



Effect of ionic liquid properties on lipase stabilization under microwave irradiation

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ABSTRACT

Ionic liquids (ILs) as neoteric solvents and microwave irradiation as alternative energy source are becoming two important tools for many enzymatic reactions. However, it is not well understood what properties of ILs govern the enzyme stabilization, and whether the microwave irradiation could activate enzymes in ILs. To tackle these two important issues, the synthetic activities of immobilized *Candida antarctica* lipase B (Novozyme 435) were examined in more than twenty ILs through microwave heating. Under microwave irradiation, enhanced enzyme activities were observed when the enzyme was surrounded by a layer of water molecules. However, such enhancement diminished when the reaction system was dried. To understand the effect of IL properties, the enzyme activities under microwave irradiation were correlated with the viscosity, polarity and hydrophobicity ($\log P$) of ILs, respectively. The initial reaction rates bear no direct relationship with the viscosity and polarity (in terms of dielectric constant and E_T^N) of ILs, but have a loose correlation (a bell curve) with $\log P$ values. The enzyme stabilization by ILs was explained from aspects of hydrogen-bond basicity of anions, dissolution of the enzyme, ionic association strength of anions, and substrate ground-state stabilization by ILs.

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1. Introduction

The use of non-aqueous media has offered many advantages to the biocatalysis field, including better substrate dissolution, improved enzyme's thermal stability, enhanced enzyme selectivity, and more synthetic strategies [1–3]. However, the volatility and toxicity of many conventional organic solvents are obvious drawbacks of this approach. To substitute these organic solvents, a new type of non-volatile solvents known as ionic liquids (ILs), has recently gained serious attention in biocatalysis [4], mainly due to their extremely low vapor pressures and designable physical/chemical properties. Although many researchers have reported high enzyme activity and stability in ILs, it is still not quite clear what properties of ILs affect the catalytic behaviors of enzymes.

In addition to factors such as water activity, pH, excipients and impurities [5], several solvent properties of ILs are often related to the enzyme's destabilization, such as polarity [6–8], hydrogen-

bond basicity [9–11], anion nucleophilicity [12], IL network [13,14], ion's kosmotropicity [15–23], and viscosity [8,24]. However, none of these properties are solely responsible for enzyme functions in ILs; in many cases, multiple factors have to be considered. To further understand the solvent effects on enzyme stabilization, the *first objective* of this study is to investigate the general properties governing the enzyme's activity in ILs.

The *second objective* of this study is to examine whether microwave irradiation (vs. conventional conductive heating) induces enzyme activation in ILs. Microwave irradiation has become a routine heating device employed in various chemical reactions, and is an energy-efficient heating method for sealed-vessel processes [25]. However, its applications as an energy source for enzymatic synthesis are rather limited [26,27]. The biggest controversy in the field of microwave-promoted reactions is whether the rate acceleration is caused by the non-thermal effect of microwaves. This controversy is partly due to the fact that many earlier reactions were simply cooked in household microwave ovens without accurate control of temperatures. Many investigators argued that the reaction-rate increase in response to microwave irradiation is caused by the superheating of solvents beyond their normal boiling-points [28,29]. Other investigators, however, proposed the existence of non-thermal effect based on

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Table 1
Initial lipase activities in organic solvents and ILs at 40 °C under microwave irradiation, and correlations with physical properties of solvents^a

