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Bioorganic Chemistry 32 (2004) 504-515

BIOORGANIC CHEMISTRY

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### The role of conformational flexibility of enzymes in the discrimination between amino acid and ester substrates for the subtilisin-catalyzed reaction in organic solvents

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> Received 26 January 2004 Available online 6 July 2004

#### Abstract

To investigate how the conformational flexibility of subtilisin affects its ability to discriminate between enantiomeric amino acid and ester substrates for the subtilisin-catalyzed reaction in an organic solvent, the flexibility around the active site and the surface of subtilisin was estimated from the mobility of a spin label bound to subtilisin by ESR spectroscopy. Many studies on enzyme flexibility focus on the active site. Both the surface and active site flexibility play an important role in the enantioselectivity enhancement of the enzyme-catalyzed reaction. It was found, however, that the different behavior observed for the enantioselectivity between the amino acid and ester substrates could be correlated with the flexibility around the surface rather than the flexibility around the surface of subtilisin. In other words, for the ester substrates, the greater flexibility around the surface of subtilisin induced by a conformational change resulting from the presence of an additive such as DMSO is essential for the enantioselectivity enhancement. This model is also supported by the Michaelis–Menten kinetic parameters for each enantiomeric substrate. Our findings provide insight into the enantioselectivity enhancement for the resolution of enantiomers for enzyme-catalyzed reactions in organic solvents. © 2004 Elsevier Inc. All rights reserved.

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#### 1. Introduction

One of the most challenging problems in organic syntheses is the production of the enantiomerically pure compounds such as pharmaceutical and agricultural chemicals because only one of the enantiomers is generally biologically active. Recently, biocatalysts have received much attention as enantioselective catalysts for the resolution of racemic mixtures [1]. Enzyme-catalyzed reactions can normally be performed under much milder conditions (i.e., room temperature and near neutral pH) than their chemical counterparts. It is also known that many enzymes like lipases or the proteases (e.g., subtilisin and chymotrypsin) display enzymatic activity in organic solvents [2]. Hence, the use of the enzymatic reactions is becoming increasingly prevalent in organic syntheses. For the last decade, many strategies have been developed to achieve the high enantioselectivity including the use of additives in the reaction medium [3], different organic solvents [4], changing the reaction temperature [5], and protein engineering by site-directed mutagenesis [6] or by the direct chemical modification of the enzyme [7]. Among these strategies, the addition of a compound or a solvent to the reaction medium is the most popular because of its simplicity. In a recent review, various additives have been successfully used to achieve high enantioselectivity and/or high activity of enzymes [8].

For enzyme-catalyzed reactions in organic solvents, however, enantioselectivity is not always high enough to obtain chiral compounds, probably because these target compounds are not the natural substrates for the enzyme with structures significantly different from those of the natural ones. These non-physiological substrates require sufficient flexibility and/or a conformational change of the enzyme before binding can take place at the active site. Thus, the effect of a change in the flexibility and/or the conformation of the enzyme on the enantioselectivity is important to understand so that high enantioselectivity can be achieved for the reactions using these non-natural substrates such as pharmaceutical and agricultural chemicals.

There is clearly a difference in the origin of the enhanced enantioselectivity for amino acid and ester substrates in subtilisin-catalyzed reactions that use additives in an organic solvent. The different behavior is discussed in terms of the relationship between the kinetic parameters for each enantiomer and the conformational flexibility of the active site and the surface of subtilisin. The flexibility of the active site and surface of subtilisin is estimated from the mobility of the spin label bound to subtilisin by ESR spectroscopy. For model reactions, two popular additives, water or dimethyl sulfoxide (DMSO), were selected to elucidate the effect of the conformational flexibility of subtilisin on its enantioselectivity. The addition of DMSO, which may correspond to greater flexibility around the surface as well as the active site of subtilisin, is found to improve the enantioselectivity and/or the enzymatic activity for subtilisin-catalyzed reactions. The greater flexibility of the enzyme may be the critical step for generation of its enantioselectivity and/or activity, especially for the ester substrates. This is the first report that the flexibility around the surface of subtilisin brought on by additives is involved in the control of the enantioselectivity, although there are a few reports describing the relationship between the enantioselectivity and the flexibility in the active site of the enzyme [9].

