JOURNAL OF THE CHEMICAL SOCIETY

Chemical Communications

Number 24
1992

Enhanced Reactivity and Enantioselectivity in Lipase-catalysed Hydrolysis of 12-Crown-4 Ester *via* Crown Ether–Metal Complexation

Hiroshi Tsukube,*[a] Akio Betchaku,[b] Yuji Hiyama[b] and Toshiyuki Itoh*[b]

[a] *Department of Chemistry, College of Liberal Arts & Science, Okayama University, Okayama 700, Japan*
[b] *Department of Chemistry, Faculty of Education, Okayama University, Okayama 700, Japan*

Lipase from *Pseudomonas* was first demonstrated to catalyse hydrolysis of a crown ether ester derivative; reaction rate and enantioselectivity are significantly enhanced by crown ether–metal complexation.

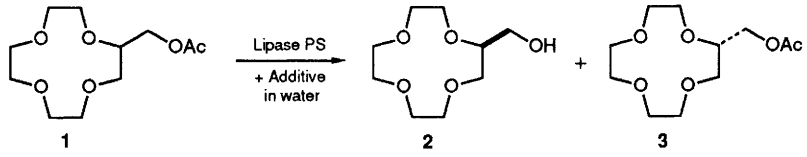
Lipases have recently been recognized as useful catalysts for asymmetric reactions.[1] Since lipase-catalysed reactions are often slow and partially stereoselective, several approaches have been used to improve their reaction performances. Optimization of reactions, modification of substrates, use of nonaqueous media and enantioselective inhibition have been investigated extensively.[2] We now report that lipase is an effective catalyst for resolution of racemic crown ether compounds. The rate and enantioselectivity in lipase-catalysed hydrolysis of 12-crown-4 ester were interestingly enhanced *via* crown ether–metal complexation. Although crown ethers have been reported to be complexing agents for several proteins,[3] this is the first example of a crown ether-type substrate for an enzymatic reaction. A combination of biological enzyme and synthetic crown ether offers new promising possibilities both in the synthesis of chiral host molecules and in the improvement of lipase-catalysed asymmetric reactions.

Enantioselective hydrolysis of racemic 12-crown-4 ester **1** was catalysed by lipase from *Pseudomonas*, though esterase from porcine liver and lipase from *Aspergillus* were not effective for chiral discrimination. Typically, racemic ester **1** (50 mg) was suspended in an aqueous solution (7 ml) and crude lipase powder (Amano PS, 25 mg) was added. The suspension was stirred at 35 °C. The extent of conversion was analysed by thin-layer chromatography (hexane–EtOAc–MeOH = 1 : 8 : 1). When the conversion was near 35–50%, the reaction was terminated by adding $CHCl_3$. The product alcohol **2** and remaining ester **3** were extracted with $CHCl_3$ and isolated by flash chromatography (silica gel, hexane–EtOAc = 1 : 6). The enantiomeric excess (e.e. %) of alcohol **2** was determined by ^{19}F NMR analysis of the cor-

Table 2 Lipase-catalysed hydrolysis of chiral 12-crown-4 ester

Entry	Ester	Additive	*t*/h	Conv. (%)
1	(*S*)-**1**	None	23	11
2	(*R*)-**1**	None	23	51
3	(*S*)-**1**	NaCl (0.1 mol dm^{-3})	7	22
4	(*R*)-**1**	NaCl (0.1 mol dm^{-3})	7	61
5	(*S*)-**1**	Na^+ buffer (0.1 mol dm^{-3})	7	19
6	(*R*)-**1**	Na^+ buffer (0.1 mol dm^{-3})	7	47

Table 1 Lipase-catalysed hydrolysis of racemic 12-crown-4 ester **1**



1 **2** **3**

Entry	Additive	*t*/h	Rate[a]	e.e. (%) of **2** (conv, %)	*E*[b]
1	None	7	1.0	44(20)	3
2	LiCl (0.1 mol dm^{-3})	28	0.6	53(47)	3
3	NaCl (0.1 mol dm^{-3})	6	2.0	73(35)	10
4	KCl (0.1 mol dm^{-3})	15	1.5	32(65)	3
5	Tris buffer (0.1 mol dm^{-3}, pH 7.2)[c]	10	1.7	36(48)	3
6	Na^+ buffer (0.1 mol dm^{-3}, pH 7.2)[d]	8	2.0	42(46)	4
7	K^+ buffer (0.1 mol dm^{-3}, pH 7.2)[e]	8	2.1	42(49)	4

