Photodynamic activity and potential usability of 14-carboxyl hypocrellin B

Yuewei Zhao, Jie Xie and Jingquan Zhao*

Key Laboratory of Photochemistry, Center for Molecular Science, Institute of Chemistry The Chinese Academy of Sciences, Beijing 100080, People's Republic of China. E-mail: zhaojq@iccas.ac.cn

Received (in Montpellier, France) 18th November 2002, Accepted 9th January 2003
First published as an Advance Article on the web 14th April 2003

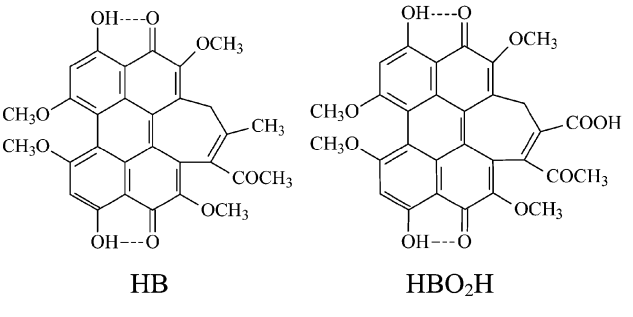
Not only the photosensitivity properties but also the amphiphilicity (both hydrophilicity and lipophilicity) are important factors for hypocrellins to be clinically applicable to photodynamic therapy of vas capillary diseases. A chemically modified derivative, 14-carboxyl hypocrellin B (HBO_2H), proved to possess far better amphiphilicity than its parent hypocrellin B (HB) and hypocrellin A (HA). In this paper, the photophysical and photochemical properties of HBO_2H were investigated by spectrophotometric methods and electron paramagnetic resonance (EPR) and its photodynamic action on mouse endothelial cells was also confirmed. In oxygen-free DMSO solution, semiquinone anion radicals ($HBO_2H^{\bullet -}$) are photogenerated *via* electron transfer between the excited triplet HBO_2H and electron donors or ground state HBO_2H (Type I mechanism). When oxygen is present, superoxide anion radicals ($O_2^{\bullet -}$) are generated *via* electron transfer from $HBO_2H^{\bullet -}$ to the ground state oxygen molecules. Singlet oxygen ($^1O_2$) can be produced *via* energy transfer from the triplet state HBO_2H to the ground state oxygen molecules (Type II mechanism). The quantum yield of singlet oxygen was estimated to be 0.63 in $CHCl_3$ with 0.76 for HB as a reference. Furthermore, investigations on the competition and transformation between $^1O_2$ and $HBO_2H^{\bullet -}$ suggested that the relative importance of Type I and Type II reactions would depend on the oxygen content in the target tissue.

Introduction

Photodynamic therapy (PDT) is a relatively new disease treatment to eradicate localized early-stage tumors and for palliation of more advanced disease where metastasis has occurred.[1–3] It is believed that PDT relies on the cytotoxic singlet oxygen ($^1O_2$) (Type II reaction) or free radicals (Type I reaction) produced by irradiation of the selectively adsorbed photosensitizer molecules on a neoplastic tissue. At present, Photofrin™ is the only clinically approved photosensitizer, however, it still has some limitations similar to its parent hematoporphyrin derivatives (HPD).[4,5] Furthermore, more and more people have realized that one phototherapeutic agent could not be universally used for all kinds of diseases and that a new strategy would be to develop specific photodynamic medicines according to the particularities of each disease. Therefore, finding alternative photosensitizers is still a subject of world-wide interest.

Hypocrellin A and B (HA and HB) belong to a new class of photosensitive pigments,[6,7] which derive their names from *Hypocrella bambusae* (*B. et Br*) sacs, growing abundantly in the northwestern region of China. Compared to HPD, these liposoluble 4,9-dihydroxy-3,10-perylenequinone derivatives do have some advantages.[8,9] There have been many reports on the photogeneration and the mechanism of formation of $^1O_2$ and active radical species by photosensitization of these perylenequinonoid derivatives.[10–18] Many investigations have collectively provided a compelling rationale for the development of hypocrellins as PDT photosensitizers.[11,13,19,20] However, there are some problems to resolve before hypocrellins can be clinically applicable.[21,22] First of all, the light absorption of most of hypocrellins appears mainly over 450–550 nm, which is quite far from the phototherapeutic window (600–900 nm) of tumors. Secondly, the liposoluble hypocrellins show very good cell-uptake but tend to self-aggregate in blood and may then block the vascular net; on the other hand, the completely water-soluble derivatives[19] show very poor cell-uptake and very low photodynamic activity. Therefore, a proper amphiphilicity is necessary for hypocrellin dyes to be clinically applicable.[23] Generally, there are two strategies to improve amphiphilicity: preparation of water-soluble liposomal hypocrellin medicines and synthesis of more hydrophilic derivatives by chemically modifying the hypocrellin parent (HA or HB). The latter possesses the advantages of definite structures as well as good chemical and physical stabilities.

