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A Highly Fluorescent Hydrophilic Ionic Liquid as a Potential Probe for the Sensing of Biomacromolecules

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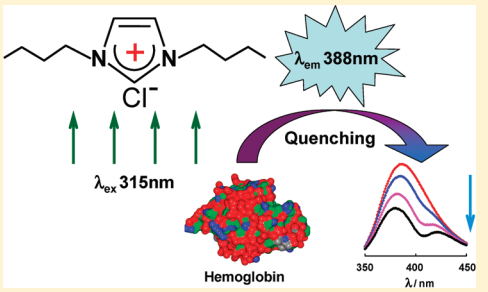
Supporting Information

ABSTRACT: With respect to the conventional imidazolium ionic liquids which generally create very weak fluorescence with quantum yields at extremely low levels of 0.005−0.02, a symmetrical hydrophilic ionic liquid 1,3-butylimidazolium chloride (BBimCl) was found to be highly fluorescent with λ_{em} at 388 nm when excited at λ_{ex} < 340 nm. The very high quantum yield of BBimCl in aqueous medium, derived to be 0.523 when excited at 315 nm, was attributed to its symmetrical plane conjugating structure. In the presence of hemoglobin, the fluorescence of BBimCl could be significantly quenched, resulting from the coordinating interaction between the iron atom in the heme group of hemoglobin and the cationic imidazolium moiety. This feature of the present hydrophilic ionic liquid makes it a promising fluorescence probe candidate for the sensitive sensing of hemoglobin. A linear regression was observed within 3×10^{-7} to 5×10^{-6} mol L^{-1} for hemoglobin, and a detection limit of 7.3×10^{-8} mol L^{-1} was derived.



1. INTRODUCTION

Ionic liquids (ILs), especially those based on substituted imidazolium cations, are drawing extensive interest because of their potential as a recyclable alternative to the conventionally volatile organic solvents due to their unique physicochemical properties, e.g., negligible vapor pressure, nonflammability, high chemical/thermal stability, low toxicity, favorable conductivity, and controllable hydrophobicity.[1−4] In order to fully explore the potentials of ILs, the knowledge concerning their fundamental properties is quite essential for utilizing and improving the performance of these materials in various chemical processes. Recently, researchers from a few groups have studied the physicochemical properties of imidazolium ionic liquids due to their unique spatial heterogeneity resulting from the inherent polar/nonpolar segregation.[5] The investigations on solvent strength and polarity of some imidazolium ionic liquids have demonstrated that [Bmim][PF_6], [C_8mim][PF_6], and [Bmim][NO_3] were more polar than acetonitrile.[6] The viscous properties of imidazolium ILs with cosolvents were also studied by exploiting the excimer-to-monomer intensity ratio and the fluorescence anisotropy of different fluorescent probes dissolved in these ILs.[7] Solvation dynamics in imidazolium ILs were reported to display a singular, ultrafast response, followed by a slower relaxation with nonexponential behavior.[8,9]

As for the optical characteristics of the imidazolium ILs, there are yet no consistent conclusions. The optical properties of [Bmim]-[PF_6] have been carefully investigated, showing that obvious absorption bands in the range 250−300 nm were observed even after strict purification of the ILs.[10] In order to make sure whether the residual absorption in this region was due to the presence of impurities or if this could be completely removed for a pure IL, a systematic study was done to examine the optical properties of [Bmim][PF_6], [Bmim][BF_4], and [Emim][BF_4] after careful purification to ensure that these ionic liquids are free from any impurities, particularly those that might contribute to the absorption in the wavelength region of interest. It was concluded that imidazolium ILs were characterized with significant absorption in the entire UV region and a long absorption tail that extends into the visible region, attributed to the imidazolium moiety and its various associated structures.[11] A further interesting finding is that these imidazolium ILs all exhibit fluorescence when excited in the UV or early part of the visible region, and the fluorescence behaviors depend strongly on the excitation wavelength.[12,13] This dependence was attributed to the presence of energetically different associated forms of the constituent ions of the ionic liquids and the slow relaxation of the excited state in these media.[14,15]

