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1. Introduction

Enantioselective synthesis is a key process in modern chemistry, and is particularly important in the fields of pharmaceuticals, foods, and pesticides, since the different enantiomers or diastereomers of a molecule often have different biological activity. Therefore, the enantioselectivity is the most valuable feature of enzymes from the standpoint of their application as practical catalysts. In nonaqueous reaction system the enantioselectivity of enzymes has been markedly dependent upon organic solvents [1-5]. On the other hand, enzymatic reactions in hydrophilic solvents have the advantage of the solubility of a variety of substrates, including amino acid derivatives, which are poorly soluble in hydrophobic solvents [5]. However, when a hydrophilic solvent is used as a reaction medium, the enzyme molecule directly contacts with the solvent, and thereby its activity and enantioselectivity are strongly influenced by the nature of solvents [1, 2]. Moreover, as the enzyme is insoluble in nonaqueous media, which are 100% organic solvent media or aqueous solutions containing high amount of organic solvents, and is suspended, the reactivity of enzymes tends to be strongly influenced by the dispersion state of enzymes.

Ionic solvent that is liquid at room temperature has attracted increasing attention as innovative nonaqueous media for the chemical processes because of the lack of vapor pressure, the thermal stability, the high polarity, and so on [6]. Chemical and physical properties of ionic liquids can be changed by the appropriate modification of organic cations and anions, which are constituents of ionic liquids. Biotransformation in ionic liquids has extensively been studied [6, 7]. We have so far reported that protease-catalyzed esterification of amino acid and peptide synthesis are highly enhanced in ionic liquids, compared to organic solvents, and the thermal stability of proteins is markedly improved [8, 9].
In the chapter, the solvent effect of ionic liquids on the enantioselectivity of enzymes is mainly discussed on the basis of enzyme kinetics [10].

2. Effect of reaction medium on α-chymotrypsin-catalyzed esterification

As a model enzyme, bovine pancreas α-chymotrypsin has been employed as shown in Fig. 1, since it has been well investigated regarding its structure, properties, and functions [11]. α-Chymotrypsin belongs to the S1 family that is one of the most predominant families of serine protease. Peptide and synthetic ester substrates are hydrolyzed in an aqueous solution by serine protease. The S1 family contains a catalytic triad system consisting of aspartate, histidine, and serine that work together to control the nucleophilicity of the serine residue during catalysis [12]. The serine proteases are widely distributed in nature, where they perform a variety of different functions.

Figure 1. Structure of α-chymotrypsin

The binding site for a polypeptide substrate consists of a series of subsites across the surface of the enzyme, as seen in Fig. 2 [12]. In the figure, P and S are the substrate residues and the subsites, respectively. Except at the primary binding site S1 for the side chains of the aromatic substrates of α-chymotrypsin, there is no obvious, well-defined cleft or groove for substrate binding. The subsites run along the surface of the protein. The binding pocket for the aromatic side chains of the specific substrates of α-chymotrypsin is a well-defined slit in the enzyme 1 to 1.2 nm deep and 0.35 to 0.4 by 0.55 to 0.65 nm in cross section. This gives a very snug fit,
since an aromatic ring is about 0.6 nm wide and 0.35 nm thick. The binding pocket in α-chymotrypsin may be described as a hydrophobic pocket, since it is lined with the nonpolar side chains of amino acids. It provides a suitable environment for the binding of the nonpolar or hydrophobic side chains of the substrates.

