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Preparation of a novel hypocrellin derivative and its photochemical, photophysical properties

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Abstract

A novel hypocrellin derivative, 17-1,3-diaminopropane-hypocrellin B Schiff base (DAHB), was designed and synthesized. The chemical modification greatly enhanced the absorbance at solid tumor phototherapeutic window (600–900 nm). Some photochemical and photophysical parameters of DAHB were determined. The weak basicity of DAHB itself may make it have stronger affinity to acidic tumor cells than its parent HB.

Keywords: Hypocrellin derivative; Phototherapeutic window; Quantum yield; Intramolecular H proton transfer

1. Introduction

The naturally occurring perylenequinonoid pigments, hypocrellins, are well known for their antitumor and antiviral activities [1–8]. Hypocrellins were proved to have a special superiority for phototherapy to some kinds of vas capillary diseases, such as port wine stains and age-related macular degeneration (AMD) [9], occurring at the shallow surfaces no more deeper than 1 mm which coincides with the penetration depth of the light (450–550 nm) absorbed mainly by hypocrellins. On the other hand, the absorbance of hypocrellins is very low on the phototherapeutic window (600–900 nm) of solid tumors, which is a problem for clinical application [10,11]. A lot of work has been done in order to enhance their red absorbance [12–15]. It was reported that aminated hypocrellins [16,17] could realize the strong red absorption and good retention in tumor cells due to the weakly basic property [18]. As known, 1,3-diaminopropane is a double end-amino compound, so it is possible to gain hypocrellin derivatives with good absorbance at phototherapeutic window and stronger basicity due to the exposed amino group. Taking account of this, an aminated hypocrellin derivative, 17-1,3-diaminopropane-hypocrellin B Schiff base (DAHB) was synthesized in this work. Compared to its parent HB, the red absorbance of DAHB was enhanced greatly. Singlet oxygen quantum yield of DAHB was determined (0.69) with that of HB (0.76) as the reference. Some fundamental photophysical parameters of DAHB have been determined in this paper.

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2. Experimental

2.1. Materials and general methods

HA was crystallized twice from chloroform–petroleum ether and HB obtained quantitatively by dehydration of HA. 9,10-diphenyl-anthracene (DPA), tetrahydrofuran (THF) and 1,3-diaminopropane were purchased from Aldrich Chemical Company. Other agents of analytical grades were purchased from Beijing Chemical Plant. Phosphate buffer saline (PBS) solution (pH 7.4) was composed of 1.4 mM KH_2PO_4, 6.4 mM Na_2HPO_4, 137 mM NaCl and 2.6 mM KCl. The working solutions were prepared immediately and water was freshly distilled before use. The solutions were purged with oxygen and argon according to experimental requirements.

UV–visible absorption spectra were obtained on an UV-2001 diode array spectrophotometer (Hitachi, Japan) and fluorescence emission spectra on an F-4500 spectrofluorimeter (Hitachi, Japan). The fluorescence lifetime was measured on an FLS-920 single photon counting apparatus (Edinburgh, UK). Fluorescence decays were collected for a maximum of 10,000 counts in the peak channel. All measurements were carried out at room temperature.

The fluorescence quantum yield (Φ) DAHB was calculated according to Eq. (1) [19] with HB as the standard ($\Phi_{HB} = 0.058$ in benzene [20]), where OD refers to optical density at excitation wavelength (475 nm), subscripts s and x refer to HB and DAHB, respectively, and A to the integrated area of the emission spectra at the same sensitivity scale. The concentrations of HB and DAHB were maintained at nearly equal OD (0.05) at 475 nm, and the emission spectra were recorded from 485 to 900 nm

$$\frac{\Phi_x OD_s}{\Phi_s OD_x} = \frac{A_x}{A_s} \quad (1)$$

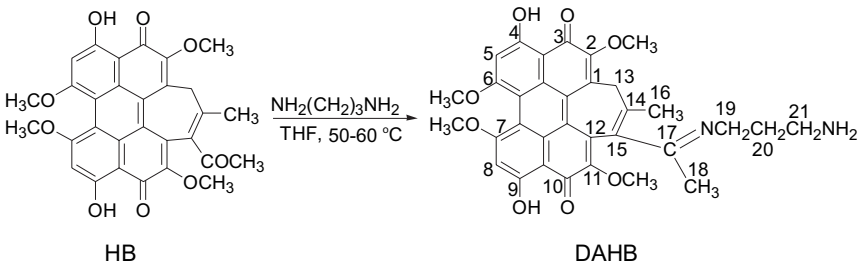
The DPA-bleaching method was used to determine the quantum yields of $^1O_2$ according to the reference [20]. The combination of a medium-pressure sodium lamp (450 W) and a 496 nm monochromatic filter provided the 496 nm light for the photo-oxidization of DPA.

