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A novel amphiphilic 2-taurine substituted hypocrellin B (THB) and its photodynamic activity

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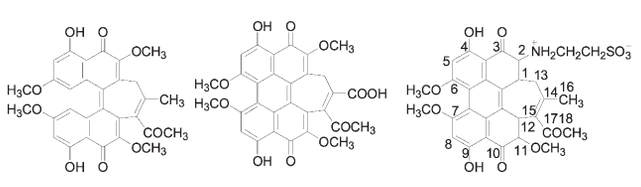
A novel 2-taurine substituted hypocrellin B (THB) was designed and synthesized in this work. Both the light absorbance in the phototherapeutic window (600–900 nm) and the amphiphilicity (a compromise between the lipophilicity and hydrophilicity) were greatly improved. The semiquinone anion radical ($THB^{\bullet -}$), superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$) (Type I mechanism) and singlet oxygen ($^1O_2$) (Type II mechanism) could be generated *via* the photosensitization of THB, confirming its photosensitization activity. In this work, it was also found that the cytochrome c reduction method is not the exclusive one for detecting $O_2^{\bullet -}$ in photosensitization systems.

Introduction

Photodynamic therapy (PDT) is a medical treatment that jointly employs light and a photosensitizer to create a cytotoxic effect on cancerous or unwanted tissues *via* photoproduced active species, $O_2^{\bullet -}$, $^{\bullet}OH$, $^1O_2$, *etc.*[1,2] As prototypes of new photosensitizers, hypocrellins (HA and HB, the latter is shown in Scheme 1) possess some attractive advantages, such as high light-induced toxicity but negligible toxicity in the dark and quick excretion from the body (within 24 h).[3–5] However, there are at least two problems preventing them from clinical application, which are low absorbance in the phototherapeutic window (600–900 nm) and their hydrophobic properties. Much work has been done to improve the absorbance and amphiphilicity,[6–13] however, a completely water-soluble derivative may lose its photodynamic activity because of low cellular uptake. To satisfy the dual requirements of good cellular uptake and fluid transport in the vascular net, a compromise between the lipophilicity and hydrophilicity is clinically necessary.[14,15]

In previous work a theoretical method was developed to estimate rationally the amphiphilicity from the molecular polarity,[16] comparable to the partition coefficients (PC) measured experimentally by the distribution between *n*-octanol and PBS solution (pH 7.4).[17] Based on the results, it was concluded that a derivative with a polarity value of 0.22 should possess the best amphiphilicity. Taurine (2-aminoethanesulfonic acid), a natural nontoxic amino acid, contains both electron-donating (amino) and strong hydrophilic (sulfonic) groups, which are expected to give contributions to the red absorption and hydrophilicity, respectively. 2-Taurine substituted hypocrellin B (THB, see Scheme 1), with a polarity value of 0.23, was designed and synthesized in the current work. It was proved that the derivative was readily soluble in not only aqueous solution but also organic solvents. The photosensitivity and photodynamic activity of THB were evaluated by EPR and spectrophotometric measurements.



Scheme 1 The chemical structures of HB, HBO_2H (14-carboxyl hypocrellin B) and THB.

Experimental

Reagents

5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), 9,10-diphenylanthracene (DPA), taurine, 2,2,6,6-tetramethyl-4-piperidone (TEMP) and 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl radical (TEMPO) were purchased from Aldrich Chemical Company. Catalase, cytochrome c (horse heart) and superoxide dismutase (SOD) were purchased from Sigma Chemical Company. Cysteine, reduced glutathione (GSH) and reduced nicotinamide adenine dinucleotide (NADH) were obtained from Biochem Technology Corporation, of the Chinese Academy of Sciences. 1,4-Diazabicyclo[2,2,2]octane (DABCO), histidine, NaN_3 and diethylenetriaminepentaacetic acid (DTPA) were purchased from Merck Chemical Company. Other reagents 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, depending on the experimental requirements.

Synthesis of THB

HB (500 mg) was added into a taurine (10 g) solution of *N*,*N*-dimethylformamide (20 mL) and tetramethylammonium hydroxide (25% in water, 20 mL) and then refluxed at 60–80 °C for 24 h. The reacted solution was mixed with water (500 mL), neutralized with 10% hydrochloric acid and then extracted by chloroform. The chloroform and aqueous phases were separately collected and then evaporated to dryness *in vacuo*. The solid from the aqueous phase was dissolved with

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pyridine (redistilled and dried) and then filtrated and dried to the solid *in vacuo*. The solid was mixed with that from the chloroform phase and then applied to a 1% KH_2PO_4–silica gel column with an eluent (ethanol–ethyl acetate–petroleum ether = 4:2:1 V/V/V) to separate the constituents. The second blue constituent was collected, chromatographed on a 1% citric acid–silica gel plate with the same developing agent, then dried *in vacuo* to obtain the product (yield 25%). The chemical structure of the THB product was confirmed by the use of ^{1}H NMR, UV-vis, IR and mass spectral data. UV-vis [($CHCl_3$), λ_{max}/nm, (log ε)]: 461 (3.95), 582 (3.90), 635 (3.87); IR [KBr, ν_{max}/cm^{-1}]: 3430, 1710, 1608, 1505; ^{1}H NMR (400 MHz, deuterated DMSO, δ): 1.99 (3H, s, 16-CH_3), 2.29 (3H, s, 18-CH_3), 2.80 (2H, m, CH_2–S), 3.99, 4.09, 4.12 (9H, s, 11-, 6-, 7-OCH_3), 4.14–4.17 (2H, m, CH_2–N), 5.18 (1H, s, 13-H_a), 6.48 (1H, s, 13-H_b), 6.77, 6.78 (2H, s, 5-, 8-H); MALDI-TOF: 622 $(M+1)^+$.

