ChemComm

Alcohol dehydrogenase is active in supercritical carbon dioxide

Tomoko Matsuda,*[a] Tadao Harada[a] and Kaoru Nakamura[b]

[a] *Department of Materials Chemistry, Faculty of Science and Technology, Ryukoku University, Otsu, Shiga 520-2194, Japan*
[b] *Institute for Chemical Research, Kyoto University, Uji, Kyoto 611-0011, Japan*

Received (in Cambridge, UK) 22nd May 2000, Accepted 14th June 2000
Published on the Web 5th July 2000

Alcohol dehydrogenase from *Geotrichum candidum* was found to be active in supercritical carbon dioxide at 10 MPa; high activities and excellent enantioselectivities were observed for the asymmetric reduction of aromatic and cyclic ketones.

Supercritical fluids have been used as solvents for organic synthesis as well as for extraction and chromatography by taking advantage of both their gas-like low viscosities and high diffusivities and their liquid-like solubilizing power.[1] Moreover, the tunability of these properties is unique to supercritical fluids; small changes in pressure or temperature lead to significant changes in density-dependent solvent properties such as the relative permittivity, the solubility parameter, and the partition coefficient.[1] Among many fluids, supercritical carbon dioxide ($scCO_2$) has the added benefits of an environmentally benign nature, nonflammability, low toxicity, high availability, and ambient critical temperature (T_c = 31.0 °C) that make it suitable for biotransformations.[1] The attraction of combining natural catalysts with a natural solvent has been the driving force behind a growing body of literature[2] on the stability, activity and specificity of enzymes in $scCO_2$.[1] Since the first report on biotransformations in supercritical fluids in 1985 by Randolph *et al.*,[3] Hammond *et al.*[4] and Nakamura *et al.*,[5] the benefits of using supercritical fluids for biotransformations have been demonstrated by Mori *et al.*[6] and Kamat *et al.*:[7] *e.g.* improved reaction rates, control of selectivities by pressure, *etc.*

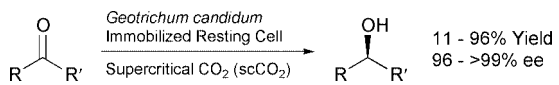
However, most of the biocatalysts used in supercritical fluids are hydrolytic enzymes[1–7] such as lipases and proteases with the exception of cholesterol oxidase,[8] and no report on the use of alcohol dehydrogenases in supercritical fluids has yet been published, despite the fact that they are an important class of enzymes for the asymmetric reduction of ketones to produce chiral alcohols. There is growing demand for such alcohols as the chiral synthones for natural products, pharmaceuticals, agrochemicals, and ferroelectric liquid crystals. Here we report the first achievement of a highly enantioselective reduction of ketones by alcohol dehydrogenase in $scCO_2$ (Scheme 1).

The resting cells of a fungus, *Geotrichum candidum*[9] IFO 5767, were employed for the reduction of ketones owing to its high reactivity with unnatural substrates and the simplicity of its growth. The whole-resting cell instead of an isolated enzyme was used for the reduction, and thus the addition of expensive coenzyme was not necessary and the solubilities of the coenzymes in $scCO_2$ did not need to be considered. The cell was immobilized on a water-absorbing polymer[9c] to spread it on the large surface of the polymer. At first, the reduction of *o*-fluoroacetophenone in $scCO_2$ at 10 MPa was conducted, which resulted in (*S*)-1-(*o*-fluorophenyl)ethanol at 81% after 12 h. A control experiment to prove that the reduction did not proceed before the supercritical condition was also conducted.[10] The time course of the reaction (Fig. 1) shows that the yield increased with the reaction time, which proved that the alcohol dehydrogenase catalyzed the reduction even in the supercritical condition.

The substrate specificity was investigated, and as listed in Table 1, the biocatalytic reduction in $scCO_2$ proceeded for various ketones. Acetophenone, acetophenone derivatives, benzyl acetone and cyclohexanone were used as substrates, and it was found that all of them were reduced by the alcohol dehydrogenase in $scCO_2$. The effects of fluorine substitution at the *ortho*, *para* and α positions of acetophenone were obvious. Compared with the unsubstituted analogue, substitution at the *ortho* or α position increased the yield, whereas substitution at the *para* position decreased the yield. Systematic study of the relationship between the effect of the fluorine substituent and the effect of properties of $scCO_2$ as well as isolation of the enzyme responsible for the reduction, tasks which are in progress in our laboratory, would give a better understanding of the fluorine recognitions by the protein.