![Diagram of binding site for polypeptide substrate.](image)

In nonaqueous media, α-chymotrypsin can function as a synthetic catalyst for esterification, transesterification, and peptide synthesis, and α-chymotrypsin-catalyzed esterification of N-acetyl-tryptophan (Ac-Trp-OH) with ethanol (EtOH) is proceeded as shown in Fig. 3. The esterification obeys ping-pong kinetics [12, 13]. The enzyme and substrate first associate to form a noncovalent enzyme-substrate complex (E⋅Ac-Trp-OH) held together by physical forces of attraction. This is followed by the attack of the hydroxyl of serine (Ser-195), which is one of amino acid residues in a catalytic triad system, on the substrate to give acyl enzyme intermediate (Ac-Trp-E) releasing water. The acyl enzyme intermediate and ethanol associate

![Diagram of α-Chymotrypsin-catalyzed esterification of N-acetyl-tryptophan (Ac-Trp-OH) with ethanol (EtOH) to N-acetyl-tryptophan ethylester (Ac-Trp-OEt).](image)
to form an acyl enzyme-ethanol complex (Ac-Trp-E⋅EtOH). This is followed by the nucleophilic attack of ethanol on the carbonyl group in Ac-Trp-E to give N-acetyl-tryptophan ethyl ester (Ac-Trp-OEt). It is assumed that the esterification proceeds through the steady state approximation. The rate of N-acetyl-tryptophan (Ac-Trp-OH) is given as

$$\frac{d[Ac-Trp-OH]}{dt} = k_1[E Ac-Trp-OH] - k_{-1}E Ac-Trp-OH$$

where E and E⋅Ac-Trp-OH are α-chymotrypsin and enzyme-substrate complex, respectively.

Similarly, the rates of E, enzyme-substrate complex (E⋅Ac-Trp-OH), acyl enzyme intermediate (Ac-Trp-E), acyl enzyme-ethanol complex (Ac-Trp-E⋅EtOH), H\(_2\)O, and N-acetyl-tryptophan ethyl ester (Ac-Trp-OEt) are as follows:

$$\frac{d[E]}{dt} = k_1[E Ac-Trp-OH] + k_2[Ac-Trp-OEtOH] - k_{-1}[E Ac-Trp-OH]$$

$$\frac{d[E Ac-Trp-OH]}{dt} = k_1[E Ac-Trp-OH] - (k_1 + k_2)[E Ac-Trp-OH]$$

$$\frac{d[Ac-Trp-E]}{dt} = k_2[E Ac-Trp-OH] + k_3[Ac-Trp-E]EtOH + k_4[Ac-Trp-E EtOH]$$

$$\frac{d[H_2O]}{dt} = k_2[E Ac-Trp-OH]$$

$$\frac{d[Ac-Trp-OEt]}{dt} = k_4[Ac-Trp-E EtOH]$$

Since all enzyme present is either free or complexes,

$$[E]_0 = [E] + [E Ac-Trp-OH] + [Ac-Trp-E] + [Ac-Trp-E EtOH]$$

where \([E]_0\) is the total concentration of enzyme in the system. Assuming the steady state approximation on the rate of E⋅Ac-Trp-OH, from Eq. 3

$$\frac{d[E Ac-Trp-OH]}{dt} = k_1[E Ac-Trp-OH] - (k_1 + k_2)[E Ac-Trp-OH] = 0$$

$$K_{S_1} = \frac{[E Ac-Trp-OH]}{[E Ac-Trp-OH]^*} = \frac{k_1 + k_2}{k_1}$$

where \(K_{S_1}\) is the dissociation constant of E⋅Ac-Trp-OH. Assuming \(d[H_2O]/dt = d[Ac-Trp-OEt]/dt\), from Eqs. 6 and 7,
\[ [\text{Ac - Trp - E} \cdot \text{EtOH}] = \frac{k_2}{k_4} [\text{E} \cdot \text{Ac - Trp - OH}] \]  

(11)

Assuming the steady state approximation on the rate of \( \text{Ac-Trp-E} \cdot \text{EtOH} \), from Eq. 5

\[
\frac{d[\text{Ac - Trp - E} \cdot \text{EtOH}]}{dt} = k_3 [\text{Ac - Trp - E} \cdot \text{EtOH}] - (k_3 + k_4) [\text{Ac - Trp - E} \cdot \text{EtOH}] = 0
\]

