African Journal of Microbiology Research Vol. 6(32), pp. 6114-6121, 23 August, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.101
ISSN 1996-0808 ©2012 Academic Journals

Review

Stereoselective nitrile hydratase

Yingqiang Shen[1, 2], Fangchuan Du[1, 2], Weifang Gao[1], Anming Wang[1]* and Chanyu Chen[1]

[1]Research Center for Biomedicine and Health, Hangzhou Normal University, Hangzhou 311121, China.
[2]College of Biological and Environmental Sciences, Hangzhou Normal University, Hangzhou 310036, China.

Accepted 6 August, 2012

Nitrile hydratase, catalyzing the hydration reaction of nitrile to amides, is an important enzyme which has been used in the industrial production. Various studies on nitrile hydratase revealed its crystal structure and also proposed some hypothesis of its catalytic mechanism. Recent reports suggested the stereoselectivity of nitrile hydratase, which is regarded as a potential character to enrich its application. However, the isolated organisms are limited as only few genera identified contain this special stereoselective enzyme. This review described the corresponding strain for producing the enzyme, the structure and catalytic mechanism for stereoselectivity of the enzyme.

Key words: Stereoselectivity, nitrile hydratase, substrate specificity, asymmetric synthesis.

INTRODUCTION

Nitrile containing compounds, abundant in nature, are important intermediates in the chemical industry (Yamada and Kobayashi, 1996). Today, the use of biological systems for the production of commodity or specialty chemicals is an attractive alternative to traditional chemical methods, because of the high yields that can often be obtained, the mild reaction conditions required, and the specialized activities possessed by some enzymes (Wu et al., 1999). Early in 1980, Yamada et al. found that *Arthrobacte*r sp. J1 could produce acetamide in the culture broth when grown on acetonitrile (Asano et al., 1980). In fact, the transformation of nitriles to the corresponding amides is catalyzed by nitrile hydratases (EC 4.2.1.84). From then, the nitrile hydratase has drawn an increasing attention of researchers from all over the world. Various microbes are isolated, and many of them are applied to the commercial process (Nagasawa and Yamada, 1989). Extensive use has been made of nitrile hydratase to transform acrylonitrile into acrylamide in the actual production of acrylamide, with a production capacity of 30,000 tons per year. *Rhodococcus rhodochrous* J1, instead of *Rhodococcus* sp. N-774 and *Pseudomonas chlororaphis* B23, has been used as a 3rd-Generation industrial stain (Yamada and Kobayashi, 1996). Since the application of this process, it has presented an obvious advantage in contrast with the conventional chemical process for synthesis of the amides.

In the last few years, asymmetric catalysis has become one of the most active fields of research and it plays a key role in modern synthetic organic chemistry, with synthetic catalysts and enzymes being the two available options (Reetz, 2011). In the biocatalysis of the nitrile hydratases, some nitrile hydratases show a special catalytic property of stereoselectivity. This catalytic feature can be used in asymmetric synthesis, which could improve the purity of the desired amide product. For the different function between a pair of enantiomers, it is often the first question needed to consider in obtaining the desired enantiomer. The use of whole microorganisms and/or their enzymatic systems alone to carry out stereospecific and stereoselective reactions has taken on great significance. These reactions have been proven useful in the asymmetric synthesis of molecules with important biological activities (Borges et al., 2009). However, there are few general reviews describing the potential aspect and application of the stereoselective nitrile hydratases. This review summarises related achievement of nitrile hydratases, emphasizing on its stereoselectivity. The development direction of stereoselective nitrile hydratase was also proposed and

*Corresponding author. E-mail: waming@hznu.edu.cn. Tel: +86-571-28865978. Fax: +86-571-28865630.

outlooked.

MICROBIAL SOURCE OF STEREOSELECTIVE NITRILE

Nitrile hydratases exhibit broad substrate specificity, which are important characteristics for their development and application potential as industrial catalysts. Nowadays, more and more researches demonstrate the stereoselective of nitrile hydratases. Generally, in the two-enzyme cascade conversion from nitriles to corresponding acids, the amidase bears the higher enantioselectivity (Choi et al., 2008, Wang et al., 2010; Trott et al., 2002). Wieser and Nagasawa pointed out the enantioselectivity of a nitrile hydratase which has been generally detected with the purified enzyme (Wiesner and Nagasawa, 2000). They carried out the inhibition of the amidase by a specific inhibitor and obtained the transient or final accumulation of a chiral amide in case of a low amidase activity. These stereoselective nitrile hydratase were isolated from a few genera of bacteria such as: *Pseudomonas*, *Agrobacterium*, *Rhodococcus*, *Moraxella* and *Serratia*. Substrate specificity and E-value were also shown in the Table 1.

