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Influence of ionic liquids under controlled water activity and low halide content on lipase activity

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Abstract−Room-temperature ionic liquids (ILs) can be used as reaction media for nonaqueous biocatalysis. However, the purity of ILs should be considered to understand the influence of ILs on enzyme activity. The major impurities in ILs are water and halide. In the transesterification of benzyl alcohol with vinyl acetate, the optimal water activities for lipases in [Omim][Tf_2N] were similar to those in organic solvents. The chloride impurity in [Omim][Tf_2N] seriously influenced the activity of lipase. In this work, the effect of ILs on lipase activity was investigated under controlled initial water activity and low halide content. The activity of lipase was highly dependent upon the anion structure of ILs. The initial reaction rate of lipases followed the order $[Tf_2N]^-$>$[PF_6]^-$>$[TfO]^-$>$[SbF_6]^-$≈$[BF_4]^-$. All tested lipases showed the highest activities in ILs containing [Tf_2N] anion. Particularly, [AAIM][Tf_2N] was shown as a suitable reaction medium for biocatalysis. Lipozyme IM showed the highest activity in this IL among tested ILs. Thermal stability of lipase was also investigated. The higher thermal stability of Novozym 435 was obtained in hydrophobic and water-immiscible ILs such as [Bmim][Tf_2N], [Edmim][Tf_2N], and [Bmim][PF_6].

Key words: Ionic Liquid, Lipase, Transesterification, Halide, Water Activity

INTRODUCTION

Room-temperature ionic liquids (ILs) are organic salts that do not crystallize at room temperature. Unlike traditional solvents, ILs are comprised entirely of ions [1]. The interest in ILs stems from their potential as "green solvents" because of their non-volatile character and thermal stability which makes them attractive alternatives for volatile organic solvents [2]. In chemical processes, ILs exhibit excellent physical characteristics including the ability to dissolve polar and nonpolar organic, inorganic, and polymeric compounds. Moreover, the number of combinations of anions and cations encompassed by ILs is vast, and their associated synthetic flexibility has led to ILs being referred to as 'designer solvents' [3]. Recently, it has been reported that ILs can be used as alternative reaction media for biocatalysis. Erbeldinger et al. [4] first described the use of anhydrous ILs as an enzymatic reaction medium, where ILs were simply used to replace organic solvents. Biocatalytic reactions in anhydrous ILs are promising technology because of higher selectivity, faster reaction rates and greater enzyme stability [5-7].

Several groups have disagreed on enzyme activity in a particular IL. For example, Schöfer et al. [6] and Itoh et al. [8] reported that *Candida antarctica* lipase B, *Candida rugosa* lipase, and porcine liver lipase had no activity in the transesterification of phenylethanol derivatives in [Bmim][PF_6], but other groups observed good activity of lipases for the same or similar transesterification in [Bmim][PF_6] [5,7]. One possible reason for these inconsistencies in enzyme activity may be the impurities in ILs, because most authors have not reported purity of ILs synthesized and used. The major impurities in ILs are water and halide ions.

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When an enzymatic reaction is performed in nonaqueous media, water plays an important role in enzyme dynamics. Water also participates in the reactions which lead to denaturation of enzyme [9]. Generally, the enzymes have optimal water activity to catalyze synthetic reaction in nonaqueous media. Therefore, the water activity in ILs can influence the activity of enzymes in ILs. Since most ILs are hygroscopic and can absorb a considerable amount of water [10], the water content or water activity in the ILs must be carefully considered.

In the synthesis of imidazolium ILs, 1-alkyl-3-methylimidazolium halides precursors are usually first made, then the halides are exchanged with the desired anions. An incomplete exchange leaves halide anions behind [5]. The unreacted halides, such as chloride, bromide, and iodide ions, left in ILs can inhibit enzyme activity, as Lee et al. [11] reported. The activity of lipase from *Rhizomucor miehei* exponentially decreased with increasing Cl^- content in [Omim][Tf_2N], and the activity of lipase in [Omim][Tf_2N] mixture containing 2% [Omim][Cl] was only about 2% of the activity in pure [Omim][Tf_2N]. Thus, the purity of ILs should be considered to understand the influence of ILs on enzyme activity.

In this work, the effect of water activity (a_w) and chloride impurity on the activity of lipase in ILs was investigated. The influence of ILs structure on lipase activity was studied under controlled initial a_w and low halide content. In addition, thermal stability of lipase in ILs was studied with varying anion structure of ILs. Fig. 1 shows the structure of ILs studied in this work.