Spectral measurements

The absorption spectra were recorded on a Shimadzu UV-1601 spectrophotometer. For irradiation, a 450 W medium pressure sodium lamp was used as the light source with a long pass filter to eliminate light of wavelengths shorter than 470 nm. The EPR measurements were performed at room temperature on a Bruker EPR 300E spectrometer at 9.8 GHz, X-band with 100 kHz field modulation (Faellanden, Switzerland). Unless otherwise indicated, the instrumental settings were: microwave power, 10.02 mW (1.02 mW for the TEMPO signal); modulation amplitude, 0.958 G; sweep width, 100 G; receiver gain, 1.0×10^5 (1.0×10^4 for the TEMPO signal). A 532 nm YAG-900 laser (Spectra-Physics Laser, Mountain View, CA, USA) was used as the light source in the EPR measurements. 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. EPR signals were recorded under direct irradiation of the samples in the cavity and manipulated with an IBM/PC computer. The kinetics of spin adduct generation were studied by recording the peak height of an EPR spectrum every 40 s.

Result and discussion

Taurine is a major constituent of the free amino acid pool in most animal tissues and occurs abundantly in membranes;[18] it was proved to be essential for certain aspects of mammalian development.[19] The good biological compatibility is one of the reasons that it was chosen as a substituent to modify hypocrellins. THB shows an absorption peak at 635 nm (From Fig. 1), the intensity of which is nearly comparable to those at 582 and 465 nm. That is to say, the structure modification of HB was successful in enhancing the red absorption. Furthermore, THB could be readily dissolved not only in PBS solution and water but also in DMSO, acetone and ethanol, meaning that the derivative possesses a very good amphiphilicity, as expected.

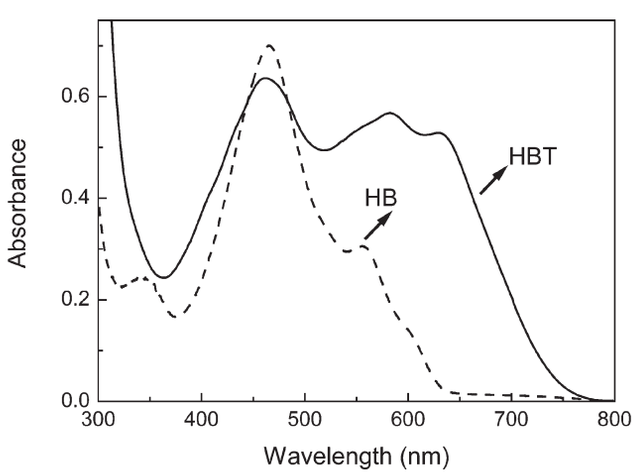

Fig. 1 Absorption spectra of HB and THB in chloroform ($C_{HB} = C_{THB} = 15$ μM).

Photoproduction of active species by THB

Semiquinone anion radicals (THB$^{\bullet-}$). Semiquinone anion radicals are believed to play an important role in photodynamic action to an anoxic tissue, such as a tumor cell. Illuminating an argon-saturated DMSO solution of THB (1 mM), an EPR signal ($g = 2.0041$) was observed (Fig. 2, trace B) with the intensity depending on oxygen (Fig. 2, trace C), the concentration of THB (Fig. 2, inset a) and irradiation time (Fig. 2, inset b). Control experiments (Fig. 2, trace D) confirmed that irradiation and THB were both essential for the signal.

As is well-known, the semiquinone anion radical of hypocrellins can be photogenerated *via* electron transfer between ground state and excited triplet state molecules (Type I mechanism).[8–10] Considering that the cationic radical is not detectable in these conditions,[20–22] the EPR signal observed above can be temporarily attributed to the semiquinone anion radical (THB$^{\bullet-}$) formed by reactions (1) and (2). The enhancement effect (Fig. 2, curve A) of reductants (NADH, GSH, *etc.*, abbreviated as D), by donating an electron to triplet THB [reaction (3)],[23] provides further support for the assignment. When oxygen and DMPO were both present, the EPR signal disappeared while that for DMPO–superoxide adducts appeared immediately, manifesting the anionic nature of the free radicals.

$$\mathrm{THB} \xrightarrow{h\nu} {}^1\mathrm{THB} \xrightarrow{\mathrm{isc}} {}^3\mathrm{THB} \quad (1)$$

$$^3\mathrm{THB} + \mathrm{THB} \rightarrow \mathrm{THB}^{\bullet-} + \mathrm{THB}^{\bullet+} \quad (2)$$

$$^3\mathrm{THB} + \mathrm{D} \rightarrow \mathrm{THB}^{\bullet-} + \mathrm{D}^{\bullet+} \quad (3)$$