Pseudomonas putida 5B

Pseudomonas putida 5B contains a nitrile hydratase capable of stereoselective hydrolysis of 2-(4-chlorophenyl)-3-methylbutyronitrile at more than 90% enantiomeric excess to the (*S*)-amide (Figure 1) (Fallon et al., 1997).

The preferred enantiomer is influenced by both nitrile hydratase and amidase selectivity in the hydrolysis of other substrates such as 2-(6-methoxy-2-napthyl)-propionitrile, and 2-(4-isobutylphenyl)-propionitrile. This enzyme is relatively stable, maintaining its activity below 35°C, and shows abroad activity optimum between pH 7.2 and 7.8 (Payne et al., 1997). Later, Wu et al. (1997) described production of stereoselective 5B nitrile hydratase in *Escherichia coli* at 6-fold higher levels than in 5B. The high yield of this active stereoselective enzyme can be applied to some special commercially important processes.

As the recombinant technology progressed, active nitrile hydratase from *P. putida* 5B was produced in *E. coli* at a high level. An open reading frame down stream of the structural genes, which encodes a protein (P14K) of 127 amino acids, is essential for optimal production of active 5B nitrile hydratase in transformed *E. coli.* Nitrile hydratase has activity only in the presence of P14K protein while P14K itself shows no nitrile hydratase-likeactivity (Payne et al., 1997). They have also described one specific application, which is the co-expression of three genes encoding nitrile hydratase α- and β-structural subunits and P14K derived from *P. putida* to construct a *P. pastori s*train with stereoselective nitrile hydratase activity. Such a strain can be used as an industrial biocatalyst for the specific conversion of one enantiomer of a nitrile racemic mixture to the corresponding amide.

Rhodococcus sp. SP361

Cohen et al. (1992) studied the enantioselectivity of an immobilised whole cell system which is derived from *Rhodococcus* sp. SP361. They proposed the hydratase catalysis process which exhibited (*R*)-selectivity as shown in Figure 2, during incubation of (±)-2-(4-iso-butylphenyl)-propionitrile with SP361. Both the enantiomeric excess and absolute configuration of the product are determined by the hydratase enzyme.

Agrobacterium tumefaciens d3

Bauer's group isolated a new gram-negative strain *Agrobacterium tumefaciens* d3. The nitrile hydratase and the amidase were both shown to convert preferentially the *S* enantiomer of their respective substrate (Bauer et al., 1994). In their studies, nitrile hydratase were purified and completely separated from the amidase activity from *A. tumefaciens* d3 (Bauer et al., 1998). The nitrile hydratase had an activity optimum at pH 7.0 and an optimum temperature of 40°C. The purified nitrile hydratase showed higher enantioselectivity than resting cells in which the amidase was inhibited. The highest enantiomeric excesses were found for the amides formed from 2-phenylpropionit-rile, 2-phenylbutyronitrile and ketoprofen nitrile. This organism is a good candidate for the production of (*S*)-amides when the action of the amidase is inhibited.

Rhodococcus erythropolis AJ270

The nitrile hydratase from *Rhodococcus* sp. AJ270 exhibits excellent regioselectivity when it catalyzed the hydrolysis of aromatic dinitriles and a variety of aliphatic dinitriles (Meth-Cohn and Wang, 1997). According to the enantioselective hydrolysis of both racemic trans- and cis-2-arylcyclopropanecarbonitriles with enantiomeric excesses as high as >99%, Wang's group proposed that a readily reachable reactive site can be embedded within the spacious pocket of the 1S-enantioselective nitrile hydratase while the amidase might comprise a relatively deep-buried and size-limited 1S-enantioselective active site to explain the enantioselectivity (Wang and Feng, 2002, Wang et al., 2001). The nitrile hydratase from *Rhodococcus* sp. AJ270 were purified and used in the enantioselective hydrolysis (Song et al., 2007). The enantiomeric excess value of the enzyme when hydrating trans-2,2-dimethyl-3-phenylcyclopropanecarbonitrile was

Table 1. Some stereoselective nitrile hydratases for given substrates.