Recently, great strides have been made in the photodynamic therapy of common diseases, especially for some kinds of vas capillary conditions, such as port wine stains and age-related macular degeneration (AMD), for which nearly no alternative methodology is currently available. Hypocrellins possess special advantages for the photodynamic therapy of this kind of diseases occurring at shallow tissue depths (1–2 mm), which is exactly the penetration depth of light with wavelengths from



Scheme 1 The chemical structures of HB and 14-carboxyl hypocrellin B

 DOI: 10.1039/b211388k

450 to 550 nm, exactly the same as the spectral range of hypocrellins. For efficient photodynamic therapy of vascular diseases, it is necessary for the photosensitizers to be not only transported fluidly in the vascular net but also taken up efficiently by the cell. In consideration of this point, a chemical derivative of HB, HBO_2H (Scheme 1), derived by oxidization of the 14-position methyl of hypocrellin B to carboxyl, has been chosen as a candidate for better amphiphilicity. Although this derivative was synthesized some years ago in our research group, up to now the photosensitization mechanism and the efficiency of HBO_2H have not been investigated. In the current work, the photogeneration mechanism of the active species *via* the photosensitization of HBO_2H was studied by using electronic paramagnetic resonance (EPR) and spectrophotometric methods; the photodynamic action on mouse endothelial cells was also determined.

Material and methods

Reagents

HB was prepared as described previously.[24] 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), 9,10-diphenylanthracene (9,10-DPA), 2,2,6,6-tetramethyl-4-piperidone (TEMP) and 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl radical (TEMPO) were all purchased from Aldrich Chemical Company. Catalase 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, the Chinese Academy of Sciences. 1,4-Diazabicyclo[2,2,2]octane (DABCO) was purchased from Merck Chemical Company. Other agents of analytical grades were purchased from Beijing Chemical Plant. Water was freshly distilled before use. The solutions were purged with oxygen, air and argon according to experimental requirements.

Synthesis of 14-carboxyl hypocrellin B (HBO_2H). HBO_2H was synthesized according to the literature[25] and its characterization by IR, H-NMR and MS (FAB) proved to be in good agreement with the reported values.

Spectroscopic measurements

The absorption spectra were recorded on a Shimadzu UV-1601 spectrophotometer. The variation of the absorbance with irradiation time in the presence of an electron donor was used to monitor the photo-induced reduction of HBO_2H. A 450 W medium pressure sodium lamp was used as light source and a long pass filter was employed to eliminate light of wavelengths shorter than 470 nm. Fluorescence emission spectra were obtained on a Hitachi F-4500 spectrofluorimeter.

The DPA-bleaching method was used to determine the quantum yields of $^1O_2$, as described in detail by Diwu and Lown[10] 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. The oxidative reaction of DPA was carried out on a 'merry go round' apparatus and monitored by observing the decrease of the absorption peak of DPA at 374 nm.

EPR measurements

The EPR measurements were performed at room temperature using a Bruker EPR 300E spectrometer. Unless otherwise indicated, the instrumental settings were: microwave power of 10.02 mW (3.17 mW for the TEMPO signal); modulation amplitude, 1.012 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 (Spectro-Physics Lasers, Mountain View, CA, USA) was used as the light source. Samples were injected into quartz capillaries designed specially for EPR analysis. Anaerobic or aerobic samples were prepared by purging the reactive volume with argon or oxygen for 30 min in the dark. EPR signals were recorded 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 20 s.

The values of partition coefficients (PC) and IC_{50} of HBO_2H were determined according to the method described in ref. 23.

Table 1 Partition coefficients (PC) and half inhibition concentration (IC_{50}) (0.5 μg mL^{-1}) of hypocrellin dyes

Compound	PC	IC_{50}
HA	41.6[23]	0.063
HB	46.4[23]	0.079
HBO_2H	11.6	0.096

Results and discussion

The measurement of partition coefficients (PC) and half inhibition concentration (IC_{50})

The partition coefficient value of HBO_2H is 11.6 (Table 1) and this is a great improvement compared to that of the parent hypocrellins.[23] It is easily soluble in ethanol and dimethylsulfoxide (DMSO). The IC_{50} values were determined by using mouse endothelial cells with the photosensitizers dissolved in DMSO. It can be seen that the photodynamic action for HBO_2H is relatively weaker than that for HA or HB, which is a typical feature for water-soluble photosensitizers. That is, the more hydrophilic a photosensitizer is, the weaker is the photodynamic action. However, the photodynamic activity for HBO_2H is high enough for clinical therapy of vas capillary diseases due to the very high photodynamic activity of hypocrellins for these diseases. Furthermore, fresh photosensitizer molecules can be continuously provided by blood flow during treatment.

