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Enantiocontrol in the Bakers' Yeast Reduction of Trifluoroacetylbiphenyl Derivatives

Tamotsu FUJISAWA,* Seiji TANAKA, Yoshio ONOGAWA, and Makoto SHIMIZU

Department of Chemistry for Materials, Mie University, Tsu, Mie 514-8507, Japan

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Abstract: The bakers' yeast reduction of trifluoroacetyl derivatives was examined in the presence of various esters of methanethiosulfonate. The yeast reduction of 4-bromo-4'-trifluoroacetylbiphenyl using the cyclohexylmethyl methanethiosulfonate resulted in the *R* product with the highest enantiomeric excess of 96% ee, which is compared with the reduction without the additive to give the corresponding alcohol with 84% ee. On the other hand, in the case of 4-hyrdoxy-4'-trifluoroacetylbiphenyl, the highest enantioselectivity was observed also using cyclohexylmethyl methanethiosulfonate to give the *R*-product with 90% ee, in contrast with the reduction without the additives to give the same product with 77% ee.

Keywords: bakers' yeast; asymmetric reduction; trifluoroacetylbiphenyls; methanethiosulfonate

Enzymes are widely accepted as useful catalysts in organic synthesis, and are capable of catalyzing asymmetric transformations [1]. In this regard, the bakers' yeast reduction of prochiral ketones has been one of the most useful methods preparing various chiral alcohols through recognition of the prochiral face of carbonyl compounds [2]. The most important advantage of this microbial method is the ready availability of bakers' yeast. However, such biocatalytic reactions have strong substrate specificity. A few methods have been reported to improve the enantioselectivity in the bakers' yeast reduction of β-keto ester derivatives. These involve the improvement of fermentation conditions [3], immobilization of bakers' yeast [4], addition of an enzyme inhibitor [5], and chemical modification of the substrates [6], although the rate of the reduction is usually decreased in such cases. From the perspective of synthetic utility, it would also be convenient to use a whole cell, rather than isolated enzyme(s). We report here a new method for the chemical modification of enzyme(s) in intact bakers' yeast with methanethiosulfonate reagents to attempt disulfide link formation.

Bakers' yeast (*Saccharomyces cerevisiae*) produces more than 6,000 enzymes [7], including many oxidoreductases [2a]. Of these, a yeast alcohol dehydrogenase in bakers' yeast has been reported to contain 36 cysteine residues and to have four active sites [8]. The thiol group of the cysteine residue has been reported to play a role in catalysis; for example, with the D-glyceraldehyde 3-phosphate dehydrogenase from Bacillus stearothermophilus, NAD bonded by hydrogen bonding. A thiol group is located near the nicotine amide. This thiol group reacts with the enzyme to form a hemiacetal, and hydride transfer then gives an acylenzyme [9]. The thiol group also participates in disulfide bonding by oxidation. There have been many reports on the reactivity of thiol: *i.e.*, it reacts with vinyl compounds [10], monosulfides [11] and disulfides [12]. Disulfides can spontaneously disproportionate [13]. These mean sulfur compounds may affect the thiol moieties in bakers' yeast to change the

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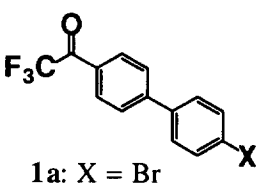
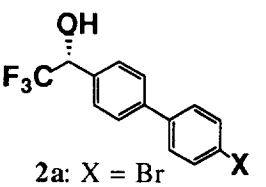
shape of the reactive site. Therefore, higher enantioselectivity may be achieved using a sulfur compound. Recently we have been interested in the effect of a sulfur-containing substituents on substrates as a modifier of enantiofacial discrimination. A sulfur atom that has been introduced to the substrate in bakers' yeast reduction [14] may interact with the thiol group in bakers' yeast. Based on this hypothesis, sulfur compounds may modify the enzyme so as to alter the receptor site and enhance the reductive ability. Accordingly, the optical purity may be improved. We have also reported that the addition of sulfur compounds improves the enantiofacial selectivity and reactivity in the course of the bakers' yeast reduction of β-keto esters [15]. There have been other reports on enhancing reactivity by modifying thiol groups in enzymes. For example, the Ellman reagent [16] has been shown to modify enzymes. It readily reacts with thiol groups in enzymes to yield disulfide links. When the Ellman reagent reacts with a thiol in chicken pepsin, it affects the hydrolysis of synthetic peptide: *i.e.*, the bulkiness of the ester part of the Ellman reagent increases the activity [17]. Further, chemically modified mutant enzymes have been reported to be better catalysts than the parent-type enzymes [18]. By chemically modifying an enzyme with methanethiosulfonate reagents [19], subtilicin Bacillus lentus forms a disulfide, and the hydrolysis of a substrate is reportedly enhanced: *i.e.*, upon the formation of a disulfide, reactivity increases as the side-chain grows.