Although imidazolium ILs have been demonstrated to be fluorescent induced by the imidazolium moiety, their fluorescence efficiencies were very low; i.e., the measured fluorescence quantum yield of purified ILs ([Bmim][PF_6], [Bmim][BF_4], and [Emim][BF_4]) were estimated to lie in between 0.005 and 0.02 when excited at 360 nm. The ultraweak fluorescent imidazolium ILs can hardly find practical applications for fluorescence studies, especially as fluorescent probes in sensing biomacromolecues.[13] However, in our present study, an imidazolium-based IL with symmetrical plane conjugating structure, i.e., 1,3-butylimidazolium chloride (BBimCl, as illustrated in Scheme 1), was found to be highly fluorescent. Its fluorescence intensity could be significantly quenched after addition of hemoglobin, resulting from the coordination interaction between the iron atom in the heme group of hemoglobin and the cationic ionic liquid

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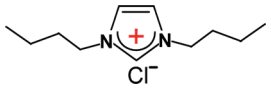
Scheme 1. Chemical Structure of 1,3-Butylimidazolium Chloride (BBimCl)



moiety. The high fluorescence efficiency and hydrophilic nature of the IL makes itself a promising probe candidate for the sensitive sensing of hemoglobin.

2. EXPERIMENTAL SECTION

2.1. Chemicals and Apparatus. *N*′-Butylimidazole and *n*-chlorobutane (Cheng Jie Chemical Co. Ltd., Shanghai, China) were used without further purification. Hemoglobin (Hb, H2500, Sigma, St. Louis, MO) was used as received. Other reagents used, including NaOH, HCl, acetone, acetonitrile, acetic ether, and ethanol (Sinopharm Chemical Reagent Co., Shanghai, China), were at least of analytical reagent grade unless specified, and 18 MΩ/cm deionized water was used throughout.

The fluorescence spectra were recorded by using an F-7000 fluorescence spectrophotometer (Hitachi High-Tech, Japan) equipped with a 1.0 cm quartz cuvette. The excitation and emission slits were both set at 5.0 nm, with a scan speed of 1200 nm min^{-1}. The fluorescence decay profiles were measured by a FluoroMax4 spectrofluorimeter (HORIBA Jobin Yvon Inc., France) with the time-correlated single-photon counting (TCSPC) technique. A 281 nm NanoLED was used as the excitation source. pH measurements were performed with an Orion 868 digital pH meter (Thermo Electron Co., USA). The absorption spectra were recorded on a U-3900 spectrophotometer (Hitachi high-tech, Japan) by using a 1.0 cm quartz cell.

2.2. Preparation of the Ionic Liquid 1,3-Butylimidazolium Chloride (BBimCl). 125.0 g of *N*′-Butylimidazole and 92.0 g of *n*-chlorobutane were added into a 500 mL three-necked round-bottom flask, and the mixture was stirred under argon protection for 48 h at 80 °C, until a pale yellow viscous product, i.e., BBimCl, was obtained. Afterward, the product was washed with 100 mL of acetic ether six times, followed by drying at 70 °C under a vacuum for 12 h. Prior to use, the obtained BBimCl was further decolorized/purified in a column packed with chromatographic silica gel (100−200 mesh), silica gel G (>260 mesh), and activated charcoal using absolute ethyl alcohol as the solvent, as described in the literature elsewhere.[16]

The ^{1}H NMR spectra of BBimCl recorded in D_2O were as follows: δ_{CH_3}, 6H, triplet, 0.85 ppm; δ_{CH_2}, 4H, quintet, 1.21 ppm; δ_{CH_2}, 4H, quintet, 1.76 ppm; δ_{CH_2}, 4H, triplet, 4.21 ppm; δ_H, 2H, singlet, 7.98 ppm; δ_H, 1H, singlet, 9.849 ppm.

3. RESULTS AND DISCUSSION

3.1. Fluorescence Behaviors of the Hydrophilic BBimCl. Figure 1 illustrated the fluorescence behaviors of the 100% BBimCl. It is obvious that the recorded fluorescence for the pure ionic liquid was strongly dependent on the excitation wavelength. This observation is consistent with those reported for the other imidazolium ionic liquids.[12] When excited at λ_{ex} < 340 nm, BBimCl exhibited an emission spectrum centering on 388 nm. However, the fluorescence maximum started to red-shift with progressive decreasing of the overall fluorescence intensity with a longer excitation wavelength at λ_{ex} > 340 nm. The existence of energetically different