Strain	Substrate	Selectivity (E-value)	References
A. tumefaciens d3	2-phenylpropionitrile 2-phenylbutyronitrile 3-Ph-propionitrile 2-(4-chlorophenyl)-3-propionitrile 2-(4-methoxyphenyl)-3-propionitrile	S(253) S(58) S(43) S(18) S(8)	Bauer et al. (1998)
Rhodococcus equi A4	2-(6-methoxy-2-naphtyl)propionitrile 2-(4-methoxyphenyl)-3-propionitrile 2-(2-methoxyphenyl)-3-propionitrile 2-(4-chlorophenyl)-3-propionitrile	S(41) S(19) S(7) S(5)	Přepechalová et al. (2001)
P.putida NRRL18668	2-(4-chlorophenyl)-3-methylbutyronitrile	S(50)	Wiesner and Nagasawa, (2000)
Rhodopseudomonas palustris HaA2	2-phenylpropionitrile 2-Phenylbutyronitrile	S(>100) S(53)	Van Pelt et al. (2011)
Rhodopseudomonas palustris CGA009	2-phenylpropionitrile 2-Phenylbutyronitrile	S(>100) S(95)	
P. putida 2D-11-5-1b	2-(4-chlorophenyl)-3-metyhlbutyronitrile 2-(4-isobutylphenyl)-propionitrile	S(ca.63) R(ca.13)	Anton et al. (1997)
P. putida 13-5S-ACN-2a	2-(4-chlorophenyl)-3-metyhlbutyronitrile	S(ca.48)	
Rhodococcus sp. AJ270	2-phenylbutyronitrile 3-Benzoyloxypentanedinitrile Naproxennitrile	R(ee 83%) S(ee 68.2%) S(80)	Wang (2005) Song et al. (2007) Van Pelt et al. (2011)
Rhodococcus sp. HT40-6	Mandelonitrile	S(ee 95%)	Tamura (2000)
Serratia liquefaciens MOB/IM/N3	2-(4-chlorophenyl)-3-methylbutyronitrile	S	Anton et al. (1997)
Moraxella sp. 3L-A-1-5-1a-1	2-(4-chlorophenyl)-3-methylbutyronitrile 2-(4-isobutylphenyl)propionitrile	S R	Martinková and Kren (2002)
Pseudomonas sp. 2D-11-5-1c	2-(6-methoxy-2-naphtyl)-propionitrile	S	Anton et al. (1997)

Table 1. count'd

Pseudomonas sp. 2G-8-5-1 a	2-(6-methoxy-2-naphtyl)-propionitrile	S	Anton et al. (1997)
P. aureofaciens MOB C2-1	2-(6-methoxy-2-naphtyl)-propionitrile 2-(4-isobutylphenyl)-propionitrile	R S	Anton et al. (1997)

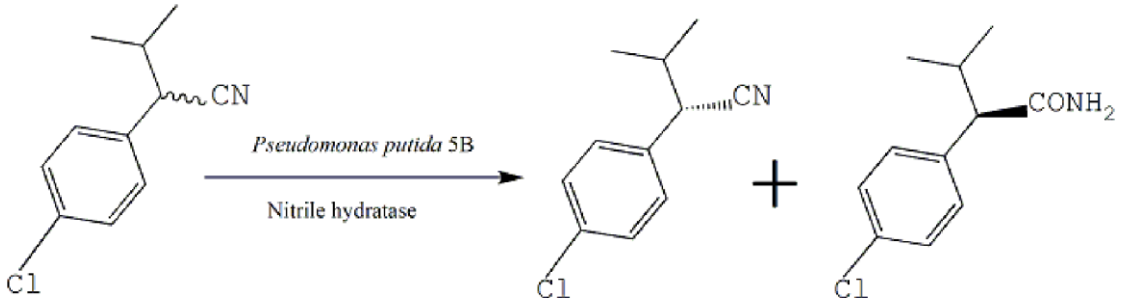

Figure 1. Reaction scheme for the biotransformation of 2(4-chlorophenyl)-3-methylbutyronitrile to (*S*) enantioselective amide by nitrile hydratase from *Pseudomonas putida* 5B (ee > 90%).

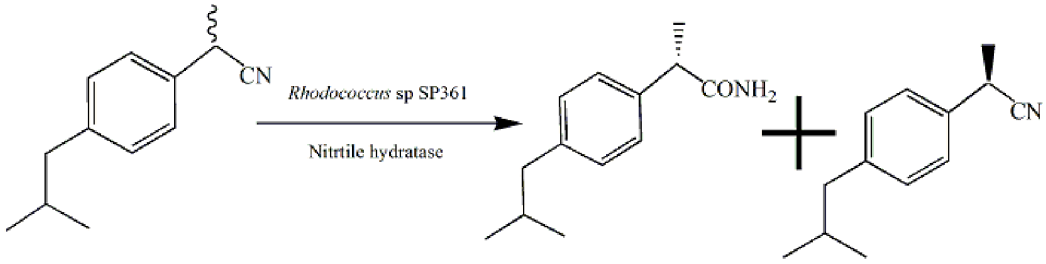

Figure 2. Reaction scheme for the biotransformation of 2-(4-iso-butylp-henyl)-propionitrile to (*R*) enantioselective amide by nitrile hydratase from *Rhodococcus* sp. SP361 (ee = 32 to 35%).

