Journal of Photochemistry and Photobiology B: Biology 94 (2009) 171–178

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

A novel hypocrellin B derivative designed and synthesized by taking consideration to both drug delivery and biological photodynamic activity

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ARTICLE INFO

Article history:
Received 19 February 2008
Received in revised form 3 September 2008
Accepted 26 November 2008
Available online 3 December 2008

Keywords:
Hypocrellins
EPR spectra
Photodynamic therapy (PDT)
Singlet oxygen
Photosensitization activity

ABSTRACT

For making hypocrellins clinically applicable for phototherapy to vascular diseases, it is mainly focused onto finding a derivative which can be transported fluently in blood system but without serious loss of the inherent activity of its parents. Based on this consideration, a novel 17-3-amino-1-propane-sulfonic acid-HB Schiff-base (NSHB) was designed and synthesized in this work. As expected, NSHB is readily dissolved in phosphate buffered saline (PBS) or any other aqueous solvent in a concentration which is suitable for intravenous injection, while the quite higher partition coefficient (5:1) is beneficial to the affinity to biological targets. Based on EPR measurements, it is proved that the photosensitization activity of NSHB to photo-generate semiquinone anion radicals and superoxide anion radical (O_2^{-}) is even higher than its parent HB, while the ability to generate singlet oxygen ($^1O_2$) is not seriously reduced. In addition, nearly comparable PDT activity to A549 cells for NSHB and HB confirms that the molecular design is successful and NSHB is readily delivered into target tissues via blood circulation after intravenous injection. Furthermore, the quantum yield of $^1O_2$ for NSHB is as 12.5 times as that for HB under red light (600–700 nm), which is beneficial to phototherapy to solid tumors.

1. Introduction

Photodynamic therapy (PDT) is known for its advantage in treating various tumors and viruses [1–3]. Besides, it has been clinically applied to some common vas capillary diseases recently, such as port wine stains (PWS) and age-related macular degeneration [4–6]. Compared to PDT methodology, development on PDT drugs is far slower although some of potential drugs are researched [7]. For a long period of time, treating solid tumors had been the main goal of PDT. Therefore, PDT drugs (photosensitizers) have been usually evaluated on the view of treating solid tumors, such as, the requirement of a photosensitizer to possess strong absorbance on red light. In fact, each kind of diseases possesses its individual character, so the drugs should not be evaluated by a common standard. For example, the requirement of strong light absorption on the "phototherapeutic window" (600–900 nm) is necessary to treat large-sized solid tumors effectively for that the red light can penetrate tissues deeper than the light of shorter wavelengths. On the other hand, vas capillary diseases are usually occurred under the shallow surface no deeper than 1 mm, therefore, strong red-light absorption is not necessary but a drawback.

Among all the studied photosensitizers, hypocrellins (including HA and HB, the latter is shown in Fig. 1) are known for their high photosensitization activity [8–11] but super-low dark toxicity [12–15], however, the low red-light absorption has been long believed as a drawback so a lot of works have been done to improve the red light absorption [11,16–22]. For PDT to vas capillary diseases, drug has to be sent by intravenous injection into the target tissue via blood circulation system. However, hypocrellins are hardly soluble in water and may aggregate into clusters in blood and block the vascular system. To make hypocrellins clinically applicable, some works have been done to improve the water-solubility by chemical modification, i.e., introducing polar substituent groups to hypocrellins [23–29], from which a general rule derived is that for improving water-solubility one has to sacrifice the biological PDT activity. For example, sulfonated derivative of HA and HB are completely soluble in aqueous solution but lose the biological activity completely [30]. It is understandable that cell-uptake of drugs is a prerequisite for photodynamic activity to a diseased target, while a water-soluble drug is less accessible to the lipidic membranes [15]. Previously, to use taurine instead of sulfonic acid as the substituent group, i.e., the two carbon-atom chain for increasing the lipophility, taurine-HB derivative (THB, see Fig. 1) was synthesized [23] and also proved to be still water-soluble but also photodynamically active to tumor cells. However, the biological activity is still far lower than its parent HB [31]. Besides, it is

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doi:10.1016/j.jphotobiol.2008.11.008

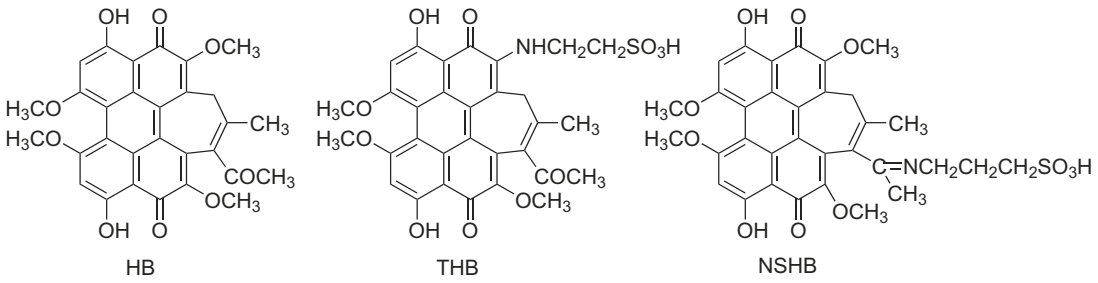

Fig. 1. Structures of HB, THB and NSHB.

recognized that derivatives with the substituent bound to the perylenequinonoid ring possess far lower singlet oxygen ($^1O_2$) quantum yields [23,32,33] while 17-substituted Schiff-base derivative preserves high singlet oxygen yield [34,35]. It is expected that a derivative of HB substituted at the 17 site with the group of one more carbon-atom than taurine should possess higher lipophility and singlet oxygen productivity. In this work, based on theoretical estimation of the molecular polarity [36], a new derivative of hypocrellin B, 17-3-amino-1-propane-sulfonic acid-hypocrellin B Schiff-base (NSHB, see Fig. 1), was designed and synthesized. Indeed, as expected, the derivative possesses an improved amphiphilicity and a singlet-oxygen quantum yield much higher than THB but comparable to its parent.

