The photophysical characteristics of 2-butylamino-2-demethoxy-hypocrellin B

Hua-yang Lee a, Zhi-xiang Zhou b, Shen Chen a, Man-hua Zhang a, Tao Shen a,∗

a Key Laboratory of Photochemistry, Center for Molecular Science, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, PR China
b Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, PR China

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Abstract 2-Butylamino-2-demethoxy-hypocrellin B (BAHB), is an efficient photodynamic sensitizer, excited singlet-state energies are determined from the overlap of the normalized from absorption and emission spectra to be 176.4 kJ mol−1 (ES CT) and 202.8 kJ mol−1 (ES 1), respectively. The quantum yields of BAHB fluorescence are 0.0098 (Φ f1) and 0.0076 (Φ f2), respectively. The energies and quantum yields of excited triplet states of BAHB are examined from the external effect of heavy atom on its fluorescence emission in the mixture of n-propyl iodide and benzene. The energies of excited triplet states of BAHB are 134.4 ± 10.5 kJ mol−1 (E T CT) and 158.6 ± 10.5 kJ mol−1 (E T 1), respectively. The quantum yields of excited triplet states of BAHB are 0.73 ± 0.04 (Φ T CT) and 0.56 ± 0.04 (Φ T 1), respectively. Meanwhile, the kinetic constants of fluorescence emission, intersystem crossing and internal conversion are also calculated from relative equations.

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1. Introduction

Amino-substituted hypocrellins are new kinds of efficient photodynamic sensitizers. Especially, 2-butylamino-2-demethoxy-hypocrellin B (BAHB) has shown the perfect photoinduced anticancer ability to tumor cells, such as Hela cancer cell, murine ascitic hepatoma cells, human pancreatic cancer cell and line Capan-1 cells[1–4]. Wu et al. [1,3] has reported the photopotentiation factor of BAHB for killing Hela cancer cell is 200–400-fold and much higher than that of HB (only 10-fold for HB). The quantum yield of O2(Δg) generated by BAHB is only 56% of HB and the ability of O2− generation by BAHB is 2.7 times stronger than that of HB. Although the investigation of the mechanism of the excellent photodynamic action of BAHB is undergoing, it is believed that both O2(Δg) and O2− are involved in the photodynamic action of BAHB. It is well known that the generation of O2(Δg) by BAHB is through the energy transfer process from the excited triplet state of BAHB to ground state of oxygen and the generation of O2− by BAHB is originated from the electron transfer between semiquinone anion radical and ground state of oxygen (the semiquinone anion radical is also produced from its excited state). The electron and energy transfer abilities of BAHB in its photodynamic mechanism have intimate relationship with its energies and quantum yields of excited state[3]. However, so far, little has been reported about the photophysical characteristics of excited states of BAHB, especially, excited triplet states, since BAHB does not exhibit appreciable phosphorescence even at low temperature. Hu and Jiang reported an efficient method for determination of triplet state energy and quantum yield of HB by the effect of external heavy atom on its fluorescence and singlet oxygen quantum yields [8]. In the present work, using this method along with the examination of absorption and fluorescence spectra, for the first time, we reported the energies and quantum yields of the excited states of BAHB and the kinetic constant of fluorescence emission, intersystem crossing and internal conversion.

2. Materials and methods

2.1. Chemicals

2-Butylamino-2-demethoxy-hypocrellin B was synthesized by the amination of HB (Scheme 1) and characterized...
by IR, $^1$H-NMR, $^{13}$C-NMR, FAB-MS, and UV-Vis spectrometers [1,5]. 9,10-Diphenyl-anthracene (DPA, A.R.) was purchased from Aldrich, USA. $\pi$-Propyl iodide (C.P.), benzene (A.R.) and other solvents were purchased from Beijing Chemical Plant China, and further purified before use.

2.2. Measurements

2.2.1. Spectrometer measurement

Ultraviolet-visible (UV-Vis) absorption spectra were recorded on a Shimadzu UV-160-A spectrometer; fluorescence emission spectra were measured on HITACHI-F4500 spectrometer, and lifetime of fluorescence was determined on HORIBA NAES-1100 time-resolved fluorescence spectrometer.

