

Stereochemical Control of Yeast Reduction of α -Keto Esters in an Organic Solvent

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Yeast reduction of α -keto esters in water afforded the corresponding (*R*)-hydroxy ester while the antipodes were obtained in the reduction in benzene. To elucidate the mechanism for stereochemical control of yeast reduction, seven enzymes responsible for the reduction have been isolated from bakers' yeast and kinetic parameters for enzymatic reductions have been measured. It was found that the (*R*)-producing enzymes have smaller *K*_ms than those of the (*S*)-producing enzymes. When the reaction is run in benzene, however, the produced α -hydroxy ester does not undergo further decomposition. The inhibition of enzymatic decomposition in an organic solvent is also accounted for by low concentration of α -hydroxy ester in aqueous phase surrounding the bakers' yeast.

Keywords: Stereochemical control / Microbial reduction / Organic solvent

Microbial reductions have widely been used to synthesize chiral alcohols because of their easiness in treatment and mild reaction conditions. Unfortunately, however, enantioselectivities of microbial reductions are not usually satisfactory, and it is necessary to improve low enantioselectivity of yeast reduction. We have been developed several methods to control stereochemistry of yeast reduction without changing microbes, *i.e.*, modification of substrate, addition of an inorganic salt, addition of an inhibitor to the specific enzyme, and thermal treatment. Here, we report that the use of an organic solvent affects largely the stereochemistry of yeast reduction of α -keto esters. For example, ethyl 2-oxohexanoate (**1d**) was reduced to (*S*)-ethyl 2-hydroxyhexanoate ((*S*)-**2d**) in 99% e.e. and to the

antipode, (*R*)-**2d**, in 86% e.e. by the reaction in water and benzene, respectively. The same tendency was observed in the reduction of **1f**. Here, although both reactions in water and in benzene afford the same isomer, (*R*) products, enantioselectivity increases from 19% e.e. to 90% e.e. by changing the medium from water to benzene. Enantioselectivity of the reduction shifts to (*R*)-side in nonpolar organic solvent such as benzene or hexane compared to that in water.

To elucidate the mechanism the effect of organic solvent on stereochemical control of yeast reduction, enzymes were isolated from bakers' yeast and kinetic parameters of these α -keto ester reductases were measured. Five reductases (YKER-I, -IV, -V, -VI, and -VII) are found to be responsible to the reduction of **1d**.

BIOORGANIC CHEMISTRY – Bioorganic Reaction Theory –

Scope of research

Biochemical reactions are studied from the viewpoint for physical organic chemistry. Specifically, the reaction mechanism and stereochemistry of NAD-dependent oxidoreductases are explored. Stereospecific redox transformations mediated by certain biocatalysts such as microbes, enzymes, cultured tissues are also studied. The results will be applied to develop new organic reactions.



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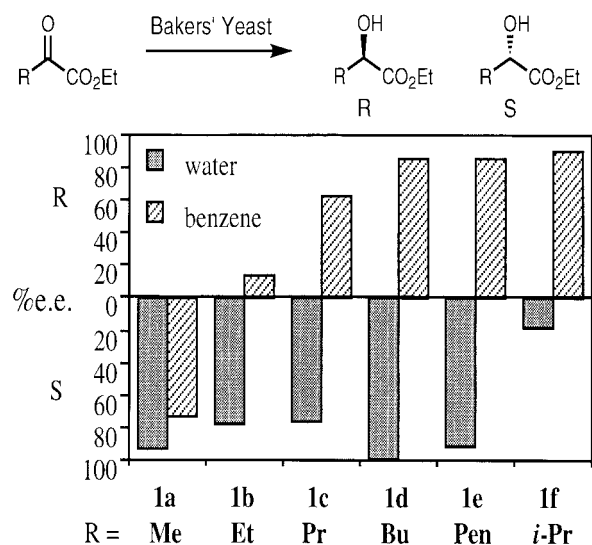


Figure 1. Enantioselectivity of bakers' yeast reduction of α -keto esters.

Among them, two enzymes (YKER-V and -VII) afforded (*S*)-**2d** and the other three enzymes (YKER I, -IV, and -VI) gave (*R*)-**2d**. Kinetic study reveals that YKER-IV and -VI have smaller K_m (0.14 mM and 1.03 mM, respectively) than the other enzymes. Then, the reduction of **1d** in benzene is catalyzed mainly by YKER-IV and -VI affording (*R*)-**2d**, whereas the reduction in water is also contributed by YKER-V and -VII. Concentration of α -keto ester in yeast cell plays an important role for the stereochemistry of reduction in an organic solvent system. Since the substrate is more soluble in benzene than in water, the substrate concentration in the aqueous phase around yeast cell. Although stereoselectivity of the reduction in benzene is, thus, explainable quantitatively (e.e. of yeast reduction = 86% (*R*) and that of YKER-IV = 88% (*R*)) on the basis of enzyme activity, that in water can be reproduced only qualitatively: e.e. of yeast reduction (99% (*S*)) exceeds that of enzymatic reduction (31% (*S*) and 84% (*S*) for

YKER-V ($K_m = 5.7$ mM) and -VII ($K_m = 27$ mM), respectively). The phenomenon can be accounted for by asymmetric decomposition of the (*R*)-product. During yeast reduction in water, certain α -hydroxy esters (**2c**, **2d**, and **2e**), the products, are decomposed under the catalysis of bakers' yeast. Thus, the (*R*)- α -hydroxy esters are decomposed preferentially remaining the antipodes unaffected. The asymmetric decomposition is one of the tricks for stereochemical control of yeast reduction of α -keto esters in water. For example, when racemic **2d** was incubated with bakers' yeast in water, the *R*-isomer disappeared after 2 h and only the *S*-counterpart was recovered. On the other hand, the enzymatic decomposition of (*R*)-**2d** was not observed when the reaction was run in benzene under the same conditions as those for the reduction in benzene. Suppress of asymmetric decomposition in benzene is also accounted for by the decrease in substrate concentration in the aqueous phase. Apparent K_m for the decomposition was determined to be 2.2 mM by measuring initial rate of the decomposition of **2d** catalyzed by whole bakers' yeast cell in water. Since the concentration of **2d** in the aqueous phase is much smaller than K_m for the decomposition, the rate of decomposition of the produced α -hydroxy esters dramatically depressed in benzene.

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