(12)

\[
K_{S_2} = \frac{[\text{Ac - Trp - E} \cdot \text{EtOH}]}{[\text{Ac - Trp - E}]} = \frac{k_3 + k_4}{k_3}
\]

(13)

where \( K_{S_2} \) is the dissociation constant of \( \text{Ac-Trp-E} \cdot \text{EtOH} \). From Eq. 13

\[
[\text{Ac - Trp - E}] = \frac{k_{S_2} [\text{Ac - Trp - E} \cdot \text{EtOH}]}{[\text{EtOH}]} \]

(14)

From Eq. 8

\[
[\text{E}] = [\text{E}]_0 - [\text{E} \cdot \text{Ac - Trp - OH}] - [\text{Ac - Trp - E}] - [\text{Ac - Trp - E} \cdot \text{EtOH}]
\]

(15)

Concerning the equation obtained from Eqs. 10, 11, 14, and 15, \( (k_2 + k_3)[\text{EtOH}] \) is much greater than \( k_2K_{S_2} \) because of excess \( \text{[EtOH]} \). Consequently,

\[
[\text{E} \cdot \text{Ac - Trp - OH}] = \frac{|k_4}{k_3} \frac{[\text{E}]_0 [\text{Ac - Trp - OH}]}{[\text{Ac - Trp - OH}]} \]

(16)

The reaction rate \( v \) is

\[
v = \frac{d[\text{E} \cdot \text{Ac - Trp - OH}]}{dt} = \frac{d[\text{H}_2\text{O}]}{dt} = k_4 [\text{E} \cdot \text{Ac - Trp - OH}] = \frac{k_{\text{cat}} [\text{E} \cdot \text{Ac - Trp - OH}]}{K_M + [\text{Ac - Trp - OH}]}
\]

(17)

where

\[
k_{\text{cat}} = \frac{k_2k_4}{k_2 + k_4}
\]

(18)

\[
K_M = \frac{k_3k_{S_2}}{k_3 + k_4} = \frac{(k_3 + k_4)k_4}{k_3(k_2 + k_4)}
\]

(19)

Thus, the reaction rate \( v \) corresponds upon Michaelis-Menten type model.
3. Effect of solvent on activity and enantioselectivity of α-chymotrypsin

The performances of enzymes such as activity and specificity in nonaqueous media markedly depend upon the nature of solvents [1, 2]. In order to assess the effect of reaction media on kinetic parameters of α-chymotrypsin, the reaction rates in ionic liquids and organic solvents containing 5% \((v/v)\) water were measured at different concentrations of substrates at 25 °C. Figure 4 shows the structures of solvents used in this study. In the figure, 1-ethyl-3-methylimidazolium tetrafluoroborate, 1-ethyl-3-methylimidazolium bis(fluorosulfonyl)imide, 1-butyl-3-methylimidazolium hexafluorophosphate, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide, and tetrahydrofuran are abbreviated as [C2mim][BF4], [C2mim][FSI], [C4mim][PF6], [C4mim][TFSI], and THF, respectively. The kinetic parameters, \(k_{\text{cat}}\) and \(K_M\), were derived by correlating resultant reaction rates with Hanes-Woolf plot, which is the plot of \([\text{Ac-Trp-OH}] / \nu\) against \([\text{Ac-Trp-OH}]\) giving intercepts at \(K_M / k_{\text{cat}} [E]_0\) and \(-K_M\) [13].

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Structure</th>
<th>m. p. (°C)</th>
<th>Water miscibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>[C2mim][BF4]</td>
<td><img src="image1" alt="Structure" /></td>
<td>15.0</td>
<td>Miscible</td>
</tr>
<tr>
<td>[C2mim][FSI]</td>
<td><img src="image2" alt="Structure" /></td>
<td>-12.9</td>
<td>Partially miscible</td>
</tr>
<tr>
<td>[C4mim][PF6]</td>
<td><img src="image3" alt="Structure" /></td>
<td>6.5</td>
<td>Immiscible</td>
</tr>
<tr>
<td>[C4mim][TFSI]</td>
<td><img src="image4" alt="Structure" /></td>
<td>-16.2</td>
<td>Immiscible</td>
</tr>
<tr>
<td>THF</td>
<td><img src="image5" alt="Structure" /></td>
<td>-108.5</td>
<td>Miscible</td>
</tr>
</tbody>
</table>