84.7. The genes encoding an enantioselective nitrile hydratase from *Rhodococcus erythropolis* AJ270 have also been cloned and the active nitrile hydrase has been produced in *E. coli*.

The recombinant enzyme was highly enantioselective in the synthesis of *S*-(+)-3-benzoyloxy-4- cyano butyramide from the prochiral substrate 3-benzoyloxyglutaronitrile (Song et al., 2008).

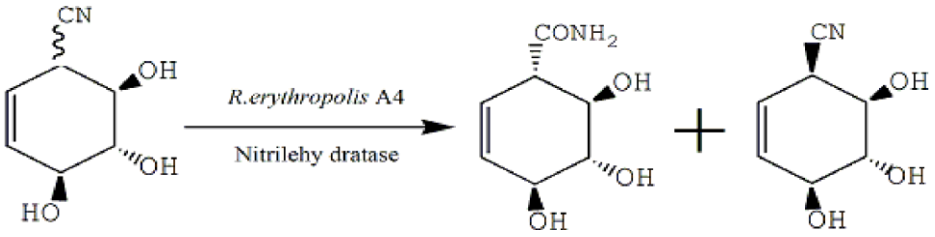

Figure 3. Reaction scheme for the biotransformation of 1-cyano-2,3,4-trihydroxycyclohex-5-ene to (*S*)enantioselective amidase by nitrile hydratase from *Rhodococcus equi* A4 (ee = 40%).

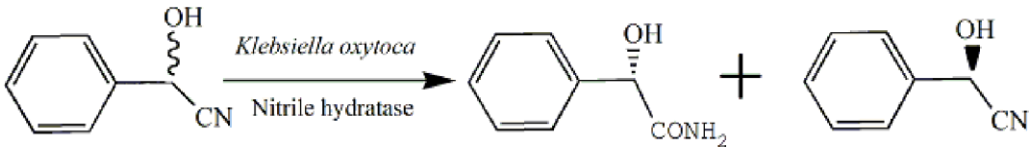

Figure 4. Reaction scheme for the biotransformation of rac-mandelonitrile to (S)-mandeloamide by nitrile hydratase from *Klebsiella oxytoca* (ee > 89%).

Rhodococcus equi A4

The nitrile hydratase from *Rhodococcus equi* strain A4 has been reported to catalyze enantioselectively the hydrolysis of (*R,S*)-2-(4-methoxyphenyl)-propionitrile and (*R,S*)-2-(4-chlorophenyl)-propi-onitdle (Martínková et al., 1996). Further study of this strain revealed some details about the properties of the nitrile hydratase. The enzyme preferentially hydrated the *S*-isomers of racemic 2-(2, 4-met-hoxyphe-nyl) propionitrile, 2-(4-chlorophenyl) propionitrile and 2-(6-methoxynaphthyl) propionitrile (Přepechalová et al., 2001). Nicola D'Antona et al. have also reported that the nitrile hydratase is (*S*)-enantioselective towards bulky aliphatic substrates (Figure 3) (D'Antona et al., 2010). This is the first case of a nitrile hydratase that is enantioselective towards bulky aliphatic substrates.

Klebsiella oxytoca

Ewert et al. (2008) reported bacteria isolated from *K. oxytoca* which could carry out the enantioselective conversion of racemic-arylnitriles to their amide products (Figure 4). (*S*)-enantioselective nitrile hydratase and a putative (*S*)-selective amidase was induced and produced in the isolated microorganism.

Others

In addition, recently, VanPelt et al. (2011) reported a diverse range of purified cobalt containing nitrile hydratases from *Rhodopseudomonas palustris* HaA2 (HaA2), *R. palustris* CGA009 (009), *Sinorhizobium meliloti* 1021 (1021), and *Nitriliruptoralk aliphilus* (iso2). These strains were screened for the first time for their enantioselectivity towards abroad range of chiral nitriles. The nitrile hydratases from both *R. palustris* strains (HaA2 and 009) showed a very high (*S*)-selectivity for 2-phenylpropionitrile, while the nitrile hydratases from *S. meliloti* and *N. alkaliphilus* (1021 and iso2) showed a lower selectivity. Through the analysis of experimental data, they put forward that at least one bulky group in close proximity to the α-position of the chiral nitriles seemed to be necessary for enantioselectivity with all nitrile hydratases aforementioned.

In some patents, the stereoselective nitrile hydratases are also used. An invention, related to a method for producing an optically active α-hydroxy acid or α-hydroxyamide from acyanohydrin via treating with a microorganism. *Rhodococcus* sp. HT40-6, is introduced to produce (*S*)-mandelamide (Tamura, 2000), and it works well.

