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Stereochemical Control in Microbial Reduction. 8. Stereochemical Control in Microbial Reduction of β-Keto Esters

Kaoru NAKAMURA,* Yasushi KAWAI,† Shinzaburo OKA,† and Atsuyoshi OHNO†
Department of Chemistry, Shiga University of Medical Science, Seta, Ohtsu 520-21
†Institute for Chemical Research, Kyoto Unversity, Uji, Kyoto 611
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Stereochemistry of the reduction of β-keto esters with bakers' yeast is controlled by the addition of a certain α,β-unsaturated carbonyl compound (or its reduced form). Glucose also exerts the same effect. The additives tend to shift the stereochemistry of the reduction toward the production of D-hydroxy ester. Namely, methyl 3-oxopentanoate and ethyl 3-oxo-6-heptenoate were reduced to the corresponding D-hydroxy esters with excellent stereoselectivities and chemical yields. The enones are supposed to inhibit the enzymes that produce the L-hydroxy ester, whereas glucose plays a role to activate the enzymes that produce the D-hydroxy ester.

Since chiral alcohols are versatile and convenient building blocks in the syntheses of biologically important compounds, asymmetric reduction of ketones has been studied quite extensively.[1] Namely, the reduction mediated by a microbe has been a promising candidate to synthesize chiral alcohols in an excellent stereoselectivity and the research in this field has been expanded tremendously within recent one or two decade(s).[2] In most cases so far reported, the results are satisfactory with high chemical yields and high stereoselectivity. However, it should be emphasized that there was a lot of examples that afforded unsatisfactory results; low chemical yields and/or low stereoselectivities. Usually, almost all of these results are discarded unreported.

At the same time, a microbe does not necessarily afford the compound of desired configuration for further use in syntheses. In this case, we have to control or reverse the stereochemistry of the reduction. There are several methods to improve or control the stereoselectivity of the reduction. The first and most common method is to screen microbes to find a suitable one for a particular purpose.[3–5] This is a purely biological method and the result can not be predicted on the basis of chemistry. The second is the modification of the substrate.[6–8] In most cases in this category, steric bulk of two substituents on the carbonyl group is differentiated so that the enzyme (or enzymes) can recognize the *re*- and *si*-faces of the substrate easily, and the results are more or less chemically predictable. The third and brand-new method reported from our laboratory is to modify the reaction conditions.[9,10] Immobilization of a microbe into a hydrophobic network of a polymer is a typical example in this category. It has been reported that there is a relationship between the hydrophobicity of medium in the vicinity of microbe and the preferability to the configuration in the product hydroxy ester.[9,10]

In this paper, we would like to report the fourth method: the addition of a third reagent to the reaction system affects the stereochemical course of the reduction and the stereoselectivity can be improved up to satisfactory levels.[11]

Results and Discussion

There are two possibilities for an unsatisfactory stereoselectivity. One of them is the case in which only one dehydrogenase is responsible for the reduction of a substrate and the structure of the substrate is not suitable to exert its enantio- (or diastereo)-facial difference in the pocket of the enzyme. A low stereoselectivity seen in the reduction with an isolated dehydrogenase is the extreme of this category.[12,14] The other is the case in which two or more dehydrogenases are operating simultaneously but some enzymes produce the (*R*)-alcohol while others contribute to produce the (*S*)-alcohol, although each reduction proceeds with a high stereoselectivity.[15] There is a good chance to obtain a racemic mixture from a microbial reduction system. If the low stereoselectivity observed in a microbial reduction is due to the latter situation, there is a chance for a chemist to improve the stereoselectivity: inhibition or activation of an appropriate enzyme (or enzymes) will result in higher stereoselectivity. Based on the concept mentioned above, we looked for an inhibitor and an activator for particular enzymes responsible for the reduction of β-keto esters with bakers' yeast.