[a] Relative rate based on conversion h^{-1}. [b] $E = \ln[1 - c\{1 + e.e.(P)\}]/[1 - c\{1 - e.e.(P)\}]$; e.e.(P) = e.e. % of the product alcohol **2**. [c] 0.1 mol dm^{-3} Tris(hydroxymethyl)aminomethane–0.1 mol dm^{-3} HCl. [d] 0.1 mol dm^{-3} NaH_2PO_4–0.1 mol dm^{-3} NaOH. [e] 0.1 mol dm^{-3} KH_2PO_4–0.1 mol dm^{-3} KOH.

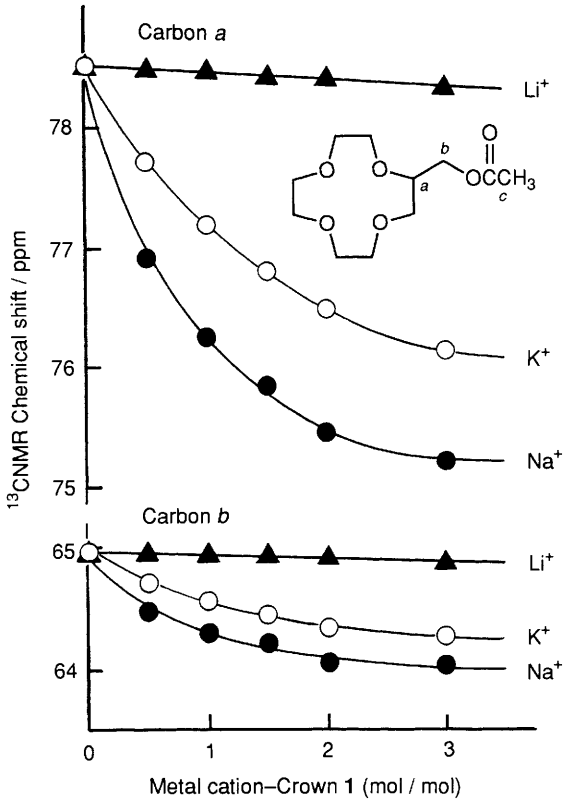


Fig. 1 Metal-induced changes in ^{13}C NMR chemical shifts of 12-crown-4 ester **1**. *Reagents*: crown, 0.025 mmol; metal iodide, 0–0.075 mmol; MeOH–[$^2H_4$]MeOH (2:3), 0.5 ml. The signal for carbon *c* rarely shifted in the presence of metal salts examined.

responding (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetate.† The *E* resolution values were calculated as reported earlier[4] and are summarized in Table 1.

Lipase from *Pseudomonas* itself catalysed hydrolysis of racemic 12-crown-4 ester **1** to give (*S*)-alcohol **2** with modest enantioselectivity (E = 3).[5] The reaction rate and enantioselectivity depended largely on the nature of the additive. The highest *E* value observed was 10 when the hydrolysis was carried out in a 0.1 mol dm^{-3} NaCl solution. KCl and LiCl showed a marked contrast. Addition of these salts significantly influenced reaction rates but rarely enhanced enantioselectivity. These observations indicate that the crown ether **1**–Na^+ complex is a suitable substrate for the lipase-catalysed enantioselective hydrolysis. Table 1 shows that the counter-anion is another important factor in determining enantioselectivity. Since an enhanced *E* value was not observed in the Na^+-buffer solution, the crown ether **1**–Na^+ cation complex may accompany the counter-anion into the reactive pocket of the lipase.

We also carried out lipase-catalysed hydrolysis of chiral 12-crown-4 esters and found that the lipase employed favoured the (*R*)-enantiomer of the 12-crown-4 ester **1** (see Table 2). The hydrolysis of both (*R*)- and (*S*)-esters was significantly promoted by the Na^+ cation. The greatest rate was observed when the (*R*)-ester was hydrolysed in a 0.1 mol dm^{-3} NaCl solution. This may explain the Na^+-enhanced enantioselectivity observed in the lipase-catalysed hydrolysis of the racemic 12-crown-4 ester. Crown ether–metal complexation was for the first time demonstrated to control the enzymatic reaction.

