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Unexpected Stereorecognition in Nitrilase-Catalyzed Hydrolysis of β-Hydroxy Nitriles

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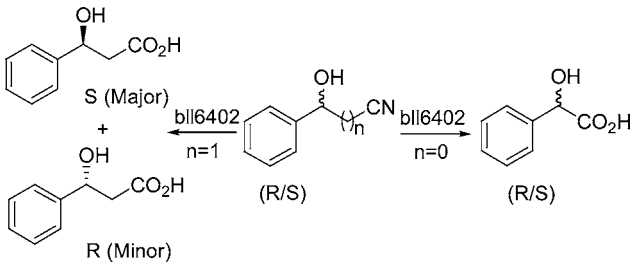
ABSTRACT



Biocatalytic enantioselective hydrolysis of β-hydroxy nitriles to corresponding (*S*)-enriched β-hydroxy carboxylic acids has been achieved for the first time by an isolated nitrilase bll6402 from *Bradyrhizobium japonicum* USDA110. This offers a new "green" approach to optically pure β-hydroxy nitriles and β-hydroxy carboxylic acids. The observed remote stereorecognition is surprising because this nitrilase shows no enantioselectivity for the hydrolysis of α-hydroxy nitriles such as mandelonitrile.

Optically pure β-hydroxy carboxylic acids and their derivatives are precursors of β-blockers and 1,3-amino alcohols, which are important intermediates for the synthesis of natural products, antibiotics, and chiral auxiliaries.[1−3] In addition, chiral β-hydroxy carboxylic acids are widely used to prepare copolyesters for film, fiber, molding, and coating applications.[4] The utility of chiral β-hydroxy carboxylate compounds as useful synthons has stimulated the development of new methodologies for their construction, and a variety of methods have been reported.[5−10] A straightforward approach to optically pure β-hydroxy carboxylic acids is enantioselective hydrolysis of β-hydroxy nitriles, which are readily accessible by the cyanization of α-haloketones with sodium cyanide followed by $NaBH_4$ reduction[11] or ring opening of epoxides by sodium cyanide.[12] However, chemical hydrolysis of nitriles usually requires strong basic or acidic conditions and an elevated reaction temperature that often result in the undesirable elimination of OH for nitriles with a β-hydroxy group, yielding unsaturated byproducts.[13] To solve these problems, biocatalytic hydrolysis of nitriles becomes the choice because the reaction can be performed at a neutral pH condition and room temperature, offering the possibility of hydrolyzing nitriles bearing functionalities that cannot

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tolerate harsh conditions. An additional advantage of biocatalytic hydrolysis of nitriles is its high chemo-, regio-, and stereoselectivity.[14−17] In this context, there have been a few reports on the biocatalytic hydrolysis of β-hydroxy nitriles to corresponding β-hydroxy carboxylic acids and amides using microorganisms possessing nitrile hydratase and amidase activity.[13,18−21] In these studies, the microorganisms with nitrilase activity were also tested but showed no or extremely low activity toward the hydrolysis of β-hydroxy nitriles.[13,18−20] To the best of our knowledge, there are only two reports dealing with the hydrolysis of β-hydroxy nitriles with isolated nitrilases. One is the report from DeSantis et al., in which the desymmetrization of 3-hydroxyglutaronitrile has been achieved with genetically engineered nitrilases.[22] The other one is our recent studies on nitrilase ZmNIT2 from maize (*Zea mays*), which catalyzed the hydrolysis of β-hydroxy nitriles to a mixture of β-hydroxy carboxylic amides and acids, with the amides being the major products (63−88% yields).[23] Herein, we present the results on the enantioselective hydrolysis of β-hydroxy nitriles to (*S*)-enriched β-hydroxy carboxylic acids catalyzed by an isolated nitrilase from *Bradyrhizobium japonicum* USDA110.