EXPERIMENTAL PROCEDURE

1. Materials

Novozym 435 (*Candida antarctica* type B lipase immobilized on acrylic resin) and Lipozyme IM (*Rhizomucor miehei* lipase im-

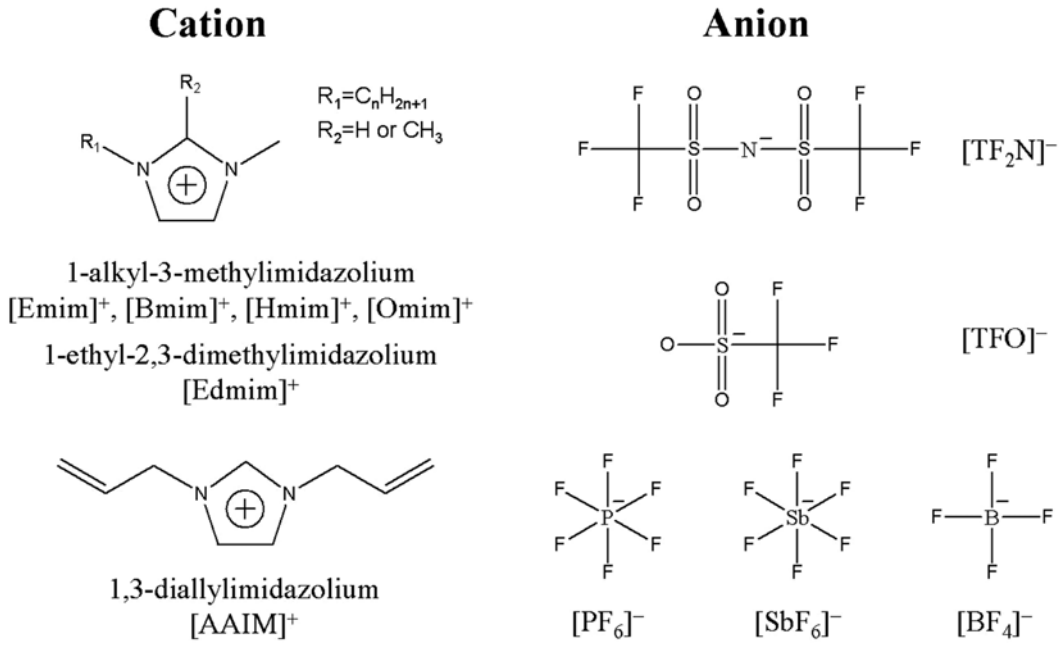

Fig. 1. Structures of ionic liquids studied in this work.

mobilized on anion exchange resin) were provided by Novo Nordisk (Bagsvaerd, Denmark). Commercial CRL (*Candida rugosa* lipase, Type VII) was purchased from Sigma (St. Louis, USA). Benzyl alcohol, vinyl acetate, and benzyl acetate were purchased from Aldrich (Steinheim, Germany). All ILs except [AAIM][Tf_2N] were synthesized and purified by C-TRI (Suwon, Korea) and had a residual chloride content of less than 30 ppm. [AAIM][Tf_2N] was kindly donated by Prof. H. Ohno at Tokyo University of Agriculture and Technology.

2. Lipase-catalyzed Transesterification under Controlled Initial Water Activity

Water activities of benzyl alcohol, vinyl acetate, and ILs were separately equilibrated over saturated salt solutions (KOH: a_w=0.082 at 25 °C, CH_3COOK: 0.225, $MgCl_2$: 0.328, K_2CO_3: 0.432, NaBr: 0.576, $CuCl_2$: 0.67, $NaNO_3$: 0.743, KCl: 0.843, $NaHPO_4$: 0.97; KOH: a_w=0.063 at 40 °C, $MgCl_2$: 0.316, $Mg(NO_3)_2$: 0.484, $CuCl_2$: 0.67, $NaNO_3$: 0.710, KCl: 0.823, K_2SO_4: 0.964) for 10 days. The lipases were equilibrated for two days. Substrate solutions were prepared by dissolving benzyl alcohol and vinyl acetate in 1 mL ILs. Enzyme reactions were started by pouring the enzyme into vials containing substrate solution, and placing these vials in a shaking incubator. Periodically, 10 μL aliquots were taken and diluted with acetonitrile (90 μL) to analyze by HPLC.