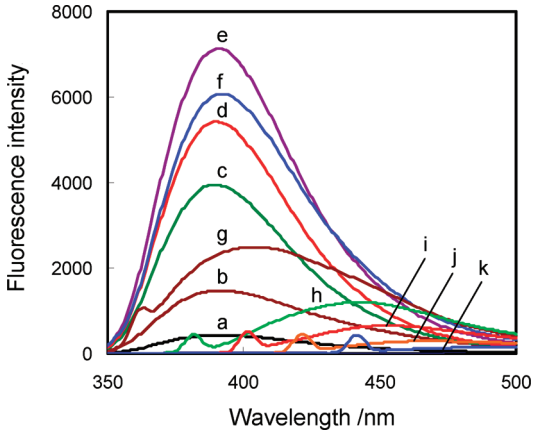


Figure 1. Excitation-wavelength-dependent emission spectra of pure BBimCl, excited at (a) 250 nm, (b) 270 nm, (c) 290 nm, (d) 320 nm, (e) 340 nm, (f) 350 nm, (g) 360 nm, (h) 380 nm, (i) 400 nm, (j) 420 nm, and (k) 440 nm.

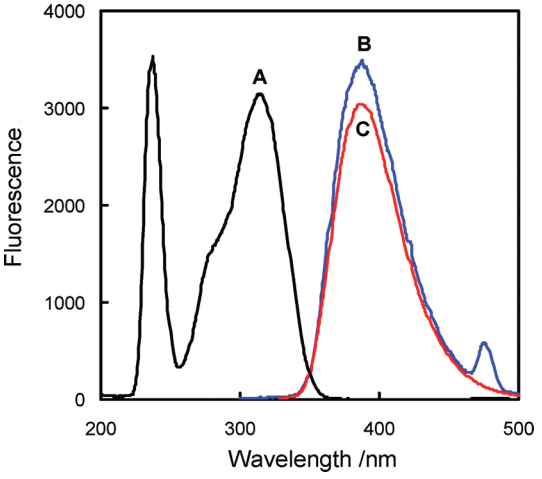


Figure 2. Fluorescence spectra of the BBimCl aqueous solution (0.01 mol L^{-1}): (A) the excitation spectrum; (B,C) the emission spectra excited at 240 and 315 nm, respectively.

associated species of imidazolium ILs was suggested to be accounted for by this excitation-wavelength-dependent phenomenon.[12]

Previous studies have demonstrated that the imidazolium ILs exhibit ultraweak fluorescence,[13] and the mechanisms for the creation of fluorescence have already been exploited.[11−13] Thus, in the present work, we will focus mainly on the unique highly intensive fluorescence nature of the BBimCl. Our experimental results further revealed that the aqueous solution of BBimCl was also highly fluorescent, as illustrated in Figure 2. It could be seen that two excitation bands (Figure 2A), with corresponding absorption bands in Figure 3, were observed and similar emission spectra with a maximum wavelength at 388 nm were obtained when excited at 240 and 315 nm, respectively (Figure 2B and C).

It has been demonstrated that the fluorescence of imidazolium ILs stems from the imidazolium moiety;[11] it is thus reasonable to ascribe the highly fluorescent property of the hydrophilic BBimCl to its symmetrical alkyl substitutes.[17] The imidazolium rings in the previously investigated imidazolium-based ILs, e.g., BmimX and EmimX (X = Cl, PF_6, BF_4), were all asymmetrical due to the different alkyl substitutes. In the case of BBimCl, the same substitutes of the alkyl group, i.e., butyl, linked to the N atoms in imidazolium

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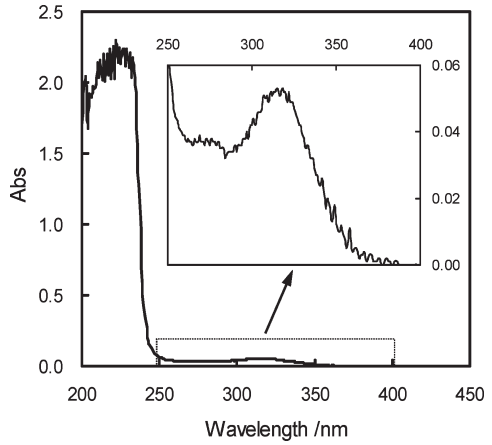

Figure 3. Absorption spectra of the BBimCl aqueous solution (0.1 mol L^{-1}) recorded by using a 10 mm cuvette.

ring resulted in not only an increase in the symmetry but also an increase in the degree of planarity and rigidity,[18] which contributed substantially to the improvement of the conjugations of the $\pi-\pi^*$ bond and eventually gave rise to a high fluorescence efficiency.