MOLECULAR STRUCTURE OF NITRILE HYDRATASES

Nitrile hydratase comprises α and β subunits, each with a molecular mass around 23 KDa (Kobayashi et al., 1992). The α subunit of nitrile hydratase consists of a long extended N-terminal arm and a globular C-terminal domain (Figure 5a and b) (Huang et al., 1997), which is wrapped by a long loop of 30 residues of the β subunit. The C-terminal part of the β chain forms a domain consisting of a β roll and one very short helix (Figure 5a and c) (Huang et al., 1997). The enzyme is typically an $\alpha_2\beta_2$ heterotetramer.

Nitrile hydratase also contains a non-heme iron ion or

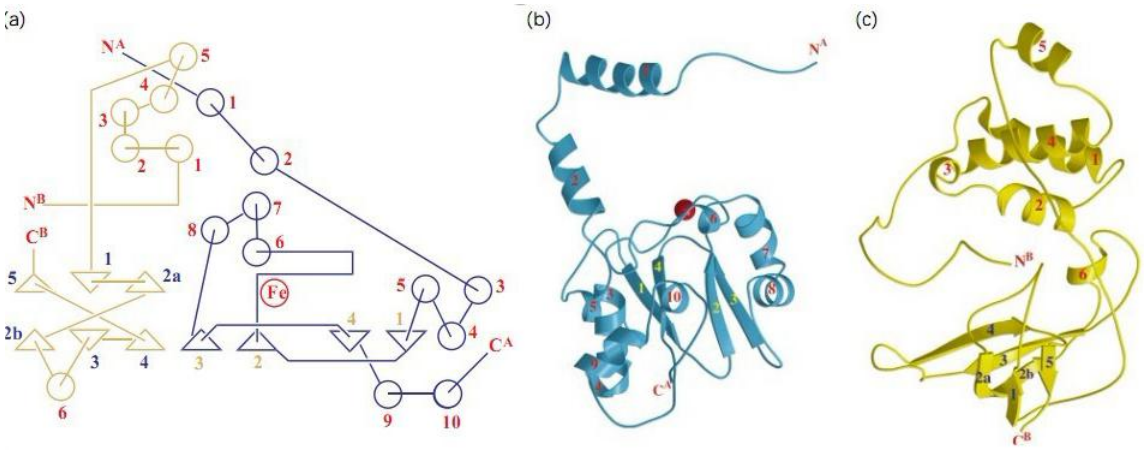

Figure 5. Subunit topology and structure of nitrile hydratase. (a) Topology diagram for the α and β subunits of nitrile hydratase; the α subunit is shown in blue and the β subunit in yellow; and the location of the iron centre is indicated by a red sphere. (b) Schematic view of the α subunit. (c) Schematic view of the β subunit (Huang et al., 1997).

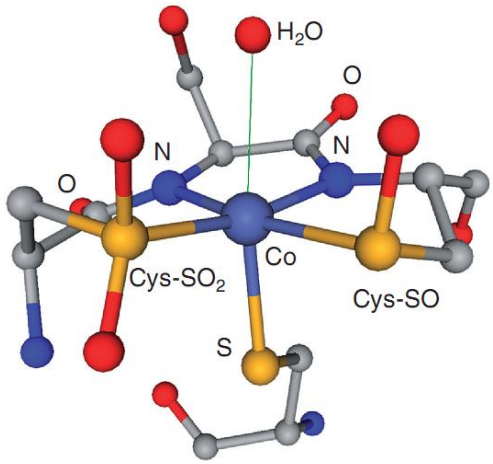

Figure 6. Active site structure of Co-type nitrile hydratase (Yano et al., 2008).

non-corrin cobalt ion at its catalytic center. As known from Co-type nitrile hydratase, the Fe type shows unique photo reactivity. The enzyme is inactivated in the dark and the activity is recovered by light irradiation (Nagasawa et al., 1990). Although these two kinds of enzyme are highly homologous, the metal iron is special to them. A Fe-type nitrile hydratase was cultivated in Co-supplemented medium, and the obtained Co-substituted enzyme exhibited rather weak nitrile hydratase activity than the native Fe-type nitrile hydratase(Nojiri et al., 2000).

The metal binding domain is highly conserved in both type of nitrile hydatase with an amino acids sequence of α-subunit of VC(T/S)LCSC(Y/T) (Figure 6). The cobalt-containing nitrile hydratase contains threonine and tyrosine as third and eighth amino acid residues of metal binding domain which are replaced by serine and threonine respectively in iron-containing nitrile hydratase (Prasad and Bhalla, 2010). In all structures published to date, the trivalent metal ion is six-coordinate, with three cysteine sulfur, two amide nitrogen, and one water molecule (Mitra and Holz, 2007).