Fig. 1 illustrates the Li^+-, Na^+- and K^+-induced changes in the ^{13}C NMR chemical shifts of the signals for the crown ring and sidearm carbons of the crown ether **1**. Addition of the Na^+ cation to the solution of crown ether **1** caused significant changes in the signals of the crown ring carbons, while the signals for the sidearm carbons shifted slightly upon complexation. Thus, the Na^+ cation was bound to the 12-crown-4 ring and not coordinated by the ester-functionalized sidearm. Since K^+ and Li^+ cations induced smaller spectral changes, crown ether **1** was supported to readily form the Na^+ complex and to be specifically hydrolysed in the active site of lipase.‡ These results reveal clearly that the crown ether derivative has the potential to regulate enzymatic reactions *via* metal complexation. Chiral crown ethers have been broadly utilized in asymmetric sensing, separation and reaction processes,[6] and have been prepared by elaborate stereoselective syntheses. Our approach can be considered a new and facile method for the optical resolution of racemic crown compounds as well as for the improvement of enzyme-catalysed organic syntheses.

The authors are grateful to Professors K. Naemura of Osaka University and K. Nakamura of Kyoto University for valuable discussions. This research was supported in part by a Grant-In-Aid for Scientific Research on Priority Area (No. 04225223) from the Ministry of Education, Science, and Culture, Japan. The provision of lipases from Amano Pharmaceutical Co. is also appreciated.

Received, 3rd September 1992; Com. 2/04748I

References

1 J. B. Jones, *Tetrahedron*, 1986, **42**, 3351; A. M. Klibanov, *Acc. Chem. Res.*, 1990, **23**, 114; G. M. Whitesides and C. H. Wong, *Angew. Chem., Int. Ed. Engl.*, 1985, **24**, 617; C.-S. Chen and C. J. Sih, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 695.
2 Recent examples: S.-H. Wu, Z.-W. Guo and C. J. Sih, *J. Am. Chem. Soc.*, 1990, **112**, 1990; T. Itoh, Y. Takagi and S. Nishiyama, *J. Org. Chem.*, 1991, **56**, 1521,
3 B. Odell and G. Earlam, *J. Chem. Soc., Chem. Commun.*, 1985, 359; D. N. Reinhoudt, A. M. Eendebak, W. F. Nijenhuis, W. Verboom, M. Kloosterman and H. E. Schoemaker, *J. Chem. Soc., Chem. Commun.*, 1989, 399.
4 C.-S. Chen, Y. Fujimoto, G. Girdaukas and C. J. Sih, *J. Am. Chem. Soc.*, 1982, **104**, 7294.
5 (*S*)-Alcohol **2** was derived from (*S*)-(+)-isopropylideneglycerol by a modification of the published method: T. Miyazaki, S. Yanagita, A. Itoh and M. Okahara, *Bull. Chem. Soc. Jpn.*, 1982, **55**, 2005.
6 D. J. Cram, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 1009; J. M. Lehn, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 90; S. Aoki, S. Sasaki and K. Koga, *Tetrahedron Lett.*, 1989, **30**, 7229; M. Zinic, L. Frkanec, V. Skaric, J. Trafton and G. W. Gokel, *J. Chem. Soc., Chem. Commun.*, 1990, 1726; J. S. Bradshaw, P. Huszthy, C. W. McDaniel, C. Y. Zhu, N. K. Dalley, R. M. Izatt and S. Lifson, *J. Org. Chem.*, 1990, **55**, 3129; C. Moberg, K. Warnmark, I. Csoregh and A. Ertan, *J. Org. Chem.*, 1991, **56**, 3339.

† ^{19}F NMR experiments were performed at the SC-NMR Laboratory of Okayama University. The e.e.% of the remaining ester **3** was determined similarly after LiOH-hydrolysis.

‡ Fast atom bombardment mass spectrometry (FAB MS) experiments supported that crown ether **1** preferred the Na^+ ion to the K^+ and Li^+ cations. Complexation of crown ether **1** (0.0033 mol dm^{-3}) with LiCl, NaCl and KCl (0.0033 mol dm^{-3}, each) in glycerol–H_2O (2:1) was studied by measuring the relative peak heights of [crown–M^+] ions: [**1**–Li^+], 1.0; [**1**–Na^+], 5.5; [**1**–K^+], 2.6.