Photophysical properties

The absorption and fluorescence spectra of HBO_2H are shown in Fig. 1. Comparing the chemical structure of HBO_2H with that of HB (Scheme 1), it can be seen that the only structural change is the substitute group at the 14-position of the seven-member ring moiety, while the skeleton of perylenequinone is kept unchanged. Due to this change, the water-solubility of HBO_2H is enhanced greatly (Table 1); the absorption and the fluorescent spectra are mainly unchanged but the molar extinction coefficient of HBO_2H is smaller.

Photo-induced generation of semiquinone anion radical by HBO_2H

Just as with the parent HB, photosensitization of HBO_2H can generate the semiquinone anion radical ($HBO_2H^{\bullet -}$), which is stable in anaerobic and aprotic solution, such as DMSO, and easily detectable by EPR measurement, with $g = 2.0036$ [Fig. 2(A)], or by UV-Vis spectrophotometry in the presence of electron donors. It should be indicated that the cation radical $HBO_2H^{\bullet +}$ cannot be detected under our experimental conditions because of the too short lifetime. In fact, it was reported that only in solvents with high ionization potentials, such as Freon-113, and at low temperature is detection possible.[26]

The EPR signal intensity of $HBO_2H^{\bullet -}$ was dependent on the irradiation intensity and time [Fig. 2(A), inset a] as well as the concentration of HBO_2H [Fig. 2(B), inset b]. When the concentration of HBO_2H reached 2 mM, the EPR signal of $HBO_2H^{\bullet -}$ could be observed even in an air-saturated system [Fig. 2(B)].

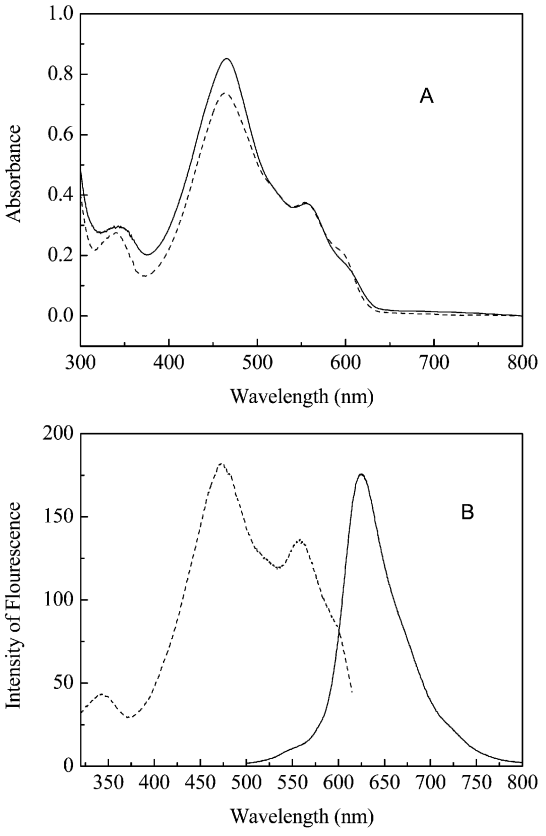


Fig. 1 (A) Absorption spectra of HB and HBO_2H in chloroform: (—) HB, 30 μM; (—) HBO_2H, 100 μM. (B) Fluorescence emission and excitation spectra of HBO_2H in chloroform: (...) $\lambda_{em} = 640$ nm; (—) $\lambda_{ex} = 465$ nm; OD about 0.1 at 470 nm.