Two different states of bakers' yeast were employed as the microbe. One is the raw bakers' yeast (RBY) and the other is the dry bakers' yeast (DBY). Although the effect of the additive is much larger for RBY than for DBY, the activity of RBY changes from a lot to another and sometimes the stereoselectivity of reduction deviates up to 10% from a run to another when the specificity is relatively low. The activity of RBY also decreases on storage and it is difficult to confirm the reproducibility in stereoselectivity of the reduction with RBY. Therefore, we employed the result from DBY to discuss fundamental trends of the reduction, but RBY is recommended for practical purpose,

Table 1. Effect of the Amount of Bakers' Yeast on the Reduction of Methyl 3-Oxopentanoate[a]

Yeast/g	Configuration	e.e./%[b]	Chem. yield/%[c]
RBY (1)	*R* (D)	54	19
RBY (2)	*R*	51	37
RBY (3)	*R*	44	33
RBY (5)	*R*	33	35
DBY (1)	*R*	10	50
DBY (2)	*R*	7	41
DBY (4)	*R*	12	46

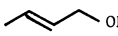
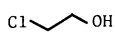
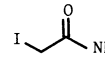
a) Substrate, 1 mmol; Water 20 ml. b) Reproducibility in the reaction with RBY and DBY are about 10% and 1%, respectively. c) Reproducibility in the reaction with RBY and DBY are about 5% and 1%, respectively.

because the latter affords a better stereoselectivity, an enantiomer excess (e.e.).

As listed in Table 1, the relative amount of DBY against the substrate does not affect the stereoselectivity of the reduction meaningfully, whereas that of RBY results in decreases in e.e. Thus, it seems probable that DBY and RBY are different in levels of various enzymes. In other words, plural enzymes are operating in the reduction system and the effect of an additive can be expected for this system.

The effect of α,β-unsaturated carbonyl and hydroxy compounds on the e.e. of product was studied in the reduction of methyl 3-oxopentanote with DBY and the results are summarized in Table 2. The bakers' yeast was preincubated for 30 min in the presence of an

Table 2. Reduction of Methyl 3-Oxopentanoate with Bakers' Yeast in the Presence of Carbonyl or Hydroxy Compound[a]

Additive	Yeast	Configuration	e.e./%[b]	Chem. yield/%[c]
	DBY	*R* (D)	68	51
	RBY	*R*	89	22
	DBY	*R*	61	71
	RBY	*R*	78	50
	DBY	*R*	60	68
	RBY	*R*	89	67
	DBY	*R*	44	67
	RBY	*R*	66	68
	DBY	*R*	44	63
	RBY	*R*	68	63
	DBY	*R*	39	61
	RBY	*R*	66	34
	DBY	*R*	23	67
	RBY	*R*	35	31
	DBY	*R*	22	58
	RBY	*R*	61	58
	DBY	*R*	21	67
	RBY	*R*	35	36
	DBY	*R*	16	59
	RBY	*R*	31	30
	DBY	*R*	16	51
	RBY	*R*	41	48
	DBY	*R*	13	66
	RBY	*R*	25	31
None	DBY	*R*	12	46
	RBY	*R*	37	38
	DBY	*R*	4	24
	RBY	*R*	20	34

a) DBY, 4 g (or RBY, 2 g); water, 20 ml; substrate, 1 mmol; additive, 0.5 mmol. b) The reproducibilities in the reduction with RBY are about 2% at >80% e.e., 5% at >50% e.e., and 10% at <50% e.e., respectively. Those with DBY are about <1%. c) The reproducibilities in the reduction with RBY and DBY are about 5% and 1%, respectively.

Table 3. Effect of Glucose on the Stereochemistry of the Reduction of Methyl 3-Oxopentanoate with Dry Bakers' Yeast[a)]

DBY/g	Glucose/g	Configuration	e.e./%	Chem. yield/%
2	0	*R* (D)	7	41
2	1	*R*	23	43
4	0	*R*	12	46
4	1	*R*	31	61

a) Substrate, 1 mmol; water, 20 ml.

additive (or additives), then a substrate was added to the reduction system. It should be mentioned that e.e. of methyl 3-hydroxypentanoate obtained from the reduction without an additive is only 12%, which means that the enzyme(s) which affords the D-product (D-enzyme) and that (those) which affords the L-product (L-enzyme) are nearly equally active for this substrate. In contrast, the addition of methyl vinyl ketone or 2-cyclohexen-1-one improves the stereoselectivity up to 60—70% e.e. Allyl alcohol exerts the same effect, but the substitution of one methyl group on any carbon of allyl alcohol decreases the effect sharply as seen in the reductions in the presence of *trans*-2-buten-1-ol, 3-buten-1-ol, and 2-methyl-2-propen-1-ol. Propargyl alcohol is ineffective either. Thus, molecular shape plays a crucial role in exerting the effect on stereochemistry of the reduction. Acrylic acid and its methyl ester are also effective, though the effect is a little bit smaller than those of ketones. It is interesting to note that the specificity decreases in the presence of iodoacetamide, which indicates that this compound shifts the stereoselectivity toward the L-side. The results from the reduction with RBY is also listed in Table 2 for comparison.