Figure 5 shows the \(k_{\text{cat}}\) in ionic liquids and THF at 5% \((v/v)\) water and 25 °C. The \(k_{\text{cat}}\) is known as the turnover number or molecular activity as it represents the maximum number of substrate molecules that the enzyme can turn over to product in a set time. The \(k_{\text{cat},L}\) for L-enantiomer was much greater than \(k_{\text{cat},D}\) for D-enantiomer in ionic liquids and THF. α-Chymotrypsin absolutely favors L-enantiomer in aqueous solutions. Consequently, the structural specificity of native enzyme remained to some extent in ionic liquids and THF. The \(k_{\text{cat}}\) was strongly dependent upon the kind of solvents. The \(k_{\text{cat},L}\) for L-enantiomer in [C2mim][BF4] was the greatest, while the \(k_{\text{cat},D}\) for D-enantiomer in [C4mim][FSI] was the greatest. [C2mim][BF4] can act as a thermal stabilizer for proteins in aqueous solutions [14]. The \(k_{\text{cat}}\) is first-order rate constant that refers to the properties and reactions of the enzyme-substrate, enzyme-intermediate, and enzyme-product complexes. It is suggested that the solvent dependence of \(k_{\text{cat}}\) is due to the conformational changes in the enzyme. As a result, the \(k_{\text{cat},L} / k_{\text{cat},D}\) in [C2mim][BF4] was the greatest, while that in [C4mim][PF6] was the smallest, as shown in Fig. 6.
Figure 5. The $k_{\text{cat}}$ in ionic liquids and THF at 5% (v/v) water and 25 °C.

Figure 6. The $k_{\text{cat},L}/k_{\text{cat},D}$ in ionic liquids and THF at 5% (v/v) water and 25 °C.

Figure 7 shows the $K_M$ in ionic liquids and THF at 5% (v/v) water and 25 °C. The $K_{M,L}$ for L-enantiomer was the smallest in [C$_4$mim][PF$_6$], while the $K_{M,D}$ for D-enantiomer was small in [C$_2$mim][BF$_4$] or [C$_4$mim][TFSI], compared with that in other solvents. The $K_M$ is an apparent dissociation constant that may be treated as the overall dissociation constant of all enzyme-bound species. In addition, $K_M$ corresponds upon the substrate concentration when the reaction rate $v$ is half of maximum reaction rate. Consequently, it is easy to form enzyme-bound species for L-enantiomer in [C$_4$mim][PF$_6$] and for D-enantiomer in [C$_2$mim][BF$_4$] and [C$_4$mim][TFSI]. As a result, the $K_{M,L}/K_{M,D}$ in [C$_4$mim][PF$_6$] was the smallest, while that in [C$_2$mim][FSI] was the greatest, as seen in Fig. 8.
Figure 7. The $K_M$ in ionic liquids and THF at 5% (v/v) water and 25 °C.

Figure 8. The $K_{M,L}/K_{M,D}$ in ionic liquids and THF at 5% (v/v) water and 25 °C.