The finding that the alcohol dehydrogenase is active in $scCO_2$ is significant, but not sufficient for practical use; high enantioselectivity of the reduction is also necessary for synthetic purposes. In our case, very high enantioselectivities (> 99% ee) were obtained for the reduction with the majority of the substrates tested, while slightly lower enantioselectivities (96, 97% ee) were observed for a few of them. The enantioselectivities obtained in this system are superior to or at least equal to those for most other biocatalytic and chemical systems.[11]

In a typical experiment, *G. candidum* IFO 5767 was grown as described previously.[9d] The freshly prepared cell (0.25 g wet wt) was suspended in H_2O (0.75 mL) and propan-2-ol (0.050 mL), and immobilized on water absorbing polymer (BL-100®, 0.125 g) as described previously.[9c] In the experiment, we used a stainless steel pressure-resistant vessel (Taiatsu Techno, Co., Osaka, TVS-N2 type, 10 mL) equipped with a stop valve (Whitey Co. SS3NBS4G), manometer (Taiatsu Techno, Co., Osaka, 15 MPa), and HPLC pump (Jasco PU-1580 pump) connected to a cooler (−5 °C) and CO_2 gas cylinder. In this vessel, the immobilized cell, a magnetic stirrer, and a ketone (0.017 mmol, placed in a glass tube to prevent it from contacting the biocatalyst before achieving the supercritical conditions) were charged. Then the vessel was warmed to 35 °C, and CO_2 preheated to 35 °C was introduced until a pressure of 10 MPa was reached. The mixture was stirred at 35 °C for 12 h, and the CO_2 was liquefied at −10 °C and then the gas pressure was



Scheme 1

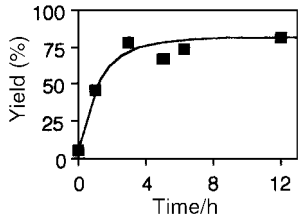
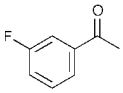
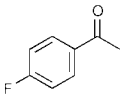
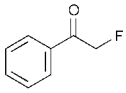
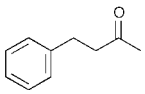


Fig. 1 Time course of reduction of *o*-fluoroacetophenone in $scCO_2$ at 10 MPa.

DOI: 10.1039/b004069j

Table 1 Reduction of various ketones by immobilized cell in $scCO_2$

Substrate	Yield (%)	Ee (%)	Config.
	51	> 99	*S*
	81	> 99	*S*
	53	> 99	*S*
	11	97	*S*
	96	96	*R*
	22	> 99	*S*
	61	> 98	*S*
	96	—	—

Conditions are described in text.

released. The resulting residue was dissolved in ether, and the mixture was put on Extrelut and quickly eluted with ether. Chemical yield and ee were measured using a chiral GC-column (Chirasil-DEX CB; 25 m; He 2 mL min^{-1}). The absolute configurations were determined by comparing the GC retention times with those of authentic samples.

In conclusion, the alcohol dehydrogenase from *G. candidum* was found to be active in $scCO_2$ at 35 °C and 10 MPa and to catalyze asymmetric reduction of various ketones with excellent enantioselectivities. For practical use, there has so far been some hesitation to use alcohol dehydrogenases in spite of their high enantioselectivities because of the difficulties in extracting the product from aqueous solvents. We believe that this report opens up new possibilities for asymmetric reduction by an enzyme with a natural and easily-removable solvent.

The authors greatly appreciate the advice and technical support in assembling the $scCO_2$ reactor given by Professor Okamoto at Kyoto Institute of Technology, Professor Okahata and Dr Mori at Tokyo Institute of Technology, Dr Ozaki at Osaka University and Professor Hori and Dr Tabata at Fukui University. This work was supported by a Grant-in-Aid for Encouragement of Young Scientists (No. 12740400) and a Grant-in-Aid for Scientific Research (C) (No. 11640602) from the Ministry of Education, Science, Sports and Cultre of Japan. The authors are grateful to Osaka Yuki Kagaku Kogyo Co., Ltd. for providing BL-100® (water-absorbing polymer).

Notes and references

1 A. J. Mesiano, E. J. Beckman and A. J. Russell, *Chem. Rev.*, 1999, **99**, 623; Y. Ikushima, *Adv. Colloid Interface Sci.*, 1997, **71–72**, 259; E. Cernia and C. Palocci, in *Methods in Enzymology*, ed. J. N. Abelson and M. I. Simon, Academic Press, San Diego, 1997 , vol. 286, p. 495; S. V. Kamat, E. J. Beckman and A. J. Russell, *Crit. Rev. Biotechnol.*, 1995, **15**, 41; O. Aaltonen and M. Rantakylä, *CHEMTECH*, 1991, 240; P. G. Jessop, T. Ikariya and R. Noyori, *Chem. Rev.*, 1999, **99**, 475; A. Baiker, *Chem. Rev.*, 1999, **99**, 453; J. F. Brennecke and J. E. Chateauneuf, *Chem. Rev.*, 1999, **99**, 433; T. Moriyoshi, T. Kita and Y. Uosaki, *Ber. Bunsenges. Phys. Chem.*, 1993, **97**, 589.