#### 2. Materials and methods

#### 2.1. Materials

Subtilisin Carlsberg (serine protease from *Bacillus licheniformis*, EC 3.4.21.14) was purchased from Sigma Chemical and used without further purification. Isooctane, 1-butanol, and dimethyl sulfoxide were purchased from Wako Pure Chemical Industries, Japan. Organic solvents were dried over molecular sieves for more than 24 h before use. Each enantiomer of Z-Ala-OEt 1 [10], ethyl 2-(4-ethylphenoxy)propionate **2**, and methyl mandelate **3** [10] was prepared according to known methods. The synthesis, resolution, and esterification of **2** were carried out by literature procedures [3b,10,11].

#### 2.2. Subtilisin-catalyzed transesterification in isooctane containing the additives

In a typical subtilisin-catalyzed transesterification, each enantiomer of substrates 1-3 (0.01–0.025 mmol) and 1-butanol (0.06–0.15 mmol, 6 equiv.) was dissolved in dry isooctane (2 ml). To the solution, a small amount of DMSO or water was added, followed by ultrasonic dispersion, and then subtilisin (10 mg) was added. The suspension was shaken (170 strokes/min) at 37 °C. At an appropriate time interval, aliquots were withdrawn and the supernatant was analyzed by HPLC on a chiral column (Daicel Chiralcel OJ) to determine the initial rate. All the experimental data points gave a straight line with a correlation coefficient >0.98.

#### 2.3. Michaelis–Menten kinetic parameters

The kinetic study was carried out by measuring the initial rates of the transesterification of each enantiomer of substrates 1 and 2. The amount of each enantiomer was varied from 0.006 to 0.05 mmol and the amount of 1-butanol was 0.15 mmol. The enantiomer and 1-butanol were dissolved in dry isooctane (2 ml). To the solution, a small amount of DMSO or water was added, followed by the ultrasonic dispersion, which was followed by the addition of subtilisin (10 mg). At an appropriate time interval, aliquots were withdrawn and the supernatant was analyzed by HPLC on a chiral column (Daicel Chiralcel OJ) to determine the initial rate at each substrate concentration. The values of  $K_{\rm m}$  and  $V_{\rm max}$  were obtained from the Michaelis–Menten equation. All the experimental data points obtained from the Lineweaver–Burk plot gave a straight line with a correlation coefficient >0.97. The values were reproducible to about  $\pm 5\%$  on repeated runs.

#### 2.4. ESR measurement

Subtilisin was spin-labeled at the active site Ser-221 with 1-oxy-2,2,6,6-tetramethyl-4-piperidinyl ethoxyphosphorofluoridate (TEMPO-4-EPF) purchased from Sigma Chemical as described by the literature [12]. Met spin-labeled subtilisin was prepared by protecting the active site Ser-221 with the inhibitor (PMSF), and then labeling at Met residues with the synthesized compound, 4-(2-iodoacetamide)-TEMPO. The spin-labeled subtilisin was separated from the free label by the dialysis for 10 h, followed by the lyophilization (-50 °C, 24 h). A control sample of spinlabeled subtilisin in the appropriate solvent was used to tune the ESR signal. Subsequently, a new sample was placed into the sample cavity, and the signal was quickly fine tuned after the sample reached the room temperature. For the same solvent conditions of the subtilisin-catalyzed transesterification, all the ESR spectra were recorded at room temperature on a Bruker EMX081 spectrometer with a microwave power of 6.3 mW, a microwave frequency of 9.6 GHz, and a sweepwidth of 300 G (resolution 1024 points).

#### 3. Results and discussion

3.1. Additive effects on the enantioselectivity and activity of subtilisin for amino acid or ester substrates in transesterification reactions

To investigate the enantioselectivity for subtilisin-catalyzed transesterification in organic solvents, we chose the amino acid derivative (Z-Ala-OEt 1) or the carboxylic esters, ethyl 2-(4-ethylphenoxy)propionate 2, and methyl mandelate 3, as substrates (Scheme 1). For these reactions, subtilisin used preferentially the L enantiomer of 1, the S enantiomer of 2, and the R enantiomer of 3. Substrates 2 and 3 are important intermediates for useful agricultural and pharmaceutical chemicals. The reactions were carried out in dry isooctane containing a small amount of water [13] or dimethyl sulfoxide (DMSO) [3c,14] as additives because these additives are known to improve the enantioselectivity and/or enzymatic activity. Moreover, in hydrophilic or aromatic solvents such as diethyl ether and tetrahydrofuran, or benzene, subtilisin displayed extremely low enzymatic activity (data not shown).