Figure 9 shows the $k_{cat}/K_M$ in ionic liquids and THF at 5% (v/v) water and 25 °C. The $(k_{cat}/K_M)_L$ for L-enantiomer was the greatest in $[C_2\text{mim}][BF_4]$, while the $(k_{cat}/K_M)_D$ for D-enantiomer was the greatest in $[C_2\text{mim}][FSI]$. The $k_{cat}/K_M$ is an apparent second-order rate constant that refers to the properties and the reactions of the free enzyme and free substrate. Thus, it sets a lower limit on the rate constant for the association of enzyme and substrate, and corresponds upon the catalytic efficiency of enzymes.
Figure 10 shows the \( \frac{k_{\text{cat}}}{K_M} / \frac{k_{\text{cat}}}{K_M} \) in ionic liquids and THF at 5% (v/v) water and 25 °C. The \( \frac{k_{\text{cat}}}{K_M} / \frac{k_{\text{cat}}}{K_M} \) in [C\(_2\)mim][BF\(_4\)] was 65000, while that in [C\(_2\)mim][FSI] was 200. The \( k_{\text{cat}} / K_M \) is referred to as the specificity constant, and determines the specificity for competing substrates [12]. Consequently, the \( \frac{k_{\text{cat}}}{K_M} / \frac{k_{\text{cat}}}{K_M} \) corresponds upon the enantioselectivity.

Figure 10 reveals that the enantioselectivity of α-chymotrypsin in ionic liquids can be forced to span a 325-fold range simply by switching from one solvent to another under otherwise identical conditions. Thus, D-enantiomer can sufficiently be employed as a substrate of α-chymotrypsin in ionic liquids, although α-chymotrypsin catalyzes biotransformation of L-enantiomer in nature.
4. Effect of water content on activity and enantioselectivity of α-chymotrypsin

A common thread in all studies of enzymes in anhydrous solvents is that the amount of water associated with the enzyme is a key determinant of the properties (e.g. activity, stability, and specificity) that the enzyme exhibits [1]. Moreover, water can act as a substrate in reactions using hydrolytic enzymes. In order to assess the effect of water content on kinetic parameters of α-chymotrypsin, the reaction rate in ionic liquids and organic solvents containing 1% (v/v) water was measured at different concentrations of substrates at 25 °C.

Figure 11 shows the $k_{cat}$ in ionic liquids and THF at 1% (v/v) water and 25 °C. The $k_{cat}$ depended upon the kind of solvents. The $k_{cat,L}$ for L-enantiomer was much greater than $k_{cat,D}$ for D-enantiomer in ionic liquids and THF, similar to the case at 5% (v/v) water. The $k_{cat,L}$ for L-enantiomer in [C$_2$ mim][FSI] was the greatest, while the $k_{cat,D}$ for D-enantiomer in [C$_2$ mim][BF$_4$] was the greatest. The $k_{cat,L}$ for L-enantiomer in [C$_2$ mim][BF$_4$] at 1% (v/v) water was about 81 times smaller than that at 5% (v/v) water, while the $k_{cat,L}$ for L-enantiomer in [C$_2$ mim][FSI] at 1% (v/v) water exhibited 18-fold decrease, compared to that at 5% (v/v) water. When a certain amount of water is added into the nonaqueous enzymatic reaction system, some water is bound to the enzyme, and thereby has a large influence on the enzyme performance, while the other amount of water is dissolved in the solvent [1]. Water associated with the enzyme activates the enzyme by increasing the internal flexibility of the enzyme molecule, since water acts as a plasticizer to increase the flexibility [15]. On the other hand, the $k_{cat}$ is the kinetic parameter that refers to the properties and reactions of the enzyme-substrate, enzyme-intermediate, and enzyme-product complexes due to the conformational changes in the enzyme. Accordingly, the water content markedly affected the conformation of enzymes in

![Figure 11. The $k_{cat}$ in ionic liquids and THF at 1% (v/v) water and 25 °C.](image-url)
ionic liquids. As a result, the $k_{\text{cat,L}} / k_{\text{cat,D}}$ in [C$_2$ mim][FSI] was the greatest, while that in [C$_2$ mim][BF$_4$] was the smallest, as shown in Fig. 12. Especially, the $k_{\text{cat,L}} / k_{\text{cat,D}}$ in [C$_2$ mim][FSI] increased with decreasing water content, whereas that in [C$_2$ mim][BF$_4$] drastically dropped.

![Figure 12](image_url)

Figure 12. The $k_{\text{cat,L}} / k_{\text{cat,D}}$ in ionic liquids and THF at 1% (v/v) water and 25 °C.

