SPECTROSCOPIC STUDIES ON THE INTERACTION BETWEEN IMIDAZOLIUM IONIC LIQUID AND HUMAN SERUM ALBUMIN

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Introduction

Human serum albumins (HSA) are the most abundant proteins in the circulatory system of human. Many drugs and other small molecules bind reversibly to albumin and other serum components, which then function as carriers[1,2]. Consequently, it is important to study the interactions of small molecules with this protein. It has been confirmed that ILs were toxic to aquatic organisms, animals and human cells[3,4,5]. Here the interaction between ionic liquid (1-butyl-3-methylimidazolium bromine, [Bmim][Br]) and HSA were investigated with spectroscopy including fluorescence emission, UV absorption spectra and circular dichroism (CD) spectra. The quenching mechanism of fluorescence of HSA by [Bmim][Br] was discussed. The binding constant (*K*) and the number of binding sites (*n*) was measured by fluorescence quenching method. The interaction between [Bmim][Br] and HSA were obtained from UV absorption spectra and CD spectra.

Materials and methods

The fluorescence quenching measurements were carried out by Shimadzu 2501 spectrofluorometer. Fluorescence spectra were recorded in the range of 300-450 nm at the excitation wavelength of 295 nm at 293K. The solution, containing 5.0×10^{-6} mol L^{-1} HSA, was titrated by successive additions of [Bmim][Br] (from 1.25×10^{-6} to 7.5×10^{-6} mol L^{-1}). The accumulated volumes were 0, 5, 10, 15, 20, 25, 30, and 35 μL. All measurements were performed in triplicate.

The UV−vis absorption spectra were recorded by using a Shimadzu UV−2450 spectrophotometer with the wavelength range of 200-350 nm. The operation were carried out at room temperature. Tri-HCl buffer solution was used to sweep baseline, and a 2 ml of HSA (2×10^{-6} mol L^{-1}) was titrated with [Bmim][Br] solution (2×10^{-6} mol L^{-1}), respectively.

CD measurements were carried out with a J-810 spectropolarimeter (Jasco, Tokyo, Japan) with 1.0 cm path length at 293K in a thermostat bath and repeated four times for each sample. CD spectra were recorded in a wavelength range of 200–250 nm with the scan band width (1 nm) and scan rate (500 nm/min). The [Bmim][Br] concentration were 2×10^{-6} mol L^{-1} at pH 7.4 under a constant nitrogen flow, which was used to purge the ozone generated by the light source of the instrument. The spectra was controlled by Jasco's Spectra Manager TM software of the instrument.

Results and discussion

At the excitation wavelength of 295 nm, the fluorescence quenching spectra of HSA with [Bmim][Br] were shown in Fig. 1. It was obvious that HSA had a strong fluorescence emission peak at 350 nm after being excited with a wavelength of 280 nm. The fluorescence intensity of HSA gradually decreases as increasing the concentration of [Bmim][Br]. Moreover, there was no slight shift at the maximum emission wavelength of HSA after gradually addition of [Bmim][Br]. This result indicates that the addition of [Bmim][Br] could influence the hydrophobic of tryptophan residue[6].

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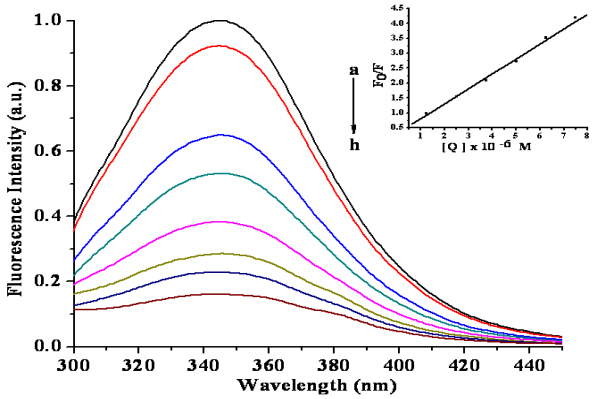

Fig.1. The fluorescence quenching spectra of HSA in the presence of various concentrations of [Bmim][Br]. The concentration of [Bmim][Br] corresponding to 0, 1.25, 2.5, 3.75, 5, 6.25, 7.5 and 8.75× 10^{-6} mol L^{-1} from curve a–h; C_{HSA} = 5.0 × 10^{-6} mol L^{-1}; T = 293K; λ_{ex} = 295nm; pH = 7.4.

Fluorescence quenching can be dynamic, resulting from collisional encounters between the fluorophore and quencher, or static, resulting from the formation of a ground state complex between the fluorophore and quencher[7]. Static and dynamic quenching can be distinguished from their different binding constants dependenct on temperature and viscosity, or preferably by lifetime measurement[8].

In order to demonstrate the quenching mechanism induced by [Bmim][Br], we assumed that the process belongs to dynamic quenching. The fluorescence quenching data were analyzed according to the Stern-Volmer equation (1)[9]

