Asian Journal of Chemistry Vol. 22, No. 6 (2010), 4744-4748

Influence of Water-Miscible Ionic Liquid 1-Butyl-3-methylimidazolium Tetrafluoroborate [BMIM][BF_4] on Acetylcholinesterase Inhibition-Based Spectrometric Assay for Parathion Methyl

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The influences of water-miscible ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) on acetylcholinesterase (AChE) inhibition-based organophosphorous assay were investigated. On the one hand, water-miscible [BMIM][BF_4] competitively removes the essential hydration shell from the AChE molecule and thus enzyme activity is inhibited slightly. On the other hand, the existence of [BMIM][BF_4] improves the inhibition binding between AChE and para-thion methyl. 5.1 % [BMIM][BF_4] leds to the best inhibition rate. Ionic liquids mixed aqueous solutions show the potential to provide a promising and effective medium in organophosphorous assay with AChE.

Key Words: Ionic liquid, Acetylcholinesterase, 1-Butyl-3-methylimidazolium tetrafluoroborate, Organophosphorous.

INTRODUCTION

Ionic liquids are the focus of many investigations because of their high ionic conductivity, low flammability, negligible vapour pressure and chemical stability[1]. Enzymes show high activity, selectivity and stability with onic liquids as solvent, which makes them good candidates as an environmentally attractive media in chemical and biochemical reactions[2,3].

Organophosphorous compounds can irreversibly inhibit acetylcholinesterase (AChE) that is essential for the functioning of the central nervous system, often causing respiratory paralysis and death. It becomes crucial to study the activity of AChE and the inhibition by organophosphorous[4]. A classical spectrophotometric assay method-Ellman's method has been the most widely used for AChE activity assay or organophosphorous determination[5]. The principle of this method is based on the fact that the substrate acetylthiocholine (ATCh) is hydrolyzed by the AChE to produce thiocholine and then thiocholine reacts with the chromophore 5, 5-dithio-*bis*-2-nitrobenzoic acid (DTNB) to produce a yellow 5-thio-2-nitrobenzoic acid. The rate of colour productions is measured at 412 nm by a spectrophotometer.

The goal of the present study is to investigate the influence of ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) on the performance of Ellman's method.

EXPERIMENTAL

Acetylthiocholine (ATCh) chloride and AChE (Type C3389, 500 U/mg from electric eels) were purchased from Sigma-Aldrich (St. Louis, USA). Parathion methyl was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany) and [BMIM][BF_4] from Chengjie Chemical Co., Ltd. (shanghai, China). All other chemicals from commercial sources were of analytical grade. Ultra-pure water was prepared from Milli-Q.

Parathion methyl solution was prepared in acetone. 50 mM phosphate buffer solution (PBS, pH 7.5) contains Na_2HPO_4 and KH_2PO_4 was employed for Ellman's assay.

For spectrophotometric measurements, a Nicolet Evolution 500UV/VIS spectrometer (Thermo Spectronic, USA) was used.

Effect of [BMIM][BF_4] on AChE activity and its inhibition by parathion methyl: AChE activity and its inhibition by parathion methyl were determined according to the modified Ellman method using ATCh as substrate[5]. In brief, the reaction mixture consisting of 780 μL 50 mM PBS (pH 7.5), 100 μL 8 mM ATCh, 100 μL 2.2 mM DTNB and 20 μL 0.5 U/mL AChE was prepared in a final volume of 1 mL. A blank with the same composition except ATCh was employed. AChE activity was expressed as ΔA, the change in absorbance at 412 nm wavelength after 10 min enzyme reaction against the blank signal.

The inhibition of parathion methyl was determined by incubating AChE and parathion methyl firstly before enzyme reaction. Parathion-methyl (200 μL) solution in acetone was dried by nitrogen firstly. Then 20 μL AChE was incubated with or without dried pesticide for designed time. The degree of inhibition was calculated as the relative decrease of ΔA using the formula:

$$\text{Inhibition } \% = [(\Delta A_0 - \Delta A_i)/\Delta A_0] \times 100$$

where ΔA_0 is the AChE activity without parathion methyl inhibiton and ΔA_i the one with parathion-methyl inhibition.

Effect of [BMIM][BF_4] on AChE activity and its inhibition by parathion methyl was studied by using 780 μL 50 mM PBS (pH 7.5) with different concentration [BMIM][BF_4] instead of pure PBS in the reaction solution.