Figure 13 shows the $K_M$ in ionic liquids and THF at 1% (v/v) water and 25 °C. The $K_{M,L}$ for L-enantiomer was the smallest in [C$_2$ mim][FSI], while the $K_{M,D}$ for D-enantiomer was the smallest in THF. The $K_{M,D}$ in [C$_2$ mim][BF$_4$] at 1% (v/v) water was eleven times greater than that at 5% (v/v) water, whereas the $K_{M,D}$ in THF at 1% (v/v) water was seven times less than that at 5% (v/v) water. The $K_{M,L}$ in [C$_2$ mim][FSI] at 1% (v/v) water was twenty-nine times less than that at 5% (v/v) water. Therefore, the solvent effect on $K_{M,L}$ and $K_{M,D}$ was dependent upon the kind

![Figure 13](image_url)

Figure 13. The $K_M$ in ionic liquids and THF at 1% (v/v) water and 25 °C.
of solvents. As a result, the $K_{ML}/K_{MD}$ in THF was extremely large, compared with $[\text{C}_2\text{mim}]\text{[BF}_4\text{]}$ or $[\text{C}_2\text{mim}]\text{[FSI]}$, as seen in Fig. 14.

Figure 14. The $K_{ML}/K_{MD}$ in ionic liquids and THF at 1% (v/v) water and 25 °C.

Figure 15 shows the $k_{cat}/K_M$ in ionic liquids and THF at 1% (v/v) water and 25 °C. The ($k_{cat}/K_M)_L$ for L-enantiomer in $[\text{C}_2\text{mim}]\text{[FSI]}$ was the greatest, while the ($k_{cat}/K_M)_D$ for D-enantiomer in $[\text{C}_2\text{mim}]\text{[BF}_4\text{]}$ was the greatest. The ($k_{cat}/K_M)_L$ in $[\text{C}_2\text{mim}]\text{[FSI]}$ at 1% (v/v) water was 1.7 times greater than that at 5% (v/v) water, and the ($k_{cat}/K_M)_D$ in $[\text{C}_2\text{mim}]\text{[BF}_4\text{]}$ at 1% (v/v) water was twice greater than that at 5% (v/v) water. In other solvent systems, the ($k_{cat}/K_M)_L$ and ($k_{cat}/K_M)_D$ decreased with a decrease in water content. Especially, the ($k_{cat}/K_M)_L$ in $[\text{C}_2\text{mim}]\text{[BF}_4\text{]}$ at 1% (v/v) water was 175-fold less than that at 5% (v/v) water, and the ($k_{cat}/K_M)_D$ in $[\text{C}_2\text{mim}]\text{[FSI]}$ at 1% (v/v) water was 97-fold less than that at 5% (v/v) water.

Figure 15. The $k_{cat}/K_M$ in ionic liquids and THF at 1% (v/v) water and 25 °C.
Figure 16 shows the enantioselectivity in ionic liquids and THF at different water contents. The enantioselectivity of α-chymotrypsin in [C\textsubscript{2}mim][BF\textsubscript{4}] increased with an increase in water content, while that in [C\textsubscript{2}mim][FSI] decreased with increasing water content. The optimum water content for the enzyme performance is due to the balance between kinetic rigidity and thermodynamic stability of enzyme structures, and is called essential water [16]. The kinetic rigidity is relaxed by increasing water content, while native enzyme structure gradually changes through thermodynamic stability. For instance, the activity increases with an increase in the flexibility of rigid enzyme in ionic liquids, and it decreases with an increase in disturbance of enzyme structure [9]. Since the water solubility of [C\textsubscript{2}mim][FSI] is much lower than that of [C\textsubscript{2}mim][BF\textsubscript{4}], the distribution of water to the enzyme molecule in [C\textsubscript{2}mim][FSI] is superior to that in [C\textsubscript{2}mim][BF\textsubscript{4}]. Consequently, the disturbance of enzyme structure at 5\% (v/v) water might be enhanced to some extent in [C\textsubscript{2}mim][FSI] system, since the water solubility of [C\textsubscript{2}mim][FSI] is 3.45\%. On the other hand, [C\textsubscript{2}mim][BF\textsubscript{4}] system exhibited the same tendency in the relation between the water content and the enantioselectivity as THF system. [C\textsubscript{2}mim][BF\textsubscript{4}] and THF systems were probably in the relax of kinetic rigidity at 5\% (v/v) water, since [C\textsubscript{2}mim][BF\textsubscript{4}] and THF are water soluble.