In spite of the excitation wavelength dependence when excited with a visible light, a fixed fluorescence maximum wavelength of 388 nm was observed for BBimCl when using a UV light beam, i.e., with a wavelength of below 340 nm, for the excitation. At the same time, its interesting feature of highly intensive fluorescence inspired us to explore the feasibility of adopting this IL as a useful fluorescence probe for the sensing of biomacromolecules.

3.2. The Effect of Ambience on the Fluorescent Behaviors of BBimCl. For solute molecules with different dipole moments in electronically excited state and ground state, excitation by photon would cause a redistribution of charges leading to conformational changes in the excited state, and eventually resulting in an increase or decrease of the dipole moment of the excited state as compared to the ground state, which is manifested by the solvatochromic shift of the emission maximum.[19]

The effects of solvent properties on the fluorescence Stokes shift ($\Delta\nu = \nu_{ex} - \nu_{em}$) of BBimCl were studied based on the Lippert−Mataga equation:

$$\Delta\nu = \frac{2}{hc}\cdot\Delta f\cdot\frac{(\mu_{ex}{}^* - \mu_g)^2}{\alpha^3} + \text{constant} \qquad (1)$$

$$\Delta f = \left(\frac{\varepsilon-1}{2\varepsilon+1} - \frac{n^2-1}{2n^2+1}\right) \qquad (2)$$

where $\mu_{ex}{}^*$ and μ_g are the dipole moments of the excited and ground states, h is Planck's constant, c is the speed of light, α is the radius of the cavity in which the BBimCl resides, and the orientation polarizability Δf is a function of dielectric constant (ε) and refractive index (n) of the solvent.

Table 1 summarizes the excitation and emission maximum wavelengths and the Stokes shift values for BBimCl in various solvents. It can be seen that remarkable Stokes shifts were obtained in all of the tested solvents, suggesting an obvious difference of the BBimCl dipole moments in the ground and excited states. The Stokes shifts were also increased with the increase of solvent orientation polarizability. In addition, the linear regression calculation from the Lippert−Mataga plot of BBimCl in different solvents (Figure 4) showed dependence with a slope of 3081.6 cm^{-1} for the aprotic solvent (acetonitrile, acetone, dichloromethane, trichloromethane, and toluene).

Due to the specific solvent−solute interactions (mainly hydrogen bonding) between BBimCl and protic solvents (H_2O and ethanol), no linear correlation was observed between the Stokes shift and the solvent orientation polarizability. The pronounced Stokes shift in water (5906 cm^{-1}) and ethanol (5003 cm^{-1}) implied that the BBimCl molecule is more sensitive to solvent polarizability in the presence of H-binding solvents.

Emission spectra of the BBimCl in dichloromethane containing various amounts of ethanol were shown in Figure 5. The addition of a small amount of ethanol results in substantial spectral shifts. 5% of ethanol or less causes a shift in the emission maximum from 350 to 380 nm. A further increase of the ethanol concentration up to 50% leads to only a minor additional shift to 384 nm. The specific spectral shift that occurred at low ethanol concentrations implied that the shift is a result of specific solvent effects induced by the H-bonding of ethanol to the imidazolium groups, rather than general solvent effects because the amount of ethanol added is too small to affect the refractive index or dielectric constant of the solvent. The effect of specific solvent−solute interactions induced by a H-bond was further convinced by the investigations on the fluorescence behaviors of BBimCl in dichloromethane containing cyclohexane. No emission spectral shift was observed when varying the cyclohexane content within a range of 0−50% (v/v), attributed to the fact that no H-bond was formed between cyclohexane and the imidalizolium moiety.

Figure 6 illustrated the pH dependence of the fluorescent emission of a BBimCl aqueous solution (0.01 mol L^{-1}) at 388 nm (λ_{ex} = 315 nm). A significant increase on the fluorescence intensity was observed by increasing the pH from 1 to 6. The fluorescence is thereafter leveled off within pH 6−9, followed by a sharp decline when further increasing the pH value up to 13. In the region of pH < 6, the majority of the IL moiety is believed to be existing in protonated form; thus, the formation of the intramolecular exciplex decreased substantially due to the inability of the protonated imidazolium groups to donate charge to the butyl acceptor group. In a more acidic medium, a red-shift of the emission maximum, i.e., from 388 nm at pH 6 to 406 nm at pH 1, is observed attributed to the heavy protonation of the imidazolium group.