3. Influence of Chloride Impurity on Lipase Activity

[Omim][Tf_2N] containing chloride impurity of varying contents was made by mixing of [Omim][Tf_2N] and [Omim][Cl]. Water activities of the mixtures and CRL were equilibrated to 0.432 for 10 and 2 days, respectively. Substrate solutions were prepared by dissolving 0.1 M benzyl alcohol and 0.1 M vinyl acetate in 1 mL of IL mixtures. Enzyme reactions were carried out with the same procedures described in section 2.2.

4. Thermal Stability of Novozym 435

All ILs were dried in vacuum oven at 80 °C for two days before use. The residual water content in ILs was confirmed to be lower than 0.1% (w/w) by Karl-Fischer Titration (765F Coulometer, Metrohm) using HYDRANAL-Coulomat AK reagent. The water activities of ILs were lower than 0.1. Novozym 435 of 5 mg was incubated in 1 mL ILs at 70 °C for determined time intervals. Enzyme reactions were started by adding 1.0 M benzyl alcohol and 1.0 M vinyl acetate into vials containing lipase and ILs, and placing these vials in a shaking incubator at 40 °C. Periodically, 10 μL aliquots were taken and diluted with acetonitrile (90 μL).

5. HPLC Analysis

The concentrations of benzyl alcohol and benzyl acetate were measured by HPLC. Separation was accomplished with a Shimadzu HPLC system (Model LC-10A, Japan) equipped with a reverse-phase C_{18} column (SYMMETRY, Waters, USA) and a UV detector (Model SPD-10A, Japan, 250 nm). The mobile phase consists of acetonitrile/water containing 100 μL phosphoric acid per liter (50/50, v/v) with a flow rate of 1.0 mL/min.

RESULTS AND DISCUSSION

1. Influence of Water Activity on Lipase Activity

The lipase-catalyzed transesterification of benzyl alcohol with vinyl acetate was selected as a model reaction system to investigate the influence of a_w in ILs on the enzyme activity. This reaction did not occur in the absence of the enzyme at 40 °C. The enzyme reaction irreversibly produces benzyl acetate and acetaldehyde.

The optimal initial rate of Novozym 435 in [Omim][Tf_2N] was observed at a_w=0.2. The initial rate decreased with increasing a_w, when a_w was higher than 0.2 (Fig. 2).

Figs. 3 and 4 show the dependence of initial reaction rates on a_w in the transesterification of benzyl alcohol catalyzed by Lipozyme IM and CRL by using [Omim][Tf_2N] as reaction media, respectively. Maximum initial rates of Lipozyme IM and CRL were found

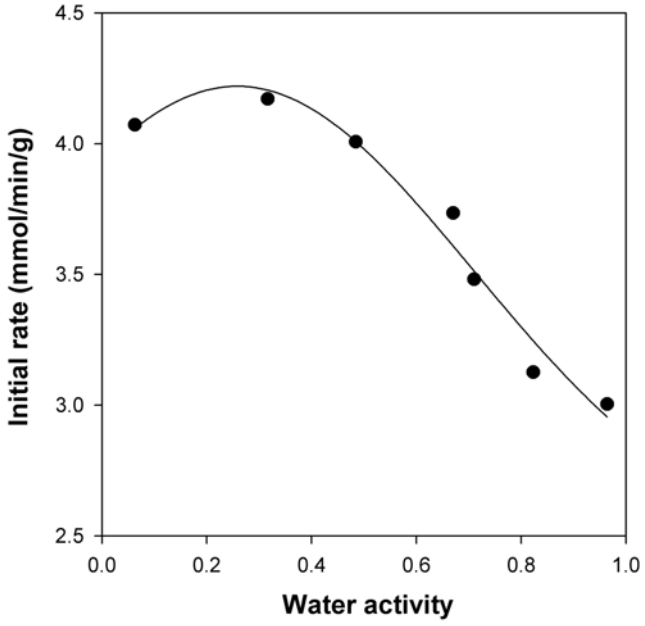

Fig. 2. Influence of water activity on the Novozym 435-catalyzed transesterification in [Omim][Tf_2N]. Reaction conditions: 1.0 M benzyl alcohol, 1.0 M vinyl acetate, 5 mg Novozym 435, 40 °C.

