Review

Biocatalysis in Organic Synthesis: The Use of Nitrile- and Amide-hydrolyzing Microorganisms

Takeshi Sugai, Takahiro Yamazaki, Masahiro Yokoyama, and Hiromichi Ohta

Department of Chemistry, Keio University, 3-14-1 Hiyoshi, Yokohama 223, Japan

This review covers recent examples of synthetic transformation of nitriles and amides by microbial enzyme systems. A variety of substrates and products involving enantiomerically enriched forms of chiral substances are referred to. The stereochemical course of enzyme-catalyzed hydrolysis is briefly commented on. Special emphasis is placed upon the range of functional groups that are acceptable by enzymes and/or survive under the transformation, as well as the advantages as a synthetic tool for conversion under mild conditions.

Key words: nitrile; amide; hydrolysis; microorganisms; organic synthesis

Nitriles (cyano compounds) are important in synthetic organic chemistry, as precursors that provide amides and carboxylic acids by hydrolysis. Even a methyl group located in saturated hydrocarbon or attached to an aromatic ring, a functional group with the lowest oxidation level is converted to a cyano group by ammoxidation. In contrast, they are conventionally prepared by introduction of one carbon as cyanide ion (CN⁻), two carbon homologation by the use of a cyanoacetate equivalent, and three carbon homologation with acrylonitrile as the Michael acceptor of radical species.

Hydrolysis of nitriles proceeds in two steps. In the step to amides, proton (H⁺) and/or metal cationic species (M⁺) work to activate the carbon–nitrogen triple bond, which facilitates the addition of water molecules. In the following step from amides to carboxylates, the C–N bonds are more resistant to the hydrolytic cleavage than the conventional C–O bonds of esters. For this reason, the total hydrolytic procedure requires as harsh conditions as heating at an acidic or alkaline pH. This situation has made the selective transformation difficult, especially with the molecules bearing other acid- or alkaline-labile functionalities (Fig. 1).

So far, to overcome this problem, enzyme-mediated transformations have been developed. Three enzymes, nitrile hydratase, amidase, and nitrilase, which catalyzes direct conversion of nitriles to carboxylates, have been disclosed. It is needless to say that a distinctive advantage is that the enzyme-catalyzed hydrolysis proceeds under as mild conditions as a neutral pH and room temperature. Selected articles and recent reviews have covered the topics on enzymologically interesting aspects as well as the industrial applications on the production of commodity chemicals of enzyme-mediated hydrolyses of nitriles, and those catalyzed by whole-cell microorganisms. In this review, we focus upon the examples that illustrate the utility of enzyme- and microorganism-mediated conversion of nitriles and amides from the standpoint of synthetic organic chemistry.

1. Conversion of Mononitriles and Monoamides

After a pioneering work by Yamada and Asano, many kinds of enzymes of microbial origin that can hydrolyze not only simple aliphatic nitriles, but also aromatic nitriles, have been found to be widespread in nature, although most nitriles are non-natural synthetic compounds. In this decade, studies on substrate specificity have been ex-

---

1. Address correspondence to this author.

2. The step of conversion from nitriles to amide is not a 'hydrolysis,' but a hydration. However, many previous papers use the term 'hydrolysis' on hydration of nitriles, and in this review we also use 'hydrolysis' for this step.

---

![Scheme 1](image1)

![Fig. 1](image2)
tensive, and through these studies, a number of substituted benzonitriles were converted to the corresponding amides and carboxylic acids (Fig. 2).\textsuperscript{17–221} Benzonitriles with a variety of para-substituents are accepted by microbial hydrolytic systems and the corresponding carboxylates are efficiently prepared. The isolated nitrile hydratase affords the corresponding amides as the major products.\textsuperscript{17,181}

*meta*-Substituted substrates are also converted to carboxylates. In the case of more sterically hindered ortho-substituted benzonitriles, the hydrolysis became slow. Even when using whole cells of microorganisms, amides could be isolated by shortening the incubation period.\textsuperscript{221}

A number of heteroaromatic substrates are also available to afford amides and carboxylates (Fig. 2).\textsuperscript{13,17,20–281} Some of which are of industrial value, such as nicotinic acid, nicotinamide, or pyrazinocarboxylic acid. One carbon homologation of the aromatic substrate also works: substituted benzyl cyanides and higher homologs could be hydrolyzed to carboxylates (Fig. 2).\textsuperscript{12,19,21,27,291}