The fluorescence lifetime and the fluorescence emission quantum yield are typical and important spectroscopic parameters of the singlet state,[20] and they depend strongly on the interaction of a molecule with its environment. Table 2 illustrates the fluorescence quantum yield of BBimCl in water, ethanol, and acetonitrile by using quinine sulfate as the standard fluorophore with a quantum yield of Φ_{std} = 0.55.[21] It showed great differences in the fluorescence quantum yield of BBimCl in various solvents. Higher quantum yields in protic solvent, i.e., 0.523 in water and 0.517 in ethanol, were achieved with respect to a low yield of 0.148 in aprotic acetonitrile, contributing to the H-bonding formation. It is obvious that the fluorescence quantum yield of BBimCl is much higher than those reported for the other imidazolium ILs, estimated at a level of 0.005−0.02.[11] These observations suggested that BBimCl could be an excellent candidate as a sensitive probe for biomolecules not only because of its highly fluorescent nature in protic solvent, e.g., water, but also for the convenience in practical applications by adopting water as a suitable solvent in addition to the fluorescence stability in neutral medium.

The temporal fluorescence behavior of BBimCl in aqueous medium (0.01 mol L^{-1}) was also investigated by exciting at 281 nm. The fluorescence decay profile was best fitted to a two-exponential decay function, as illustrated in Figure 7. It was found

Table 1. The Excitation/Emission Maximum Wavelengths and Stokes Shift Values for BBimCl in Various Solvents Together with Some of the Physical Parameters of the Solvents Used

solvent	λ_{ex}/nm	ν_{ex}/cm^{-1}	λ_{em}/nm	ν_{em}/cm^{-1}	$\Delta\nu$/cm^{-1}	ε	n	Δf
water	315	31746	387	25840	5906	80.4	1.333	0.320
acetonitrile	270	37037	310	32258	4779	37.5	1.344	0.305
acetone	325	30769	381	26246	4523	20.7	1.359	0.284
ethanol	320	31250	381	26247	5003	24.3	1.362	0.288
dichloromethane	280	35714	320	31250	4464	9.08	1.424	0.218
trichloromethane	316	31646	363	27548	4097	4.81	1.447	0.148
toluene	370	27027	431	23202	3825	2.38	1.497	0.015

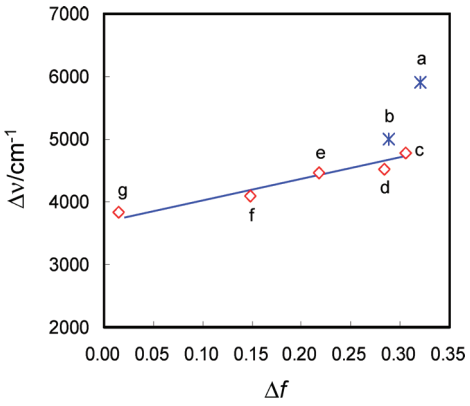

Figure 4. Lippert−Mataga plot of BBimCl in various solvents: (a) water; (b) ethanol; (c) acetonitrile; (d) acetone; (e) dichloromethane; (f) toluene; (g) trichloromethane.

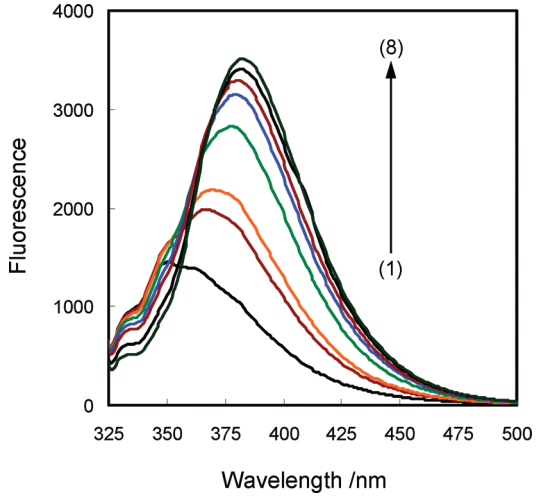

Figure 5. Emission spectra of BBimCl in dichloromethane−ethanol solutions, with ethanol contents (v/v) of (1) 0%, (2) 1.5%, (3) 2.5%, (4) 5%, (5) 10%, (6) 15%, (7) 30%, and (8) 50%.