2. Materials and methods

2.1. Materials

HA was isolated from fungus sacs of *hypocrella bambusae* and recrystallized three times from acetone before use. HB was prepared by dehydration of HA in alkaline solution and purified by recrystallization twice from acetone [37]. THB was synthesized according to the literature [23]. 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO), 2,2,6,6-tetramethyl-4-piperidone (TEMP), 9,10-diphenylanthracene (DPA) and 3-amino-1-propane-sulfonic acid were purchased from Aldrich Chemical Company. Catalase and superoxide dismutase (SOD) were purchased from Sigma Chemical Company. Reduced nicotinamideadenine dinucleotide (NADH) was obtained from BiochemTechnology Corporation, of the Chinese Academy of Science. 1,4-Diazabicyclo[2,2,2]octane (DABCO) was purchased from Merck Chemical Company. Other reagents of analytical grades were purchased from Beijing Chemical Plant. Phosphate buffered 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 or argon according to experimental requirements.

2.2. Preparation of NSHB

HB of 100 mg and 3-amino-1-propane-sulfonic acid of 600 mg were dissolved in 50 mL DMF/NaOH·H_2O (2 M, 1:1), stirred and refluxed at 120 °C for 3 h in dark (Scheme 1). Then the solvent was evaporated under reduced pressure. The residue was applied to a 1% KH_2PO_4–silica gel column with an eluant (dichloromethane: methyl alcohol = 5:1 (V/V)) to separate into constituents. The brown–green constituent was collected, chromatographed on 1% citric acid–silica gel plate with the same developing agent to obtain 12 mg NSHB (yield 10%) which was characterized below. UV–vis [(DMSO), λ_{max}(nm), (log ε)]: 500 (4.29), 593 (3.91), 640 (4.10); IR [KBr, ν_{max}, cm^{-1}]: 3440, 2930, 1608, 1512; ^1H NMR (300 MHz, d_6-DMSO, δ, ppm): 16.59, 16.53 (s, 2H, H-Phenol), 6.72, 6.60 (s, 2H, 5,8-H), 4.12, 4.06, 4.03, 4.00 (m, 12H, 2,6,7,11-OCH_3), 3.94 (m, 2H, 19-CH_2), 3.90, 2.60 (d, 2H, 13-CH_2), 3.83 (m, 2H, 21-CH_2), 2.24 (s, 3H, 18-CH_3), 2.06 (m, 2H, 20-CH_2), 1.82 (s, 3H, 16-CH_3). *m/z* (ESI): 648.2 (M−1).

2.3. Spectral measurements

The absorption spectra were recorded on a Shimadzu UV-1601 spectrophotometer. All measurements were carried out at room temperature. EPR measurements were recorded with X-band (9.8 GHz) on a Bruker model E500 spectrometer equipped with ST4102 cavity at room temperature. Unless otherwise indicated, the instrumental settings were: microwave power, 10.02 mW; modulation frequency, 100 KHz; modulation amplitude, 1 G; sweep width, 100 G; and receiver gain, 1.0×10^5. A Q-switched Spectra Physics (USA) INDI series Nd:YAG laser (λ = 532 nm) was used as a light source. The energy was 10 mJ at 10 Hz repetition rate. Samples in cuvettes were purged as required with argon or oxygen for 30 min in the dark and immediately transferred to a quartz capillary designed specifically for EPR analysis. The relative free radical quantum yields were estimated with normalized absorbance at 532 nm.

2.4. Quantum yield of singlet oxygen

The DPA-bleaching method was used to determine the quantum yields of $^1O_2$ according to the Ref. [8]. The combination of a medium-pressure sodium lamp (450 W) and a filter eliminating light of wavelengths shorter than 470 nm were used for the photo-oxidization of DPA. During the measurements, the integrated areas of the absorption spectra of HB, THB and NSHB from 470 nm to 800 nm were adjusted to be the same.

$^1O_2$ generated by red light was measured with Red light Treatment Instrument (Institute of Electronics, Academia Sinica China) as the light source in 90% of the total power output at 600–700 nm [38].

2.5. Partition coeffecients

Photosensitizers were diluted to a concentration of 20 μM in 2 mL *n*-octanol, and then 2 mL PBS solution (pH 7.4) were added. The mixture were sonicated for 2 min, and then centrifuged for 10 min to separate the phases. The concentration of photosensitizers in each phase was measured spectrophotometrically based on Lambert–Beer law. Partition coefficient was calculated by the ratio of photosensitizer concentration in *n*-octanol to that in PBS solution.

2.6. Solubility in PBS solution

Photosensitizers were dissolved in PBS solution (pH 7.4) in a series of concentrations. Then the solutions were measured for