These observations imply that the radicals may be generated *via* self-electron transfer between the excited triplet and ground state species in the absence of electron donors [eqns (1) and (2)]:

$$HBO_2h \xrightarrow{h\nu} {}^1HBO_2H \xrightarrow{ISC} {}^3HBO_2H \quad (1)$$

$${}^3HBO_2H + HBO_2H \rightarrow HBO_2H^{\bullet+} + HBO_2H^{\bullet-} \quad (2)$$

When NADH, a typical electron donor (D), was added to the solution and irradiated, the signal intensity of the radicals

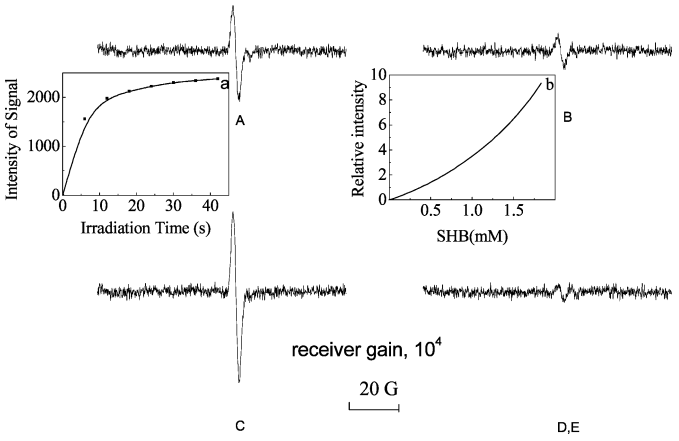


Fig. 2 (A) Photo-induced EPR spectrum from a deoxygenated DMSO solution of HBO_2H (1 mM) under illumination for 30 s. (B) Photo-induced EPR spectrum from an air-saturated DMSO solution of HBO_2H (2 mM) under illumination for 30 s. (C) Same as in A except that NADH (2.5 mM) was added. (D) Same as in A but without HBO_2H or illumination. (E) Same as in A except that oxygen was bubbled through the solution after illumination.

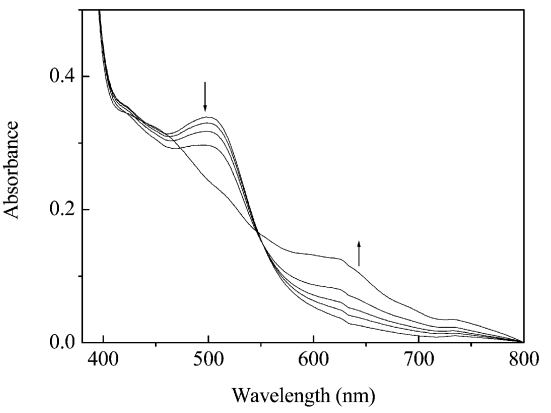


Fig. 3 Absorption spectra in deoxygenated DMSO solution of HBO_2H (1 mM) and NADH (50 uM) upon irradiation for 5, 10, 15, 20 and 25 s. The arrows indicate the direction of changes.

was enhanced greatly [Fig. 2(C)] with a similar spectral shape to that in Fig. 2(A). No EPR signal was observed without HBO_2H or illumination [Fig. 2(D)], which implies that the EPR signal was generated *via* photosensitization of HBO_2H. This also implies that the electron transfer from D to triplet HBO_2H generated the anion radicals. The addition of other electron donors such as GSH and ascorbic acid gave similar results [eqn. (3)]:

$${}^3HBO_2H + D \rightarrow HBO_2H^{\bullet-} + D^{\bullet+} \quad (3)$$

When oxygen was bubbled through the irradiated HBO_2H solution, the EPR signal of $HBO_2H^{\bullet-}$ disappeared completely [Fig. 2(E)]. Furthermore, when DMPO was added to the oxygen-saturated solution, the EPR signal of DMPO–$O_2^{\bullet-}$ adducts (details discussed below) appeared immediately, accompanied by a significant decrease or disappearance of the $HBO_2H^{\bullet-}$ radicals. This confirms that the EPR signal in Fig. 2(A) originated from $HBO_2H^{\bullet-}$. In accordance with these observations, the EPR signal shown in Fig. 2(A) can be safely assigned to the semiquinone anion radicals of HBO_2H.

With NADH in the argon-saturated DMSO solution, the appearance of $HBO_2H^{\bullet-}$ could be easily detected spectrophotometrically, as shown in Fig. 3. Under irradiation, the absorption peaks of HBO_2H at 500 nm decreased while new peaks appeared at 630 nm and increased with longer irradiating time, accompanied by a isosbestic point at 547 nm within the spectral region examined (300–700 nm). The photoproducts were very stable in the absence of oxygen. When oxygen was bubbled into the solution for half an hour in the dark after irradiation, the absorption spectrum of the photoproducts disappeared completely with an 85% recovery of the absorbance of HBO_2H. Since a strong reducing agent and argon were both necessary for the reaction, it is reasonable to assign the intermediate to the reduced form of HBO_2H, $HBO_2H^{\bullet-}$. Both EPR and spectrophotometric results prove that semiquinone anion radicals ($HBO_2H^{\bullet-}$) can be photogenerated through one-electron reduction of HBO_2H.