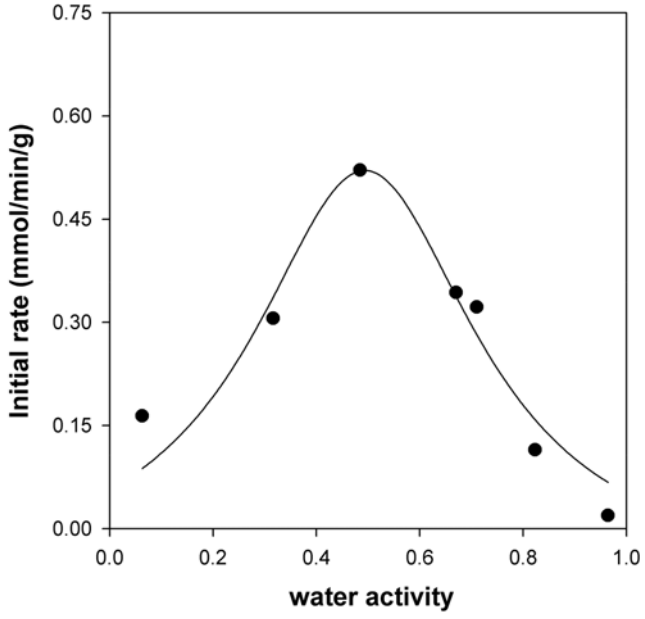

Fig. 3. Influence of water activity on the Lipozyme IM-catalyzed transesterification in [Omim][Tf_2N]. Reaction conditions: 0.2 M benzyl alcohol, 0.2 M vinyl acetate, 5 mg Lipozyme IM, 40 °C.

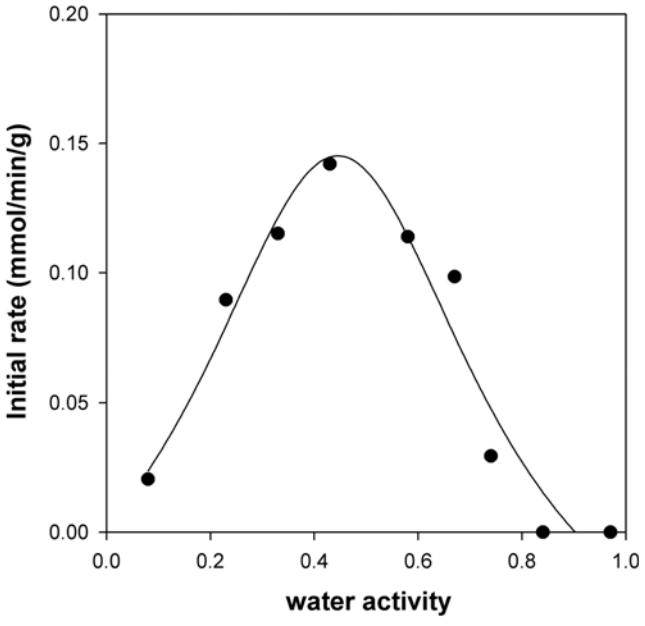

Fig. 4. Influence of water activity on the CRL-catalyzed transesterification in [Omim][Tf_2N]. Reaction conditions: 0.2 M benzyl alcohol, 0.2 M vinyl acetate, 5 mg CRL, 25 °C.

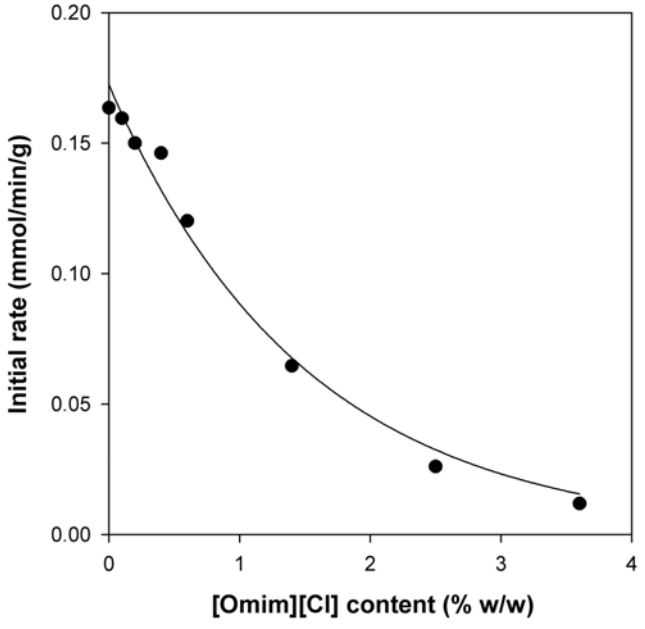

Fig. 5. Initial rates of the CRL-catalyzed transesterification in [Omim][Tf_2N] with varying chloride content. Reactions conditions: 0.1 M benzyl alcohol, 0.1 M vinyl acetate, 10 mg CRL, 25 °C, a_w=0.432.