5. Effect of reaction temperature on enantioselectivity of α-chymotrypsin in ionic liquids

Enzymatic reactions, as well as chemical reactions, obey the Arrhenius correlation between reaction rate constant and temperature, although the temperature range is quite limited. Accordingly, it is considered that the enantioselectivity is strongly influenced by the reaction temperature. In order to estimate the effect of reaction temperature on α-chymotrypsin-catalyzed esterification in ionic liquids, the kinetic parameters in ionic liquids and THF containing 5\% (v/v) water were investigated at 40 °C.
Figure 17 shows the $k_{cat}$ in ionic liquids and THF at 5% (v/v) water and 40 °C. The $k_{cat}$ was dependent upon the kind of solvents. Comparing the $k_{cat}$ at 40 °C with that at 25 °C, the $k_{cat, L}$ in THF at 40 °C exhibited about 720-fold decrease, whereas the $k_{cat, D}$ in THF at 40 °C shows about 480-fold increase. This indicates that thermal perturbation to enzyme structures at THF is large. On the other hand, the $k_{cat, L}/k_{cat, D}$ in [C$_2$mim][BF$_4$] was the greatest, while that in [C$_2$mim][FSI] was the smallest, as shown in Fig. 18. Especially, the $k_{cat, L}/k_{cat, D}$ in [C$_2$mim][FSI] and THF decreased with increasing temperature, whereas that in [C$_2$mim][BF$_4$] slightly increased.

![Figure 17](image1.png)

*Figure 17. The $k_{cat}$ in ionic liquids and THF at 5% (v/v) water and 40 °C.*

![Figure 18](image2.png)

*Figure 18. The $k_{cat, L}/k_{cat, D}$ in ionic liquids and THF at 5% (v/v) water and 40 °C.*

Figure 19 shows the $K_M$ in ionic liquids and THF at 5% (v/v) water and 40 °C. The $K_{M, L}$ in [C$_2$mim][FSI] was the smallest, while the $K_{M, D}$ in [C$_2$mim][BF$_4$] and THF was seventy times less than that
in [C$_2$ mim][FSI]. The $K_{M,L}$ in THF at 40 °C was 5.6 times greater than that at 25 °C, whereas the $K_{M,D}$ in [C$_2$mim][FSI] at 40 °C was seven times greater than that at 25 °C. As a result, the $K_{M,L}/K_{M,D}$ in THF was much greater than that in [C$_2$mim][BF$_4$] or [C$_2$mim][FSI], as seen in Fig. 20.

Figure 19. The $K_M$ in ionic liquids and THF at 5% (v/v) water and 40 °C.

Figure 20. The $K_{M,L}/K_{M,D}$ in ionic liquids and THF at 5% (v/v) water and 40 °C.