The fifth and seventh amino acid are post-translationally modified to cysteine-sulfinic acid and cysteine-sulfenic acid, respectively (Nagashima et al., 1998). This characteristic distinguishes the active centers of nitrile hydratase from that of other metalloenzymes (Yano et al., 2008).

THE POSSIBLE CATALYTIC MECHANISM

The resolved structure characterization of nitrile hydratases could provide some clue to make clear the catalytic mechanism of the enzyme. Up to date, there have been three main possible ways to explain the enzymatic reaction of the hydrolysis of nitriles to their corresponding amides such as: (i) inner-sphere mechanism, (ii) outer-sphere mechanism, and (iii) second-outer sphere mechanism.

In the inner-sphere mechanism, the nitrile substrate binds directly to the metal center of the enzyme. An activated hydroxide nucleophilic attacks the nitrile carbon, which increased its electrophilicity by coordinating the Lewis acidic metal in the active site pocket. According to the structure of the enzyme (Kobayashi and Shimizu, 1998; Miyanaga et al., 2001; Hourai et al., 2003; Huang et al., 1997), two oxidized Cys ligands, the Ser ligand, and a strictly conserved Tyr residue may play a critical role in this catalysis process. The sulfenate oxygen of α-Cys114-SO(H) activates the water molecule and forms the hydroxide to attack the nitrile carbon. α-Ser113 affects the electronic state of the Fe center as well as the structure around the catalytic cavity including the

hydrogen-bond networks, but it is not essential for catalytic activity. The phenol oxygen of β-Tyr72 affects the electronic state (perhaps the Lewis acidity) of the Fe center (Yamanaka et al., 2010). Hopmann et al. (2007) used quantum chemical models of the nitrile hydratase active site to investigate this mechanism. The metal is suggested to provide electrostatic stabilization to the anionic imidate intermediate, thereby lowering the reaction barrier.

Different from that in the inner-sphere mechanism, outer-sphere mechanism and second outer-sphere mechanism, the nitrile group of substrate does not displace a hydroxide ligand from the coordination sphere of the metal center. The hydroxide nucleophile attacks the nitrile carbon directly; in the second outer-sphere mechanism, the metal-bound hydroxide causes deprotonation of a free water molecule near the active site, forming hydroxide to attack the nitrile carbon. Prasad and Bhalla (2010) suggested that the coordination structures of the M(III) sites of the Fe– and Co–nitrile hydratases are very similar or identical. They assumed that the M(III) centres of these two metal ions function in very similar ways.

Different nitrile hydratases are specific for the different nitrile substrates. Antona's group synthetized several novel cyano-cyclitols, and they found that both the efficiency and enantioselectivity of the biotransformations were strongly dependent upon the structures of the nitrile substrates (D'Antona et al., 2010). A major hindrance for substrate to interact with active site is generated by β-Phe37 residue and this region may be responsible for the stereoselectivity of nitrile hydratase (Peplowski et al., 2008). The enzyme has various cavities which lead to the active site and the entrances to these cavities, and extension of the N-terminal might be relevant to substrate specificity. The possible substrate binding mechanisms are breathing and flip-flop, involving the opening and closing of the cavities and mouths (Mitra and Holz, 2007). Prasad and Bhalla (2010) concluded that the architecture of the interior of channel may be responsible for the tuning of catalytic efficiency of certain nitrile hydratases for their stereospecificity.

CONCLUSIONS AND FUTURE PERSPECTIVES

Biological catalysis is in the ascendant, and biosynthesis will certainly contribute more profit. In the past several decades, the applications of nitrile hydratase achieved excellent benefits. The discovery of the stereoselectivity enriches the application of this enzyme. However, only a handful of enantioselctive biotransformations catalyzed by nitrile hydrateses have been reported and further investigated. The application of enzymes in synthetic chiral compound has suffered from the limited substrate scope. With the technology of genetic engineering and structural biology, some details of stereoselective nitrile hydratase should be revealed, and the enantioselectivity of nitrile hydratase needs to be improved and enhanced to meet the demand of stereoselective biocatalysis with the high purity. The special catalytic properties of nitrile hydrates will further broaden the scope of this enzyme in industry.

ACKNOWLEDGEMENTS

This work was financially supported by the National Natural Science Foundation of China (20906016, 21076053), and Technology Research and Development Program of Hangzhou (20120232B13).

REFERENCES

Anton DL, Fallon RD, Stieglitz B, Witterholt VG (1997). Process for the preparation of enantiometric 2-alkanoic acid amides from nitriles. United States Patent 5593871.

Asano Y, Tani Y, Yamada H (1980). A new enzyme nitrile hydratase which degrades acetonitrile in combination with amidase. Agric.Biol.chem. 44:2251-2252.