Table 1 summarizes the initial rates for each enantiomer of 1–3 and the enantioselectivity (ratio of the initial rate of the correctly binding enantiomer to the incorrectly binding one:  $V_{\rm L}/V_{\rm D}$  for 1,  $V_S/V_R$  for 2, and  $V_R/V_S$  for 3) observed for the subtilisincatalyzed transesterification in isooctane containing various amount of water or DMSO. In the absence of additives, substrates 1–3 were not processed by subtilisin. As shown in Table 1, when a small amount of water was added to the reaction medium, the initial rate between the amino acid substrate and the ester substrate differs. For the model reaction using 1, the addition of water brought about the high enantioselectivity with an increase of the initial rate for the correctly binding L enantiomer, whereas the subtilisin-catalyzed reaction of 2–3 displayed extremely low



Scheme 1.

enzymatic activity (data not shown). The initial rate for 2–3 was too slow to obtain reproducible values.

Changing the additive from water to DMSO improved the enantioselectivity and enzymatic activity with all substrates 1-3. As shown in Table 1, when DMSO was added to the reaction medium, the initial rate for the correctly binding enantiomers of 1-3 increased, as compared with that for the incorrect ones. As a result, there was an enhancement of the enantioselectivity. For the transesterification of the correctly binding L enantiomer of 1, the addition of DMSO produced a significant increase in the initial rate, as compared with that for substrates 2–3. Upon the addition of the optimum amount of DMSO (0.30 vol%) to the reaction using 1, subtilisin displayed excellent enantioselectivity  $V_{\rm L}/V_{\rm D} = 26$ . The marked enhancement of the enantioselectivity was confirmed in model reactions using other amino acid substrates (for example,  $V_1/V_1$ )  $V_{\rm p} = 105$  for Z-Met-OEt,  $V_{\rm L}/V_{\rm p} = 171$  for Z-Phe-OEt, and  $V_{\rm L}/V_{\rm p} = 928$  for Z-Leu-OEt). For all the reactions, a steep decrease in the initial rate was observed by addition of an excess of DMSO (0.60 vol), although with the addition of more water (0.45-1.0 vol%) to these reactions, the enantioselectivity and the initial rate for each enantiomer of 1 remained almost constant  $V_1/V_p = ca. 85$ ,  $V_1 = ca. 30$ , and  $V_p = ca. 0.35$  (data not shown). DMSO may cause a conformational change of subtilisin.

# 3.2. Relationship between the enantioselectivity and activity and the conformational flexibility of subtilisin estimated from the ESR measurement of a spin-labeled enzyme

For subtilisin-catalyzed transesterification in an organic solvent, the variation of the enantioselectivity and/or enzymatic activity by the presence of additives is found

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Table 1

Enantioselectivity<sup>b</sup> Substrate Additive<sup>a</sup> Amount Initial rate (nmol  $h^{-1}$  mg<sup>-1</sup>) (vol%)  $V_{
m correctly biding enantiomer} V_{
m incorrectly biding enantiomer}$ 1 Water 0 0 0 10 0 0.150 0.300 24 0.35 69 32 0.450 0.38 84 DMSO 0 0 0 0.150 20 2.8 7.1 0.300 257 10 26 0.375 212 14 15 0.450 151 8.8 17 0.600 0 0 2 DMSO 0 0 0 0.150 0.21 0.16 1.3 0.300 1.0 0.77 1.3 0.375 15 3.2 4.7 20 2.1 9.5 0.450 0.500 14 2.1 6.7 0.600 1.4 1.0 1.4 3 DMSO 0 0 0 0.150 6.8 1.2 5.7 24 5.2 0.300 4.6 0.375 37 5.2 7.1 21 4.0 5.3 0.450 0.500 3.5 1.9 1.8 0.600 0 0

The variation of the initial rate for each enantiomer and the enantioselectivity for the subtilisin-catalyzed transesterification of 1-3 with 1-butanol in isooctane with additives

<sup>a</sup> For the addition of water, the subtilisin-catalyzed reaction of **2–3** displayed extremely low enzymatic activity.

