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Asymmetric Reduction of α,β-Unsaturated Ketones with a Carbon-Carbon Double-Bond Reductase from Baker's Yeast

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Abstract

A novel carbon-carbon double-bond reductase was isolated from the cells of baker's yeast. The reduction of α,β-unsaturated ketones catalyzed by this enzyme affords the corresponding saturated (*S*)-ketones selectively.

Keywords: Asymmetric reactions; Enzymes and enzyme reactions; Reduction; Ketones

Although a number of isolated enzymes have been used widely in the field of asymmetric synthesis [1], little is known about carbon-carbon double-bond reductases. Only a few investigations on the isolation and characterization of enone reductases from plant cell cultures have been reported [2]. Asymmetric reduction of enones with a microbe has been studied and utilized as a method for preparing chiral ketones [3-12]. In many cases so far, however, the microbial reaction gives a mixture of ketone, saturated alcohol, and allylic alcohol, in which several enzymes may catalyze the reduction of C=C and C=O double bond competitively [3-7, 9-12]. Although this disadvantage is expected to be overcome by the use of an isolated enzyme, no study on the isolation of enzyme from a microbe has been reported. Here, we would like to report the purification and characterization of a novel carbon-carbon double-bond reductase from the cells of baker's yeast.

The reductase was isolated from a cell free extract of baker's yeast (Oriental Yeast, Japan) by anion-exchange (DEAE-Toyopearl) and hydrophobic (Butyl-Toyopearl) column chromatography, as the same method reported previously for the isolation of keto ester reductases [13, 14]. All purification steps were run at 4 °C and the enzyme solution containing 10% glycerol was stored at -20 °C. Activity was measured by employing 4-(3-chlorophenyl)-3-methyl-3-buten-2-one (**1b**) as a substrate. This reductase utilizes

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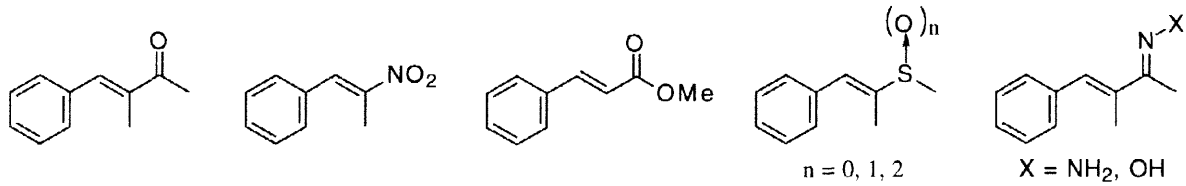
NADPH as the coenzyme preferentially. Since each chromatogram appeared as a single peak, there is no doubt that only one enzyme catalyzes the reduction of the carbon-carbon double bond of the enone in a whole cell of baker's yeast.

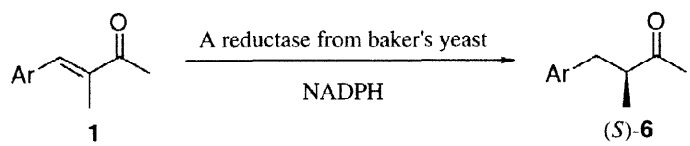
In microbial reductions of carbon-carbon double bonds, it has been deduced that the reductions require the conjugation of a carbonyl [3-12] or nitro [15, 16] group as a strong electron-withdrawing group. In order to clarify the scope and limitation of this novel reduction, various olefins **1-5** were subjected to the enzymatic reduction. In addition to the enone **1**, nitroolefin **2** can also be a substrate and gives the corresponding saturated nitro compound as the product but only in modest enantiomeric purity (48% e.e.). Other olefins with an electron-withdrawing group such as sulfide, sulfoxide, sulfone, hydrazone, oxime, and ester functions gave no product.



1 **2** **3** **4** **5**

To obtain further information about the reduction with this reductase, several enones, **1a-e**, were prepared by the same method as reported previously [12] and reduced with this enzyme at 35 °C in the presence of NADPH. After usual extraction, enantiomeric excesses and absolute configurations of the product **6a-e** were determined by GLC on a chiral capillary column [12]. The results are listed in Table 1.