RESULTS AND DISCUSSION

The effect of [BMIM][BF_4] concentration on AChE activity and its inhibiton rate by parathion methyl was studied first (Fig. 1). Several concentrations of [BMIM][BF_4] from 0 to 10.3 % were tested. Similar to the effects produced by standard organic solvents used in non-aqueous enzymology, catalytic activity of the enzymes decreases with the increase of water-miscible ionic liquid concentration[6] (Fig. 1A). Interestingly, the effect of [BMIM][BF_4] concentration on AChE inhibiton rate by parathion methyl presents a Gauss curve (Fig. 1B). 5.1 % [BMIM][BF_4] led to the biggest inhibition rate. The compromise between two factors could be employed to explain it. On the one hand, water-miscible [BMIM][BF_4] competitively

removes the essential hydration shell from the AChE molecule and thus its activity is inhibited partly. On the other hand, the existence of [BMIM][BF_4] improves the inhibition binding between AChE and parathion methyl[7]. The similar result was reported also by Zhang and Malhotra[8] by using ionic liquid ethylpyridinium hexafluorophosphate [EtPy][PF_6].

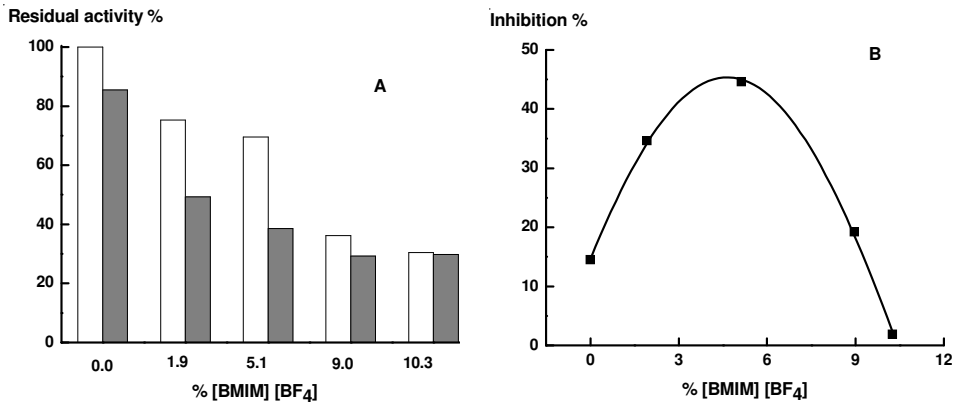

Fig. 1. (A) Influence of the [BMIM][BF_4] concentration on AChE activity and its inhibition by parathion methyl. (□) Percentage of residual AChE activity in the presence of various concentration of [BMIM][BF_4] in whole reaction volume. (■) Percentage of residual AChE activity in the presence of various concentrations of [BMIM][BF_4] in whole reaction volume with 1 μg/mL dried parathion methyl to inhibit AChE firstly before enzyme reaction; (B) Effect of [BMIM][BF_4] concentration on the inhibiton rate of AChE by 1 μg/mL dried parathion methyl

Fig. 2A shows the calibration plot of reaction rate *vs.* substrate concentration. The reaction rate is fast for ATCh at low concentration and it approaches to be a maximum (V_{max}) at high concentration where the saturation occurs with all of enzymes present as enzyme-substrate (ES) complex. The value of V_{max} in the absence of [BMIM][BF_4] is bigger than the one in the presence of [BMIM][BF_4]. Lineweaver-Burk plots of enzyme activity in the presence/absence of [BMIM][BF_4] are shown in Fig. 2B. The linear plots of rate-1 *versus* substrate concentration-1 indicate that inhibition of enzyme by [BMIM][BF_4] is mixed-type inhibition where a reduction of both the maximum reaction rate and the Michaelis constant are observed[9]. In such a case, the binding of substrate to the enzyme's reactive center as well as further reaction of the enzymes substrate-complex with the enzyme and product are inhibited. Without [BMIM][BF_4], the substrate concentration needed to achieve half of V_{max} (K_m) is 0.158 mM with a V_{max} of 0.268 mM/min. In the presence of [BMIM][BF_4], K_m and V_{max} is shift to 0.350 mM and 0.174 mM/min. AChE activity was inhibited in the presence of [BMIM][BF_4], which is consistent with the result of Fig. 1.