<sup>b</sup>Ratio of the initial rate of the correctly binding enantiomer to the incorrectly binding one.

to be significantly dependent on the structure of the substrate. One could speculate that the observed difference in the enantioselectivity and/or enzymatic activity between the amino acid and ester substrates is primarily attributed to the additiveinduced flexibility of subtilisin. Thus, in order to investigate how the conformational flexibility of subtilisin affects the discrimination between the amino acid and ester substrates, the mobility of a spin label bound to the active site Ser-221 of subtilisin was examined in isooctane containing the various amounts of water or DMSO by ESR spectroscopy. Fig. 1 shows the ESR spectra in isooctane containing 0 and 0.45 vol% of DMSO, in which two parts of the spectrum are arbitrarily labeled  $H_a$ and  $H_i$ , respectively. According to our method [3c,4d], the degree of the enzyme's flexibility can be monitored by the change in the ratio of the signal height,  $H_i/(H_a + H_i)$ , because each signal  $H_a$  and  $H_i$  represents the anisotropy and the isotropy of the spin label, respectively. Thus, the increase in the  $H_i/(H_a + H_i)$  value reflects an increase in the mobility of the spin label. Fig. 2A shows the variation of the



Fig. 1. The ESR spectra of the spin-labeled subtilisin in isooctane; (A) with no additive and (B) in the presence of DMSO (0.45 vol%).

 $H_i/(H_a + H_i)$  value for the spin label bound to Ser-221 as a function of the amount of two additives, water and DMSO. The increase in the amount of each additive is found to increase the flexibility around the subtilisin's active site. Unfortunately, however, as judged from the result that two additives gave the change of the  $H_i/(H_a + H_i)$  value in the same manner, the observed difference in the enantioselectivity and/or enzymatic activity between the amino acid and ester substrates cannot be explained by the flexibility around the active site of subtilisin.

To investigate the change in the subtilisin's flexibility outside the active site, we measured the ESR spectra of the spin-labeled subtilisin bound to the Met residues (119, 125, 135, or 199), which are found on the surface of subtilisin. The Met spin-labeled subtilisin was prepared by protecting the active site Ser-221 with the inhibitor (PMSF), in order not to label Met-222 in the active site of subtilisin by the Met spin label (see Section 2). The MALDI-TOF MS spectrum of the Met spin-labeled



Fig. 2. The variation of the  $H_i/(H_a + H_i)$  value for the spin-labeled subtilisin as a function of the amount of two additives ( $\bigcirc$ , water;  $\bullet$ , DMSO). (A)  $H_i/(H_a + H_i)$  value for the spin-label bound to Ser-221, (B)  $H_i/(H_a + H_i)$  value for the spin-label bound to Met around the surface of subtilisin.

subtilisin suggested that only one spin label reacted with subtilisin, although the position of the spin label (Met 119, 125, 135, or 199) was not determined. Thus, taking into consideration that the spin label combined with the Met residues at random, the  $H_i/(H_a + H_i)$  value represents the average flexibility around the four Met residues.

The variation of the  $H_i/(H_a + H_i)$  value as a function of the amount of two additives is depicted in Fig. 2B. In the absence of additives, subtilisin displays little flexibility around its surface  $(H_i/(H_a + H_i) = 0.4)$  as well as in its active site (see Fig. 2A;  $H_i/(H_a + H_i) = 0.1$ ). This finding suggests that the subtilisin molecule is very rigid with little flexibility in isooctane without the additives. In fact, as seen in Table 1, the reaction condition without the additives causes a serious decrease in the enzymatic activity. This decrease is likely due to inability of substrate to fit into the rigid binding pocket of subtilisin because of the steric bulk of the substrate.

Interestingly, the addition of 0.15 vol% of DMSO produced greater flexibility around the surface of subtilisin  $(H_i/(H_a + H_i) = 0.69)$  than is observed for the addition of water  $(H_i/(H_a + H_i) = 0.54)$  (Fig. 2B). However, there was little difference in the flexibility around the active site of subtilisin using either water or DMSO as additives (see Fig. 2A;  $H_i/(H_a + H_i) = 0.25$  for water,  $H_i/(H_a + H_i) = 0.29$  for DMSO). With the addition of more than 0.30 vol% of DMSO, the isotropic signal  $(H_i)$  became too saturated to obtain the reliable  $H_i/(H_a + H_i)$  value. With water, the  $H_i/(H_a + H_i)$  value changed in proportion to the amount of water added to the isooctane. This result can be explained by the assumption that the addition of DMSO has a denaturing ability on subtilisin through the specific interaction between the surface of the subtilisin and the ionic S=O group of DMSO. In view of this assumption, the larger flexibility around the surface rather than the active site of subtilisin is required to overcome the steric problems of the ester substrate fitting into the binding pocket of subtilisin. The flexibility seems to be essential for a stable association between substrate and enzyme. Therefore, the larger flexibility caused by the addition of DMSO is assumed to produce the increase in enzymatic activity in model reactions using 2-3. In the absence of DMSO or water, subtilisin displayed extremely low activity due to the loss of flexibility. The critical difference in the variation of the enantioselectivity and the enzymatic activity for the amino acid and ester substrates may be due to the flexibility around the surface of subtilisin.