When a substituent is introduced into the α-position of a benzyl cyanide, the substrate becomes a ‘chiral’ substance: an enantioselective hydrolysis possibly affords nitriles, amides, and carboxylates in enantiomERICALLY enriched forms via a kinetic resolution\textsuperscript{19,21,30–401} (Scheme 2). In the case that only nitrilase works on the racemic nitrile, the products are nitrile and carboxylates both in enantiomERICALLY enriched forms,\textsuperscript{301} however, in most cases the products are a mixture of nitrile, amide, and carboxylate. Since the products are related to antiinflammatory agents and synthetic pyrethroids, a large-scale preparation involving the use of bio-reactors mediated by immobilized microorganisms has been developed.\textsuperscript{36,371} Examples of nitriles, amides, and carboxylates thus produced are shown in Fig. 3. The kinetic resolution of the related substrate, α-aryloxypropionitrile, has also been reported.\textsuperscript{411}

Kinetic resolution of simple aliphatic nitrile yielded poor

---

**Fig. 2.** References: a 141; b 191; c 201; d 211; e 221; f 111; g 171; h 181; i 121; j 131; k 271; l 231; m 241; n 251; o 261; p 281; q 291.
results: enantiomerically enriched carboxylate could be obtained in only limited cases, such as (S)-2-methylhexanoic acid. In contrast, good stereoselectivity in the amidase-catalyzed hydrolysis of racemic 2,2-dimethylcyclopropanecarboxamide was observed, which serves for a preparation of an intermediate for silastatin (Fig. 3). 

Cyanohydrins (z-hydroxyanilide) can be prepared either via chemical or biocatalyst-mediated reaction from aldehydes, and are important precursor of z-hydroxy acids. Indeed, the microbial approach to the preparation of z-hydroxy acids from racemic cyanohydrin has been studied.

As cyanohydrin, the starting material, is prone to racemize in a aqueous solution via an equilibrium between aldehyde and hydrogen cyanide, the use of highly enantioselective hydrolytic enzyme such as *Acinetobacter faecalis* ATCC 8750 made a 'dynamic kinetic resolution' possible; only (R)-mandelic acid was obtained from the racemic form of mandelonitrile. In contrast, *Rhodococcus rhodochrous* IFO 15564 hydrolyzes both enantiomers of mandelonitrile, the optical resolution of cyanohydrin before the microbial hydrolysis provides the both enantiomers of mandelic acid. To obtain only one enantiomer of hydroxynitrile, another combination of an enantioselective nitrile-hydrolyzing enzyme and an enantioselective oxynitrilase has been proposed. The examples of hydroxynitrile hydrolysis thus prepared are shown in Figure 4. Hydrolysis of β- and γ-hydroxy nitriles only proceeded with a rather low enantioselectivity. However, from a screening of microorganisms, an enantioselective amidase was established for the production of l-carnitine.

Aminonitriles and amioamides were also available as the substrates of nitrile hydrolyzing enzymes (Fig. 4). The key step for the preparation of amino acid in an enantiomerically enriched form is the stereoselective hydrolysis catalyzed by amino acid amidase (Scheme 4). The first step of the total conversion, hydrolysis of nitriles, is also biologically catalyzed. For example, 'Pronase', an amidase immobilized on a polymer support can hydrolyze aminonitrile; the conversion of nitrile to amide is catalyzed by the basic amino functional group involved in the polymer supports at an alkaline pH. An interesting amidase, d-aminopeptidase, which catalyzes the hydrolysis of various secondary amides, in contrast to other amidases, has been reported.

Poly-hydroxylated nitriles with carbohydrate frameworks were accepted by nitrile-hydrolyzing microorganisms. The activity of amidase was greatly affected by the steric hindrance of the amide intermediates as in Fig. 5.