The strong correlation of the signal with the concentration of THB (Fig. 2, inset a) suggests compelling evidence for the electron transfer [eqn. (2)], however, for concentrations higher than 1.5 mM, the signal intensity decreased because of back-electron-transfer and autoquenching processes.[11]

THB$^{\bullet-}$ in aqueous solution can also be detected spectrophotometrically. When a deoxygenated PBS solution (pH 7.4) of

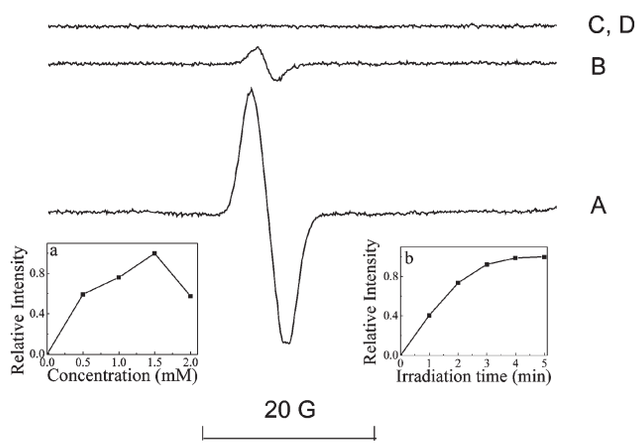

Fig. 2 Trace A: EPR spectrum for an argon-saturated DMSO solution of THB (1 mM) in the presence of NADH (2 mM) after illumination for 2 min. Trace B: EPR spectrum from an argon-saturated DMSO solution of THB after illumination for 2 min. Curve C: same as trace B except that oxygen was bubbled through the solution after illumination. Trace D: same as trace B except that THB or illumination was omitted. Insets: variation of THB$^{\bullet-}$ EPR intensity against its concentrations (a) or illumination time (b).

THB (0.1 mM) and NADH (2 mM) was irradiated, the solution color changed from blue to green then to pink-orange. Fig. 3(A) and 3(B) show the absorption spectra for the color changes from blue to green (within 10 s) and from green to pink-orange (14–30 s), respectively. It can be seen clearly that the changes in the absorption peaks are accompanied by the appearance of isobestic points. The absorption bands at 505 nm and 583 nm can be assigned to $THB^{\bullet -}$ and hydroquinone ($THBH_2$), respectively.[24] The generation mechanism of $THBH_2$ was postulated as eqns. (4) and (5). When oxygen was bubbled into the solution for half an hour in the dark after irradiation, the photoproducts disappeared completely with an 85% recovery of the absorbance of THB.[25]

$$THB^{\bullet -} + THB^{\bullet -} \xrightarrow{H^+} THBH_2 + THB \qquad (4)$$

$$THB^{\bullet -} + D \xrightarrow{H^+} THBH_2 + D^{\bullet +} \qquad (5)$$

The spin counteraction of TEMPO with $THB^{\bullet -}$ was also used to detect the photoproduction of $THB^{\bullet -}$, providing compelling evidence for the generation of $THB^{\bullet -}$ in aqueous solution.[26] The decay of the TEMPO signal obeys first-order kinetics (Fig. 4).

Superoxide anion radical ($O_2^{\bullet -}$). DMPO is commonly used as a spin trap for detecting superoxide ($O_2^{\bullet -}$) and hydroxyl ($^{\bullet}OH$) radicals.[27] Detection of $O_2^{\bullet -}$ was carried out in DMSO for it is relatively stable in aprotic solvents. When an oxygenated DMSO solution containing THB (0.1 mM) and

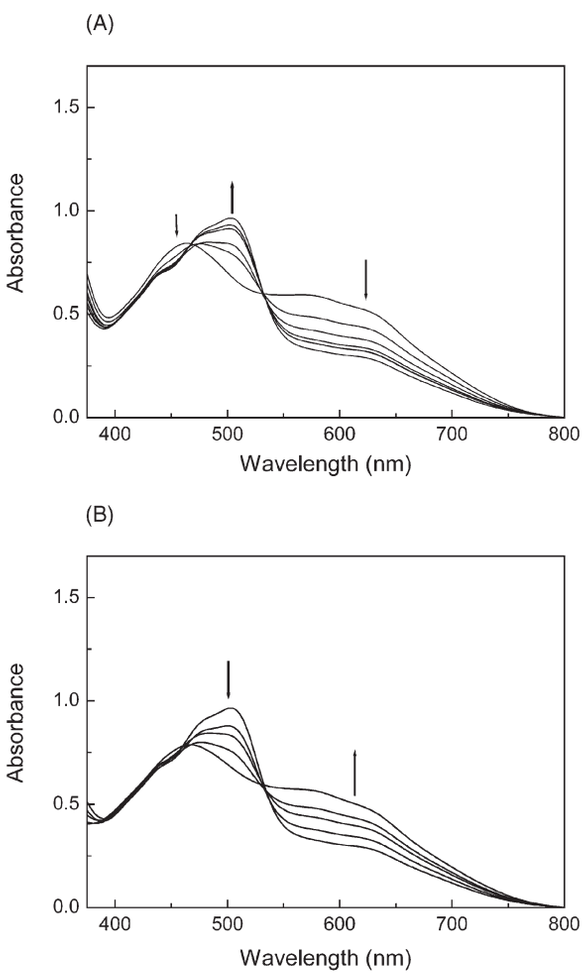

Fig. 3 Absorption spectra from argon-saturated PBS (pH 7.4) containing THB (100 μM) and NADH (2 mM) upon illumination for (A) 0, 2, 4, 6, 8, 10 s and (B) 14, 18, 22, 26, 30 s. The arrows indicate the direction of absorption changes.