# 3.3. Effect of the conformational flexibility on the discrimination between enantiomers of amino acid and ester substrates as assessed by kinetic studies

To elucidate the mechanistic details of the enhancement of the enantioselectivity and/or the enzymatic activity brought about by the conformational flexibility of subtilisin, Michaelis–Menten kinetic parameters were determined for the subtilisin-catalyzed transesterification of 1 and 2 in isooctane containing a small amount of water (Table 2) or DMSO (Tables 3 and 4). The data show that for the correctly binding L enantiomer of 1 (Tables 2 and 3), the increase in the amount of additives, corresponding to an increased flexibility of subtilisin, brings about an increase in  $V_{max}/K_m$ value where the  $K_m$  value is almost unchanged and the  $V_{max}$  value increases. The small effect of the subtilisin flexibility on the  $K_m$  value can probably be attributed to the ready accommodation of the L enantiomer in the active site of subtilisin, because

Configuration	Water (vol%)	$K_{\rm m}({\rm mM})$	$V_{\rm max}~({\rm mmol}~{\rm h}^{-1}~{\rm mg}^{-1})$	$V_{\rm max}/K_{\rm m}({\rm h}^{-1})$
L	0.150	15.9	$4.21 \times 10^{-5}$	$1.3  imes 10^{-4}$
L	0.300	14.0	$9.37 \times 10^{-5}$	$3.3 \times 10^{-3}$
L	0.450	13.7	$1.19 \times 10^{-4}$	$4.3 \times 10^{-3}$
D	0.150	a	а	а
D	0.300	151	$1.13 \times 10^{-5}$	$3.7 \times 10^{-5}$
D	0.450	151	$1.14 \times 10^{-5}$	$3.8 \times 10^{-5}$

The Michaelis–Menten parameters for the subtilisin-catalyzed transesterification of substrate 1 with 1butanol in isooctane containing a small amount of water

<sup>a</sup> The initial rate for the D enantiomer of **1** is too slow to obtain the reproducible value.

#### Table 3

Table 2

The Michaelis–Menten parameters for the subtilisin-catalyzed transesterification of substrate 1 with 1butanol in isooctane containing a small amount of DMSO

Configuration	DMSO (vol%)	$K_{\rm m}({\rm mM})$	$V_{\rm max}~({\rm mmol}~{\rm h}^{-1}~{\rm mg}^{-1})$	$V_{\rm max}/K_{\rm m}({\rm h}^{-1})$
L	0.150	14.3	$7.74 \times 10^{-5}$	$2.7 \times 10^{-3}$
L	0.300	11.6	$8.51 \times 10^{-4}$	$3.7  imes 10^{-2}$
L	0.375	11.6	$7.04 \times 10^{-4}$	$3.0 \times 10^{-2}$
L	0.450	12.0	$5.10 \times 10^{-4}$	$2.1 \times 10^{-2}$
D	0.150	40.9	$2.54 \times 10^{-5}$	$3.1 \times 10^{-4}$
D	0.300	30.6	$7.30 \times 10^{-5}$	$1.2 \times 10^{-3}$
D	0.375	23.9	$8.32 \times 10^{-5}$	$1.7 \times 10^{-3}$
D	0.450	38.0	$7.67 \times 10^{-5}$	$1.0 \times 10^{-3}$

Configuration	DMSO (vol%)	$K_{\rm m}({ m mM})$	$V_{\rm max}~({\rm mmol}~{\rm h}^{-1}~{\rm mg}^{-1})$	$V_{\text{max}}/K_{\text{m}}(\text{h}^{-1})$		
S	0.300	109	$7.51 \times 10^{-5}$	$3.4 \times 10^{-4}$		
S	0.375	87.4	$8.77 \times 10^{-5}$	$5.0 \times 10^{-4}$		
S	0.450	68.1	$1.10 \times 10^{-4}$	$8.1 \times 10^{-4}$		
S	0.500	80.1	$9.71 \times 10^{-5}$	$6.1 \times 10^{-4}$		
R	0.300	157	$2.51 \times 10^{-5}$	$8.0 \times 10^{-5}$		
R	0.375	147	$4.31 \times 10^{-5}$	$1.4  imes 10^{-4}$		
R	0.450	143	$3.56 \times 10^{-5}$	$1.2 \times 10^{-4}$		
R	0.500	151	$2.84 \times 10^{-5}$	$9.4 \times 10^{-5}$		