that the major component (α_1, 96.7%) of the decay possessed a lifetime of 14.96 ns (τ_1), and that for the other component (α_2, 3.3%) was 7.269 ns (τ_2). The average lifetime (τ) was calculated to be 14.70 ns on the basis of the following equation:[22]

$$\tau = \frac{\alpha_1 \cdot \tau_1^2 + \alpha_2 \cdot \tau_2^2}{\alpha_1 \cdot \tau_1 + \alpha_2 \cdot \tau_2} \quad (3)$$

3.3. The Fluorescence Quenching of BBimCl by Hemoglobin.

Figure 8 illustrated the variation of the fluorescence intensity of BBimCl in aqueous medium as a function of hemoglobin (Hb) concentration. The fluorescence spectra were recorded using an excitation wavelength at 315 nm. The fluorescence quenching is described by the well-known Stern−Volmer equation:

$$\frac{F_0}{F} = 1 + k_q\tau_0[Q] = 1 + K_{SV}[Q] \quad (4)$$

F_0 and F are the fluorescence quantum efficiency in the absence and presence of Hb, K_{SV} is the Stern−Volmer quenching constant, K_q is the bimolecular quenching constant, τ_0 is the unquenched lifetime, and [Q] is the Hb concentration.

The Stern−Volmer curves at 293 and 313 K showed linear relationships. The quenching constants derived from the fitted results were 1.833×10^5 L mol^{-1} at 293 K and 1.718×10^5 L mol^{-1} at 313 K, with correlation coefficients of 0.9983 and 0.9995, respectively. The decline of the Stern−Volmer quenching constant K_{SV} with the increase of temperature implied that the quenching mechanism of the BBimCl−Hb interaction might be attributed to compound formation rather than dynamic collision.

The iron atom in the heme group of hemoglobin coordinates with four pyrrole nitrogen atoms of protoporphyrin IX and one nitrogen atom in imidazole of histidine,[23] thus leaving the sixth coordinating position of the iron atom vacant, which offers a potential to coordinate with other suitable ligands.[24] It has been proved that imidazole is a strong covalent coordinating ligand with an iron atom in the heme group.[25−27] Our previous investigations have also demonstrated that the free coordinating position of the penta-coordinated ferrous atom in the heme group of hemoglobin was able to interact with the cationic moiety of imidazolium ILs, which is the driving force for facilitating fast extraction of Hb with imidazolium IL.[28] In this respect, it is most probable that the quenching of BBimCl fluorescence in the presence of hemoglobin is due to the interaction/coordination reaction between the iron atom in the heme group of hemoglobin and the imidazolium cation.

The association constants (K_α) and the number of binding sites (n) can be derived from the regression curve on the basis of the following equation:[29]

$$\log\left(\frac{F_0 - F}{F}\right) = \log K_\alpha + n \log[\mathrm{Hb}] \quad (5)$$

F and F_0 are the fluorescence intensities of BBimCl in the presence and absence of Hb. K_α and n were derived to be 5.10×10^5, 2.40×10^5 and 1.09, 1.03 at 313 and 333 K, respectively. The obtained K_α values indicate that there is obviously a strong interaction between BBimCl and Hb.

In order to further verify this hypothesis, the fluorescence behaviors of BBimCl before/after addition of apohemoglobin, whose molecular structure is highly similar to those of hemoglobin but contains no heme group, were investigated. No variations

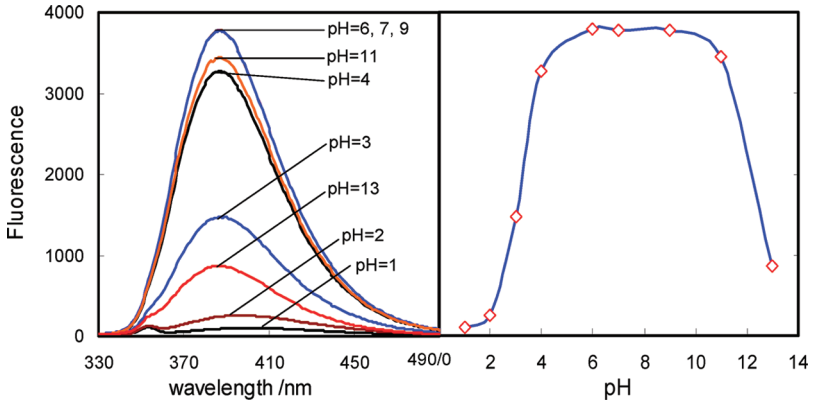

Figure 6. Effect of pH on the fluorescence intensity of BBimCl aqueous solution (0.01 mol L^{-1}). The spectra were recorded with excitation and emission at 315 and 388 nm, respectively.

