ELSEVIER

Journal of Molecular Catalysis B: Enzymatic 5 (1998) 129–132

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Highly stereoselective reduction of ketones by *Geotrichum candidum*

Kaoru Nakamura *

Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan

Received 26 September 1997; accepted 19 November 1997

Abstract

Reduction of ketones by a crude alcohol dehydrogenase from *Geotrichum candidum* with a reductant, 2-alkanol, affords the corresponding (*S*)-alcohols of excellent ee in high yield. The substrate specificity of the biocatalyst is very wide: ketones including β-keto esters, aromatic and aliphatic ketones and trifluoromethyl ketones are reduced enantioselectively in $>99\%$ ee in almost all reductions. The stereochemical course observed in the reduction of methyl ketones is different from that of trifluoromethyl ketones in spite of high enantioselectivity of the reaction.

Keywords: Enzyme; Reduction; Microbial; Trifluoromethyl; Enantioselective; Ketone

1. Introduction

Microbial reductions have been used widely for synthesizing chiral alcohols [1–3]. However, usually, a microbe reduces a carbonyl compound with unsatisfactory enantioselectivity since a microbe generally possesses several enzymes which reduce artificial ketones into different configurational alcohols. Several methods have been developed to remove this drawback. For example, stereochemical control of microbial reduction to obtain an optically pure alcohol of the desired configuration has been achieved by the use of organic solvents [4–7], additives [6–8], or inhibitor of unnecessary enzyme(s) [9,10]. On the other hand, if an isolated enzyme is used for a reaction, selectivity is usually high [11–13], although isolated enzymes are not always available or affordable. An alternative way to access an enzymatic system is the use of crude enzymes such as acetone powder [14], microbial dried cell dehydrated by using acetone, the preparation of which is facile in contrast to isolation of enzymes.

2. Reduction of ketones by the acetone powder of *Geotrichum candidum* [15]

We found that the acetone powder from *Geotrichum candidum* IFO4597, (APG4) catalyzes reduction of many kinds of ketones in the presence of reductants giving chiral alcohols with extremely high enantioselectivities. For example, when methyl-3-oxobutanoate was reduced by APG4 in the presence of 2-hexanol

* Corresponding author.

1381-1177/98/$19.00
PII: S1381-1177(98)00017-4

Table 1
Comparison of the acetone powder system with the resting cell system on reduction of methyl-3-oxobutanoate[a]

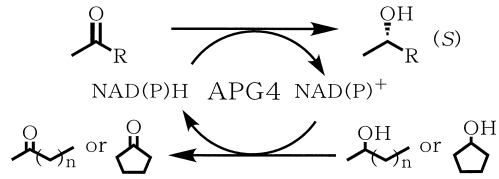
Catalysts	Yield (%)	ee (%) (config.)
Resting cell	97	39(*S*)
APG4	0.9	–
APG4 + NAD^+	1.6	–
APG4 + NAD^+ + pentanol	> 99	> 99(*S*)

[a][Substrate] = 30 mM, [Water] = 3 ml, [Cell] = 0.5 g or [APG4] = 20 mg, [NAD^+] = 6 μmol, [pentanol] = 50 μl.

and a small amount of a coenzyme, NAD^+, methyl-(*S*)-3-hydroxybutanoate of > 99% ee was obtained in > 99% yield, while the reduction with a resting cell system afforded the corresponding (*S*)-alcohol of 39% ee. Comparison of the reduction of methyl-3-oxobutanoate by APG4 with that by the resting cell is shown in Table 1. The optical purity of the product is markedly improved by the use of the acetone powder.

The reaction scheme is shown in Scheme 1. As the substrate, a ketone is reduced to an alcohol, $NAD(P)^+$ is formed, which in turn is reduced to NAD(P)H by the coupled oxidation of 2-alkanol. Racemic 2-alkanol is used for the reaction, but (*S*)-2-alkanol is oxidized selectively to reduce the substrate to (*S*)-alcohol.

Besides the high selectivity of the reduction, there are many advantages in the use of APG4: wide substrate specificity, preservability of the biocatalyst, high ratio of substrate to biomass, redox coupler system by 2-alkanol, flexibility in the choice of coenzyme and reductant, easiness of preparation, experimental reproducibility and freedom from asepsis. First and most important of all, the substrate specificity of the acetone powder system is very wide in spite of the high enantioselectivity of the reduction. As shown in Table 2, the reduction of ketones from α-keto-esters and acetophenone derivatives to aliphatic ketones results in high yield and excellent ee. Next, preservation of the acetone powder for extended periods is possible. Storage of this powder in a freezer preserves the enzyme activity for more than 1 year, whereas the resting



