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PRELIMINARY NOTE

Modification of Catalytic Activity of Hydrolytic Enzymes with Fluorine-containing Molecules

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SUMMARY

Studies on the new usage of fluorine compounds have been undertaken to improve the catalytic activity of hydrolytic enzymes. Some modified enzymes have been found to transform prochiral diethyl 2-fluoro-2-substituted malonates to chiral ethyl 2-fluoro-2-substituted malonates with high optical purity.

In recent years the opportunities for asymmetric synthesis provided by the wide range of catalytic activities of enzymes have been increased to cover the field of halogen-containing compounds hardly assimilated by microorganisms [1-3].

In studies of the behaviors of fluorine compounds towards microbial systems, we have reported the potential use of microbial transformations which afforded optically active fluorinated materials [4-6]. The importance of enzymatic immobilization and modification have been recognized as techniques for modifying the catalytic activities and/or capabilities of enzymes [7-9]. However, little research concerning the catalytic activities of modified enzymes towards fluorine-containing molecules, has been reported. In some cases, immobilization of an enzyme on polytetrafluoroethylene was

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effected with retention of *ca.* 4% of the soluble enzyme activity [10], but with [N-(4-fluorophenyl)-N-phenyl carbamoyl]-α-chymotrypsin there was no retention of catalytic activities [11,12].

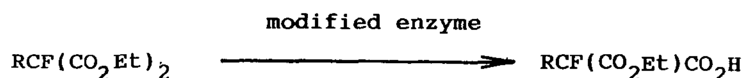
In our continuing effort to demonstrate affinities of fluorinated compounds to enzymes, we now present some results establishing the catalytic activity of the first stable fluorine-containing enzymes. These involve transforming prochiral diesters into optically active monoesters with high optical purity (>99 %ee) and are considered to be significant in demonstrating a new usage of fluorine-containing materials.

Modification of hydrolytic enzymes

To attempt the enzymatic modification with fluorine-containing molecules, 2-trifluoromethyl propenoic acid chloride, hexafluoropropene and perfluoroalkanoic acid chlorides were allowed to react with enzymes of microbial origin in an N,N-dimethylformamide-water system at room temperature. After 1 day of stirring, the solvent was removed under dynamic vacuum under 30°C. Proton or Carbon NMR spectroscopy would be difficult to apply for the determination of covalent attachment of fluorine-containing groups because of the difficulty in recognising the resonances of the attached group among the many originating from groups present in the enzyme. Therefore, we measured the Fluorine NMR spectra and the results shown in Table 1 suggest that the fluorinated molecules were attached to the enzymes.

Catalytic activity of modified enzymes

The above results encouraged us to study the catalytic activities of the modified hydrolytic enzymes in asymmetric hydrolysis of diethyl 2-fluoro-2-substituted malonates.



$$RCF(CO_2Et)_2 \xrightarrow{\text{modified enzyme}} RCF(CO_2Et)CO_2H$$

TABLE 1

Fluorine NMR spectrum of modified enzymes

Modified enzyme	^{19}F NMR[a]	
	CF_3 chemical shift	
lipase-MY[b]- $C(O)(CF_3)C{=}CH_2$	-12.1	
lipase-MY - HFP		-0.01
cellulase[c]- $C(O)(CF_3)C{=}CH_2$	-13.2	
cellulase - HFP		-0.01
Pig liver esterase - $C(O)(CF_3)C{=}CH_2$	-6.7	
Pig liver esterase - HFP		-0.02
α-*Chymotrypsin* - $C(O)(CF_3)C{=}CH_2$	-12.0	
α-*Chymotrypsin* - HFP		-0.03

[a] δ ppm from CF_3CO_2H as an external standard in water : The ^{19}F NMR spectra were recorded by using a Varian EM-390 spectrometer. [b] *Candida cylindracea*
[c] *Trichoderma viride*

TABLE 2
Asymmetric hydrolysis of $RCF(CO_2Et)_2$

Modified enzyme[a] or native enzyme[h]	Product R	Method[g]	Time (h)	Yield (%)	$[\alpha]_D$/MeOH	Optical purity %ee
lipase-MY - $C(O)(CF_3)C=CH_2$[b]	Me	A	14	94	-22.5 (c 2.20)	99
lipase-MY - $C(O)(CF_3)C=CH_2$[c]	Me	A	48	53	-17.0 (c 1.86)	74
lipase-MY - $C(O)CF_3$[d]	Me	A	15	30	-19.1 (c 2.14)	83
lipase-MY - $C(O)C_4F_9$[e]	Me	A	24	no reaction		
lipase-MY - HFP[f]	Me	A	96	34	-16.1 (c 1.96)	70
lipase-MY (native)	Me	B	6	87	-20.9 (c 2.81)	91
lipase-MY - $C(O)(CF_3)C=CH_2$	H	A	18	70	+13.9 (c 3.59)	100
lipase-MY - HFP	H	A	142	30	+13.5 (c 2.67)	98
lipase-MY (native)	H	B	6	79	+11.4 (c 1.56)	82
cellulase - $C(O)(CF_3)C=CH_2$	Me	A	120	70	+12.6 (c 0.20)	55
cellulase - HFP	Me	A	73	no reaction		
cellulase (native)	Me	B	6	42	+13.1 (c 2.24)	56
Pig liver esterase - $C(O)(CF_3)C=CH_2$	Me	A	23	no reaction		
Pig liver esterase (native)	Me	B	6	34	-4.88 (c 0.70)	24
α-*Chymotrypsin* - $C(O)(CF_3)C=CH_2$	Me	A	120	10	-7.54 (c 1.45)	33
α-*Chymotrypsin* (native)	Me	B	6	82	-16.0 (c 1.70)	70

[a] 5 g of lipase-MY(*Candida cylindracea*)/10 mmol of $R_fC(O)Cl$ or HFP/10 mmol of substrate. [b] $H_2C=C(CF_3)C(O)$- (3.5 mmol) group attached on lipase-MY (5 g). [c] $H_2C=C(CF_3)C(O)$- (6.5 mmol) group attached on lipase-MY (3 g). [d] CF_3CO_2H (3.9 mmol) was recovered. [e] $C_4F_9CO_2H$ (3.3 mmol) was recovered. [f] hexafluoropropene [g] Method A : 50 ml of buffer solution (pH = 8.0); Method B : 50 ml of buffer solution (pH = 7.3). [h] lipase-MY : Meito Sangyo Co. Ltd. ; cellulase(*Trichoderma viride*) : Yakult Pharmaceutical Industry Co. Ltd. ; *Pig liver esterase* : Sigma Co. Ltd. ; α-*Chymotrypsin* : Sigma Co. Ltd.

The results shown in Table 2 clearly demonstrated that lipase-MY modified with 2-trifluoromethyl propenoic acid chloride and/or hexafluoropropene acted on the transformation of diesters into optically active monoesters at 40-41 °C. The asymmetric hydrolysis of diethyl 2-fluoro-2-methylmalonate by modified lipase-MY (*Candida cylindracea*) proceeded smoothly to afford (S)-(-)-ethyl 2-fluoro-2-methylmalonate (>99 %ee) [5], and that by modified cellulase (*Trichoderma viride*) afforded the enantiomer, (R)-(+)-ethyl 2-fluoro-2-methylmalonate. However, in the case of 2-fluoromalonic acid diethyl both modified esterase and cellulase gave only (R)-(+)-2-fluoromalonic acid monoethyl ester [13].

With respect to these results, we believe that the presently reported microbial approach provides a useful modification with fluorine-containing materials and is an advantageous biotechnical way for the generation of new catalytic functions and abilities in enzymes.

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