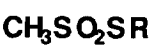
Fluorine-containing optically active alcohols have recently attracted attention in connection with ferroelectric liquid crystals [20] and as tools for metabolic studies [21]. Thus, we examined the yeast reduction of trifluoroacetylbiphenyl derivatives [22] with the chemical modification of the enzyme(s) in intact bakers' yeast with methanethiosulfonate reagents to form disulfide links, and determined the effect on the enantioselectivity of the products.



1a: X = Br
1b: X = OH



2a: X = Br
2b: X = OH



3

A yeast suspension with additives in phosphate buffer (pH 7.0) was stirred for 1 h, and the substrate was then added. Since the concentration of the additives may affect the stereochemistry, the optimal amount of the additive, n-hexyl methanethiosulfonate **3** (R = n-Hex), per gram of bakers' yeast was examined using 4-bromo-4'-trifluoroacetylbiphenyl **1a** as a substrate. The reduction without the additive proceeded for 16 h to give the corresponding alcohol **2a** in *R* form [23] with 84% ee. When from 0.02 to 0.20 mmol/g of the additive was used, the yield decreased, but the enantiomeric excess increased up to 94% ee. The best results were obtained with 0.10 mmol of the additive, which gave the corresponding secondary alcohol with 94% ee in a yield of 60%.

Next we examined the effect of the length of the alkyl group in the thiol part on enantioselectivity, as shown in Table 1, under the optimal conditions described above. The bakers' yeast reduction of 4-bromo-4'-(trifluoroacetyl)biphenyl **1a** in the absence of alkyl methanethiosulfonate showed moderate enantiofacial discrimination to give the corresponding

Table 1. Bakers' Yeast Reduction of 4'-Substituted 4-(trifluoroacetyl)biphenyl Derivatives **1** in the Presence of Alkyl Methanthiosulfonates **3**

entry	Substrate	Additive R in 3	Time (h)[a]	Yield (%)[b]	% ee[c]	entry	Substrate	Additive R in 3	Time (h)[a]	Yield (%)[b]	% ee[c]
1	**1a**	------	16	75	84	11	**1b**	-------	2	71	77
2		Et	40	41	84	12		Et	2	67	76
3		n-Pr	42	35	88	13		n-Pr	2.5	61	77
4		n-Bu	43	68	91	14		n-Bu	2	77	77
5		n-Pen	43	58	93	15		n-Pen	2	77	82
6		n-Hex	43	60	94	16		n-Hex	2.5	81	89
7		n-Oct	42	81	80	17		n-Oct	2	85	77
8		i-Bu	40	31	94	18		i-Bu	2	61	84
9		i-pen	40	88	90	19		i-pen	2	65	86
10		c-HexCH$_2$	42	71	96	20		c-HexCH$_2$	2	80	90

a) Reaction time is disappearance of starting material. b) Isolated by preparative TLC. c) The enantiomeric excess of the products was determined by HPLC using a chiral stationary column (Dicel OD).

alcohol **2a** in 75% yield (entry 1), whereas the enantioselectivity improved when sulfur compounds were used to modify the thiol group of a reductase in the yeast. Using esters with normal alkyl chains, the enantioselectivity increased as the length of the alkyl part increased, and the greatest improvement was observed for the n-hexylthio ester (entry 6). The greater effect of chain length over steric volume is demonstrated by the use of esters with branched alkyl thiol groups, such as a secondary alkyl group. The highest enantiomeric excess was observed for the cyclohexylmethylthio ester, which gave a product with 96% ee, although a long reaction time was required (entry 10).

The bakers' yeast reduction of 4-hydroxy-4'-trifluoroacetylbiphenyl **1b** in the absence of a sulfur compound gave the corresponding (R)-alcohol **2b** [23] with 77% ee (entry 11), and the reaction was completed in 2 h. In this case, the suspension of yeast and additives in phosphate buffer (pH 7.0) was stirred for 6 h before addition of the substrate. The addition of methanethiosulfonate reagents improved the enantioselectivity, although the reaction time was almost the same as in the reduction without additives, and higher enantioselectivity (up to 89% ee) was achieved using *n*-hexyl methanethiosulfonate (entry 16). The highest enantioselectivity was observed using cyclohexylmethyl methanethiosulfonate, which gave the *R*-product with 90% ee (entry 20).

Enantioselectivity was enhanced by chemical modification of an enzyme. Methanethiosulfonate is known to react with thiol in the enzyme to give a disulfide [24]. Thus, disulfide formation leads to the enhancement of optical purity. Six to eight carbons of alkyl esters in the additives are most effective in the reduction of trifluoroacetyl derivatives. This may be because the hydrophobic trifluoromethyl group interacts with the hydrophobic alkyl group.

These results demonstrate the considerable potential that such chemical modification offers in improving enantioselectivity. Interestingly, chemical modification of the intact yeast enzyme(s) can change the reactive sites involved in bakers' yeast reduction.

In summary, disulfide formation in reductases by the addition of methanethiosulfonate can modify the enantioselectivity of trifluoromethyl carbinols in the bakers' yeast reduction of trifluoroacetyl derivatives. This is the first example of the control of enantioselectivity through the chemical modification of enzymes in bakers' yeast.

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