2.2.2. Determination of the quantum yield of singlet oxygen

The photooxidation of 9,10-DPA to its endoperoxide derivative by singlet oxygen (Scheme 2) is usually used to detect singlet oxygen generated from photosensitizers [6]. In order to determine the quantum yield of $O_2$ ($^{1}A_g$) generated by BAHB, we used 9,10-DPA bleaching method with HB in benzene solution as standard. Samples were placed in the series of Pyrex glass cells of the same specification, and irradiated with a 500 W tungsten–halogen lamp on a marry-go-around apparatus. Filters are combined to select wavelengths around 460 and 600 nm, respectively, in which only the sensitizers absorb significantly. The concentration of samples was adjusted to give the same optical density at the selected wavelength (460 or 600 nm). The reaction was followed spectrometrically via observing the decrease in

374 nm absorption peak of DPA (where BAHB and $\pi$-propyl iodide have the smallest absorption) as a function of irradiation time. The irradiation time was controlled so that the conversion rate for the BAHB-sensitized photo-oxidation of DPA was within 5–10%.

2.2.3. Determination of the energy and quantum yield of excited state

The energies and quantum yields of excited singlet state of BAHB were investigated through the measurements of its absorption and fluorescence spectra in benzene solution. Based on the Stoke’s shift, the energy gap of zero vibration energy level, $E_{0\rightarrow0}$ between $S_{\text{CT}}$ and $S_0$ was measured by the curve crossing point of absorption and emission spectrum (excited wavelength is 620 nm), and also the energy gap of zero vibration energy level of $E_{1\rightarrow0}$ between $S_1$ and $S_0$ was measured by the curve crossing point of excitation spectra (detected wavelength is 625 nm) and fluorescence emission spectra (excited wavelength is 460 nm) [7]. The fluorescence quantum yield of BAHB was measured and calculated according to the standard of HB ($\Phi_0 = 0.058$ in benzene) [6]. The concentration of BAHB and standard solution was adjusted to the same absorbance at excited wavelength (460 nm). The dissolved oxygen was removed by bubbling with high purity nitrogen for 30 min before measurement.

The energies and quantum yields of excited triplet-state of BAHB were estimated through the heavy atom effect on its fluorescence emission and quantum yields of singlet oxygen generation in benzene solution, and the DPA bleaching method [6,8] was used to examine the generation quantum yields of singlet oxygen.

A set of solutions consisting of BAHB and CH$_3$CH$_2$CH$_2$I (BAHB 1 x $10^{-3}$ M, CH$_3$CH$_2$CH$_2$I 10.5x M, x is the multiple of 0.5) in benzene were used to determine the relative quantum yields of fluorescence at 611 and 732 nm, respectively. A set of solution consisting of BAHB (0.8 x $10^{-3}$ M), DPA (1.2 x $10^{-3}$ M) and CH$_3$CH$_2$CH$_2$I (0.5x M, x is the multiple of 0.5) in benzene was carefully prepared to ensure the apparent external heavy atom effect could be observed, the relative quantum yields of singlet oxygen generated by the sensitizer were determined at two different wavelength bands: around 460 nm or around 600 nm by the
DPA-bleaching method. The energies of BAHB triplet states (T_C and T_1) were estimated via the rate constant of BAHB fluorescence quenching by CH_3CH_2CH_2I in benzene solution [9]. The quantum yield of triplet states of BAHB was measured by the relationship between the relative fluorescence quantum yield of BAHB and the relative quantum yield of singlet oxygen generated by BAHB in the different CH_3CH_2CH_2I concentration of benzene solutions [8].

