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Asymmetric synthesis of a nitroalkane by the use of novel nitroalkene reductases from baker's yeast

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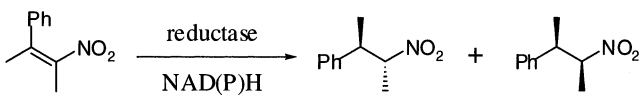
Abstract—Two kinds of novel nitroalkene reductases were isolated from baker's yeast. Reduction of a trisubstituted nitroalkene by these reductases afforded the corresponding nitroalkane with excellent enantioselectivity, moderate diastereoselectivity, and in good yield.

Chiral nitro compounds are important synthetic intermediates, which can be easily converted into chiral amines, and chiral amines are useful compounds in organic synthesis. Asymmetric reduction of nitroalkenes with microbes affords chiral saturated nitro compounds. The baker's yeast reduction of β,β-disubstituted nitroalkenes is known to afford the (*R*)-nitroalkanes enantioselectively,[1,2] while α,β-disubstituted nitroalkenes are transformed into almost racemic saturated nitroalkanes under similar reduction conditions.[3–7] Recently, we have reported that the reduction of trisubstituted nitroalkenes affords saturated nitroalkanes with excellent enantioselectivities, moderate diastereoselectivities and in good yield.[7] On the other hand, we have already demonstrated the advantage of the use of an isolated enzyme compared with the whole cell reaction, in which the enzyme catalyzes a reaction without any side reactions and performs a reaction in excellent stereoselectivity.[8–11] However, no investigation on the isolation of a nitroalkene reductase has been reported. Herein, we report the first purification and characterization of novel nitroalkene reductases from the cells of baker's yeast, and the asymmetric reduction of a nitroalkene with these reductases.

The reductases were isolated from a cell-free extract of baker's yeast (Oriental Yeast, Japan) by column chromatography such as anion exchange, hydrophobic and gel-filtration, as the same method reported previ-

Table 1. Enzymatic reduction of (*Z*)-3-phenyl-2-nitro-2-butene (**1**) with YNAR-I and II[a]



1 (2*R*,3*R*)-**2** (2*S*,3*R*)-**3**

Enzyme	Coenzyme	Relative activity	d.e.[b] (%)	e.e.[c] (%)	e.e.[d] (%)
YNAR-I	NADPH	1.00	31	>98	97
	NADH	0.60	29	>98	97
YNAR-II	NADPH	1.00	35	>98	97
	NADH	0.54	34	>98	97

[a] Conditions: acetate buffer, 100 mM, pH 5.0.
[b] Excess of **2** over **3**.
[c] e.e. of **2**.
[d] e.e. of **3**.

Keywords: enzymes and enzyme reactions; asymmetric reactions; nitro compounds.
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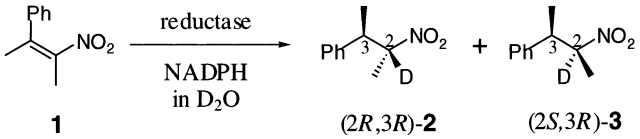
Table 2. Enzymatic reduction in D_2O[a]



1 (2*R*,3*R*)-**2** (2*S*,3*R*)-**3**

	Deuterium content (%)	
Enzyme	C-2	C-3
YNAR-I	52	0
YNAR-II	59	0

[a] The reaction was in more than 99% D_2O.

ously.[8,12,13] All purification steps were run at 4°C and the enzymes were stored at −20°C. Activity was measured by employing (*Z*)-3-phenyl-2-nitro-2-butene (**1**) as a substrate. Two enzymes, yeast nitroalkene reductase-I and -II (YNAR-I and -II), were isolated and the stereoselectivities of the reduction are summarized in Table 1. Both enzymes preferentially utilize NADPH as the coenzyme, but NADH also serves as the coenzyme to a certain extent. Although diastereoselectivities in the reduction of (*Z*)-3-phenyl-2-nitro-2-butene with these enzymes were moderate, enantioselectivities of these enzymes were satisfactory (more than 98% e.e.), giving (2*R*,3*S*)-3-phenyl-2-nitrobutane (**2**) in good yield. Compared with the whole cell reaction, the diastereoselectivities were slightly improved by the use of an isolated enzyme.[7]

McAnda et al. reported that the mechanism of the yeast reduction of nitrostyrenes using deuterium labeling experiments.[6] They concluded that the yeast mediated reduction of the nitroalkenes proceeds in two stages: a reversible non-stereoselective protonation at the α-carbon followed by a stereoselective addition of a hydride from a coenzyme at the β-carbon. Since they studied the mechanism of the reduction using a whole cell system, a detailed mechanism of the nitroalkene reductase remained unclear. To elucidate the hydrogenation process with these enzymes, nitroalkene **1** was subjected to the enzymatic reduction in D_2O. The incorporation of deuterium in the product was determined by ^1H and ^2H NMR spectrum analyses. The results summarized in Table 2 reveal that the product **2** contains a deuterium at the α-position of the nitro group (C-2). It has thus been elucidated that the hydrogen incorporated into the β-position of the nitro group by the enzymatic reduction is derived from NADPH and that into the α-position comes from water or a certain amino acid residue in the active site of the enzyme. The reduction with these enzymes affords the product in excellent enantioselectivity and modest diastereoselectivity, indicating that stereoselective addition of hydride from NADPH occurs at the β-position of the nitro group, followed by less stereoselective protonation at the α-position.

The diastereoisomers thus obtained were readily separable from each other by column chromatography on silica gel, allowing enantiomerically pure isomers of **2** and **3** to be obtained without difficulty. Nitroalkene reductases, the new class of enzymes reported here, are potentially useful as catalysts for obtaining chiral nitro compounds in excellent stereoselectivity.

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