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Enzymatic resolution of 2-fluoro-2-arylacetic acid derivatives

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Abstract

Optical resolutions of 2-fluoro-2-arylacetic acids, ArC*FRCOOH (R = CN, H, and Me), were performed by enantioselective hydrolysis of the corresponding esters using *Candida rugosa* lipase (CRL). The enantioselectivity of commercial CRL toward the esters was greatly improved when commercial CRL was treated with 2-propanol solution. In the enantioselective hydrolysis of these esters, as represented by ArC*SMCOOR (**S** and **M** are small and medium substituents, respectively), the active site of the 2-propanol-treated CRL recognized fluorine as **S** in both PhCF(CH_3)COOR and *p*-TolCF(CN)COOR but recognized fluorine as **M** in PhCHFCOOR.

Keywords: Enantioselective hydrolysis; Lipase; *Candida rugosa*; Chiral derivatizing agent

1. Introduction

2-Fluoro-2-arylacetic acids are very effective chiral derivatizing agents (CDAs) for enantiomeric excess (ee) determinations by ^{19}F NMR spectroscopy [1–4] (Fig. 1). Recently, α-cyano-α-fluorophenylacetic acid (**1**, CFPA), the most efficient CDA reported to date, was developed by our group [2,4]. However, this agent is rather difficult to prepare in optically pure form on a practical scale (ca. 10 g) required for convenient general use. To develop an effective procedure for resolution of these CDAs, we examined a two-step method based on enzymatic hydrolysis of precursor esters followed by recrystallization of their amine salts of the derived acids. Since the amine salt of α-cyano-α-fluoro-*p*-tolylacetic acid (**2**, CFTA) proved to be more easily crystallized than that of CFPA, we focused on the enzymatic resolution of CFTA.

Among 20 commercial hydrolytic enzymes examined using (±)-CFTA ethyl ester as the substrate, *Candida rugosa* lipase (CRL, lipase OF) was found to be the most promising. However, the enantioselectivity achieved was clearly insufficient for practical use. Colton et al. [5] recently reported that pretreatment of CRL with 2-propanol improved enantioselectivity in the hydrolysis of 2-arylpropanoic acid esters. This strategy was successfully applied to the resolution of CFTA ethyl ester, producing an 8-fold increase in enantioselectivity. The resolution of

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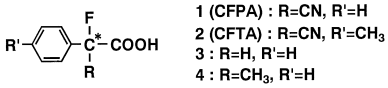

Fig. 1. 2-Fluoro-2-arylacetic acid derivatives as chiral derivatizing agents.

2-fluoro-2-arylacetic acid derivatives **3** and **4** are also efficiently carried out using the 2-propanol-treated CRL.

Colton et al. [5] proposed an empirical rule of the enantiopreference of hydrolysis reaction catalyzed by 2-propanol-treated CRL, based on the size of three substituents at chiral center. Our results suggest that the observed enantiopreference of 2-propanol-treated CRL toward 2-fluoro-2-arylacetic acid structures fitted the model and that the stereochemical recognition of fluorine depended on the structure of the substrate.

2. Materials and methods

2.1. Materials

CRL (lipase OF) was obtained from Meito Sangyo (Japan). The ethyl ester of α-cyano-α-fluoro-*p*-tolylacetic acid (**2**) was prepared starting with *p*-xylyl cyanide. Ethoxycarbonylation with diethyl carbonate and sodium hydride followed by electrophilic fluorination with $FClO_3$ afforded the ethyl ester of **2**. α-Fluorophenylacetic acid (**3**) was purchased from Sigma (USA). The methyl ester of **3** was synthesized by esterification with diazomethane. The methyl ester of α-fluoro-α-phenylpropanoic acid (**4**) was prepared starting with atrolactic acid, obtained from Aldrich Chemical (USA). Esterification with diazomethane followed by fluorination with diethylaminosulfur trifluoride afforded the methyl ester of **4**. The esters of **2**, **3**, and **4** were fully characterized by spectral data.