Solvent	Halide ^a (ppm)	Initial activity ($\mu\text{mol}/\text{min}/\text{mg E}$)	Dielectric constant ϵ_r (T in °C)	E_T^N (25 °C)	H-bond basicity (β) (25 °C)	Viscosity (cP) (T in °C)	C_p (J/g K) (T in °C)	$\log P$
1	<i>t</i> -BuOH	None	12.47 (25) ^b	0.395 ^h	0.95 ^t	4.312 (25) ^b	2.97 (25) ^b	0.35 ⁿ
2	1-BuOH	None	17.84 (20) ^b	0.85 ^t	0.85 ^t	2.544 (25) ^b	2.39 (25) ^b	0.84 ⁿ (0.80 ^k)
3	DCM (CH ₂ Cl ₂)	None	8.93 (25) ^b	0.309 ^f	-0.014 ^f	0.413 (25) ^b	1.19 (25) ^b	1.25 ^{l,n} (0.633 ^l)
4	THF	None	7.52 (22) ^b	0.207 ^h	0.523 ^t	0.456 (25) ^b	1.72 (25) ^b	0.46 ⁿ (0.49 ^k)
5	Acetonitrile	None	36.64 (20) ^b	0.460, ^f 0.451 ^h	0.370 ^f , 0.31 ^r	0.369 (25) ^b	2.23 (25) ^b	-0.34 ⁿ (-0.33 ^k)
6	[EMIM][Tf ₂ N]	600 Br ⁻	12.3 (25) ^{d,e}	0.676 ⁱ		34 (25) ^o		-1.18 ^w (-1.05 to -0.96 ^w)
7	[BMIM][Tf ₂ N]	260 Br ⁻	11.7 (25) ^d	0.642 ^g	0.243 ^f	52 (20) ^o	1.05 (25) ^q	0.11±0.01 (-0.96 to -0.21 ^w , 0.33 ^y)
8	[HMIM][Tf ₂ N]	140 Cl ⁻		0.654 ⁱ		79.5 (70) ^{s1}		0.64±0.01 (0.15 to 0.22 ^w , 0.65 ^y)
9	[BMIM][PF ₆]	160 Cl ^{-x}	11.4 (25) ^c	0.667 ^g	0.207 ^f , 0.21 (20) ^s	207 (25) ^o	1.14 (25) ^q	-1.66 ^w (-2.39±0.27, ^{m,y} -2.38±0.25 ^{r1} , -2.06 ^y)
10	[EMIM][OAc]	350 Br ⁻		0.814 ^v		162 (20) ^o		-2.53±0.02
11	[EMIM][TFA]	2830 Br ⁻				35 (20) ^o		-2.75±0.28
12	[HMIM][TFA]	510 Cl ⁻						-2.30±0.21
13	[BMIM][dca]	<i>n/d</i>						-2.32±0.02
14	[EMIM][BF ₄]	2420 Br ⁻	12.8 (25) ^c	0.71 ^j		34 (25) ^o	1.28 (100) ^p	-2.57±0.06
15	[BMIM][BF ₄]	160 Cl ^{-x}	11.7 (25) ^c	0.673 ^g	0.376 ^f	119.78 (25) ^p	1.66 (100) ^p	-2.51±0.04 (-2.44±0.23 ^{r1} , -2.52 ^w)
16	[OMIM][BF ₄]	9160 Br ⁻ 150 Cl ^{-x} 6050 Br ⁻	0.10 0.53 0.31			439 (20) ^{aa}		-1.34±0.09 (-1.14 ^v)
17	[EtPy][TFA]	620 Br ⁻	0.08					-2.57±0.11
18	[EtPy][Tf ₂ N]	480 Br ⁻	1.14					-0.90±0.06
19	[BuPy][Tf ₂ N]	590 Cl ⁻	0.89			76 (25) ^z		-0.26±0.08
20	[BMPyo][Tf ₂ N]	800 Br ⁻	0.91					
21	[Amm110]Cl	<i>n/d</i>	0.16	0.569 ^v		572 (25) ^u		
22	[Amm110][dca]	<i>n/d</i>	0.89	0.543 ^v		512 (25) ^u		
23	[Bu ₃ MeN][beti]	870 Br ⁻	0.29	0.832 ^v		687 (25) ^u		
24	[Bu ₃ MeN][Tf ₂ N]	600 Br ⁻	0.35	0.628 ^v				
25	[OctMe ₃ N][beti]	460 Br ⁻	0.15	0.569 ^v				
26	[Oct ₃ MeN][Tf ₂ N]	300 Br ⁻	0.32	0.451 ^v				
27	[Et ₃ HexN][Tf ₂ N]	640 Br ⁻	0.61	0.569 ^v				