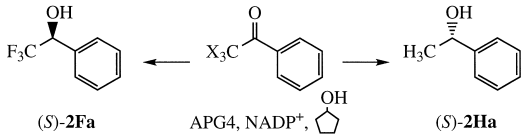
Scheme 1.

cell of the microbe is usually active only for a few days. The use of APG4 also resolves the problem of the excessively high weight of the biocatalyst compared to the substrate, usually required in biocatalysis [16]. The ratio of the resting cell mass/substrate (50/1) is largely improved in the ratio of APG4/substrate (1/1). Although the system requires a coenzyme, only a small amount is sufficient for the reduction, since the redox coupler system can recycle it more than 100 times with the aid of easily available and cheap alcohols such as 2-alkanol. The flexibility in the structures of the reductant and coenzyme is also an advantage. The reductant can be any 2-alkanol from isopropanol to 2-octanol, and both NAD^+ and $NADP^+$ are effective as coenzymes. Other advantages are easiness of the preparation of APG4, which does not require any special equipment or professional technique, and the powder is available within 1 h. Once APG4 is prepared, the catalyst from the same batch can be used for many experiments over a long time, which leads to experimental reproducibility. Since some of the

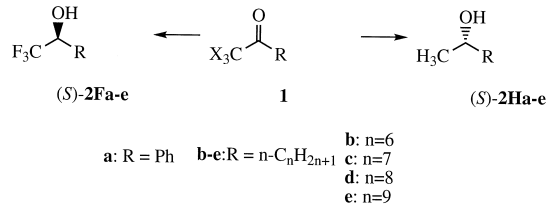
Table 2
Reduction of ketones by the acetone powder system[a]

Substrate	Yield (%)	ee (%) (config.)
Methyl-3-oxobutanoate	> 99	> 99(*S*)
Ethyl-3-oxobutanoate	99	> 99(*S*)
Neopentyl-3-oxobutanoate	> 99	> 99(*S*)
Acetophenone	89	99(*S*)
o-Chloroacetophenone	> 99	> 99(*S*)
m-Chloroacetophenone	95	> 99(*S*)
p-Chloroacetophenone	80	99(*S*)
2-Octanone[b]	87	> 99(*S*)
6-Methyl-5-heptene-2-one	90	99(*S*)

[a][Substrate] = 30 mM, [Water] = 3 ml, [APG4] = 20 mg, [NAD^+] = 6 μmol, [cyclopentanol] = 50 μl.
[b]Isopropanol is used instead of cyclopentanol.



Scheme 2.



Scheme 3.

cell components are destroyed during the preparation of APG4, asepsis is not required.

3. Reduction of trifluoromethyl ketones [17]

When 2,2,2-trifluoroacetophenone (**1Fa**) was subjected to the reduction with the APG4 system, the corresponding alcohol, 2,2,2-trifluoro-1-phenylethanol (**2Fa**), was obtained in 98% ee with $>99\%$ chemical yield. The absolute configuration of the product was determined to be *S* by comparison of its optical rotational value with the literature value [18]. The reduction of acetophenone (**1Ha**), unfluorinated analogue, with the APG4 system affords the corresponding (*S*)-1-phenylethanol ((*S*)-**2Ha**) in excellent ee. Surprisingly, different configurational alcohols were obtained by subjecting the trifluorinated ketone and the corresponding hydrogen analogue to the same reduction system as shown in Scheme 2. (The absolute configuration of (*S*)-**2Fa** and (*S*)-**2Ha** are opposite according to definition.)

The system for reduction of methyl ketones requires a catalytic amount of a coenzyme which can be recycled by adding excess amount of 2-propanol or cyclopentanol (reducing agent) to the system. Similarly, the reduction of **1Fa** also requires a catalytic amount of a coenzyme and a reducing agent. Combination of $NADP^+$ and cyclopentanol as a coenzyme and a reducing agent, respectively, affords the best result, although the enzyme can also use NAD^+ and 2-propanol as a coenzyme and a reducing agent, respectively.

As shown in Table 3, the reduction of other 1,1,1-trifluoro-2-alkanones also gave excellent results. As expected from the difference in the stereochemical course between the reductions of **1Fa** and **1Ha**, the stereochemical course of the reduction of fluorinated compounds is opposite to that of the unfluorinated compounds. For example, the reduction of 1,1,1-trifluoro-2-decanone (**1Fd**) yields (*S*)-1,1,1-trifluoro-2-decanol (**2Fd**) in $>99\%$ ee, whereas the reduction of 2-decanone (**1Hd**), the corresponding unfluorinated analogue, yields (*S*)-2-decanol (**2Hd**) also in $>99\%$ ee (Scheme 3).