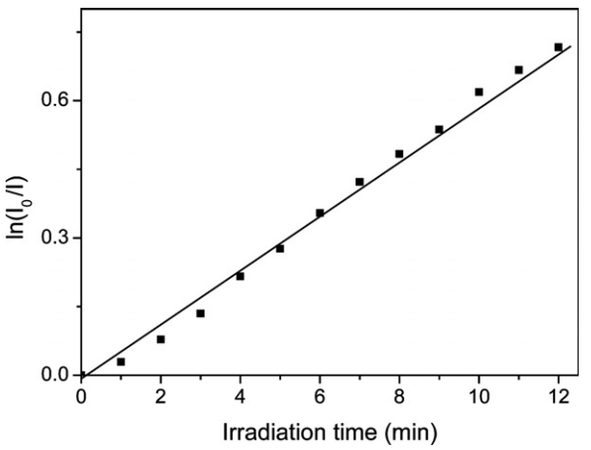

Fig. 4 Spin counteraction of TEMPO (10 μM) by THB (1 mM) photosensitization detected by the decrease of TEMPO signal intensity in deoxygenated PBS solution.

DMPO (40 mM) was irradiated, an EPR signal appeared (Fig. 5, trace B) with the g factor and coupling constants ($g = 2.0056$, $\alpha^N = 12.48$ G, $\alpha_\beta^H = 10.36$ G, $\alpha_\gamma^H = 1.35$ G) being in good agreement with previously reported values for the DMPO–$O_2^{\bullet -}$ adduct.[27,28] Control experiments confirmed that THB, oxygen and irradiation were all essential for appearance of the EPR signal (Fig. 5, trace A). SOD (30 μg mL^{-1}) inhibited the EPR signal by about 50% (Fig. 5, trace D), whereas thermally denatured SOD had no effect on the EPR spectrum, confirming that the EPR signal in Fig. 5, trace B originates from the DMPO–$O_2^{\bullet -}$ adduct. NADH (2 mM) greatly intensified the signal of DMPO–$O_2^{\bullet -}$ (Fig. 5, trace C). The mechanism for formation of DMPO–$O_2^{\bullet -}$ in the presence of NADH is suggested to be:[23]

$$^3THB + NADH \rightarrow THB^{\bullet -} + NAD^{\bullet} \ (+H^+) \qquad (6)$$

$$THB^{\bullet -} + O_2 \rightarrow THB + O_2^{\bullet -} \qquad (7)$$

$$NAD^{\bullet} + O_2 \rightarrow NAD^+ + O_2^{\bullet -} \qquad (8)$$

An $^1O_2$ scavenger (DABCO or histidine) did not have any effect on the DMPO–$O_2^{\bullet -}$ signal, suggesting that $^1O_2$ is not involved in the formation of $O_2^{\bullet -}$. Fig. 6 shows plots of the EPR signal intensity of $O_2^{\bullet -}$ for THB and HB against illumination time, from which it can be seen that THB

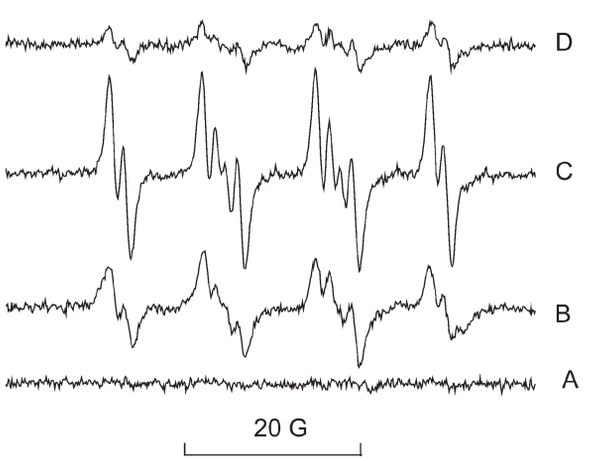

Fig. 5 EPR spectra of DMPO–$O_2^{\bullet -}$ adducts. Trace A: control experiment (no light or THB). Trace B: produced upon irradiation of an oxygenated DMSO solution of THB (100 μM) and DMPO (40 mM). Trace C: same as trace B but in the presence of NADH (2 mM). Trace D: same as trace B but in the presence of SOD (30 μg mL^{-1}).