at 0.5 and 0.4, respectively. These results are similar to those reported for the enzymatic reaction in organic solvents, solvent-free systems, and ILs [9,12-14].

2. Influence of Chloride Impurity on Lipase Activity

Fig. 5 shows the initial rates of CRL in the various mixtures of [Omim][Tf_2N] and [Omim][Cl] under the same a_w (0.432). CRL showed no activity in pure [Omim][Cl]. The activity of CRL exponentially decreased with increasing Cl^- content in [Omim][Tf_2N]. It was seen that the activity of lipase in the [Omim][Tf_2N] mixture containing 1% (w/w) [Omim][Cl] was only about 50% of that in pure [Omim][Tf_2N] which contains Cl^- of less than 30 ppm. Table 1 shows the influence of chloride impurity in [Omim][Tf_2N] on the activity of various lipases [11]. Unlike Novozym 435, the activities

Table 1. Influence of chloride impurity in [Omim][Tf_2N] on the activity of lipases

[Omim][Cl] content (% w/w) which shows a half activity of lipase		
Novozym 435[a]	Lipozyme IM[a]	CRL
9.8±0.6	0.27±0.02	1.1±0.1

[a]From Ref. [11].

of Lipozyme IM and CRL were highly influenced by chloride impurity in [Omim][Tf_2N]. Novozym 435 could retain enzyme activity until [Omim][Cl] content reached 20% (w/w) in the mixture of [Omim][Tf_2N] and [Omim][Cl].

3. Influence of Ionic Liquids Structure on Lipase Activity

The influence of ILs structure on the enzyme activity was investigated under the same a_w. Table 2 shows the initial rates of three different lipases (Novozym 435, Lipozyme IM, CRL) in the transesterification of benzyl alcohol by using various ILs as reaction media. The water activity was controlled to 0.063, 0.063, and 0.432 for Novozym 435, Lipozyme IM, and CRL, respectively. The three different lipases showed a similar influence of the anion and cation structure of ILs. When the ILs containing [Bmim] cation were compared, the initial rates were highly dependent on the nature of the IL anion. The initial reaction rate followed the order $[Tf_2N]^-$>$[PF_6]^-$>$[TfO]^-$>$[SbF_6]^-$≈$[BF_4]^-$.

4. Thermal Stability of Lipase in Ionic Liquids

Fig. 6 shows the thermal stability of Novozym 435 in various ILs containing different anions. Thermal stability of lipase was highly dependent on the changes of physicochemical properties by altering the anion structure of ILs. The activities of lipases were well maintained after two days incubation at 70 °C in hydrophobic and water-immiscible ILs containing [Tf_2N] or [PF_6], while the activities of lipase remarkably decreased in hydrophilic ILs.