We found that the reduction of trifluoromethyl ketones takes place through a completely different stereochemical course from that of the corresponding unfluorinated analogues is indeed noteworthy, but the mechanism of this intriguing stereochemical consequence is not clear at present. There are three plausible explanations for this phenomenon. Firstly, the bulki-

Table 3
Reduction of trifluoromethyl ketones and methyl ketones[a]

Trifluoro substrate	Yield (%)	ee (%) (config.)	Unfluorinated substrate	Yield (%)	ee (%) (config.)
1Fa	> 99	98(*S*)	**1Ha**	89	> 99(*S*)
1Fb	> 99	96(*S*)	**1Hb**	97	98(*S*)
1Fc	> 99	96(*S*)	**1Hc**	88	> 99(*S*)
1Fd	> 99	> 99(*S*)	**1Hd**	87	> 99(*S*)
1Fe	> 99	98(*S*)	**1He**	84	> 99(*S*)

[a] $NADP^+$ and cyclopentanol were used as a coenzyme and a reductant, respectively.

ness of the trifluoromethyl group affects the stereochemical course of the reduction. The trifluoromethyl group may be recognized by the enzyme as a bulky substituent, since the effective radius [19] of a trifluoromethyl moiety (2.2 Å) is similar to that of an isopropyl moiety (2.2 Å), which is larger than a phenyl moiety (1.62 Å). Secondly, the electronic property of the trifluoromethyl moiety may change the stereochemistry of the reduction. Thirdly, there may exist several dehydrogenases participating in the reduction, and the substrates of different properties (trifluoromethyl ketones and methyl ketones) may use different enzymes.

4. Conclusion

In conclusion, we present here highly stereoselective reduction of ketones by a crude alcohol dehydrogenase(s) from *G. candidum*. Since the experimental operation is facile, and the enzyme(s) can be stored for more than 2 years, we believe that the present method is very useful for asymmetric reduction of trifluoromethyl ketones. Isolation of the dehydrogenase(s), investigation of steric and electric effects of the fluorinated compounds in the enzymatic reactions and application of the system to a wide variety of substrates are in progress.

References

[1] S. Servi, Synthesis (1990) 1.
[2] R. Csuk, B.I. Glänzer, Chem. Rev. 91 (1991) 49.
[3] K. Mori, Tetrahedron 45 (1989) 3233.
[4] K. Nakamura, K. Inoue, K. Ushio, S. Oka, A. Ohno, J. Org. Chem. 53 (1988) 2589.
[5] K. Nakamura, S. Kondo, N. Nakajima, A. Ohno, Tetrahedron 51 (1995) 687.
[6] K. Nakamura, Y. Inoue, A. Ohno, Tetrahedron Lett. 36 (1995) 265.
[7] K. Nakamura, S. Takano, K. Terada, A. Ohno, Chem. Lett. (1992) 951.
[8] K. Nakamura, S. Kondo, A. Ohno, Bioorg. Med. Chem. 2 (1994) 433.
[9] K. Nakamura, Stereochemical control in microbial reduction, in: S. Servi (Ed.), Microbial Reagents in Organic Synthesis, Kluwer Academic Publishers, Forderecht, 1992, 389.
[10] K. Nakamura, Y. Kawai, N. Nakajima, A. Ohno, J. Org. Chem. 56 (1991) 4778.
[11] D.R. Dodds, J.B. Jones, J. Chem. Soc. Chem. Commun. (1982) 1080.
[12] C.-H. Wong, D.G. Druckhammer, H.M. Sweers, J. Am. Chem. Soc. 107 (1985) 4028.
[13] K. Nakamura, T. Yoneda, T. Miyai, K. Ushio, S. Oka, A. Ohno, Tetrahedron Lett. 29 (1988) 2453.
[14] C.T. Goodhue, J.P. Rosazza, G.P. Peruzzotti, in: A.L. Demain, N.A. Solomon (Eds.), Manual of Industrial Microbiology and Biotechnology, American Society for Microbiology, Washington, DC, 1986, pp. 97–121.
[15] K. Nakamura, K. Kitano, T. Matsuda, A. Ohno, Tetrahedron Lett. 37 (1996) 1629.
[16] T. Sugai, O. Katoh, H. Ohta, Tetrahedron 51 (1995) 11987.
[17] K. Nakamura, T. Matsuda, T. Itoh, A. Ohno, Tetrahedron Lett. 37 (1996) 5727.
[18] G. Holzwarth, E.C. Hsu, H.S. Mosher, T.R. Faulkner, A. Moscowitz, J. Am. Chem. Soc. 96 (1974) 251.
[19] G. Bott, L. Field, S. Sternhell, J. Am. Chem. Soc. 102 (1980) 5618.