$$\frac{F_0}{F} = 1 + K_q \tau_0 [Q] = 1 + K_{SV}[Q] \qquad (1)$$

where, F_0 and F are the relative fluorescence intensities in the absence and presence of quencher, respectively; K_q is the quenching constant of the biological macromolecule and $K_q = K_{SV}/\tau_0$, where τ_0 is the average biomolecular life-time without quencher and the fluorescence lifetime of the biopolymer is 10^{-8} s[10], K_{SV} is the Stern–Volmer quenching constant and [Q] is the concentration of quencher[11]. The biomolecular quenching constant K_q at pH 7.40 can be obtained by linear Stern-Volmer plots of F_0/F against [Q] of [Bmim][Br] at room temperatures, when [Q] ranged from 1.25 × 10^{-6} mol L^{-1} to 7.5 × 10^{-6} mol L^{-1} of [Bmim][Br]. The corresponding dynamic quenching constants (K_{SV}) for the interaction between ILs and HSA are 1.68×10^4 L·mol^{-1} (293 K, R=0.9978). Accordingly, the value of K_q was much larger than maximum scatter collision quenching constant of the biomolecule (2.0×10^{10} L·mol^{-1} ·s^{-1})[12], which indicated that the possible quenching mechanism of fluorescence of HSA by [Bmim][Br] was a static quenching procedure[13]. Namely, the fluorescence quenching of HSA results from complex formation predominantly. Further the type of interaction between HSA and [Bmim][Br] was also confirmed by UV-visible absorption spectra.

For static quenching, small molecules bind independently to a set of equivalent sites on a macromolecule, Eq.(2) can be used to estimate the binding constant of ILs-HSA complex[14].

$$\frac{\log(F_0 - F)}{F} = \log K + n\log[Q] \qquad (2)$$

Where K is the binding constant, n is the number of binding sites, and [Q] is the concentration of [Bmim][Br]. According to Eq. (2), the values of K and n can be acquired using the double–logarithm algorithm curve (log[(F_0-F)/F]) vs. log[Q]) as shown in Fig.2. Thus we can calculate binding constant K as 2.64× 10^4 mol L^{-1} and binding sites n (0.93) of [Bmim][Br] with HSA from the intercept and slope of Fig.2 (R=0.9994).

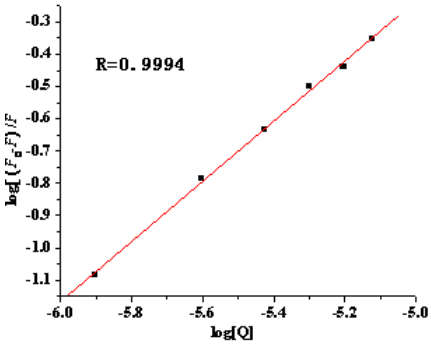

Fig. 2. The plots of log [(F0-F)/F] versus log[Q]. $C_{HSA} = 5.0 \times 10^{-6}$ mol L^{-1}; $T = 293K$; $\lambda_{ex} = 295nm$; pH = 7.4.

As shown in Fig. 3, the maximum absorption peak of HSA and [Bmim][Br] appeared at 215nm and 205nm, respectively. With the addition of [Bmim][Br], a new peak appeared at 220nm and the absorption intensity decreases obviously compared with absorbance of HSA only. The change of the UV absorption spectrum may be due to the formation of HSA-[Bmim][Br] complex[15], which meaned that the static fluorescence quenching type of HSA initiated by [Bmim][Br].

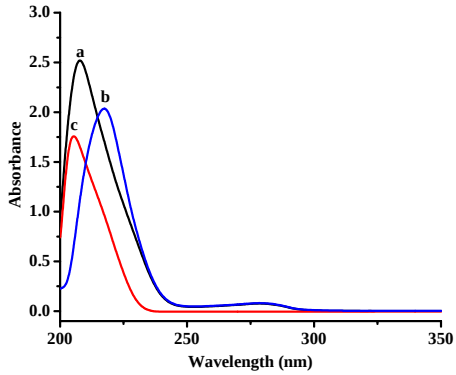

Fig. 3. UV-visible absorption spectra of [Bmim][Br], HSA and [Bmim][Br]-HSA system: (a) the absorbance spectrum of HSA only; (b) the absorbance spectrum of [Bmim][Br]-HSA when the mol ratio is 1:1 and (c) the absorbance spectrum of [Bmim][Br] only. $C_{HSA} = 2.0 \times 10^{-6}$ mol L^{-1}; $c_{[Bmim][Br]} = 2.0 \times 10^{-6}$ mol L^{-1}.

CD spectrum is particularly sensitive to secondary structure of protein[16] and so here was used to explore the structural changes of HSA by the addition of [Bmim][Br] at pH 7.40. The effect of spectrum of HSA in the absence and in the presence of [Bmim][Br] was shown in Fig. 4. As the experimental data indicate, the CD spectrum of HSA alone exhibits negative ellipticity below 250 nm and gives no signal above this wavelength. The free and asymmetric of HSA exhibited two negative bands in the UV region at 208 and 222 nm, which were both contributed to *n*-π* transition in the peptide bond of α-helical[17]. It was observed that CD bands decreased with the interaction of [Bmim][Br] and HSA, which clearly indicating the decrease of the α-helical content in protein. It was evident that interaction of [Bmim][Br] with HSA causes a slight conformational change of the protein, leading a loss of α-helical stability. A quantitative analysis exhibited a reduction of α-helical structures from 52.9% to 51.5% at the molar ratio [Bmim][Br] /HSA of 1:1.

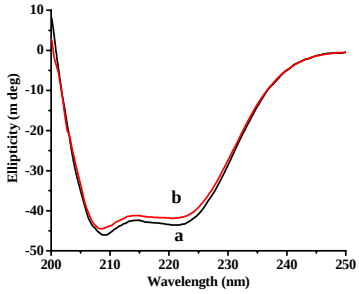

Fig.4. CD spectra of the HSA and [Bmim][Br]-HSA system: (a) the CD spectra of HSA only; (b) the spectrum of

[Bmim][Br]-HSA when the mol ratio is 1:1.*T* = 293K; pH = 7.4

Acknowledgements
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