
[25] Wang Q, Ouazzani J, Sasaki N A, *et al* 2002 A Practical Synthesis of (2*S*,3*R*,4*S*)-4-Hydroxyisoleucine, A Potent Insulinotropic α-Amino Acid from Fenugreek *Eur J Org Chem* **5** 834-9
[26] Rolland V, Rolland M, Roumestant M L, *et al* 2004 Chemoenzymatic synthesis of enantiomerically pure (2*S*,3*R*,4*S*)-4-Hydroxyisoleucine, an insulinotropic amino acid isolated from Fenugreek seeds *Eur J Org Chem* **4** 873-7
[27] Ogawa J, Yamanaka H, Mano J, *et al* 2007 Synthesis of 4-Hydroxyisoleucine by the aldolase–transaminase coupling reaction and basic characterization of the aldolase from *Arthrobacter simplex* AKU 626 *Biosci Biotech Bioch* **71** 1607-15
[28] Haefelé C, Bonfils C and Sauvaire Y 1997 Characterization of a dioxygenase from Trigonella foenum-graecum involved in 4-hydroxyisoleucine biosynthesis *Phytochemistry* **44** 563-6
[29] Shi X, Miyakawa T, Nakamura A, *et al* 2017 Engineering a short-chain dehydrogenase/reductase for the stereoselective production of (2*S*,3*R*,4*S*)-4-hydroxyisoleucine with three asymmetric centers *Sci Rep* **7** 13703
[30] Shibasaki T, Mori H and Ozaki A 2000c Cloning of an isozyme of proline 3-hydroxylase and its purification from recombinant *Escherichia coli Biotech Lett* **22** 1967-73
[31] Mori H, Shibasaki T, Yano K, *et al* 1997 Purification and cloning of a proline 3-hydroxylase, a novel enzyme which hydroxylate free L proline to cis-3-hydroxy-L-proline *J Bacteriol* **179** 5677-83
[32] Johnston R M, Chu L N, Liu M, *et al* 2009 Hydroxylation of L-proline to cis-3-hydroxy-L-proline by recombinant Escherichia coli expressing a synthetic L-proline-3-hydroxylase gene *Enzym Micro Tech* **45** 484-90
[33] Wieland T 1968 Poisonous principles of mushrooms of the genus Amanita. Four-carbon amines acting on the central nervous system and cell-destroying cyclic peptides are produced *Sci*

159 946-52
[34] Hiroshi T and Satoshi Y 2013 Roles of enzymes in anti-oxidative response system on three species of chenopodiaceous halophytes under NaCl-stress condition *Soil Sci Plant Nutr* **59** 603-11
[35] Tan E M and Ryhanen L U 1983 Proline analogues inhibit human skin fibroblast growth and collagen production in culture *J Invest Dermatol* **80** 261-7
[36] Eldridge C F, Bunge R P and Bunge M B 1988 Effect of *cis*-4-hydroxy-Lproline, an inhibitor of Schwann cell differentiation, on the secretion of collagens and noncollagenous protein by Schwann cells *Exp Cell Res* **174** 491-501
[37] Navnath B K, Kasture V M and Dhavale D D 2010 Total synthesis of natural *cis*-3-hydroxy-L-proline from D-glucose *Tetrahdron Lett* **51** 6745-7
[38] Shibasaki T, Hashimoto S, Mori H, *et al* 2000a Construction of novel hydroxyproline-producing recombinant Escherichia coli by introducing a proline 4-hydroxylase gene *J Biosci Bioeng* **90** 522-5
[39] Shibasaki T, Mori H, Chiba S, *et al* 1999 Microbial proline 4-hydroxylase screening and gene cloning *Appl Environ Microbiol* **65** 4028-31
[40] Matsuoka T, Serizawa N, Hosoya T, *et al* 1994 Isolated cultures of microorganism of Clonostachys cylindrospora, Gliocladium and Nectria gliocladioides *US Patent* 5 407 826