Recently, we have cloned and purified a nitrilase (bll6402) from *Bradyrhizobium japonicum* USDA110 and demonstrated that it was an efficient catalyst for the hydrolysis of mandelonitrile and its derivatives.[24] To further explore its synthetic application, this nitrilase was examined for the hydrolysis of β-hydroxy nitriles (Figure 1). β-Hydroxy nitriles were prepared by the cyanization of α-bromoketones with sodium cyanide followed by $NaBH_4$ reduction (see Supporting Information).[11] The obtained β-hydroxy nitriles were treated with purified nitrilase bll6402 in potassium phosphate buffer (100 mM, pH 7.0), and the reaction mixture was incubated overnight at 30 °C. The mixture was then saturated with NaCl and extracted with ethyl acetate.[25] The extract was dried over sodium sulfate, and evaporation of the solvent under reduced pressure afforded the crude products. The β-hydroxy carboxylic acids were separated from unreacted β-hydroxy nitriles by preparative thin-layer chromatography.

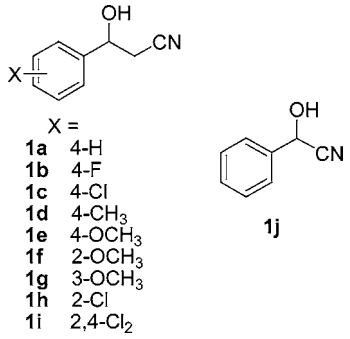
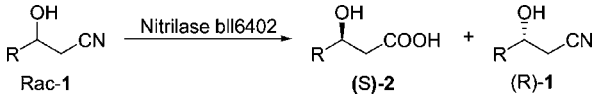


Figure 1. β- and α-hydroxy nitriles.

The ee values of both the product acids and the recovered nitriles were measured by chiral HPLC analysis, and their absolute configurations were determined by comparing the sign of optical rotation with the literature data. The enantiomeric ratios (E) were calculated using the equations proposed by Sih et al.[26] The results are presented in Table 1

Table 1. Enantioselective Hydrolysis of β-Hydroxy Nitriles Catalyzed by Nitrilase bll6402



Rac-1 (S)-2 (R)-1

entry	β-hydroxy nitrile	recovered nitrile (*R*)-**1** yield (%)[a]	recovered nitrile (*R*)-**1** ee (%)	product acid (*S*)-**2** yield (%)[a]	product acid (*S*)-**2** ee (%)	E[b]
1	**1a**	41	53	36	48	5
2	**1b**	37	74	38	60	9
3	**1c**	40	53	32	65	8
4	**1d**	35	76	40	42	5
5	**1e**	57	66	27	90	43
6	**1f**	46	75	36	43	5
7	**1g**	46	67	35	91	52
8	**1h**	40	75	32	84	27
9	**1i**	42	37	39	59	13
10	**1j**	0	—[c]	98	0	—[c]

[a] Isolated yield. [b] The enantiomeric ratio is calculated by the equation $E = \ln[(1 - c)(1 - ee(S))]/\ln[(1 - c)(1 + ee(S))]$, where c is calculated by the equation $c = [ee(S) + ee_o]/[ee(S) + ee(P)]$, ee(S) is the ee of the substrate, ee_o is the initial ee of the substrate, and ee(P) is the ee of the product.[26] [c] Not applicable.

together with the data for the hydrolysis of mandelonitrile under the same conditions.[27]

Nitrilase bll6402 catalyzed the enantioselective hydrolysis of aromatic β-hydroxy nitriles to give (*S*)-enriched β-hydroxy

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carboxylic acids with recovery of (*R*)-enriched *β*-hydroxy nitriles. The substituent on the benzene ring of *β*-hydroxy nitriles did not significantly affect the enzyme activity but exerted some effect on the enantioselectivity. Among the *para*-substituted aryl *β*-hydroxy nitriles, the hydrolysis of the substrate with the *para*-methoxy group (**1e**) showed the highest enantioselectivity with E being 43. The position of the substituent on the benzene ring also affected the enantioselectivity. For example, the enantiomeric ratios E for the substrates with a *para*- and *meta*-methoxy substituent were 43 (**1e**) and 52 (**1g**), respectively, whereas that of their counterpart with an *ortho*-methoxy group (**1f**) was only 5. In contrast, this nitrilase showed higher enantioselectivity for *β*-hydroxy nitrile with an *ortho*-chloro group (**1h**) than the one with a *para*-chloro substituent (**1c**). Thus, the enantioselectivity of this nitrilase was influenced by both the steric and electronic factors of the substituents on the benzene ring.