2. Conversion of Dinitriles and Diamides

The selective hydrolysis of simple aliphatic dinitriles worked well, to give the corresponding monoamide and monocarboxylates (cyano acids) as illustrated in Fig. 6. A number of examples of conversion of aromatic dinitriles and diamides have been reported as.
Dinitriles related to perfluorobenzene nuclei were well accepted by nitrile hydratase and the major product were cyanoamides. A benzylic dinitrile was also the precursor of cyano acid. Dinitriles of non-symmetrical structure, however, afforded a mixture of regioisomers. The possible intermediate, α-carbamoylbenzoic acid from α-dicyanobenzene was susceptible to further spontaneous hydrolysis to afford phthalic acid. An alicyclic dinitrile, trans-cyclohexane-1,4-dinitrile was also converted to a cyano acid, an important industrial precursor of tranexamic acid.

The hydrolytic systems showed a discrimination of enantiotopic groups in prochiral dinitriles. From β-substituted glutaromitriles, a sequential action of nitrile hydratase and amidase afforded the cyano acids as an
enantiomerically enriched form.\textsuperscript{33,67–70} A kinetic resolution was observed when one of the cyano groups was replaced with alkyl groups.\textsuperscript{70} In contrast, amide-carboxylates were obtained from disubstituted malononitriles.\textsuperscript{71,72} In this case, prochiral disubstituted diamides were proved to be the intermediates.

3. The Stereoselectivity of Enzyme-catalyzed Reactions

In some cases of kinetic resolution of racemic substrates, the enantiomerically enriched forms of the products are simply ascribable to the enantioselectivity of amidase\textsuperscript{73} and nitrilase.\textsuperscript{74} However, the whole cell-catalyzed reactions\textsuperscript{75,76} brought about rather complex results. In the following examples,\textsuperscript{19,33,34} only a small change of substituent greatly influenced the stereochemical preferences; a nitrile hydratase from \textit{Rhodococcus} preferentially hydrolyzes the (R)-enantiomers of the substrate bearing isobutyl group on \( p \)-position of the ary1propionitrile as shown in Scheme 5.

The selectivity is lost when the substituent was replaced with a chlorine atom and a methoxy group. Moreover, the preference is reversed when the side chain was substituted by a methyl group. The change of preference apparently affected the distribution of the products as highlighted in Scheme 5. An active site model of hydrolytic enzyme is proposed to predict a stereochemical outcome of the reaction.\textsuperscript{77}

4. Hydrolysis of Nitriles with Other Functional Groups

As mentioned before, the scope and limitations of nitrile-hydrolyzing enzymes and microorganisms in synthetic organic chemistry is commented on here. A wide variety of substrates with other functional groups have been hydrolyzed, involving neighboring double bonds, halogen atoms, hydroxyl groups, carbonyl groups, and acetals, as in Fig. 8.\textsuperscript{11–13,15,33,35,38,78,79} Some carboxylate esters, phosphonates, and boronates were well accepted by microorganisms without any effect on these functional groups.

However, some important side reactions caused by the use of whole-cell catalysis should also be mentioned (Scheme 6). For example, some species of \textit{Rhodococcus} have an epoxide hydrolase.\textsuperscript{80} Starting from a nitrile with an epoxy ring, the corresponding dihydroxy carbonate could be isolated by the concomitant action of both of epoxide hydrolase and a nitrile-hydrolyzing system.\textsuperscript{13} In other cases, the serious effect of esterase activity of \textit{Rhodococcus} have been reported.\textsuperscript{13,38,59,70} Furthermore, a \textit{Rhodococcus} has been reported to have an 'enantioselective' oxide-reduction system,\textsuperscript{81} which might cause another kinetic resolution of the primary product, a hydroxy acid from the corresponding
5. Application of Enzymatic Nitrile-hydrolyzing Systems in Multistep Chemical Syntheses

Three examples of applications as synthetic tools are illustrated in Scheme 7. In the course of the total synthesis of pyrenophorin, a β,γ-unsaturated carboxylate with (E)-configuration has been prepared starting from readily available (E)-allylic alcohol via combining one carbon homologation using cyanide and the subsequent microbial hydrolysis. There was observed no isomerization or migration of double bond. A four carbon synthetic building block containing boronate, which plays an important role for constructing a further elaborated system of (E, E)- and (E, Z)-conjugated double bonds, was developed by combining microbial hydrolysis of nitriles and palladium-catalyzed coupling of boronate.