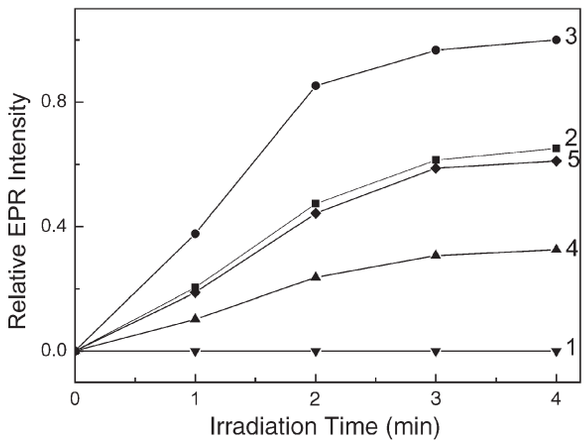

Fig. 6 Plot of the DMPO–$O_2^{\bullet -}$ signal intensity against irradiation time. Curves 1–4 are under the same conditions as A–D in Fig. 5, respectively. Curve 5 is the same as curve 2 except that THB was replaced by HB.

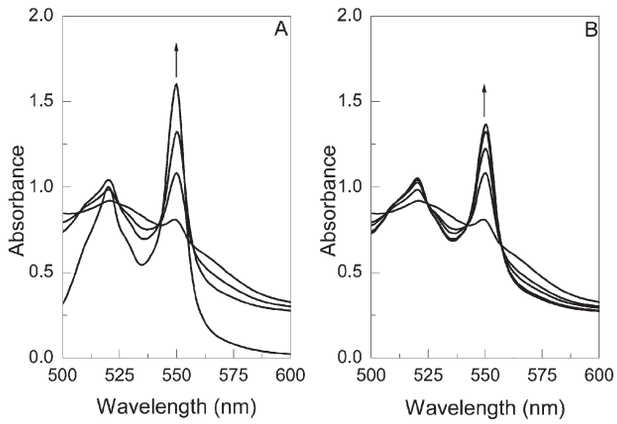

Fig. 8 (A) Absorption spectra of an argon-saturated aqueous solution (pH 7.4) containing Cyt Fe^{3+} (85 μM) and NADH (2 mM) at 0, 1, 2 and 3 min in the dark. (B) Absorption spectra of an argon-saturated aqueous solution (pH 7.4) containing Cyt Fe^{3+} (85 μM) and HB (10 μM) with illumination times of 0, 3, 6, 9 and 12 min.

possesses an even stronger ability for the photogeneration of $O_2^{\bullet -}$ than HB.

The cytochrome c method, usually used for quantitative analysis of $O_2^{\bullet -}$ by monitoring the absorbance at 550 nm,[11,29] was used to detect the formation of $O_2^{\bullet -}$ by THB. Fig. 7 shows that SOD can partially inhibit the reduction of Cyt Fe^{3+} [eqn. (9)], confirming the formation of $O_2^{\bullet -}$ and the occurrence of reaction (9). However, in a control experiment, when illuminating an argon-saturated aqueous solution (pH 7.4) of THB (or HB) (10 μM) and Cyt Fe^{3+} (85 μM), the reduction of Cyt Fe^{3+} could also take place, as shown in Fig. 8(B), while it could not occur in the absence of light or a photosensitizer. In this case, the only photoproduced reductive species were semiquinone anion radicals (*vide supra*), which could also drive the reduction of Cyt Fe^{3+} [eqn. (10)].

$$\text{Cyt Fe}^{3+} + \text{O}_2^{\bullet -} \rightarrow \text{Cyt Fe}^{2+} + \text{O}_2 \qquad (9)$$

$$\text{Cyt Fe}^{3+} + \text{HB}^{\bullet -}\ (\text{THB}^{\bullet -}) \rightarrow \text{Cyt Fe}^{2+} + \text{HB}\ (\text{THB}) \qquad (10)$$

When a reductant (NADH or GSH), expected to promote the production of semiquinone radicals as well as that of $O_2^{\bullet -}$, was introduced into the oxygenated aqueous solution of THB (10 μM) and Cyt Fe^{3+} (85 μM), the photoinduced reduction of Cyt Fe^{3+} was complete within 30 s. The tremendous increase of reduction rates when reductant (NADH or GSH) was added suggests the possibility that the reductant reduces Cyt Fe^{3+} directly [eqn. (11)], in addition to reactions (9) and (10).

$$\text{Cyt Fe}^{3+} + \text{D} \rightarrow \text{Cyt Fe}^{2+} + \text{D}^{\bullet +} \qquad (11)$$

Indeed, reaction (11) did occur at a rather high rate in the absence of photosensitizer and light, as shown in Fig. 8(A).

In summary, Cyt Fe^{3+} can be reduced by semiquinone anion radical as well as $O_2^{\bullet -}$; furthermore, it can also be reduced directly by a reductant, which means that the cytochrome c method should not be used as the exclusive one for quantitative measurement of $O_2^{\bullet -}$ formed by photosensitization processes.