In the presence of glucose, the energy-gaining and NAD(P)H-producing glycolysis system of yeast is stimulated. Consequently, the dehydrogenase-levels are distorted in yeast and the change in the stereoselectivity of the reduction can be expected. The results from the addition of glucose to the reduction system are summarized in Table 3. Indeed, it is recognized that the addition of glucose improves both the chemical yield and the stereoselectivity toward the D-side.

In order to elucidate the mechanism for the shift of stereochemistry on addition of an α,β-unsaturated carbonyl compound or its corresponding alcohol and of glucose, the kinetics of the reduction were followed in the presence or absence of these additives. The results plotted in Fig. 1 clearly show that glucose accelerates and methyl vinyl ketone retards the reduction in comparison to that without the additive. Since both glucose and methyl vinyl ketone are the additives that increase the yield of the *R* (D)-product, there is no doubt that the former *activates* the D-enzyme(s) (along the glycolysis cycle), whereas the latter *inhibits* the L-enzyme(s). The improvement of the specificity toward the D-side is not due to

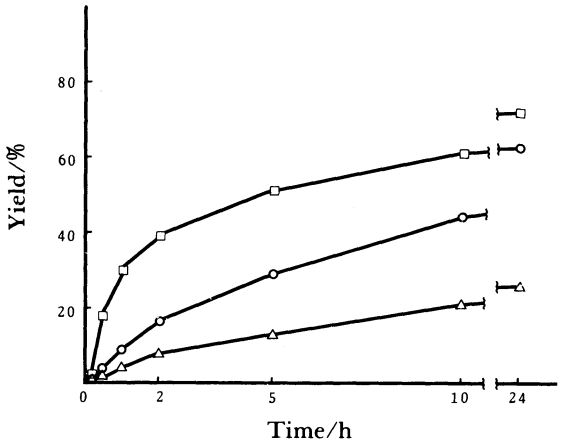


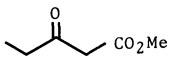
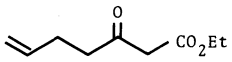
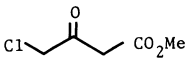
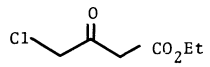
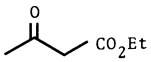
Fig. 1. Plots of the chemical yields of methyl 3-hydroxypentanoate against reaction time for the reduction of methyl 3-oxopentanoate with DBY in the presence of glucose (—□—□—) or methyl vinyl ketone (—△—△—), or absence of an additive (—○—○—).

decomposition of the *S* (L)-product.

We believe that the hydroxy compounds employed as inhibitors are once oxidized in yeast by the dehydrogenase system, than the resulted aldehyde is again trapped by an enzyme(s) in the reducing system. Since all the additives so far exerted the efficient inhibitory effect are good Michael acceptors, it is conceivable that the attack on the double bond in the α,β-unsaturated carbonyl compound by a nucleophile at the vicinity of the reaction center of dehydrogenase takes place faster than the process to reduce the substrate.[16)] It is not clear at present why these compounds do not inhibit the D-enzyme(s). Probably, the D- and L-enzymes locate in different sites in yeast so that the D-enzyme does not find a chance to encounter with these inhibitors. Another alkylating agent, 2-chloroethanol, did not work effectively.

For practical purpose, a composite use of glucose and an enone in the reduction with RBY will afford the most favorable result. This idea has been tested for various β-keto esters and the results are summarized in Table 4. It is obvious that the present method is quite useful in obtaining the D-hydroxy esters. Although the reduction of ethyl acetoacetate did not result in the reversion of configuration, it should be noted that, unlike other β-keto esters, the stereoselectivity of the

Table 4. Stereochemical Results for the Reductions of Various β-Keto Esters with Raw Bakers' Yeast[a]