As we previously reported, nitrilase bll6402 showed no enantioselectivity for the hydrolysis of α-hydroxy nitriles such as mandelonitrile.[24] Therefore, the observed stereodiscrimination in the hydrolysis of *β*-hydroxy nitriles was surprising because it was in contrast to the usual observation that a chiral carbon atom at the *β*-position to the reaction center would be recognized with much more difficulty than the one at the α-position.[18,23]

To test the role that the *β*-hydroxy group plays in the enantioselective hydrolysis of *β*-hydroxy nitriles, 3-phenylbutyronitrile (in which the hydroxy group was replaced with a methyl group) was treated with nitrilase bll6402. However, 3-phenylbutyronitrile was not hydrolyzed by nitrilase bll6402 under the same conditions. This indicates that the hydroxy group of *β*-hydroxy nitriles not only promotes the stereodiscrimination at the *β*-position but also plays a critical role in determining the enzyme activity.

Nitrilase possesses a cysteine residue in its catalytic site, and the proposed reaction mechanism for the hydrolysis of nitriles involves the formation of an enzyme−substrate complex where the thiol residue of the enzyme forms a covalent bond with nitrile carbon. Addition of a H_2O molecule to the complex generates a tetrahedral intermediate. Loss of ammonia from this tetrahedral intermediate gives an acyl−enzyme intermediate, which is further hydrolyzed to produce carboxylic acid and release the enzyme for the next catalytic cycle.[28,29] Hydrogen bond formation, which is a very common feature in enzyme catalysis, occurs when a polar amine/hydroxy group either from the substrate or from the enzyme residue is in close proximity to another amine/hydroxy group from a similar environment.[30,31] The observed stereorecognition in the hydrolysis of *β*-hydroxy nitriles might be due to hydrogen bonding between the *β*-hydroxy group with a NH_2 or a OH group of enzymes. The substrate is thus anchored on the surface of the enzyme by hydrogen bonding so that the CN group of the (*S*)-enantiomer is in closer proximity to the cysteine residue of the catalytic site than that of the (*R*)-enantiomer. This allows the formation of an enzyme−substrate complex favorable for the (*S*)-enantiomer over (*R*)-configured *β*-hydroxy nitriles, thus leading to (*S*)-configured *β*-hydroxy carboxylic acids as the major enantiomer. Because the crystal structure of nitrilase bll6402 is not known, further studies are needed to determine the effect of substrate−enzyme hydrogen bonding on activity and enantioselectivity.

In conclusion, kinetic resolution of aromatic *β*-hydroxy nitriles to the corresponding enantiomerically enriched *β*-hydroxy carboxylic acids and *β*-hydroxy nitriles has been achieved by an isolated nitrilase from *Bradyrhizobium japonicum* USDA110. In addition, nitrilase bll6402 shows surprisingly unusual stereorecognition in the hydrolysis of *β*-hydroxy nitriles, whereas the hydrolysis of α-hydroxy nitriles is not enantioselective. Nitrilase-catalyzed enantioselective hydrolysis is superior to the lipase-catalyzed kinetic resolution of *β*-hydroxy nitriles because the latter is usually followed by chemical hydrolysis that is difficult to be performed without side reactions.[10] Therefore, nitrilase-catalyzed enantioselective hydrolysis offers a new "green" approach to optically pure *β*-hydroxy nitriles and *β*-hydroxy acids, although a further improvement in enantioselectivity is needed.

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Supporting Information Available: General procedures for the preparation of *β*-hydroxy nitriles and their hydrolysis, characterization data, and ^{13}C NMR spectra for enantiomerically enriched *β*-hydroxy nitriles and acids. This material is available free of charge via the Internet at http://pubs.acs.org.

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