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Biocatalytic reduction of aromatic ketones with the aid of electrochemical regeneration of the coenzyme

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ABSTRACT

Halogenated aromatic ketones were reduced electrochemically using dried cell of *Geotrichum candidum* as a catalyst with high enantioselectivity. Enzymes in the dried cell catalyzed both reactions to reduce substrates and to recycle the coenzyme using electric power, so any additional enzyme or co-substrate for coenzyme recycling was not necessary.

KEYWORDS

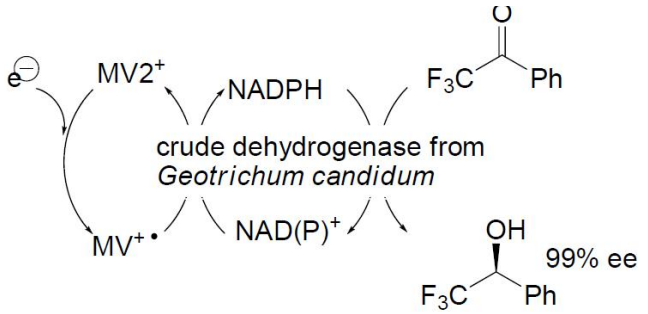
Asymmetric reduction;
Electrochemical;
Geotrichum candidum;
Acetophenone derivatives.

INTRODUCTION

Asymmetric reduction of carbonyl group is one of the most important, fundamental and practical reactions for producing chiral alcohols, which can be transformed into various functionalities to synthesize industrially important chemicals such as pharmaceuticals, agrochemicals and natural products. For catalysts, both chemical and biological catalysts have been used and biocatalysts have the advantages for being natural, having high chemo-, regio- and enantioselectivity, and being active under mild reaction conditions. However, biocatalytic reductions have a drawback that the reduction needs the reduced form of the coenzyme (NAD(P)H) which is expensive and is difficult to use it and throw it away. Since it is only the oxidation state of the cofactor that changes during the reaction, it may be regenerated *in situ* using a second redox-reaction to allow it to re-enter the reaction cycle. Usually, formate, glucose, and simple alcohols such as ethanol and 2-propanol are used to reduce the oxidized form of the coenzyme to the reduced form[1]. Recently, another methodology, the light-mediated recycling of the oxidized form of the coenzyme has been developed[2]. The third methodology for regeneration of the coenzyme is the use of electronic power and several methods of this category have been reported[3]. The numerous bioelectrocatalytic biosensors, bioreactors, and biofuel cells have been studied but a few streoselective biocatalytic reactions performed on a preparative scale have been reported. Therefore, we focused on the streoselective biocatalytic synthesis using electrochemical mediator method.

We present here asymmetric reduction of halogenated aromatic ketones catalyzed by a crude dehydrogenase from *Geotrichum candidum*, a fungi[4], with the aid of electric power.

The electrochemical recycle of the oxidized form of the coenzyme needs a mediator such as



Scheme 1 : Electrochemical aymmetric reduction of trifluoroacetophenone

methylviologen (MV^{2+}) and enzymes such as diaphorase to transfer electron from cathode to $NAD(P)^+$.

We have found that the crude dehydrogenase from *Geotrichum candidum*[4] had diaphorase activities, then the biocatalyst can catalyze both reductions of $NAD(P)^+$ to NAD(P)H and ketones to the corresponding alcohols as shown in scheme 1. Trifluoroacetophenone was reduced with excellent ee.

MATERIAL AND METHODS

Materials

NAD^+ and $NADP^+$ were obtained from Kojin Co. Crude alcohol dehydrogenase from *Geotricum candidum* IFO 4597 (APG4) was obtained according to the reference[4a]. Methyl viologen, trifluoroacetophenone, *o*-chloroacetophenone and other chemicals were purchased from Aldrich.

Electolysis

A gold plate (area, 2.0 cm^2) was dipped into mercury and a gold-amalgam (Au /Hg) surface was formed. The Au/ Hg electrode used as the working electrode gave negligible background currents at negative potentials, but the gold surface was exposed after repeated measurements and higher background currents due to the reduction of surface gold oxides resulted. At this stage, the used electrode was discarded and a new one was prepared for further experiments.