Bauer R, Hirrlinger B, Layh N, Stolz A, Knackmuss H-J (1994). Enantioselective hydrolysis of racemic 2-phenylpropionitrile and other (R,S)-2-arylpropionitriles by a new bacterial isolates. Appl. Microbiol. Biotechnol. 42:1-7.

Bauer R, Knackmuss HJ, Stolz A (1998). Enantioselective hydration of 2-arylpropionitriles by a nitrile hydratase from *Agrobacterium tumefaciens* strain d3. Appl. Microbiol. Biotechnol. 49:89-95.

Borges KB, Borges WS, PatrónR D, Pupo MT, Bonato PS, Collado IG (2009). Stereoselective biotransformations using fungi as biocatalysts. Tetrahedron: Asymmetry. 20:385-397.

Choi YH, Uhm K-N, Kim H-K (2008). Biochemical characterization of *Rhodococcus erythropolis* N'4 nitrile hydratase acting on 4-chloro-3-hydroxybutyronitrile. J.Mol.Catal.B-enzym. 55:157-163.

Cohen MA, Parratt JS, Turner N (1992). Enantioselective hydrolysis of nitriles and amides using an immobilised whole cell system. Tetrahedron:Asymmetry. 3:1543-1546.

D'Antona N, Nicolosi G, Morrone R, Kubáč D, Kaplan O, Martínková L (2010). Synthesis of novel cyano-cyclitols and their stereoselective biotransformation catalyzed by *Rhodococcus erythropolis* A4. Tetrahedron: Asymmetry. 21:695-702.

Ewert C, Lutz-Wahl S, Fischer L (2008). Enantioselective conversion of α-arylnitriles by *Klebsiella oxytoca*. Tetrahedron: Asymmetry. 19: 2573-2578.

Fallon RD, Stieglitz B, Turner Jr I (1997). A *Pseudomonas putida* capable of stereoselective hydrolysis of nitriles. Appl.Microbiol.Biotechnol. 47: 156-161.

Hopmann K, Guo JD, Himo F (2007). Theoretical investigation of the first-shell mechanism of nitrile hydratase. Inorg.Chem. 46:48-50.

Hourai S, Miki M, Takashima Y, Mitsuda S, Yanagi K (2003). Crystal structure of nitrile hydratase from a thermophilic Bacillus smithii. Biochem.Bioph.Res.Co. 312:340-345.

Huang W, Jia J, Cummings J, Nelson M, Schneider G, Lindqvist Y (1997). Crystal structure of nitrile hydratase reveals a novel iron centre in a novel fold. Structure. 5:691-699.

Kobayashi M, Nagasawa T, Yamada H (1992). Enzymatic synthesis of acrylamide a success story not yet over. Trends.Biotechnol. 10:402-408.

Kobayashi M, Shimizu S (1998). Metalloenzyme nitrile hydratase structure, regulation, and application to biotechnology. Nat.Biotechnol. 16:733-736.

Martínková L, Stolz A, Knackmuss H (1996). Enantioselectivitiy of the nitrile hydratase from *Rhodococcus equi* A4 towards substituted (R, S)-2-arylpropionitriles. Biotechnol.Lett. 18:1073-1076.