Hydroxyl radical ($^{\bullet}$OH). As shown in Fig. 9, trace A, the irradiation of an oxygenated aqueous solution (pH 7.4) containing THB (1 mM) and DMPO (50 mM) with 532 nm laser radiation led to appearance of a quartet spectrum with an intensity ratio of 1:2:2:1 and hyperfine coupling constants $\alpha^N = \alpha_\gamma^H = 14.9$ G, suggesting formation of the DMPO–$^{\bullet}$OH

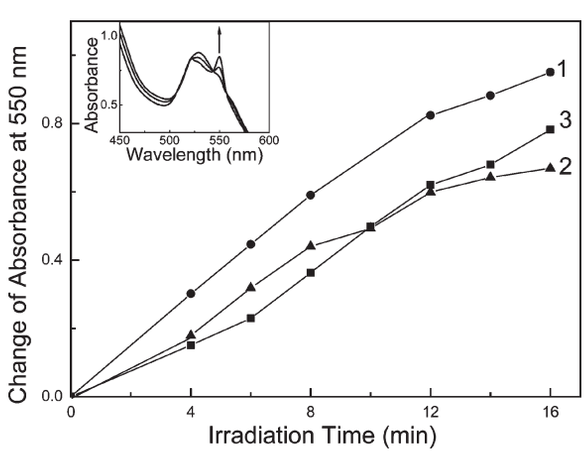

Fig. 7 Curve 1: peak absorbance at 550 nm of an oxygen-saturated aqueous solution (pH 7.4) containing THB (10 μM) and Cyt Fe^{3+} (85 μM) for a series of illumination times. Curve 2: the same as curve 1 but with SOD (45 μg mL^{-1}) added. Curve 3: the same as curve 1 but THB was replaced by HB. Inset: absorption spectra of the solution upon irradiation for 4, 6, 8 min. The arrow indicates the direction of change.

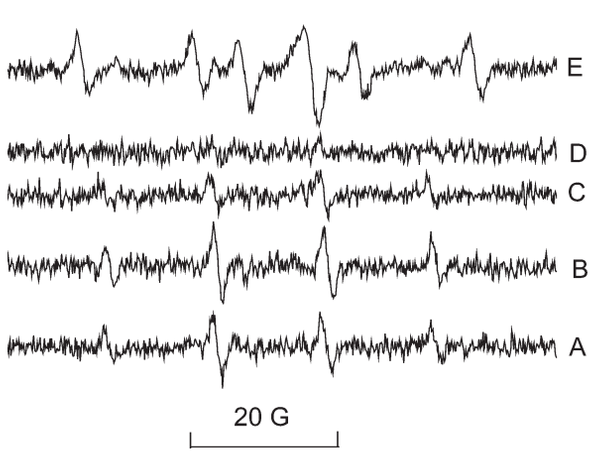

Fig. 9 Trace A: EPR spectrum of the DMPO–$^{\bullet}$OH adduct generated by irradiation of an oxygen-saturated PBS solution (pH 7.4) containing THB (1 mM) and DMPO (50 mM). Trace B: same as trace A except that SOD (25 μg mL^{-1}) was added. Trace C: same as trace A but in the presence of catalase (30 μg mL^{-1}). Trace D: spectrum of the control experiment (no light or THB). Trace E: same as trace A but in the presence of ethanol (10% V/V).

spin adduct.[30] In the absence of light, THB, oxygen or DMPO, no signal could be observed (Fig. 9, trace D).

Singlet oxygen ($^1O_2$) was not involved in the formation of DMPO–$^{\bullet}$OH[31] because histidine or DABCO (scavengers of $^1O_2$) did not have any effect on the signal intensity. When ethanol (10%, V/V) was added into the system prior to irradiation, the EPR signal of the DMPO–$^{\bullet}$CH(OH)CH_3 adduct (α^N = 15.9 G, α^H_γ = 23.0 G) was recorded (Fig. 9, trace E),[30] suggesting that the DMPO–$^{\bullet}$OH adduct forms *via* a direct reaction of $^{\bullet}$OH with DMPO instead of decomposition of DMPO–$O_2^{\bullet-}$.

Catalase (30 μg mL^{-1}) could significantly reduce the signal intensity of the DMPO–$^{\bullet}$OH adduct (Fig. 9, trace C), suggesting that the formation of $^{\bullet}$OH was H_2O_2 relevant. SOD (25 μg mL^{-1}) enhanced the intensity of the signal (Fig. 9, trace B) while DTPA (5 mM), which is a well-known chelator of iron preventing further reaction with H_2O_2,[32] greatly reduced the intensity of the DMPO–$^{\bullet}$OH signal (not shown). These findings support the formation of $^{\bullet}$OH as originating from $O_2^{\bullet-}$ and being H_2O_2 relevant, that is $^{\bullet}$OH can be produced *via* the Fenton–Haber–Weiss reaction after $O_2^{\bullet-}$ dismutation, described elsewhere.[9,11]

Singlet oxygen ($^1O_2$). When an oxygenated aqueous solution of THB (1 mM) and TEMP (50 μM) was irradiated at room temperature, an EPR spectrum of triplet peaks with equal intensity, characteristic of a nitroxide radical, was observed (Fig. 10, trace A). The hyperfine splitting constant and *g* factor are identical to those for commercial TEMPO (α^N = 16.3 G, *g* = 2.0056),[33] formed *via* reaction of TEMP with $^1O_2$. In the absence of oxygen, THB or irradiation, no EPR signal could be observed. When a typical $^1O_2$ scavenger (DABCO, histidine, NaN_3, 4 mM) was introduced into the solution, the signal of TEMPO was significantly decreased (Fig. 10, traces B–D). Furthermore, it was found that the intensity of the EPR signal increased by about fivefold when H_2O was replaced by D_2O as the solvent.[34] All of these results confirm that $^1O_2$ is formed by energy transfer from excited triplet state THB to ground state molecular oxygen (Type II mechanism).