3. Results and discussions

3.1. Characteristics of the excited singlet state

The absorption spectrum of BAHB is shown in Fig. 1A (Curve a). Based on the study of absorption spectrum of HB (Fig. 1A (Curve c)) [2,5]. The absorption of shorter wavelength at 463 nm (log ε = 4.06) is assigned to the π-π* transition and the absorption of the longer wavelength at 583 nm (log ε = 4.10) was in relation to intramolecular proton transfer between peri-hydroxyl and quinone groups, which may take on some active role in the photodynamic activity. Compared with HB, the absorption of BAHB at the longer wavelength is enhanced obviously, and raises a new absorption at 621 nm (log ε = 4.09), which resulted from the introduction of a butylamino group into the perylenequinone ring. Its curve shapes of fluorescence emission excited respectively at 460 and 620 nm are apparently different: when excited at 460 nm, the fluorescence spectrum shows double emission peaks with maxima at 613 and 733 nm (Fig. 1B (Curve d)), compared with the fluorescence spectrum of HB [10,11], the shorter wavelength emission of BAHB is similar to the emission of peri-hydroxyl perylenequinone group in HB caused by intramolecular proton transfer, while the longer wavelength emission of BAHB is a new peak caused apparently by the introduction of a butylamino group into perylenequinone ring Fig. 1A and 1B (Curves c and d)). The excitation spectra at different detecting wavelengths, 625 and 725 nm, respectively, are shown in Fig. 1B (Curves e and f). Two different excitation spectra belong to different fluorescence excited states of BAHB. When excited at 460 nm, the dual fluorescence emission is related to two excited fluorescence states, S_C and S_I [12,13]. The energy level of S_C and S_I can be estimated by the crossing points of absorption (or excitation) and fluorescence spectra. They are listed in Table 1. The fluorescence quantum yields of S_I and S_C are determined using the standard HB in benzene via Eq. (1) and listed in Table 1.

\[
\Phi_f^X = \frac{F_X}{A_X} \quad \Phi_f^S = \frac{F_S}{A_S}
\]

\[
\Phi_f^X \text{ and } \Phi_f^S \text{ are the fluorescence quantum yields of BAHB and the standard HB, respectively.}
\]

\[
A_X \text{ and } A_S \text{ are the absorptions at the excitation wavelength (460 and } 620 \text{ nm) of the BAHB and standard HB, respectively.}
\]

\[
F_X \text{ and } F_S \text{ are the area of the fluorescence peaks of the BAHB and the standard HB, respectively, at the same sensitivity scale.}
\]

Table 1. Photophysical characteristics of singlet state of BAHB along with those of HB [7,8,10] are listed in Table 1. Judged from Table 1, in contrasted with HB, the fluorescence quantum yields of BAHB reduced obviously. It is known that the fluorescence of HB mainly results from the intramolecular proton transfer between peri-hydroxyl and perylenequinoid carbonyl groups. When a butylamino group was introduced into perylenequinone structure, it reduces the intramolecular proton transfer between quinone and perihydroxyl group, and in the consequence, reduces the...
fluorescence quantum yield. Moreover, the electron donating properties of amino group may cause some extent of intramolecular charge transfer (ICT) between the butylamino and quinonoid carbonyl groups, which provides a pathway for fluorescence quenching via radiationless transition that also reduces the fluorescence quantum yields of BAHB.  

3.2. Determination of the energy of triplet state  

Intersystem crossing process will be accelerated in presence of heavy atom in solution system such as iodine and bromine [6]. This leads to the fluorescence quenching of fluorophore by heavy atom and the increase of quantum yield of triplet state. When adding CH₃CH₂CH₂I gradually to BAHB in benzene solution (1.07 × 10⁻³ M), the fluorescence quantum yield of BAHB varied with the concentration of CH₃CH₂CH₂I that satisfied the Stern–Volmer bimolecular kinetic constant logK₂ have linear relationship with ΔE₀ (i.e. ΔE₀ is the energy gap of triplet and singlet state). Based on the relationship mentioned above, we can determine the ΔE₀ of BAHB to be 42.0 ± 10.5 kJ mol⁻¹ for S_CT → T_CT and 44.21 kJ mol⁻¹ for S₁ → T₁. Since the S_CT and S₁ energy level of BAHB are 176.43 and 202.8 kJ mol⁻¹, the T_CT and T₁ energy levels of BAHB are 134.4 ± 10.5 and 158.6 ± 10.5 kJ mol⁻¹, respectively.
The efficiency of the energy transferring of BAHB. In our experimental conditions, a reasonable as-

selected irradiation wavelength (460 or 600 nm), respectively, which were absorbed only by the BAHB; the reaction was followed spectrometrically by observing the decrease in the 374 nm absorption peak of DPA as a function of irradiation time (Scheme 2), where the samples have the smallest absorption. Figs. 3 and 4 indicate that the quantum yields of singlet oxygen generated by BAHB increase obviously in the presence of heavy atom. The relative quantum yields against the BAHB solution without CH3 CH2 CH2 I were measured from the ratio of the line slope.