Martinková L, Kren V (2002). Nitrile-and amide-converting microbial

enzymes: stereo-, regio-and chemoselectivity. Biocatal.Biotransfor. 20: 73-93.
Meth-Cohn O, Wang MX (1997). Regioselective biotransformations of dinitriles using Rhodococcus sp. AJ2701. J. Chem. Soc. Perkin Trans. 21:3197-3204.
Mitra S, Holz RC (2007). Unraveling the catalytic mechanism of nitrile hydratases. J.Biol.Chem. 282:7397-7404.
Miyanaga A, Fushinobu S, Ito K, Wakagi T (2001). Crystal structure of cobalt-containing nitrile hydratase. Biochem.Biophy.Res.Co. 288: 1169-1174.
Nagasawa T, Yamada H (1989). Microbial transformations of nitriles. Trends.Biotechnol. 7:153-158.
Nagasawa T, Yamada H, Hirata M, Honda J, Hirata A, Endo I (1990). Photosensitive phenomena of nitile hydratase of *Rhodococcus* sp. N-771. Photoch.Photobio.B. 51:87-90.
Nagashima S, Nakasako M, Dohmae N, Tsujimura M, Takio K, Odaka M, Yohda M, Kamiya N, Endo I (1998). Novel non-heme iron center of nitrile hydratase with a claw setting of oxygen atoms. Nat.Biotechnol. 5:347 - 351.
Nojiri M, Nakayama H, Odaka M, Yohda M, Takio K, Endo I (2000). Cobalt-substituted Fe-type nitrile hydratase of *Rhodococcus* sp. N-771. FEBS Lett. 465:173-177.
Payne MS, Wu S, Fallon RD, Tudor G, Stieglitz B, Turner Jr IM, Nelson MJ (1997). A stereoselective cobalt-containing nitrile hydratase. Biochemistry. 36:5447-5454.
Peplowski L, Kubiak K, Nowak W (2008). Mechanical aspects of nitrile hydratase enzymatic activity. Steered molecular dynamics simulations of *Pseudonocardia thermophila* JCM 3095. Chem.Phys.Lett. 467: 144-149.
Prasad S, Bhalla TC (2010). Nitrile hydratases (NHases): at the interface of academia and industry. Biotechnol.Adv. 28:725-741.
Přepechalová I, Martínková L, Stolz A, Ovesná M, Bezouška K, Kopecký J, Křen V (2001). Purification and characterization of the enantioselective nitrile hydratase from *Rhodococcus equi* A4. Appl.Microbiol.Biot. 55:150-156.
Reetz MT (2011). Laboratory evolution of stereoselective enzymes: a profile source of catalysts for asymmetric reactions. Angew. Chem. Int. Edit. 50:138-174.
Song L, Wang M, Yang X, Qian S (2007). Purification and characterization of the enantioselective nitrile hydratase from *Rhodococcus* sp. AJ270. Biotechnol. J. 2:717-724.
Song L, Yuan HJ, Coffey L, Doran J, Wang MX, Qian S, O'Reilly C (2008). Efficient expression in *E. coli* of an enantioselective nitrile hydratase from *Rhodococcus erythropolis*. Biotechnol. Lett. 30:755-762.
Tamura K (2000) Method of producing optically active alpha-hydroxy acid or alpha-hydroxyamide. EP Patent 0711836.
Trott S, Burger S, Calaminus C, Stolz A (2002). Cloning and heterologous expression of an enantioselective amidase from *Rhodococcus erythropolis* Strain MP50. Appl.Environ.Microb. 68: 3279-3286.
Van Pelt S, Zhang M, Otten LG, Holt J, Sorokin DY, van Rantwijk F, Black GW, Perry JJ, Sheldon RA (2011). Probing the enantioselectivity of a diverse group of purified cobalt-centred nitrile hydratases. Org. Biomol.Chem. 9:3011-3019.
Wang MX (2005). Enantioselective biotransformations of nitriles in organic synthesis. Top.Catal. 35:117-130.
Wang MX, Feng GQ (2002). A novel approach to enantiopure cyclopropane compounds from biotransformation of nitriles. New. J. Chem. 26:1575-1583.
Wang MX, Liu CS, Li JS (2001). Enzymatic desymmetrization of 3-alkyl- and 3-arylglutaronitriles, a simple and convenient approach to optically active 4-amino-3-phenylbutanoic acids. Tetrahedron: Asymmetry. p.12
Wang YS, Zheng RC, Xu JM, Liu ZQ, Cheng F, Feng ZH, Liu LL, Zheng YG, Shen YC (2010). Enantioselective hydrolysis of (R)-2, 2-dimethylcyclopropane carboxamide by immobilized cells of an R-amidase-producing bacterium, Delftia tsuruhatensis CCTCC M 205114, on an alginate capsule carrier. J.Ind.Microbiol.Biot. 37:503-510.
Wieser M, Nagasawa T (2000). Stereoselective nitrile-converting enzymes. In Patel RN (ed) Stereoselective Biocatalysis, CRC Press 2000, New York pp. 461-486.
Wu S, Fallon R, Payne M (1997). Over-production of stereoselective nitrile hydratase from *Pseudomonas putida* 5B in *Escherichia coli*: activity requires a novel downstream protein. Appl. Microbiol. Biot. 48:704-708.
Wu S, Fallon R, Payne M (1999). Engineering *Pichia pastoris* for stereoselective nitrile hydrolysis by co-producing three heterologous proteins. Appl. Microbiol. Biot. 52:186-190.
Yamada H, Kobayashi M (1996). Nitrile Hydratase and Its Application to Industrial Production of Acrylamide. BIosci. Biotechnol. Biochem 60:1391-1400.
Yamanaka Y, Hashimoto K, Ohtaki A, Noguchi K, Yohda M, Odaka M (2010). Kinetic and structural studies on roles of the serine ligand and a strictly conserved tyrosine residue in nitrile hydratase. J. Biol. Inorg. Chem. 15:655-665.
Yano T, Ozawa T, Masuda H (2008). Structural and functional model systems for analysis of the active center of nitrile hydratase. Chem. Lett 37: 672-677.