The DPA bleaching method is usually applied to measure the quantum yield of $^1O_2$.[35] It was found that DPA bleaching occurred but was completely inhibited by 10 mM histidine (Fig. 11, curve 3). Control experiments proved that oxygen, hypocrellins, and light are all necessary for DPA bleaching. The $^1O_2$ quantum yield for THB in $CHCl_3$ was evaluated to be 0.15 by this method with HB as reference (ϕ = 0.76) [Fig. 11, curve 1].

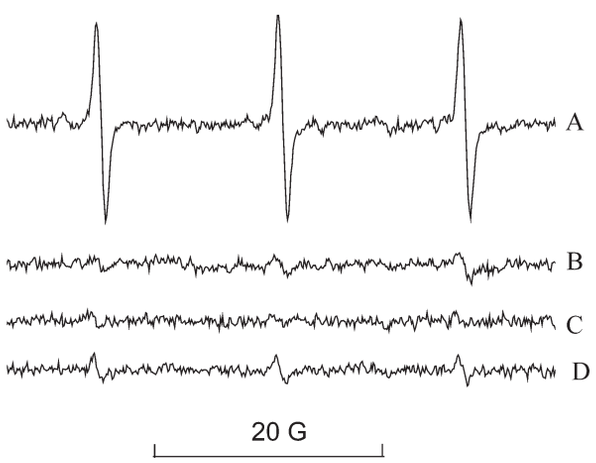

Fig. 10 Trace A: EPR spectrum produced by irradiation of an oxygenated PBS solution (pH 7.4) containing THB (1 mM) and TEMP (50 μM). Traces B, C, D: the same as trace A but in the presence of 4 mM DABCO, histidine or NaN_3, respectively.

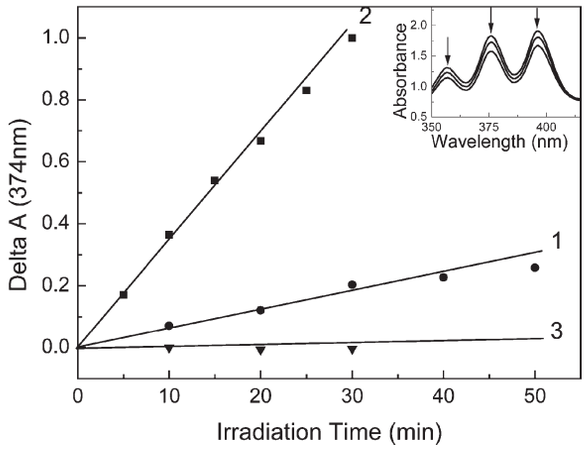

Fig. 11 DPA bleaching measured by the decrease of the absorbance at 374 nm as a function of irradiation time. Curve 1: THB in oxygen-saturated $CHCl_3$. Curve 2: same as curve 1 but THB was replaced by HB. Curve 3: same as curve 1 but in the presence of histidine (10 mM).

Comparison of PC and polarity values for several hypocrellin derivatives

The partition coefficient (PC) values for THB, HBO_2H and HB are 2.5, 11.6 and 46.4 while the polarities are 0.23, 0.20 and 0.19, respectively. Compared to HB and HBO_2H, the PC value for THB is smaller but its polarity higher, suggesting an enhanced hydrophilicity (*vide supra*) due to introduction of the strongly hydrophilic sulfonic group. On the other hand, the PC and polarity values for 13-sulfonated HB, a completely water-soluble derivative,[36] are 0.20 and 0.25, respectively.[16,17] Compared to these values, THB shows a more preferential distribution in the organic phase, which is necessary for cellular uptake. As known, hypocrellins (HA or HB) tend to aggregate and so may block the vascular net. At the moment, an applicable preparation of hypocrellins is freshly prepared liposome, however, the physical and chemical stability of the liposome suspension is not high enough (no longer than months). Moreover, the HB–liposome suspension could only be prepared at concentrations no greater than 0.5–1.0 mg mL^{-1}, which limits its application in PDT. On the other hand, THB possesses high physical and chemical stability, and good solubility in aqueous solution as well as in organic solvents. Combined with the strong red absorption, it can be seen that THB possesses most of the desired properties for a phototherapeutic medicine.

Conclusion

2-Taurine substituted hypocrellin B was designed and synthesized in the current work. Both of the amphiphilicity and red absorption in the phototherapeutic window (600–900 nm) were greatly improved. THB is readily soluble in water, PBS solution and physiological saline so that it can readily be used to prepare intravenous injections. Photogeneration of the chemically active species, semiquinone anion radical ($THB^{\bullet-}$), $O_2^{\bullet-}$, $^{\bullet}$OH (*via* Type I mechanism) and $^1O_2$(*via* Type II mechanism), were detected by EPR. The photoproductivity of $O_2^{\bullet-}$ by THB is higher but that of $^1O_2$ lower than those by HB. The photosensitization activity as well as the good physical chemistry stability and amphiphilicity suggest that this novel derivative may be clinically applicable to phototherapy. In the current work, it was also found that not only $O_2^{\bullet-}$ but also semiquinone anion radicals could reduce cytochrome c, which could also be reduced directly by a reducing agent; therefore, this method might not be the exclusive one for quantitatively detecting $O_2^{\bullet-}$ in photosensitization systems.