2.2. 2-Propanol treatment of CRL

2-Propanol treatment of CRL and determination of its hydrolytic activity were carried out according to the methods reported by Colton et al. [5]. Commercial CRL (5.0 g) was dissolved in 50 mM 2-(*N*-morpholino)ethanesulfonic acid (25 ml, pH 6.0) by stirring at 4°C for 30 min. 2-Propanol (5–35 ml) was added dropwise over 40 min and the resultant mixture was stirred for 42 h at 4°C. After removal of the precipitate by centrifugation at 3000 rpm for 30 min at 4°C, the supernatant was dialyzed against deionized distilled water (3×1 **1**) and used for hydrolysis. Hydrolytic activity was measured at pH 7.5 and 25°C using *p*-nitrophenyl acetate (PNPA) as the substrate [5,6].

2.3. Enzyme reaction

The ($\pm$)-carboxylic acid ester (100 mg) dissolved in isooctane (2 ml) was added to a solution of 2-propanol-treated CRL (10 ml, 50 units of activity by PNPA assay) and 1 M phosphate buffer (1 ml) at 25°C, and the mixture was stirred for several hours at this temperature. The progress of the kinetic resolution was monitored by HPLC as described below. The reaction was stopped when the ee of the remaining ester reached to about 80%. The remaining ester and the hydrolyzed acid were separated in the usual manner. The ee of the acid was determined after esterification with ethanol/*p*-toluenesulfonic acid or diazomethane.

2.4. Analytical methods

The enantiomers of the esters of **2**, **3**, and **4** were separated by HPLC (LC-6A system, Shimadzu, Japan) with a Daicel Chiralcel OJ column (Daicel Chemical, Japan) under the following conditions: mobile phase, *n*-hexane/2-propanol (9:1); column temperature, 30°C; detection, 254 nm. The enantiomeric ratio, *E* value, was calculated using $E = \ln[1 - c(1 + ee_P)]/\ln[1 - c(1 - ee_P)]$ and $c = ee_S/(ee_S + ee_P)$ where ee_S represents the ee of remaining ester and ee_P represents the ee of hydrolyzed acid [7].

3. Results and discussion

3.1. Commercial CRL

The ethyl ester of (±)−**2** (100 mg) was hydrolyzed with commercial CRL (40 mg, 50 units of activity by PNPA method) under the conditions described in Section 2.4. At the stage of 81% conversion, ee_S was 80% and ee_P was determined after esterification to be 19%. Thus, the enantioselectivity of the reaction ($E = 3$) was clearly insufficient to fulfill our needs. Enzymatic resolutions of the methyl esters of (±)−**3** and (±)−**4** by the same method were moderately enantioselective ($E = 12$ and 24, respectively).

3.2. Effect of 2-propanol treatment on enantioselectivity

We next examined the effect of the 2-propanol treatment on the enantioselectivity of CRL toward the ethyl ester of (±)−**2**. CRL was treated under several conditions of 2-propanol concentration as described in Section 2.4 and each 50 units of the pre-treated CRL was used for the hydrolysis of 100 mg of the ethyl ester of (±)−**2**. As shown in Fig. 2, E value dramatically changed at a 2-propanol concentration of 40% (v/v). The best result was obtained at 50% (v/v) 2-propanol, with achievement of an 8-fold improvement in the enantioselectivity ($E = 23$; $ee_S = 98\%$ and $ee_P = 68\%$ at 59% conversion). Large-scale application of this enzymatic resolution and purification of each enantiomer will be described in the near future.

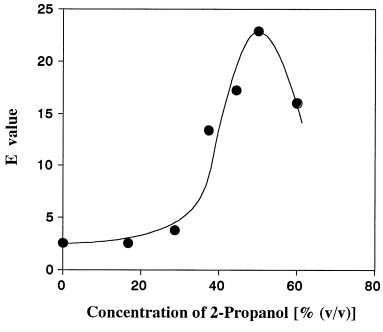

Fig. 2. Effect of 2-propanol concentration in pretreatment of commercial CRL on E value of hydrolysis reaction of the ethyl ester of (±)-**2**. Enantioselective hydrolysis of the ethyl ester of (±)-**2** (100 mg) was carried out using pre-treated CRL (50 units) under several conditions of 2-propanol concentration.

The enantioselective hydrolyses of methyl esters of (±)−**3** and (±)−**4** using the same 50% (v/v) 2-propanol-treated CRL were also improved to be the E value of 20 ($ee_S = 76\%$ and $ee_P = 86.5\%$ at 53% conversion) and 89 ($ee_S = 91\%$ and $ee_P = 97\%$ at 52% conversion), respectively. Thus, enzymatic optical resolutions of these three 2-fluoroarylacetic acid esters were accomplished efficiently using the 2-propanol-treated CRL.