[41] Hosoya T, Matsuoka T, Serizawa N, *et al* 1995 Two morphological groups derived from Clonostachys cylindrospora and their relationship to trans-4-L-proline productivity *Mycoscience* **36** 193-7
[42] Hara R and Kino K 2009 Characterization of novel 2-oxoglutarate dependent dioxygenases converting L-proline to cis-4-hydroxy-Lproline *Biochem Biophys Res Commun* **379** 882-6
[43] Bach T M H, Hara R, Kino K, *et al* 2013 Microbial production of N-acetyl cis-4-hydroxy-L-proline by coexpression of the Rhizobium L-proline *cis*-4-hydroxylase and the yeast N-acetyltransferase Mpr1 *Appl Microbiol Biotechnol* **97** 247-57
[44] Hoa B T M, Hibi T, Nasuno R, *et al* 2012 Production of N-acetyl *cis*-4-hydroxy-L-proline by the yeast Nacetyltransferase Mpr1 *J Biosci Bioeng* **114** 160-5
[45] Bell E A and Fellows L E 1966 Occurrence of 5-hydroxy-L-tryptophan as a free plant amino-acid. *Nature* **210** 529
[46] Nicolodi M and Sicuteri F 1996 Fibromyalgia and migraine, two faces of the same mechanism. Serotonin as the common clue for pathogenesis and therapy *Adv Exp Med Biol* **398** 373-9
[47] Cangiano C, Ceci F, Cascino A, *et al* 1992 Eating behavior and adherence to dietary prescriptions in obese adult subjects treated with 5-hydroxytryptophan *Am J Clin Nutr* **56** 863-7
[48] Guilleminault C M, Cathala J P and Castaigne P 1973 Effects of 5-hydroxytryptophan on sleep of a patient with a brain-stem lesion *Electroencephalogr Clin Neurophysiol* **34** 177-84
[49] Bono G, Criscuoli M, Martignoni E, *et al* 1982 Serotonin precursors in migraine prophylaxis *Adv Neurol* **33** 357-63
[50] Birdsall T C 1998 Therapeutic applications of taurine *Altern Med Rev* **3** 128-36
[51] Turner E H, Loftis J M and Blackwell A D 2006 Serotonin a la carte: Supplementation with the serotonin precursor 5-hydroxytryptophan *Pharmacol Ther* **109** 325-38
[52] Winnicka E and Kańska M 2009 Synthesis of [3-14C]-L-tryptophan and 5′-hydroxy-[3-14C]-L-tryptophan *J Radioanal Nucl Ch* **280** 79-84
[53] Boroda E, Kański R and Kańska M 2003 Synthesis of [14C]-l-tryptophan and [14C]-5′-hydroxy-l-tryptophan labeled in the carboxyl group *J Labelled Compd Rad* **46** 441-7
[54] Kang S H, Kang S Y, Lee H S, *et al* 2005 Total synthesis of natural tert-alkylamino hydroxy carboxylic acids *Chem Rev* **105** 4537-58
[55] Alcaide B, Almendros P and Aragoncillo C 2007 Beta-lactams: versatile building blocks for the stereoselective synthesis of non-beta-lactam products *Chem Rev* **107** 4437-92
[56] Tung C L, Lam H Y, Xu J, *et al* 2014 A fluorogenic probe for recognizing 5-hydroxylysine

inspired by serine/threonine ligation *Chem Commun* **50** 5298-300
[57] Baud D, Saaidi P, Monfleur A, *et al* 2015 Synthesis of Mono-and Dihydroxylated Amino Acids with New α-Ketoglutarate-Dependent Dioxygenases: Biocatalytic Oxidation of C-H Bonds *Chemcat chem* **6** 3012-7
[58] Liu L X, Peng Q L and Huang P Q 2008 A new approach for the asymmetric synthesis of (2*S*,3*S*)-3-hydroxypipecolic acid *Tetrahedron-asymmetry* **19** 1200-3
[59] Hibi M, Kasahara T, Kawashima T, *et al* 2015 Multi−enzymatic synthesis of optically pure β-hydroxy α−amino acids *Adv Synth Catal* **357** 767-74.