Table 2. Fluorescence Quantum Yield of BBimCl in Various Solvents

	λ_{ex} (nm)	I	A	Φ
quinine sulfate	313	233130	0.012	0.55
BBimCl in water	315	277065	0.015	0.523
BBimCl in ethanol	320	209369	0.012	0.517
BBimCl in acetonitrile	320	61500	0.012	0.148

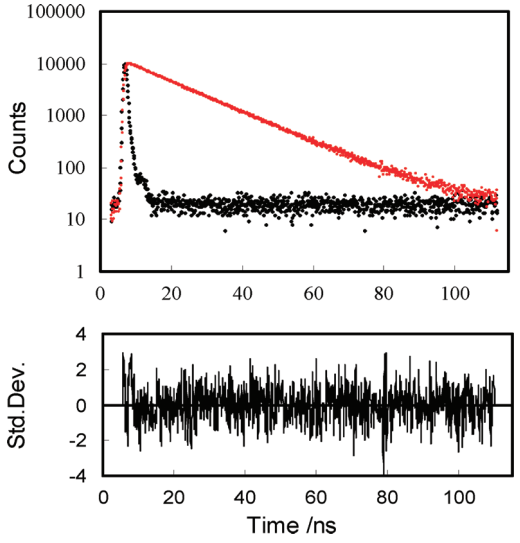

Figure 7. Fluorescence decay profile of BBimCl in a 0.01 mol L^{-1} aqueous solution: (top) the experimental decay curve; (bottom) the lamp profile. The bottom is the profile for the residuals with a χ^2 value of 1.02.

of the fluorescence of BBimCl were observed. This observation well demonstrated that the formation of a new complex between BBimCl and hemoglobin, e.g., the iron atom in the heme group, is the main reason for the alteration of the fluorescence.

Figure 9 showed the emission spectrum of BBimCl in aqueous medium (0.01 mol L^{-1}) and the absorption spectrum of the Hb (1.00 μmol L^{-1}). Fluorescence resonance energy transfer (FRET) is an electrodynamic phenomenon that occurs between the primarily excited molecule and its neighbors, and the distance dependence of FRET allows measurement of the distances between the donors and acceptors. The heavy overlap between the emission and absorption spectra indicated that excitation energy transfer might take place between BBimCl and Hb. The distance r between Hb and BBimCl was calculated by the following equation:[22]

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \quad (6)$$

E denotes the efficiency of transfer between the donor BBimCl and the acceptor Hb, F and F_0 are the fluorescence intensities of BBimCl in the presence and absence of Hb, and r is the average distance between BBimCl and Hb. R_0 is the critical distance when the efficiency of transfer is 50%, which was deduced by

$$R_0^6 = 8.79 \times 10^{-25} K^2 n^{-4} \varphi J \quad (7)$$

where K^2 is the orientation factor related to the geometry of the BBimCl and Hb of dipoles (usually $^2/_3$ for random orientation in fluid solution), n is the average refracted index of medium, φ is the fluorescence quantum yield of the BBimCl aqueous solution, and J is the effect of the spectral overlap between the emission spectrum of the BBimCl and the absorption spectrum of the Hb (Figure 9), which was calculated by the following equation:

$$J = \frac{\int_0^\infty F(\lambda)\varepsilon(\lambda)\lambda^4 \, d\lambda}{\int_0^\infty F(\lambda) \, d\lambda} \quad (8)$$

$F(\lambda)$ is the corrected fluorescence intensity of the BBimCl in the wavelength of λ, and $\varepsilon(\lambda)$ is the extinction coefficient of Hb at λ.

In the present case, n = 1.36 and φ = 0.523 for a BBimCl aqueous solution of 0.01 mol L^{-1}. J, R_0, and E were derived to be 4.48 × 10^{-13} cm^3 L mol^{-1}, 5.84 nm, and 0.13, respectively. Thus, r was deduced to be 8.02 nm. As the donor-to-acceptor distance was located in the 2−9 nm region,[22] the energy transfer from BBimCl to Hb was suggested to occur with high probability.