Substrate	Reaction condition[b]	Configuration	e.e./%[c]	Chem. yield/%[d]
	A	*R* (D)	59	54
	B	*R*	98	10
	B′	*R*	90	70
	C	*R*	98	37
	C′	*R*	96	56
	A	*R* (D)	64	77
	B	*R*	99	8
	B′	*R*	98	61
	C	*R*	97	37
	A	*S* (D)	52	74
	B	*S*	94	22
	C	*S*	78	74
	A	*S* (D)	43	62
	B	*S*	68	20
	C	*S*	85	42
	A	*S* (L)	77	66
	B′	*S*	47	66
	C′	*S*	40	68

a) RBY, 2 g; water, 20 ml; substrate, 1 mmol; glucose, 2 g. b) A, without an enone; B, with 1 mmol of methyl vinyl ketone; B′, with 0.5 mmol of methyl vinyl ketone; C, with 1 mmol of allyl alcohol; C′, with 0.5 mmol of allyl alcohol. c) The reproducibilities are about 1% at >95% e.e., 2% at >80% e.e., and 5% at >50% e.e. d) The reproducibilities are about 5%.

reduction of this substrate without additives is quite high in the L-side. The present method is not effective enough to shift the e.e. more than 40% toward the D-side. More effective device is awaited for a versatile reversion of configuration.

Experimental

Instruments. ^{1}H NMR spectra were recorded on a Varian VXR-200 spectrometer in $CDCl_3$ with Me_4Si as an internal standard. Gas chromatograms were recorded on a YANACO G-2800 gas chromatograph. HPLC was performed on a Hitachi 655 liquid chromatography with Wakosil 5SIL (4.6×150 mm) column and an IRICA 852-III spectrophotometer. Optical rotation was measured with a Perkin-Elmer 241 polarimeter.

Materials. Methyl 3-oxopentanoate, methyl and ethyl 4-chloro-3-oxobutanoates, and (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) were purchased from Aldrich Chemical Co. Ethyl 3-oxo-6-heptenoate was prepared according to the literature procedure.[17] Raw bakers' yeast (pressed bakers' yeast; RBY) and dry bakers' yeast DBY) were purchased from Oriental Yeast Co. and stored in a refrigerator. Other reagents were purchased from Nacalai Tesque Co.

Reduction of β-Keto Ester. In general, an appropriate amount of an additive was added to a suspension composed of 4 g of dry bakers' yeast and 20 ml of water. The whole mixture was stirred for 30 min at 30 °C. Then, 1 mmol of a substrate was added to the mixture and the stirring was continued at the same temperature. After 1 day, Hyflo-super cel and ethyl acetate was added to the suspension and the resulted mixture was filtered. The celite was washed with ethyl acetate and the combined filtrate was extracted with ethyl acetate. The organic portion was washed with water and brine, dried over anhydrous sodium sulfate, concentrated under reduced pressure, and the residue was subjected to preparative gas chromatography (PEG, 1.5 m, 130—170 °C), giving the corresponding hydroxy ester.

Determination of the Optical Purity. To a stirred mixture of 0.03 mmol of a β-hydroxy ester and 1 ml of benzene, were added 1.5 mmol of pyridine and 0.2 mmol of (*R*)−(+)−α-methoxy-α-(trifluoromethyl)phenylacetyl chloride in this order and the mixture was stirred overnight. Ethyl acetate and water were added to the mixture and the layers were separated. The organic portion was washed with 1 M hydrochloric acid (1 M=1 mol dm^{-3}), saturated aqueous sodium carbonate, and brine, then dried over anhydrous sodium sulfate and concentrated to give the corresponding (*R*)-MTPA ester in more than 90% yield.

The enantiomer excess in the hydroxy ester was determined by GLC (OV 1701, 25 m, 200 °C; ethyl 3-hydroxybutanoate and methyl 3-hydroxypentanoate) and HPLC (hexane/ethyl acetate=30/1; methyl and ethyl 4-chloro-3-hydroxybutanoate, and ethyl 3-hydroxy-6-heptenoate) analyses of the corresponding (*R*)-MTPA ester.

Kinetics of the Reduction. To a suspension containing 2 g of DBY in 20 ml of water 1 mmol of methyl 3-oxopentanoate was added and the whole suspension was stirred at room temperature. After appropriate time intervals, 2 ml aliquots were taken out from the suspension and poured into 5 ml of ethyl acetate containing ethyl succinate (an internal standard for GLC). The organic materials were extracted and subjected to GLC (PEG, 1 m, 100 °C). The experiment with glucose (2 g, 11 mmol) or methyl vinyl ketone (70 mg, 1 mmol) was also run in the same way as mentioned above by adding these additives to the suspension of DBY in water 30 min before the addition of

methyl vinyl ketone. The results are plotted in Fig. 1.

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