Electrochemical experiments were performed in an H-type cell separated by agar (2 % KCl) and a gas bubbling tube were fitted through the cap and the reaction was done under argon atmosphere(argon gas was bubbled before the reaction). Au amalgam electrode and Ag/AgCl in a saturated KCl solution served as a working and a reference electrodes, respectively. Pt counter electrode was immersed in another compartment in the case of electrolysis. Phosphate buffer (20 mM, pH 7.0) was used as the supporting electorolyte solution. Cyclic voltammograms were measured by three electrode system with Polarograph Model 311 and HECS 311B (Fuso Seisakusho Co,).

General procedure for reduction

A mixture of ketone (1.0-2.8 mmol), APG4(20 mg), NAD^+ (1.6 mmol) and methyl viologen(1.7 mmol) in phosphate buffer (pH 7.0, 0.1M) was reacted under electrolysis (-0.72V *vs* SCE) under argon atmosphere. Argon gas was bubbled before electrolysis and bubbling was stopped during electrolysis to avoid vaporization of the substrate and the product. After 1 day, the mixture was extracted with ether containing naphthalene as GC internal standard and the resulted ether solution was subjected to GC. GC-analysis was performed using a Shimazu GC-17A instrument with a FID detector equipped with a chiral GC-column: trifluoroacetophenone, Chirasil-DEX CB; 25 m; He 2 mL/ min; 120 °C; ketone: 2.2 min, (*S*)-alcohol 9.4 min, (*R*)-alcohol 10.0 min; *o*-chloroacetophenone; CP cycloxextrin130 °C; ketone; 2.8 min, (*R*)-alcohol 9.1 min, (*S*)-alcohol; 9.7 min.

RESULTS AND DISCUSSION

From cyclic voltammogram, the cathodic peak potential of methyl viologen was observed at -0.72 V *vs* SCE and the electrochemically catalytic current was observed in the presence of APG4 as the biocatlyst (both diaphorase and dehydrogenase) and MV^{2+} as the mediator, and NAD^+ as the coenzyme. When trifluoroacetophenone (TFA) was added to the system and the electrolysis was done for 24h, the corresponding (*S*)-alcohol was obtained as shown in TABLE 1.

Usually, dipaphorase (DP) and methyl viologen were used for the reduction of NAD^+ to NADH in electrolysis. Oxidized form of methyl viologen, MV^{2+} receives one electron from the cathode and two mol of the resultant reduced form of methyl viologen reduces one mol of NAD^+ with the aid of a diaphorase. Then the reduction requires two enzymes, a diaphorase and a dehydrogenase, the latter catalyzes reduction of a substrate ketone to the product. However, the present re-

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TABLE 1 : Enzymatic electrochemical reduction of TFA[a,b)]

Entry	MV2+(mM)	NAD(mM)	Glucose(mM)	Yield(%)	ee.(%)	Config.
1	0	1.6	56	4		
2	1.6	1.6	0	34	>99	*S*
3	1.6	0	56	0		
4	1.6	1.6	56	72	>99	*S*
5[C)]	1.6	1.6	56	22	>99	*S*
6	1.6	1.6	56	4		

a) APG4 (20 mg) was used, b) The electrolysis was performed at -0.7V vs SCE for 17 h and maximum 0.1 mA of electrolysis current was observed during the electrolysis, c) Diaphorase (10 mg) was added to the reaction mixture.

duction system does not need any diaphorase from outside since the crude alcohol dehydrogenase has considerable diaphorase activities. The reduction without DP could proceed and the product was obtained in 34% yiled (entry 2). On the contrary, the addition of DP to the mixture decreased the yield (entry 5: 22 % yield in the presence of DP). Thus, excess amounts of DP may accelerate oxidation of NADH and inhibit the reduction of the ketone. Although glucose was added to the reaction mixture, glucose was not used for reduction of NAD^+ but to remove active oxygen[5], which was produced by the reaction of reduced form of methyl viologen, $MV^{+\cdot}$ with oxygen (although the reaction was conducted under argon atmosphere, it is difficult to remove oxygen completely). Active oxygen is possible to react with the reduced form of the coenzyme. Then, complete removal of oxygen is required to proceed the reduction of the substrate. As shown in entry 1, an addition of glucose could not affect to the reduction of the ketone unless electrolysis was performed. However, the reduction proceeded under electrolysis in the presence of glucose gave the (*S*)-alcohol in a good yield. Other sugar such as galactose and inositol can also be used as additives. However, chemical yields of the (*S*)-alcohol were low (galactose = 44%, inositol = 23%) compare to that of glucose and in the case of galactose as an additive, the enantioselectivity decreased to 95% ee. The low ee observed in the reaction with galactose stems from the existence of a minor dehydrogenase that reduce the ketone to the (*R*)-alsohol because the reaction in the presence of galactose and without electrolysis afforded the (*S*)-alcohol of 48% ee with 7% chemical yield. Galactose may act as a reductive agent to reduce the substrate to the (*S*)-alcohol with low ee. Anyhow, the addition of glucose in electrochemical reduction is a convenient and practical method because it does not required nervous complete removal of oxygen, which is a drawback in the reduction reaction compare to other electrochemical reactions such as oxidations.