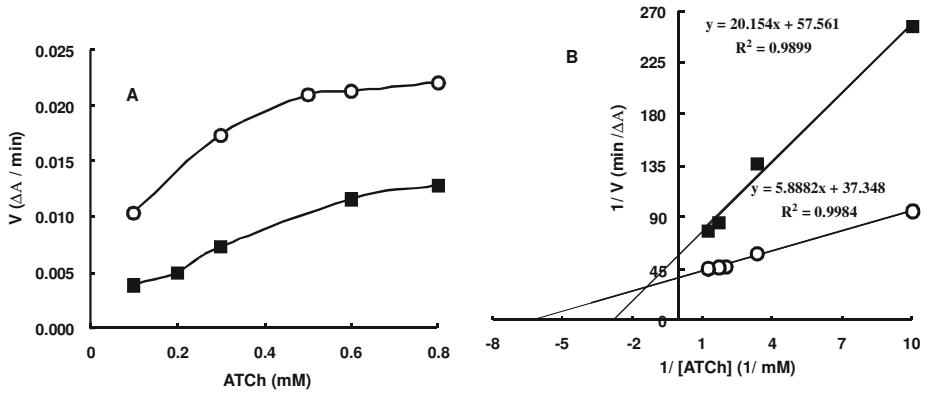

Fig. 2. (A) Enzymatic reaction rate *vs.* substrate concentration in the presence (○)/absence (■) of [BMIM][BF_4]; (B) Lineweaver-Burk plot of enzyme reaction in the presence/absence of [BMIM][BF_4]

According to Fig. 1, [BMIM][BF_4] improves the inhibition binding between AChE and parathion methyl. Although it also inhibits the catalytic activity of AChE, which is confirmed further by studying the effect of incubation time on inhibition efficiency by parathion methyl in the absence and presence of 5.1 % [BMIM][BF_4]. As shown in the Fig. 3, the inhibition rate increases along with the incubation time both in the absence and presence of 5.1 % [BMIM][BF_4]. The latter also shows higher inhibition rate than the former. The incubation time for complete inhibiton is 6 min in the absence of [BMIM][BF_4], but it only needs 4 min with the existence of [BMIM][BF_4].

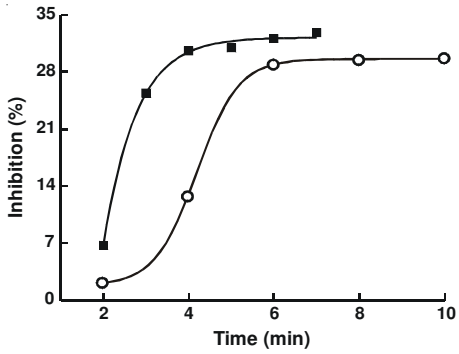

Fig. 3. Effect of incubation time on inhibition efficiency by 50 μg/mL parathion methyl in the absence (○) and presence of 5.1 % [BMIM][BF_4] (■)

Calibration curves of parathion methyl inhibiton on AChE with [BMIM][BF_4] or without [BMIM][BF_4] are shown in Fig. 4. Without [BMIM][BF_4], the calibration curve is sigmoidal as expected. The inhibition rate increases slowly within the low concentration range from 10^{-9} to 10^{-5} g/mL and does rapidly from 10^{-5} to 10^{-3} g/mL after which it doesn't increase again. Similar trend is observed for the calibration

curve of parathion methyl inhibiton on AChE with [BMIM][BF_4], but higher inhibition rates are given. It means that [BMIM][BF_4] improves the inhibition sensitivity of parathion methyl inhibiton on AChE. The inhibition rate to 1 μg/mL parathion methyl without [BMIM][BF_4] was measured three times and gave the average inhibition 15.24 ± 0.04 %. The inhibition rate to 10 μg/mL parathion methyl with [BMIM][BF_4] was measured three times and gave the average inhibition 29.88 ± 0.06 %.

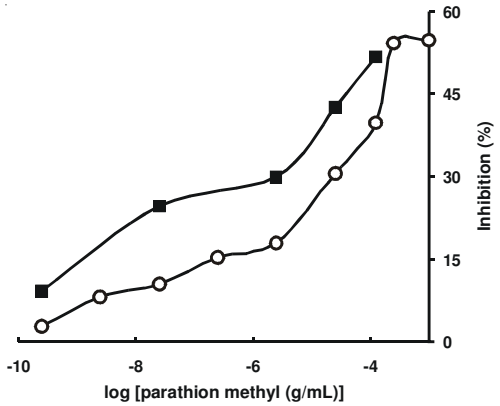

Fig. 4. Calibration curves of parathion methyl inhibiton on AChE with [BMIM][BF_4] (○) or without [BMIM][BF_4] (●) for 6 min inhibiton time

Conclusion

In this paper, the influence of [BMIM][BF_4] ionic liquid on AChE inhibition based assay was investigated. AChE activity was inhibited slightly by [BMIM][BF_4], but the inhibition degree by organophosphorous increased due to the addition of 5.1 % [BMIM][BF_4].

ACKNOWLEDGEMENT

This work was supported by National Natural Scientific Foundation of China, (Project No. 20975093).

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(*Received*: 21 October 2009; *Accepted*: 22 February 2010) AJC-8470