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References

1 T. J. Dougherty, *Photochem. Photobiol.*, 1987, **45**, 837.
2 B. W. Henderson and T. J. Dougherty, *Photochem. Photobiol.*, 1992, **55**, 145.
3 Z. J. Diwu, J. Zimmermann, T. Meyer and J. W. Lown, *Biochem. Pharmacol.*, 1994, **47**, 373.
4 J. B. Hudson, J. Zhou, J. Chen, L. Harris, L. Yip and G. H. N. Towers, *Photochem. Photobiol.*, 1994, **60**, 253.
5 G. G. Miller, K. Brown, M. Ballangrud, O. Barajas, Z. Xiao, J. Tulip, J. W. Lown, J. M. leithoff, M. J. Allalunis-Turner, R. D. Mehta and R. B. Moore, *Photochem. Photobiol.*, 1997, **65**, 714.
6 I. Singh, R. T. Ogata, R. E. Moore, C. W. J. Chang and P. J. Scheuer, *Tetrahedron*, 1968, **24**, 6053.
7 Z. J. Diwu, C. L. Zhang and J. W. Lown, *Anti-Cancer Drug Des.*, 1993, **8**, 129.
8 Y. Y. He, J. Y. An and L. J. Jiang, *Int. J. Radiat. Biol.*, 1998, **74**, 647.
9 J. H. Ma, J. Q. Zhao and L. J. Jiang, *Free Radical Res.*, 2001, **35**, 607.
10 Y. W. Zhao, J. Xie and J. Q. Zhao, *New J. Chem.*, 2003, **27**, 880.
11 Y. Y. He, J. Y. An and L. J. Jiang, *Free Radical Biol. Med.*, 1999, **27**, 203.
12 S. J. Xu, S. Chen, M. H. Zhang and T. Shen, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 2045.
13 Z. Z. Ou, J. R. Chen, X. S. Wang, B. W. Zhang and Y. Cao, *New J. Chem.*, 2002, **26**, 1130.
14 J. Moan, Q. Peng, J. F. Evensen, K. Berg, A. Western and C. Rimington, *Photochem. Photobiol.*, 1987, **46**, 713.
15 G. Oenbrink, P. Jürgenlimke and D. Gabel, *Photochem. Photobiol.*, 1988, **48**, 451.
16 J. Xie, J. H. Ma and J. Q. Zhao, *Sci. China, Ser. B: Chem.*, 2002, **45**, 251.
17 Y. Y. He, H. Y. Liu, J. Y. An, R. Han and L. J. Jiang, *Dyes Pigm.*, 2000, **44**, 63.
18 J. G. Jacobsen and L. H. Smith, *Physiol. Rev.*, 1968, **48**, 424.
19 J. A. Sturman, *Physiol. Rev.*, 1993, **73**, 119.
20 J. Mayer and R. Krasluklans, *J. Chem. Soc., Faraday Trans. 1*, 1991, **87**, 2943.
21 H. Chandra, L. Portwood and M. C. R. Symons, *J. Chem. Soc., Faraday Trans. 1*, 1989, **85**, 1801.
22 M. C. Depew, Z. L. Liu and J. K. S. Wan, *J. Am. Chem. Soc.*, 1983, **105**, 2480.
23 K. Reszka, P. Kolodziejczyk and J. W. Lown, *Free Radical Biol. Med.*, 1986, **2**, 267.
24 Y. Z. Hu, J. Y. An, L. J. Jiang and D. W. Chen, *J. Photochem. Photobiol., A*, 1995, **89**, 45.
25 Y. Y. He, J. Y. An, W. Zou and L. J. Jiang, *J. Photochem. Photobiol., B*, 1998, **44**, 45.
26 Y. Y. He, J. Y. An and L. J. Jiang, *J. Photochem. Photobiol., A*, 1998, **115**, 213.
27 J. R. Harbour and M. L. Hair, *J. Phys. Chem.*, **82**, 1397.
28 K. Lang, D. M. Wagnerova, P. Stopka and W. Damerau, *J. Photochem. Photobiol., A*, 1992, **67**, 187.
29 S. P. Sanders, S. J. Harrison, P. Kuppusamy, J. T. Sylvester and J. L. Zweier, *Free Radical Biol. Med.*, 1994, **16**, 753.
30 E. Finkelstein, G. M. Rosen and E. J. Rauckman, *Arch. Biochem. Biophys.*, 1980, **200**, 1.
31 J. B. Feix and B. Kalyanaraman, *Arch. Biochem. Biophys.*, 1991, **291**, 43.
32 B. Halliwell and J. M. C. Gutteridge, *Arch. Biochem. Biophys.*, 1986, **246**, 501.
33 Y. Lion, M. Delmelle and A. V. D. Vorst, *Nature (London)*, 1976, **263**, 242.
34 D. R. Kearns, in *Organic Chemistry*, eds. H. H. Wasserman and R. W. Murray, Academic Press, New York, 1979, p. 139.
35 Z. J. Diwu and J. W. Lown, *J. Photochem. Photobiol., A*, 1992, **64**, 273.
36 Y. Z. Hu, J. Y. An and L. J. Jiang, *J. Photochem. Photobiol., B.*, 1993, **17**, 195.