[60] Fraser T G, Hansen C and Long J K 2006 Newer antibiotics for serious Gram-positive infections *Clev Clin J Med* **73** 847-53
[61] Thomas M G, Chan Y A and Ozanick S G 2003 Deciphering tuberactinomycin biosynthesis: isolation, sequencing, and annotation of the viomycin biosynthetic gene cluster *Antimicrob. Agents Ch* **47** 2823-30
[62] Hibi M, Kawashima T, Sokolov P, *et al* 2013 L-Leucine 5-hydroxylase of *Nostoc punctiforme* is a novel type of Fe(II)/α-ketoglutarate-dependent dioxygenase that is useful as a biocatalyst Appl Microbiol Biotechnol **97** 2467-72
[63] Hibi M and Ogawa J 2014 Characteristics and biotechnology applications of aliphatic amino acid hydroxylases belonging to the Fe(II)/α-ketoglutarate-dependent dioxygenase superfamily *Appl Microbiol Biotechnol* **98** 3869-76
[64] Qin H-M, Miyakawa T, Nakamura A, *et al* 2014 Structural optimization of SadA, an Fe(II)-and α-ketoglutarate-dependent dioxygenase targeting biocatalytic synthesis of N-succinyl-L-threo-3,4-dimethoxyphenylserine *Biochem Biophys Res Commun* **450** 1458-61
[65] Qin H-M, Miyakawa T, Jia M, *et al* 2013 Crystal structure of a novel N-substituted L-amino acid dioxygenase from *Burkholderia ambifaria* AMMD *PLoS One* **8** e63996
[66] Henshaw T F, Feig M and Hausinger R P 2004 Aberrant activity of the DNA repair enzyme AlkB *J Inorg Biochem* **98** 856-61
[67] Hausinger R P 2015 Biochemical diversity of 2-oxoglutarate-dependent oxygenases C J Schofield and R P Hausinger eds *2-Oxoglutarate-Dependent Oxygenases* pp 1-58 (Cambridge, UK: The Royal Society of Chemistry)
[68] Hausinger R P 2004 Fe(II)/a-ketoglutarate-dependent hydroxylases and related enzymes *Crit Rev Biochem Mol* **39** 21-68
[69] Chowdhury R, McDonough MA, Mecinovic J, *et al* 2009 Structural basis for binding of hypoxia-inducible factor to the oxygensensing prolyl hydroxylases *Structure* **17** 981-9
[70] Clifton I J, McDonough M A, Ehrismann D, *et al* 2006 Structural studies on 2-oxoglutarate oxygenases and related doublestranded b-helix fold proteins *J Inorg Biochem* **100** 644-69
[71] Hanauske-Abel H M and Günzler V 1982 A stereochemical concept for the catalytic mechanism of prolylhydroxylase: applicability to classification and design of inhibitors *J Theor Biol* **94** 421-55
[72] Elkins J M, Ryle M J, Clifton I J, *et al* 2002 Xray crystal structure of Escherichia coli taurine/a-ketoglutarate dioxygenase complexed to ferrous iron and substrates *Biochemistry* **41** 5185-92
[73] Hegg E L and Que L J 1997 The 2-His-1-carboxylate facial triad: an emerging structural motif in mononuclear non-heme iron(II) enzymes *Eur J Biochem* **250** 625-9
[74] Zhou J, Kelly W L, Bachmann B O, *et al* 2001 Spectroscopic studies of substrate interactions with clavaminate synthase 2, a multifunctional alpha-KG-dependent non-heme iron enzyme: correlation with mechanisms and reactivities *J Am Chem Soc* **123** 7388-98
[75] Borowski T, Bassan A and Siegbahn P E M 2004 Mechanism of dioxygen activation in 2-oxoglutarate-dependent enzymes: a hybrid DFT study *Chem Eur J* **10** 1031-41
[76] Dambrova M, Liepinsh E and Kalvinsh I 2002 Mildronate: cardioprotective action through carnitine-lowering effect *Trends Cardiovas Med* **12** 275-9
[77] Bollinger J M Jr, Price J C, Hoffart L M, *et al* 2005 Mechanism of taurine: α-ketoglutarate dioxygenase (TauD) from *Escherichia coli Eur J Inorg Chem* **2005** 4245-54

[78] Krebs C, Galonic´ Fujimori D, Walsh C T, *et al* 2007 Non-heme Fe(IV)-oxo intermediates *Acc Chem Res* **40** 484-92
[79] Hopkinson R J, Hamed R B, Rose N R, *et al* 2010 Monitoring the activity of 2-oxoglutarate dependent histone demethylases by NMR spectroscopy: direct observation of formaldehyde *Chem Bio Chem* **11** 506-10
[80] Tuderman L, Myllylä R and Kivirikko K I 1977 Nutrition classics. European Journal of Biochemistry, Volume 80, 1977: Mechanism of the prolyl hydroxylase reaction. 1. Role of co-substrates *Nutr Rev* **40** 306-9