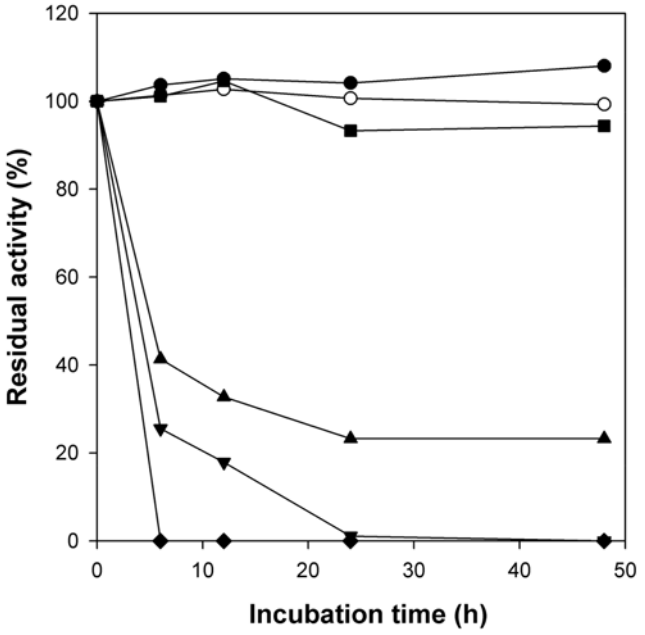

Fig. 6. Thermal stability of Novozym 435 in various ionic liquids. Reaction conditions: 1.0 M benzyl alcohol, 1.0 M vinyl acetate, 5 mg Novozym 435, 70 °C, a_w<0.1. (●) [Bmim][Tf_2N], (○) [Edmim][Tf_2N], (■) [Bmim][PF_6], (▲) [Bmim][TfO], (▼) [Bmim][BF_4], (◆) [Bmim][SbF_6].

DISCUSSION

1. Influence of Water Activity on Lipase Activity

Lower enzyme activity at high water levels is usually explained by the increasing rate of the hydrolysis reaction or enzyme aggregation. Halling [15] reported that optimal a_w values for esterifications in different organic solvents are similar. It was also concluded that a_w is the most significant variable affecting the microenvironment of enzyme. Considering the influence of a_w on Novozym 435 in nonaqueous media, the initial rate of lipase usually decreased with increasing a_w [16-18]. Similar results in ILs were reported in the transesterification of methyl methacrylate with 2-ethylhexanol using [Bmim][PF_6] as reaction media and acylation of 1-β-D-arabinofuranosylcytosine with vinyl propionate using a mixture of THF and [Bmim][PF_6] [10,19]. Our results were similar to these. How-

Table 2. Activities of various lipases in ionic liquids

	Physicochemical properties				Initial rate (mmol/min/g)		
Ionic liquids	Viscosity (cp)[a]	b	log P^c	log $S^{c,d}$	Novozym 435[e]	Lipozyme IM[f]	CRL[g]
[Emim][Tf_2N]	28	0.676	−1.18	0.193	4.08	0.132	0.150
[Bmim][Tf_2N]	52	0.644	−0.55	0.061	3.98	0.122	0.127
[Hmim][Tf_2N]	-[h]	0.654	0.16	−0.083	3.82	0.106	0.125
[Omim][Tf_2N]	93	0.630	0.79	−0.178	4.16	0.147	0.151
[Edmim][Tf_2N]	-	-	-	-	4.02	0.129	0.162
[AAim][Tf_2N]	-	-	-	-	3.00	0.181	0.155
[Bmim][PF_6]	371	0.669	−2.06	0.220	3.28	0.089	0.095
[Bmim][TfO]	90	0.656	−1.63	1.183	2.80	0.008	0.016
[Bmim][SbF_6]	-	0.673	−2.66	0.216	1.04	0.003	<0.001
[Bmim][BF_4]	154	0.670	−2.71	1.121	0.76	0.005	0.002

[a]From Ref. [26], [b]from Ref. [27], [c]from Ref. [28], [d]log scale of solubility of water vapor in ionic liquids, Reaction conditions: [e]1.0 M benzyl alcohol, 1.0 M vinyl acetate, 5 mg Novozym 435, 40 °C, a_w=0.063; [f]0.2 M benzyl alcohol, 0.2 M vinyl acetate, 5 mg Lipozyme IM, 40 °C, a_w=0.063; [g]0.2 M benzyl alcohol, 0.2 M vinyl acetate, 5 mg CRL, 25 °C, a_w=0.432; [h]no data.

ever, shifts of optimal a_w in ILs have recently been reported. In the direct esterification of geraniol with acetic acid using [Bmim][PF_6], optimal a_w was 0.6 and enzyme activity was low for both high and low a_w [20]. The analogous result was obtained in the acylation of 1-trimethylsilylethanol with vinyl acetate using [Bmim][PF_6] (optimal a_w=0.75) [21]. Although the optimal a_w can be changed by temperature, pressure, reactant concentrations, and direct water participation [22], these results showed severely different optimal a_w of Novozym 435. A possible explanation of this phenomenon is the modification of the tertiary structure of Novozym 435 in [Bmim][PF_6], because the [Bmim][PF_6] is a highly hydrophilic solvent compared to general organic solvents for biocatalysis [20].

While too low a_w cannot provide flexibility enough for Lipozyme IM and CRL to function in the IL system, too high a_w may cause the hydrolysis of product, enzyme inactivation, and agglomeration of the enzyme particles. Therefore, a_w of ILs should be considered to understand the influence of ILs properties on enzyme activity.