Thus, trifluoroacetophenone was reduced in 72% chemical yield with >99% ee. The current efficiency of the reaction is calculated to be 28.1%.

The other substrate, o-chloroacetophenone was reduced with the same method resulted in affording 13% with 46% ee. This ketone was also reduced electochemically, but with a low yield and a low enantioselectivity. Since the reaction of o-chloroacetophenone with the fungus, *Geotricum candidum* IFO 4597, afforded excellent ee[4], we can not explain undesirable result in the present time. It is possible that the another enzyme that usually does not react with the ketone will participate to the reduction. Further study is necessary to apply the present reduction system to other ketones.

CONCLUSION

Trifluoroacetophenone was reduced bio-electrochemically in excellent ee. The merit of the present system is the use of crude enzyme which has diaphorase activities in addition to the dehydrogenase which reduces trifluoroacetophenone. Thus the reduction proceeded without additional enzyme or co-substrate for the coenzyme recycling. The second merit is the activity of the present system. Usually, the electrolytic reduction using methyl viologen requires long reaction times such as 1 week [3g], however the ketone was reduced within 1 day.

REFERENCES

[1] **(a)** K.Nakamura, R.Yamanaka, T.Matsuda, T.Harada; Tetrahedron: Asymm, **14**, 2659 **(2003)**; **(b)** W.Van Der Donk, H.Zhao; Cur.Opin.Biotech., **14**, 421 **(2003)**; **(c)** F.Hollmann; A Schmidt, Biocat.Biotrans., **22**, 63 **(2004)**.

[2] **(a)** K.Nakamura, R.Yamanaka, K.Tohi, H.Hamada, H.Tetrahedron Lett., **41**, 6799 **(2000)**; **(b)** K.Nakamura, R.Yamanaka, Chem.Commun. 1782 **(2002)**; **(c)** K.Nakamura, R.Yamanaka, Tetrahedron: Asymm. **13**, 2529 **(2002)**.

[3] **(a)** S.Suye, Y.Aramoto, M.Nakamura, I.Tabata, M.Sakakibara; Enz.Microbe.Technol., **30**, 139 **(2002)**; **(b)** H.Li, R. Mustacchi, C.J.Knowles, W.Skibar, G.Sunderland, I.Dalrymple, S.A.Jackman, Tetrehedron, **60**, 655 **(2004)**; **(c)** K.Delecouls-Servat, R.Basseguy, A.Bergel; Chem.Eng.Science, **57**, 4633 **(2002)**; **(d)** R.Yuan, S.Watanabe, S.Kuwatata, H.Yoneyama, J.Org.Chem., **62**, 2494 **(1997)**; **(e)** F.Hollmann, B.Witholt, A Shimd, J.Mol.Cat.B: Enzymatic **19(20)**, 167 **(2003)**; **(f)** K.Vuorilehto, S.Lutz, C.Wandrey; Bioelectrochem., **65**, 1 **(2004)**; **(g)** R.Dicosimor, C.H.Wong, S.L.Dainel, G.M.Whitesides, J.Org.Chem., **46**, 4622 **(1981)**.

[4] **(a)** K.Nakamura, T.Matsuda; J.Org Chem., **63**, 8957 **(1998)**; **(b)** T.Matsuda, T.Harada, N.Nakajima, T.Itoh, K.Nakamura; J.Org.Chem., **65**, 157 **(2000)**.

[5] B.Bektasoglu, S.E.Celik, M.Ozyurek, K.Guclu; R.Apak.Biochem.Biophys.Res.Commun., **345**, 1194 **(2006)**.