Figure 21 shows the $k_{cat}/K_M$ in ionic liquids and THF at 5% (v/v) water and 40 °C. The ($k_{cat}/K_M$)$_L$ in [C$_2$mim][BF$_4$] was the greatest, while the ($k_{cat}/K_M$)$_D$ in [C$_2$mim][FSI] was the greatest. Comparing the $k_{cat}/K_M$ at 40 °C with that at 25 °C, the $k_{cat}/K_M$ in ionic liquids and THF decreased with an increase in temperature. Especially, the ($k_{cat}/K_M$)$_L$ in THF at 40 °C exhibited about 6000-fold decrease, compared to that at 25 °C. This result indicates that heat extremely damages the catalytic efficiency of enzymes in THF.
As seen in Fig. 22, the enantioselectivity of α-chymotrypsin in [C$_2$mim][BF$_4$] at 40 °C was lower than that at 25 °C, while that in [C$_2$mim][FSI] increased with increasing the reaction temperature. On the other hand, the enantioselectivity of α-chymotrypsin in THF drastically dropped with an increase in the reaction temperature. The temperature dependence of the enantioselectivity of protease and lipase is markedly affected by organic solvents [3]. Thus, the result indicates that the temperature dependence of the enantioselectivity is controlled by changing the reaction medium from one ionic liquid to another, similar to the case of organic solvents.

![Figure 21. The $k_{cat}/K_M$ in ionic liquids and THF at 5% (v/v) water and 40 °C.](image1)

![Figure 22. Effect of reaction temperature on the enantioselectivity of α-chymotrypsin-catalyzed esterification in ionic liquids and THF at 5% (v/v) water.](image2)
6. Relationship between catalytic efficiency and enantioselectivity of α-chymotrypsin in ionic liquids

As mentioned above, the enantioselectivity is strongly influenced by a kind of ionic liquids, water content, and reaction temperature. In order to assess the correlation between the catalytic efficiency and the enantioselectivity, the \( \frac{k_{\text{cat}}}{K_M} \) was plotted against the \( \frac{k_{\text{cat}}}{K_M} \), as seen in Fig. 23. One can observe an increase in the \( \frac{k_{\text{cat}}}{K_M} \) with increasing the \( \frac{k_{\text{cat}}}{K_M} \). The catalytic efficiency \( \frac{k_{\text{cat}}}{K_M} \) is attributable to the enzyme structure [11]. Therefore, the higher the native structure of enzymes is kept, the larger the enantioselectivity becomes.

![Figure 23. Dependence of the enantioselectivity of α-chymotrypsin in the esterification of N-Ac-Trp-OH with EtOH on the catalytic efficiency. Reaction conditions: (1) 1% (v/v) water and 25 °C in [C2mim][BF4], (2) 5% (v/v) water and 25 °C in [C2mim][BF4], (3) 5% (v/v) water and 40 °C in [C2mim][BF4], (4) 1% (v/v) water and 25 °C in [C2mim][FSI], (5) 5% (v/v) water and 25 °C in [C2mim][FSI], (6) 5% (v/v) water and 40 °C in [C2mim][FSI], (7) 5% (v/v) water and 25 °C in [C4mim][PF6], (8) 5% (v/v) water and 25 °C in [C4mim][TFSI].](http://dx.doi.org/10.5772/58998)

7. Conclusion

In this chapter, the solvent effect of ionic liquids on the enantioselectivity of α-chymotrypsin has been described. The \( k_{\text{cat}} \) and \( K_M \) in ionic liquids were sensitively influenced by the reaction conditions such as water content and reaction temperature. As a result, the enantioselectivity, \( \frac{k_{\text{cat}}}{K_M} \), changed in the wide range. The \( k_{\text{cat}} \) and \( K_M \) correspond upon the conformational changes in the enzyme and the overall dissociation constant of all enzyme-bound species, respectively. Consequently, the major change of those parameters indicates that ionic liquids strongly affect the structure of enzyme molecules and the distribution of substrates and products between the reaction medium and the active site of enzymes under a requisite condition. By using the variation of enantioselectivity in ionic liquids, the optical resolution of biological active compounds and the synthesis of nonnative chiral compounds such as D-amino acid derivatives can effectively be carried out. Furthermore, it is expected that the ionic liquid, which exhibits the enantioselectivity suitable for a certain asymmetric synthesis, is...
prepared by tailoring the constituents of ionic liquids, since chemical and physical properties of ionic liquids can be changed by the appropriate modification of organic cations and anions, which are constituents of ionic liquids.

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