Those results furthermore demonstrate that CH3CH2CH2I accelerated the intersystem crossing procedures of S1 → T1 resulting in quenching of the fluorescence of BAHB triplet state. Eq. (2) was used to estimate the quantum yields of triplet states of BAHB.

Φ = Φ0

Φ is the quantum yield of triplet state in the presence of heavy atom and in the absence of it, respectively. The values of Φ0/Φ1 can be repeated within 3–5%.

from the BAHB triplet state to ground oxygen that produces singlet oxygen, does not vary with the adding heavy atom (Eq. (2)). The quantum yield of singlet oxygen generated by BAHB only depends on the quantum yields of BAHB triplet states. Eq. (2) was used to estimate the quantum yields of triplet states of BAHB.

Table 2

<table>
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<th>λ (nm)</th>
<th>The concentration of heavy atom (mol·l−1)</th>
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<tbody>
<tr>
<td></td>
<td>0.5</td>
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<tr>
<td>460</td>
<td>1</td>
</tr>
<tr>
<td>600</td>
<td>1</td>
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Notes: Φ0 and Φ1 are the quantum yields of triplet state in the presence of heavy atom and in the absence of it, respectively. The values of Φ0/Φ1 can be repeated within 3–5%.

Table 3

<table>
<thead>
<tr>
<th></th>
<th>Φ1</th>
<th>Energy level (kJ·mol−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAHB</td>
<td>0.73 ± 0.04 (T1)</td>
<td>134.4 ± 10.5 (T2)</td>
</tr>
<tr>
<td></td>
<td>0.58 ± 0.04 (T1)</td>
<td>158.6 ± 10.5 (T2)</td>
</tr>
<tr>
<td></td>
<td>0.76 (T1)</td>
<td>168.0 (T2)</td>
</tr>
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Table 2

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<tr>
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<th>Φ1</th>
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</tr>
<tr>
<td></td>
<td>0.76 (T1)</td>
<td>168.0 (T2)</td>
</tr>
</tbody>
</table>

Notes: Φ1 is the quantum yield of triplet state.
According to Eq. (4), measuring \( \Phi_f / \Phi_f^0 \) at different concentrations of \( \text{CH}_3\text{CH}_2\text{CH}_2\text{I} \) irradiated at 460 or 600 nm in benzene as a function of the numerator of Eq. (4) will give the consequences of the quantum yields of two triplet states of BAHB from the slopes of line (Figs. 5 and 6). The quantum yield of \( T_1 \) is \( 0.73 \pm 0.04 \) and \( T_2 \) is \( 0.35 \pm 0.04 \) are shown in Figs. 5 and 6, respectively.

### 3.4. Calculating the kinetic constants of fluorescence emission, intersystem transition and internal conversion

Based on the data of research described above, we can estimate kinetic constants of fluorescence emission, intersystem crossing and internal conversion with following formula [6,7]:

\[
\Phi_{IC} = 1 - \Phi_f - \Phi_T \quad (5)
\]

\[
\Phi_f = \kappa_f \tau_S \quad (6)
\]

\[
\Phi_T = \kappa_{ST} \tau_S \quad (7)
\]

\[
\Phi_{IC} = \kappa_{IC} \tau_S \quad (8)
\]

Here, \( \Phi_{IC} \) is the quantum yield of internal conversion; \( \Phi_f \) is the quantum yield of fluorescence; \( \Phi_T \) is the quantum yield of triplet state; \( \kappa_f \), \( \kappa_{ST} \) and \( \kappa_{IC} \) are kinetic constants of fluorescence emission, intersystem crossing and internal conversion, respectively, \( \tau_S \) is the lifetime of fluorescence.

The calculated results are listed in Table 4 and the energy level and photophysical transitions are summarized in Fig. 7.

### 4. Conclusions

In our work, by the measurement of absorption and fluorescence spectra of BAHB under the external heavy atom effect of \( \text{CH}_3\text{CH}_2\text{CH}_2\text{I} \), we determined a series of physical characteristics of BAHB excited singlet and triplet states. It will be important for understanding the mechanism of photodynamic action of BAHB.

### Acknowledgements

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References