2 S.-H. Yoon, H. Nakaya, O. Ito, O. Miyawaki, K.-H. Park and K. Nakamura, *Biosci. Biotechnol. Biochem.*, 1998, **62**, 170; S. H. Yoon, O. Miyawaki, K.-H. Park and K. Nakamura, *J. Ferment. Bioeng.*, 1996, **82**, 334; Y. Ikushima, N. Saito, M. Arai and H. W. Blanch, *J. Phys. Chem.*, 1995, **99**, 8941; J. C. Erickson, P. Schyns and C. L. Cooney, *AIChE J.*, 1990, **36**, 299; N. Fontes, M. C. Almeida, C. Peres, S. Garcia, J. Grave, M. R. Aires-Barros, C. M. Soares, J. M. S. Cabral, C. D. Maycock and S. Barreiros, *Ind. Eng. Chem. Res.*, 1998, **37**, 3189; N. Fontes, E. Nogueiro, A. M. Elvas, T. C. d. Sampaio and S. Barreiros, *Biochim. Biophys. Acta*, 1998, **1383**, 165.

3 T. W. Randolph, H. W. Blanch, J. M. Prausnitz and C. R. Wilke, *Biotechnol. Lett.*, 1985, **7**, 325.

4 D. A. Hammond, M. Karel, A. M. Klibanov and V. J. Krukonis, *Appl. Biochem. Biotechnol.*, 1985, **11**, 393.

5 K. Nakamura, Y. M. Chi, Y. Yamada and T. Yano, *Chem. Eng. Commun.*, 1986, **45**, 207.

6 T. Mori and Y. Okahata, *Chem. Commun.*, 1998, 2215; T. Mori, A. Kobayashi and Y. Okahata, *Chem. Lett.*, 1998, 921.

7 S. Kamat, J. Barrera, E. J. Beckman and A. J. Russell, *Biotechnol. Bioeng.*, 1992, **40**, 158; S. V. Kamat, B. Iwaskewycz, E. J. Beckman and A. J. Russell, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 2940; S. V. Kamat, E. J. Beckman and A. J. Russell, *J. Am. Chem. Soc.*, 1993, **115**, 8845; A. K. Chaudhary, S. V. Kamat, E. J. Beckman, D. Nurok, R. M. Kleyle, P. Hajdu and A. J. Russell, *J. Am. Chem. Soc.*, 1996, **118**, 12 891.

8 T. W. Randolph, D. S. Clark, H. W. Blanch and J. M. Prausnitz, *Science*, 1988, **239**, 387; T. W. Randolph, H. W. Blanch and J. M. Prausnitz, *AIChE J.*, 1988, **34**, 1354.

9 (*a*) R. Azerad and D. Buisson, in *Microbial Reagents in Organic Synthesis, NATO ASI Series C*, ed. S. Servi, Kluwer Academic Publishers, Dordrecht, 1992, p. 421; (*b*) T. Matsuda, T. Harada, N. Nakajima, T. Itoh and K. Nakamura, *J. Org. Chem.*, 2000, **65**, 157; (*c*) K. Nakamura, Y. Inoue, T. Matsuda and I. Misawa, *J. Chem. Soc., Perkin Trans. 1*, 1999, 2397; (*d*) K. Nakamura and T. Matsuda, *J. Org. Chem.*, 1998, **63**, 8957.

10 The reaction was stopped as soon as the pressure reached 10 MPa by cooling at −10 °C, then a negligible amount of the reaction proceeded (yield 5%), which was probably due to the reaction during the time between the critical pressure and 10 MPa.

11 S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 2000, 611; S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1999, 1; S. M. Roberts, *J. Chem. Soc., Perkin Trans. 1*, 1998, 157; T. Benincori, E. Cesarotti, O. Piccolo and F. Sannicolo, *J. Org. Chem.*, 2000, **65**, 2043; Y.-J. Cherng, J.-M. Fang and T.-J. Lu, *J. Org. Chem.*, 1999, **64**, 3207; T. Ohkuma, M. Koizumi, H. Doucet, T. Pham, M. Kozawa, K. Murata, E. Katayama, T. Yokozawa, T. Ikariya and R. Noyori, *J. Am. Chem. Soc.*, 1998, **120**, 13 529.