Table 4

The Michaelis–Menten parameters for the subtilisin-catalyzed transesterification of substrate 2 with 1butanol in isooctane containing a small amount of DMSO

the L enantiomer has the correct configuration for the shape of the active site. Thus, the greater flexibility is not required for the formation of the acyl-enzyme in the reaction using the L enantiomer. In addition, the unchanged  $K_{\rm m}$  value suggests that the addition of the denaturing additive DMSO does not alter the shape of the active site. As for the reactivity of the acyl-enzyme, however, the increase in the subtilisin flexibility favors the preferred orientation of the amino acid side chains to promote effectively the reaction of the acyl-enzyme with 1-butanol. Hence, the  $V_{\rm max}$  value increases with the increased flexibility of subtilisin.

In contrast to the kinetic parameters measured for the L enantiomer of 1, there is a marked difference in the  $K_m$  value for the D enantiomer when water and DMSO are used as additives. The data in Tables 2 and 3 show that the addition of DMSO, resulting in a greater flexibility around the surface of subtilisin, produces a 0.2-fold decrease in  $K_m$  and a 7-fold increase in  $V_{max}$  than those measured when water is used as the additive. The greater flexibility around the surface of subtilisin will enable the accommodation of the D enantiomer of 1 into the active site and stabilize the complex between the substrate and subtilisin. The small flexibility brought about by addition of water is not sufficient to provide a stable association between the D enantiomer and subtilisin, thereby resulting in the large  $K_m$  value (Table 2).

For substrate 2, the dependence of  $K_{\rm m}$  and  $V_{\rm max}$  on subtilisin flexibility showed the characteristic profile, as compared with that of substrate 1 (Table 4). As can be seen in Table 4, the increase in subtilisin flexibility caused by addition of DMSO produced a decreased  $K_{\rm m}$  value and an increased  $V_{\rm max}$  value for the correctly binding S enantiomer. The large effect of subtilisin flexibility on  $K_{\rm m}$  and  $V_{\rm max}$  for the S enantiomer of 2 can be also attributed to accommodation of the substrate into the active site through the induced fit for the substrate. In sharp contrast to the S enantiomer, the  $K_{\rm m}$  and  $V_{\rm max}$  values for the incorrectly binding R enantiomer of 2 were not sensitive to the increased flexibility of subtilisin. This is probably because the R enantiomer is the non-natural substrate with a structure significantly different from that of the natural one and has an incorrect configuration for the shape of the active site of subtilisin. Thus, the different enzymatic activities ( $V_{\rm max}/K_{\rm m}$ ) for each enantiomer caused by the flexibility effect produces the high enantioselectivity (( $V_{\rm max}/K_{\rm m}$ )<sub>S</sub>/( $V_{\rm max}/K_{\rm m}$ )<sub>R</sub>) = 6.8 for 0.45 vol% DMSO).

### 4. Conclusions

Our observation is the first report that the flexibility of subtilisin can have an effect on the discrimination between amino acid and ester substrates for subtilisin-catalyzed reactions in organic solvents. This work is supported by a kinetic study as well as a study of the flexibility in the active site and the surface of the enzyme estimated from the ESR spectroscopy. The conformational flexibility of subtilisin appears to be correlated with the enhancement of enantioselectivity, although the flexibility effect depends markedly on the structure of the substrate used. In particular, for the ester substrates, the large flexibility around the surface of subtilisin with a partial conformational change induced by the presence of a denaturing additive such as DMSO is found to be significantly responsible for the enantioselectivity enhancement based on the increase in enzymatic activity for the correctly binding enantiomer. These findings should be of interest to organic chemists as a strategy to improve the enantioselectivity and/or the enzymatic activity for ester substrates used as pharmaceutical and agricultural chemicals.

#### Acknowledgments

The authors thank Prof. H. Ohta (Department of Physics, Faculty of Science, Kobe University), Miss C. Urakawa, and Miss J. Yoshikawa for the helpful suggestions regarding the acquisition of the ESR spectra.

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