2.2. Preparation of DAHB

The preparation of DAHB is given in Scheme 1.

2.2.1. Synthesis of DAHB

A solution of 200 mg HB and 10 mL 1,3-diaminopropane dissolved in 50 mL tetrahydrofuran (THF) was stirred and refluxed at 50–60 °C for 8 h in dark, and then drained the solvent on a rotary evaporator under reduced pressure. The residue was applied to a 1% KH_2PO_4–silica gel column with an eluant (ethanol/ethyl acetate/petroleum ether = 4:2:1 (V/V/V)) to separate into constituents. The green constituent was collected, chromatographed on 1% citric acid–silica gel plate with the same developing agent, and dried in vacuo to obtain the yield (20%). UV–vis [($CHCl_3$), λ_{max}, nm, (log ε)]: 490(4.01), 590(3.71), 635(3.83); IR [KBr, ν_{max}, cm^{-1}]: 3398, 2924, 1608, 1505; ^1H NMR (400 MHz, $CDCl_3$, δ, ppm): 16.53, 16.41 (s, 2H, H-phenol), 6.61 (2H, $-NH_2$), 6.51, 6.48 (s, 2H, 5,8-H), 4.14, 4.12, 4.10, 4.07 (m, 12H, 2,6,7,



Scheme 1.

11-OCH_3), 3.68 (t, 2H, 19-CH_2), 3.45 (m, 2H, 21-CH_2), 3.15, 2.96 (2H, d, 13-CH_2), 2.37 (s, 3H, 18-CH_3), 1.88 (s, 3H, 16-CH_3), 1.61 (m, 2H, 20-CH_2). *m*/*z* (MALDI-TOF): 585.4 (M + 1).

3. Results and discussion

3.1. Photophysical properties

The energy levels of the excited singlet states listed in Table 1 were determined from the absorption and fluorescence spectra. The singlet lifetime of DAHB was directly measured in a dilute benzene solution on the single photon counting apparatus. The fluorescence quantum yield (Φ) DAHB was determined to be 0.072, a little greater than that of HB (0.058).

Comparing the absorption spectrum of DAHB to that of HB (Fig. 1), it can be seen that red absorption is greatly modified with the extinction coefficient at 635 nm, nearly comparable to that at 490 nm, the main absorption of DAHB. The absorption intensification on the phototherapeutic window made it possible for DAHB to be applied as a photodynamic therapy (PDT) medicine to solid tumors. The maximum fluorescent emission peak of DAHB moves to 670 nm from that of HB (615 nm) because of red shift of the absorption spectrum.

Table 1
Absorption and photophysical parameters of HB and DAHB at room temperature in benzene

Parameter	DAHB	HB
λ^{a}_{max} (log ε)	490 (4.01)	466 (4.35)
	590 (3.71)	548 (4.08)
	635 (3.83)	580 (3.96)
λ^{F}_{max}	670	615
	725	654
E_s (kJ mol^{-1})	183.5	200.4
τ_s (ns)	1.00	1.00
Φ	0.072	0.058
$^1O_2$ quantum yield	0.69	0.76

λ_{max} refers to the wavelength at maximum absorption, ε to the molar extinction coefficient, E to energy level, τ to lifetime, subscript s to the singlet state, superscripts a and F to the absorption and fluorescence, respectively, and Φ to fluorescence quantum yield.

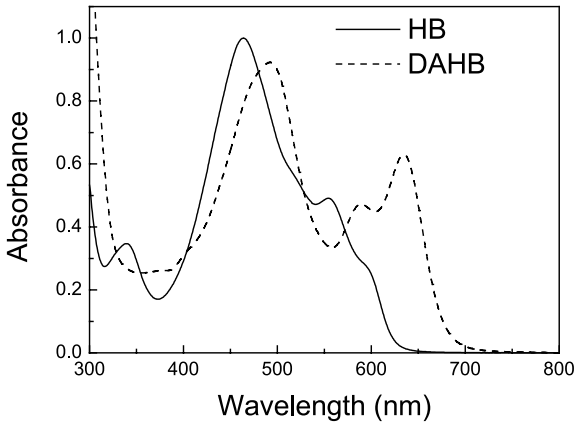


Fig. 1. The absorption spectra of HB and DAHB.