2. Influence of Chloride Impurity on Lipase Activity

There are some possible explanations for lipase inactivation by the unreacted [Omim][Cl] impurity in [Omim][Tf_2N]. First, [Bmim][Cl] has been reported to have a significant denaturing effect on some enzymes due to the intrinsically high Cl^- concentration similar to concentrated salt [23]. The high Cl^- concentration in [Omim][Cl] may cause dehydration and denaturation of enzymes. Second, enzyme inactivation may be caused by the increase of interference with the internal hydrogen bonds of an enzyme, because [Omim][Cl] shows a high hydrogen-bond basicity value of 0.9 [24], whereas enzyme-compatible ILs containing BF_4, PF_6, Tf_2N, SbF_6 anions show lower hydrogen-bond basicity values [25]. Third, since the estimated log *P* value for [Omim][Cl] of −0.27 is lower than that for [Omim][Tf_2N] of 0.79, the existence of [Omim][Cl] in the mixtures of [Omim][Tf_2N] can change the environment around lipase to the hydrophilic condition which can inactivate lipase [11]. In order to understand the enzyme-catalyzed reaction in ILs, therefore, the purity of ILs used should be considered, since the Cl^- content in ILs may be a major factor to determine the physicochemical properties of ILs and activity of enzyme.

3. Influence of Ionic Liquids Structure on Lipase Activity

Similar results for the influence of anion structure on the lipase-catalyzed esterifications using anhydrous ILs have been reported [8,19,21,29]. However, several conflicting results were also reported for the acetylation of 1-phenylethanol using *Candida antarctica* lipase B or *Pseudomonas cepacia* lipase [2,6,30]. They reported that the lipases showed higher activity in [Bmim][BF_4] than in [Bmim][PF_6]. These results may be caused by the existence of water or impurities in ILs which can change the physicochemical properties of ILs. As we previously described, the consideration of water activity and impurities in biocatalysis using ILs as reaction media is very important. Itoh and coworkers also observed that the rate of the transesterification of a chiral allylic alcohol was strongly dependent on the nature of the anion in [Bmim][X] [8]. Anions such as $[TfO]^-$ and $[BF_4]^-$ are more nucleophilic than $[PF_6]^-$ and thus may coordinate more strongly to positively charged sites in the structure of lipase causing conformation changes in the enzyme's structure [2,31]. Therefore, lipases will be more active in the ILs containing $[PF_6]^-$ than those with $[TfO]^-$ and $[BF_4]^-$. When the ILs containing [Tf_2N] anion was compared, the activity of lipase generally decreased with increasing alkyl chain length from C2 to C6. It may be caused by the limitation of mass transfer due to the higher viscosity of ILs containing cations of longer alkyl chains. However, the lipases showed the highest activity in [Omim][Tf_2N]. The [Omim][Tf_2N] is specifically hydrophobic IL and its hydrophobic nature may be suitable to increase the lipase activity, although the viscosity of this IL is higher than that of ILs containing shorter alkyl chain on cation. Considering the influence of cation structure of ILs with same anion of $[BF_4]^-$ or $[PF_6]^-$ on the lipase-catalyzed esterifications, several conflicting results have been reported. Some results showed that the activity of lipase increased with increasing the alkyl chain length of cation [6,32], while the inverse proportion of lipase activity to the alkyl chain length was also reported [21,29,30]. As the change of lipase activity with increasing alkyl chain length of cation was not so high, it is very difficult to understand the effect of cation on lipase activity. However, the increase of viscosity and hydrophobicity with increasing alkyl chain length of cation appeared to have a contradictory effect on lipase activity.

Nurok et al. [33] reported that the log scale of initial rate in the lipase-catalyzed reaction can be predicted by a two-descriptor (log *P* and dipole moment). They emphasized that log *P*, which represents the hydrophobicity of solvents, is an important descriptor to predict the enzyme activity. The initial rate in the transesterification of benzyl alcohol using ILs was correlated well with the log *P* values of ILs (Table 2). The hydrophobic ILs except [Bmim][TfO] induced higher activity of lipase, while the activity of lipase in hydrophilic ILs was very low. Lou and Zong [21] also recently found a good correlation between lipase activity in ILs and log *P* value.