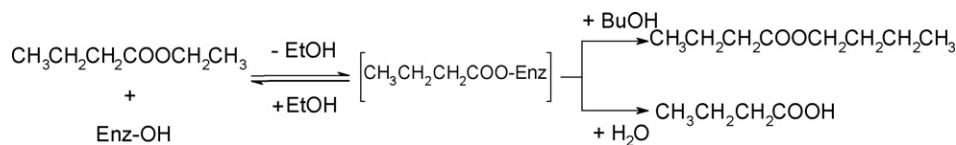
^aAll reactions were conducted in 2.0 mL solvent with 50 mg Novozyme 435; the initial rates were based on the mass of immobilized enzyme, not the actual protein content (20%); X⁻ is the impurity of halides (the type of halide is determined based on the starting materials used; *n/d* means 'not determined') meanings of ILs see section 2.1; ^bdata from Ref. [55]; ^cRef. [114]; ^dRef. [115]; ^eRef. [116] (this Ref. also reported [EMIM][Tf₂N] $\epsilon_s = 15.76$ or 14.01); ^fRef. [82]; ^gRef. [117]; ^hcalculated from $E_T(30)$ data in Ref. [118]; ⁱRef. [119]; ^jRef. [7]; ^kRef. [79,80]; ^lcalculated from hydrophobic fragmental constants in Ref. [120,121]; ^mRef. [12]; ⁿRecommended value of Sangster in Ref. [122]; ^ofrom a collection by Mantz and Trulove in Ref. [123]; ^pRef. [124]; ^qRef. [125]; ^rRef. [126]; ^sRef. [127]; ^tRef. [128]; ^uRef. [129]; ^vRef. [130]; ^wRef. [131]; ^xRef. [131]; ^ydetermined by this study using the Cannon-Fenske Routine (CFR) viscometer at 25 °C using benzyl alcohol as the reference (5.474 mPa s [132]) (based on Solvent Innovation, [Amm110]Cl has a viscosity of 495 at 20 °C; the data reported here are for dried ILs); ^zdetermined by this study; ^{aa}calculated from octanol-water partition coefficient (K_{ow}) in Ref. [133]; ^{ab}the identity of halide is unknown, and the impurity was calculated as Cl⁻ concentration; ^{ac}Ref. [134,135]; ^{ad}Ref. [136]; ^{ae}Ref. [137].

the argument that polar functional groups exhibit higher reactivity with adjacent reactants under microwaves than under conductive heating at the same temperature [30]. Nowadays, specialized microwave reactors equipped with *in situ* temperature and pressure controls are commercially available [31]. With this technology, some researchers still reported higher enzyme activities and selectivities in organic solvents under microwave irradiation [32–34]. Contradictorily, recent studies suggested that the microwave irradiation and thermal heating made no difference on the synthetic activities of *C. antarctica* lipase B (CaLB) at various temperatures (40–100 °C) [35–37].

Despite such a controversy, the utilization of ILs as media for microwave-assisted reactions is advantageous [38–40]. Organic solvents are usually flammable and volatile, which is a safety hazard for high-temperature and closed-vessel applications using

microwaves. In contrast, ILs are ideal solvents for microwave reactions because they have high boiling-points, low vapor pressures and high thermal stabilities [41,42]. In addition, typical ILs have moderately high dielectric constants (in the range of 10–15), and relatively low heat capacities (in the range of 1–2) (see Table 1 and Ref. [43]). This unique combination allows ILs to absorb microwaves efficiently and to be heated up easily. Owing to these advantages, ILs have been investigated as solvents in a number of microwave-mediated reactions [38–40,44]. However, the use of microwave irradiation for enzymatic catalysis in ILs is very limited (for example, Ref. [45]).

To achieve the above two objectives, a model reaction chosen for this study is the transesterification between ethyl butyrate and 1-butanol catalyzed by Novozyme 435 (immobilized *C. antarctica* lipase B). Scheme 1 illustrated a fast formation of an acyl-enzyme



Scheme 1. Lipase catalyzed transesterification of ethyl butyrate with 1-butanol.

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