3.3. Enantiopreference of 2-propanol-treated CRL

We examined the enantiopreferences of three enantioselective hydrolysis reactions by determining the absolute configurations of the slow-reacting esters. The configuration of the slow-reacting ester of **2** was determined by X-ray crystallographic analysis of its (*S*)-1-phenylethylamide to be the (*R*)-configuration. For the reactions of the methyl esters of **3** and **4**, we isolated the unreacted esters with more than 98% ee by using prolonged reaction times. We then compared their optical rotations, $[\alpha]_D^{25} = -118°$ (c 1.04, $CHCl_3$) for the methyl ester of **3** and $[\alpha]_D^{25} = +28.8°$ (c 1.51, EtOH) for **4**, with reported values [3,6,8]. From these data, we were able to assign the slow-reacting methyl esters of **3** and **4** the (*R*)- and (*S*)-configuration, respectively. Thus, the readily hydrolyzed isomers are esters of (*S*)-**2**, (*S*)-**3**, and (*R*)-**4**, as shown in Fig. 3.

Colton et al. [5] proposed an empirical rule for the enantiopreference of the ester hydrolysis of 2-arylacetic and 2-(aryloxy)acetic acid derivatives catalyzed by 2-propanol-treated CRL as shown in Fig. 3. Our results suggested that the 2-fluoro-2-arylacetic acid derivatives fitted the model as shown in Fig. 3. When the esters were represented by ArC*H**SM**COOR (**S** and **M** are small and medium substituents, respec-

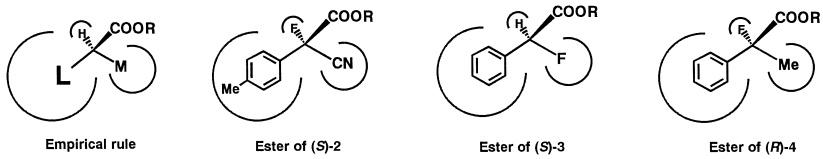

Fig. 3. Enantiopreference of hydrolysis reaction with 2-propanol-treated CRL. An empirical rule was proposed by Kazlauskas et al. (L = a large substituent; M = a medium substituent).

tively), the active site of CRL recognized fluorine as **S** in both *p*-TolCF(CN)COOH (**2**) and $PhCF(CH_3)COOH$ (**4**) but recognized fluorine as **M** in PhCHFCOOH (**3**). Thus, according to the empirical rule, the active site of CRL accepts fluorine as a replacement for hydrogen in the reactions of **2** and **4**. In contrast, since the van der Waal's radius of fluorine is slightly larger than that of hydrogen and other factors, such as stereoelectronic influence of fluorine, are also considered [9,10], the active site of CRL discriminates between the hydrogen and fluorine atom in the case of **3**. Thus, the stereochemical recognition of fluorine by CRL depends on the nature of the remaining substituents on the chiral carbon atom.

Acknowledgements

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References

[1] S. Hamman, M. Barrelle, F. Tetaz, C.G. Beguin, J. Fluorine Chem. 37 (1987) 85.
[2] Y. Takeuchi, N. Itoh, H. Note, T. Koizumi, K. Yamaguchi, J. Am. Chem. Soc. 113 (1991) 6318.
[3] S. Hamman, J. Fluorine Chem. 60 (1993) 225.
[4] Y. Takeuchi, N. Itoh, T. Satoh, T. Koizume, K. Yamaguchi, J. Org. Chem. 58 (1993) 1812.
[5] I.J. Colton, S.N. Ahmed, R.J. Kazlauskas, J. Org. Chem. 60 (1995) 212.
[6] S.N. Ahmed, R.J. Kazlauskas, A.H. Morinville, P. Grochulski, J.D. Schrag, M. Cygler, Biocatalysis 9 (1994) 209.
[7] C.S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. 104 (1982) 7294.
[8] K. Miyamoto, S. Tsuchiya, H. Ohta, J. Fluorine Chem. 59 (1992) 225.
[9] P. Kalaritis, R.W. Regenye, J.J. Partridge, D.L. Coffen, J. Org. Chem. 55 (1990) 812.
[10] D. O'Hagan, H.S. Rzepa, J. Chem. Soc., Perkin Trans. 2 (1994) 3.