Park and Kazlauskas [5] studied the relation between E_T^N of some ILs and common organic solvents and the activity of *Pseudomonas cepacia* lipase. They showed that in common organic solvents with Reichardt's polarity lower than 0.5 the activity of *Pseudomonas cepacia* lipase is in inverse proportion to solvent polarity, while in the more polar ILs, enzyme activity is in direct proportion to solvent polarity. However, E_T^N values were poorly correlated with initial rate of lipases in ILs studied.

The activities of Novozym 435 and Lipozyme IM in [Edmim][Tf_2N] were similar to those in [Emim][Tf_2N], while CRL showed higher activity in [Edmim][Tf_2N]. It means that the replacement of proton in 2-position of 1-ethyl-3-methylimidazolium with methyl group induced higher activity of CRL. As it was suggested that the acidity of the 2-position of imidazolium cation is very high [34], the decrease of acidity may decrease the interference between internal hydrogen bonds of CRL and [Edmim][Tf_2N]. On the other hand, Itoh et al. [35] reported that [Bdmim][BF_4] which lacked hydrogen at the 2-position was found to be an excellent solvent to make a lipase recycling system using vinyl acetate as acyl donor. They explained that no accumulation of an acetaldehyde oligomer in ILs containing [Bdmim] cation can induce the high reusability of lipase suspended in IL. In addition, it was known that acetaldehyde liberated during transesterification reaction deactivates CRL through the formation of a Schiff base with lysine residue [36]. The increased activity of CRL in [Edmim][Tf_2N] may be induced by the lowered inhibition effect of acetaldehyde.

Mizumo et al. [37] prepared 1,3-diallylimidazolium halides and their derivatives. These ILs showed considerably lower viscosity and higher ionic conductivities. In this work, [AAIM][Tf_2N] was

shown as suitable reaction medium for biocatalysis. Specifically, Lipozyme IM showed the highest activity in [AAIM][Tf_2N] among tested ILs.

4. Thermal Stability of Lipase in Ionic Liquids

The stability of lipase in solvents can be usually understood by log *P* value [38]. It is believed that hydrophilic organic solvents strip essential water from the lipase, which leads to the unfolding of the molecule with exposure of the inner hydrophobic residues, while hydrophobic organic solvents keep lipase flexible and active conformation [39]. Since [Bmim][BF_4] and [Bmim][SbF_6] are much hydrophilic compared to [Bmim][Tf_2N] and [Bmim][PF_6] as shown in Table 2, the stability of lipase can be partly understood by hydrophobicity. However, the activity of lipase in [Bmim][TfO] drastically decreased with increasing incubation time at 70 °C compared to [Bmim][PF_6], although the log *P* value of this IL is higher than [Bmim][PF_6]. The lower thermal stability of lipase in [Bmim][TfO] may be caused by the high water solubility in this IL, as the essential water of Novozym 435 can be stripped. Water solubility in ILs can also be correlated with thermal stability of lipase, although the thermal stability of lipase in [Bmim][SbF_6] is exceptionally very low. However, it was difficult to understand the thermal stability of lipase in ILs with one parameter of ILs.

CONCLUSIONS

The water and halide content in ILs may be the major factors to determine the physicochemical properties of ILs and activity of enzyme. Therefore, the purity of ILs used should be considered to understand the influence of ILs on enzyme-catalyzed reaction. When the water activity and halide content of ILs were similar, the activity and thermal stability of lipase were highly dependent on the anion structure of ILs. These results can be partially understood by hydrophobicity and water solubility of ILs, even though it is difficult to explain the effect of ILs on lipase activity and stability by using only one parameter.

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ABBREVIATIONS

[$Emim]^+$: 1-ethyl-3-methylimidazolium
[$Bmim]^+$: 1-butyl-3-methylimidazolium
[$Hmim]^+$: 1-hexyl-3-methylimidazolium
[$Omim]^+$: 1-octyl-3-methylimidazolium
[$Edmim]^+$: 1-ethyl-2,3-dimethylimidazolium
[$Bdmim]^+$: 1-butyl-2,3-dimethylimidazolium
[$AAIM]^+$: 1,3-diallylimidazolium
[$BF_4]^-$: tetrafluoroborate
[$PF_6]^-$: hexafluorophosphate
[$SbF_6]^-$: hexafluoroantimonate
[$TfO]^-$: trifluoromethanesulfonate
[$Tf_2N]^-$: bis[(trifluoromethyl)sulfonyl]amide

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