Another important use of nitrile-hydrolyzing enzymes is exemplified in the synthesis of an enantiomerically enriched form of lactone related to compactin. Asymmetrization of the prochiral starting dinitrile was done with Brevibacterium sp. After several steps of functional group transformation, a β-hydroxy nitrile with a homoolylic system, which is labile both under acidic and alkaline conditions, was efficiently hydrolyzed by other enzymes of different origin, Rhodococcus sp. The successful result of hydrolysis

Fig. 8. References: a 12); b 33); c 35); d 11); e 15); f 13); g 78); h 58); i 79).
was due to the change of both of functional and protective groups and the source of enzymes.

6. Typical Procedure

An experimental procedure by using *R. rhodochrous* IFO 15564 is described. To a sterilized medium (pH 7.2, 95 ml) containing glucose (1.5 g), KH₂PO₄ (40 mg), K₂HPO₄ (120 mg), MgSO₄·7H₂O (50 mg), yeast extract (100 mg), peptone (500 mg) in a 500-ml Erlenmeyer flask with two internal projections, a solution of e-caprolactam (100 mg) and Fe₂SO₄ (30 mg) in water (5 ml) was added aseptically. A loopful of microorganisms was put in and the flask was incubated at 30°C on a gyratory shaker for 2 days. The cells were harvested by centrifugation. The wet cells (5 g) were re-suspended with buffer solution (pH 6.0, 0.1 M, 50 ml) and the substrates [nitriles or amides, 50–250 mg, 0.1–0.5% (w/v)] were added. The mixture was stirred at 20–30°C and the progress of the hydrolysis was monitored by TLC and/or GLC analysis. After the removal of the cells, the filtrate or supernatant was extracted, and the extract was purified in
a conventional manner.

7. Microorganisms as Reagent

The microorganisms cited in this review are listed as follows:

- Acinetobacter sp., 51K, AK126, 30,36,37,74 Acronemum sp. D9K, 64 Agrobacterium sp., 16 A. tumefaciens d, 20 Alcaligenes faecalis ATCC 8750, 44 DSM 5335, 200 Aspergillus fumigatus, 51 Brevibac terium sp., 50 R312, 53,69,82 B. impedi ale B222, 42 Comamonas acidivorans, 42 KPO-2772, 4,40 Corynebacterium sp. C5, 65,66 Klebsiella pneumoniae, 14 Mycobacterium neoaurum ATCC 25795, 56 Ochrobactrum anthropi SCRC C1-38, 57 Pseudomonas sp., 8, 50 B21C9, 38 BC-18, 46 P. chlororaphis B23, 73 Rizobium sp. MCI2643, 20 Rhodococcus sp. (nitri lase SP409), 13,15,27,58 C311, 34 CH3 (nit ri lase SP361), 0,12,33,68,82 R. equi A4, 63 TG328, 24 R. rhodochros J-1 (Arthrobacter sp.), 4,6,17,18,22,24 AJ270, 51 IFO 15564 (R. butanico), 19,34,45,59,59,67,70,71,78,79 K22, 66 NCIB 1112, 35,61,62 PA34, 54 Tow toropsis candida GN405, 83 Some of the strains are available, 53 and of course, the appropriate microorganism would be obtained by a screening.

Conclusion

At present, although nitrile- and amide-hydrolyzing enzymes are used in the functional group transformation in an industrial scale, the application of these enzymes as the tools in multistep syntheses has not fully been exploited yet. The diversity of substrates and microorganisms, however, will give us a large future prospect for biocatalysis in organic synthesis.

Acknowledgements

The authors thank Professor N. J. Turner and Professor H. Grainger, Drs. V. Kien, L. Martin'ková, and K. Yamamoto for their valuable information.

References and Notes


Microbial Hydrolysis of Nitriles and Amides


83) Examples: *Rhodococcus equi* strain A4: correspondence to Dr. Ludmila Martinková, Institute of Microbiology, Academy of Sciences of the Czech Republic, Videnka 1083, CZ-142 20 Prague 4, Czech Republic. *Brevibacterium* R312 (renamed as a *Rhodococcus* sp., CBS 717-73); from Centraalbureau voor Schimmelcultures Baarn, The Netherlands. *Rhodococcus rhodochrous* IFO 15564: Institute for Fermentation, Osaka (IFO), 2-17-85 Juso-honmachi, Yodogawa-ku, Osaka 532, Japan. Some other strains are available from ATCC and NCTC.