3.4. BBimCl as a Fluorescence Probe for the Sensing of Hemoglobin. The results in Figure 8 illustrated that the presence of a small amount of hemoglobin (Hb) causes significant fluorescence quenching of the BBimCl aqueous solution. This observation provides a promising potential by using BBimCl as a fluorescence probe for the sensing of biomacromolecules. Investigations on the effect of the concentration of BBimCl indicated that, in the presence of a certain amount of Hb, an increment of the relative fluorescence $(F_0 - F)/F_0$ was observed with an increase of the BBimCl concentration up to 0.01 mol L^{-1}. Afterward, $(F_0 - F)/F_0$ kept virtually constant when further

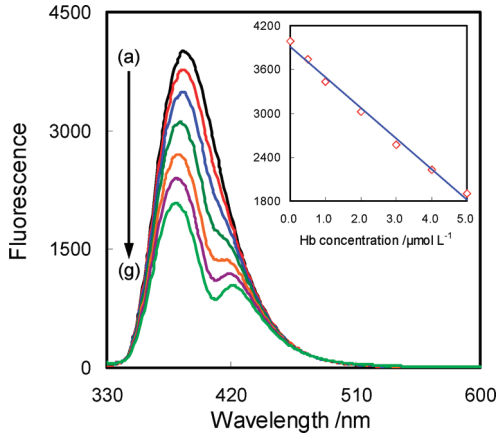

Figure 8. Fluorescence quenching of BBimCl by hemoglobin (T = 293 K, λ_{ex} = 315 nm). Hb concentration (μmol L^{-1}): (a) 0, (b) 0.5, (c) 1.0, (d) 2.0, (e) 3.0, (f) 4.0, (g) 5.0. BBimCl concentration in the aqueous solution: 0.01 mol L^{-1}.

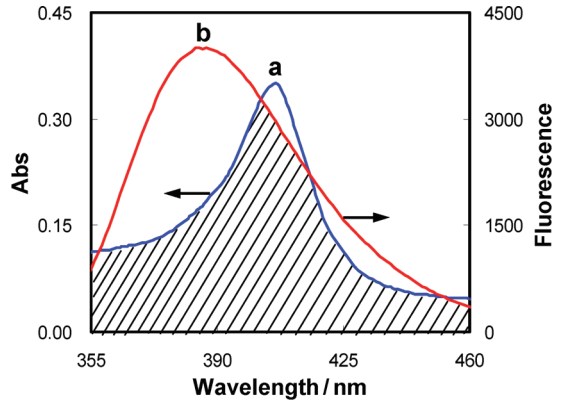

Figure 9. Spectral overlap of (a) hemoglobin absorption with (b) BBimCl fluorescence (T = 293 K). Hb: 1.00 μmol L^{-1}. BBimCl: 0.01 mol L^{-1}.

increasing the BBimCl concentration. Therefore, 0.01 mol L^{-1} BBimCl was adopted for the quantitative sensing of Hb. In this particular case, a detection limit of 7.3 × 10^{-8} mol L^{-1} was derived for the detection of hemoglobin within a concentration range of 3 × 10^{-7} to 5 × 10^{-6} mol L^{-1}. Further experiments have indicated that the presence of 5-, 10000-, 5000-, 50000-, 1000-, and 100-fold BSA, glucose, urea, Na^+, Ca^{2+}, and ATP causes no interfering effect on the quantification of hemoglobin within an error range of ±5%. The fluorescence data for 0.01 mol L^{-1} BBimCl in the presence of these species in the quantification of Hb are provided in Table S1 of the Supporting Information.

4. CONCLUSIONS

The hydrophilic ionic liquid, i.e., 1,3-butylimidazolium chloride (BBimCl), was proven to be highly fluorescent, giving rise to a fluorescence quantum yield of 0.523 in a BBimCl aqueous solution of 0.01 mol L^{-1}. This could be attributed to the unique symmetric structure of the ionic liquid. The presence of hemoglobin results in significant static quenching of the fluorescence of BBimCl, probably attributed to the interaction/coordination between the iron atom in the heme group of hemoglobin and the imidazolium cation in the symmetric ionic liquid. The present investigations not only provide useful information on the relationship between the structure of the ionic liquid and its fluorescence nature but also offer a promising probe candidate for the sensitive or even selective sensing of biomacromolecules.

■ ASSOCIATED CONTENT

Supporting Information. Table showing the fluorescence data for 0.01 mol L^{-1} BBimCl. This material is available free of charge via the Internet at http://pubs.acs.org.

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