Table 1
Asymmetric reduction of enones with a reductase from baker's yeast



1 (*S*)-**6**

	Ar	Activity (%Conv./h)	E.e.[a] (%)		Configuration
a	Ph	8.3	80	(71)	*S*
b	3-Cl-C_6H_4	15	>99	(96)	*S*
c	3-NO_2-C_6H_4	32	>99	(>95)	*S*
d	3-MeO-C_6H_4	9.1	>99	(>95)	*S*
e	4-MeO-C_6H_4	3.3	68	(61)	*S*

[a] Data from baker's yeast reduction [12] are indicated in parenthesis.

The stereoselectivity of the reduction is the same as that of the baker's yeast reduction [12], which suggests strongly that the enzyme isolated herein is solely responsible to the reduction of an enone in a whole cell. The reduction of **1** having no substituent or *para*-substituent on the phenyl ring affords (*S*)-ketone **6** in moderate stereoselectivity, whereas introduction of a substituent at the *meta*-position drastically improves the stereoselectivity. The site of a substituent plays a crucial role in the stereodiscrimination by the enzyme. On the other hand, reaction rates which were determined by GLC as percent conversion per hour gave a linear Hammett plot (Figure 1), which means that the electronic effect of the substituent is more important than its steric bulk or position on the facility of the reaction.

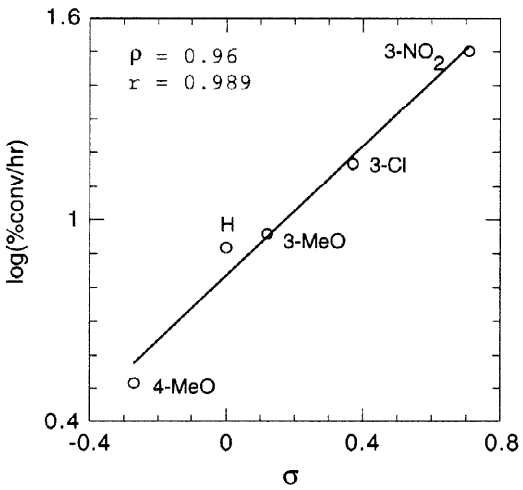
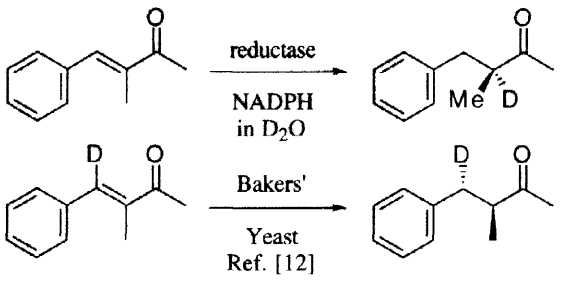


Figure 1. Hammett Plot for the Enone Reductase.

In order to elucidate the hydrogenation process with this enzyme, enone **1a** was subjected to the enzymatic reduction in D_2O (Scheme 1). The ^1H NMR spectrum of the product reveals that the product **6a** contains a deuterium at the α-position. It has thus been elucidated that the hydrogen incorporated into the β-position of the carbonyl group by the enzymatic reduction is derived from NADPH and that into the α-position comes from water (or *via* a certain amino acid residue). The whole cell reduction with a deuterium as a tracer confirmed that the stereochemistry is 100% formal *trans*-addition of hydrogens regardless of its satisfaction in enantioselectivity (Scheme 1) [12]. These results make it possible to assume the arrangement of catalytic groups at the active site of the enone reductase (Figure 2).



Scheme 1

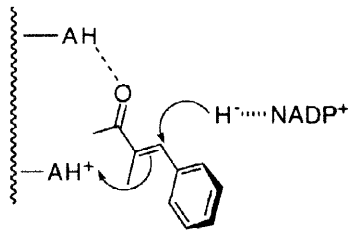


Figure 2. Arrangement of catalytic groups in the reductase.

The chemical yield becomes quantitative, provided sufficient amount of the enzyme was employed for the reaction [17]. Moreover, the enzymatic reduction affords no other products. The result reported here is believed to be a useful method for obtaining chiral ketones in excellent stereoselectivity. We will soon report detailed procedure for the purification of the enzyme.

References and Note

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[17] A typical procedure for the enzymatic reaction was carried as follows: The mixture of enone **1c** (0.5 mM), NADPH (0.5 mM), enzyme 8 units/L, and potassium phosphate buffer (10 mM, pH 7.0) was stirred for 8 h at 35 °C.