As known, the fluorescence of hypocrellins originates from the excited state intermolecular H atom transfer, and the intramolecular H bond between the *peri*-hydroxyl group and the quinonoid carbonyl group plays a crucial role during the fluorescent emission process [21]. And for the absorption spectrum of hypocrellins, the Ia band absorption was assigned to the π–π^* transition, while IIa and IIIa were thought to originate from the intramolecular H proton transfer between the *peri*-hydroxyl group and the quinonoid carbonyl group. Compared with HB, the fluorescent quantum yield of DAHB did not change much because the 17-position amination had no effect on its intramolecular H transfer (Fig. 2).

From the fluorescent excitation spectra, it could be seen that the IIa and IIIa bands contributed to the fluorescent emission more efficiently than Ia band. The Ia band may decay to the intramolecular H proton state or be transformed into excited triplet state by intersystem crossing, which further transfer energy to ground oxygen to yield singlet oxygen.

Singlet oxygen ($^1O_2$) quantum yield is an important index evaluating PDT effect of a phototherapeutic medicine for $^1O_2$ is a principal cytotoxic species during PDT [22]. From Table 1 and Fig. 2, it can be seen that the $^1O_2$ quantum yield of DAHB (0.69) is a little lower than that

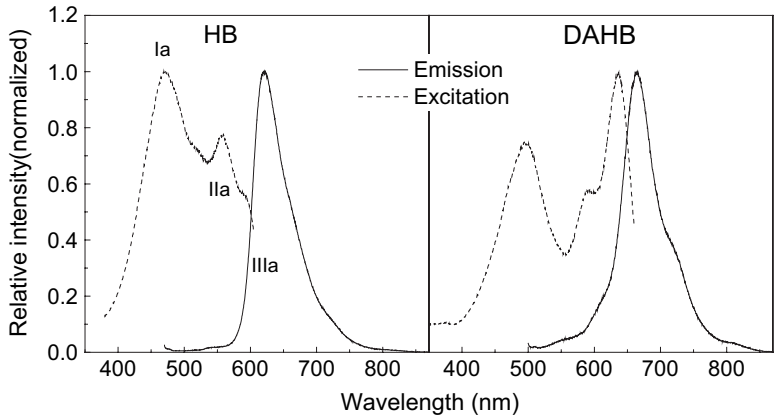


Fig. 2. The fluorescence emission and excitation spectra of DAHB and HB in $CHCl_3$ ($\lambda_{ex} = 490$ nm for DAHB, 460 nm for HB and $\lambda_{em} = 610$ nm for HB, 670 for DAHB).

of HB. During the measurements, the optical densities of the two dyes were adjusted to be the same at 496 nm. Control experiments proved that no DPA-bleaching would occur when the photosensitizer, oxygen or irradiation was absent (Fig. 3).

The introduction of amino group makes the derivative a weak alkaline molecule, which increases its affinity to the weak acidic tumor cells. From this aspect and also the intensified red absorption, DAHB may have a higher PDT effect than its parent HB.

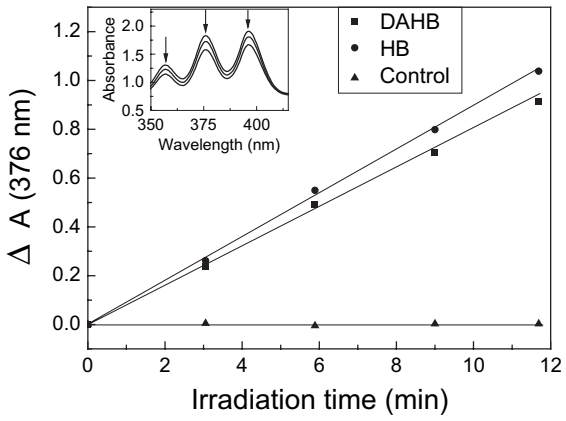


Fig. 3. Photosensitized DPA-bleaching by measuring the decrease in absorbance (ΔA) at 376 nm as a function of irradiation time in an oxygen-saturated solution of $CHCl_3$. Inset: absorption spectra during the bleaching of DPA.

4. Conclusion

To realize the clinical application for solid tumor, a novel hypocrellin, 17-1,3-diaminopropane-hypocrellin B Schiff base (DAHB), was designed and synthesized. Its absorbance on phototherapeutic window was enhanced greatly and it basically remains the ability of photogeneration of singlet oxygen for HB. The basicity after amination may make it have stronger affinity to tumor cells. The cell and animal experiments on DAHB are under investigation.

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