Generation of superoxide anion radical ($O_2^{\bullet-}$) by HBO_2H

It was mentioned above that the signal of $HBO_2H^{\bullet-}$ [shown in Fig. 2(A)] disappeared when oxygen was bubbled through the deoxygenated HBO_2H solution. When DMPO and oxygen were both present in the reaction system, a new EPR signal appeared. This multiple EPR spectrum [Fig. 4(A)] was characterized by the coupling constants $\alpha^N = 12.89$ G, $\alpha_\beta^H = 10.67$ G and $\alpha_\gamma^H = 1.56$ G, which are in good agreement with the signal of DMPO–$O_2^{\bullet-}$.[27] Adding DMPO to an argon-saturated system, only the signal of $HBO_2H^{\bullet-}$ was detected. This observation indicates that the oxidation of the semiquinone

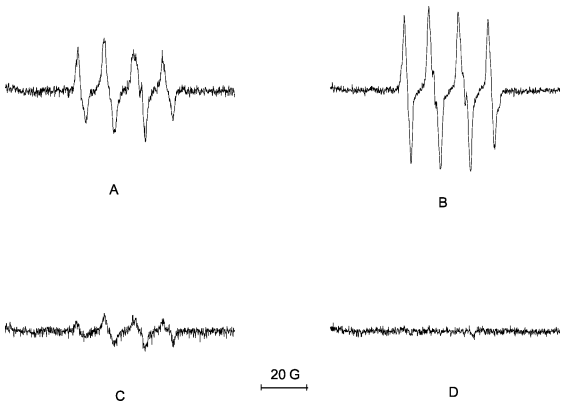


Fig. 4 (A) EPR spectrum of the DMPO–superoxide radical adduct produced on irradiation of an oxygen-saturated DMSO solution of HBO_2H (1 mM) and DMPO (50 mM). (B) Same as in A but NADH (25 mM) was added. (C) Same as in A but in the presence of SOD (25 μg mL^{-1}). (D) Same as in A but oxygen, HBO_2H or light was absent.

anion radicals by the dissolved oxygen led to formation of another radical species that could be trapped by DMPO. The reaction mechanism can be expressed as eqn. (4):

$$HBO_2H^{\bullet -} + O_2 \rightarrow O_2^{\bullet -} + HBO_2H \quad (4)$$

Superoxide radical adducts were formed [Fig. 4(A)] while illuminating an oxygen-saturated DMSO solution of HBO_2H (1 mM) and DMPO (50 mM). When oxygen or irradiation or HBO_2H was absent, the signal of DMPO–$O_2^{\bullet -}$ did not appear [Fig. 4(D)]. Addition of SOD (25 μg mL^{-1}) prior to illumination reduced the formation of DMPO–$O_2^{\bullet -}$ adducts [Fig. 4(C)], whereas thermally denatured SOD had no effect on the intensity of the EPR signal. These observations further confirm the formation of superoxide anion radicals.

Introduction of singlet oxygen ($^1O_2$) inhibitors (DABCO, NaN_3 or histidine) into the solution did not affect the EPR signal of superoxide anion radicals. This result rules out the possibility of $^1O_2$ participation in the generation of $O_2^{\bullet -}$. Moreover, catalase (50 μg mL^{-1}) and hydrogen peroxide (10 mM) had a negligible effect, thus excluding a role of H_2O_2 in the formation of $O_2^{\bullet -}$. Addition of electron donors, such as NADH (25 mM), cysteine and GSH, strengthened the EPR signal of the DMPO–$O_2^{\bullet -}$ species [Fig. 4(B)], for the electron donors could promote the formation of $HBO_2H^{\bullet -}$, which in turn contributes to the formation of $O_2^{\bullet -}$ [eqns (4) and (5)]. Besides, NAD$^{\bullet}$, the oxidized intermediate of NADH, can also participate in the formation of $HBO_2H^{\bullet -}$ and the generation of $O_2^{\bullet -}$ [eqns (6) and (7)].[28] Other reactions may also be possible, such as that shown in eqn. (8), though there are no data available for this reaction.[29]

$$^3HBO_2H + NADH \rightarrow HBO_2H^{\bullet -} + NAD^{\bullet} + H^+ \quad (5)$$

$$NAD^{\bullet} + HBO_2H \rightarrow HBO_2H^{\bullet -} + NAD^+ \quad (6)$$

$$NAD^{\bullet} + O_2 \rightarrow O_2^{\bullet -} + NAD^+ \quad (7)$$

$$^3HBO_2H + O_2 \rightarrow O_2^{\bullet -} + HBO_2H^{\bullet +} \quad (8)$$

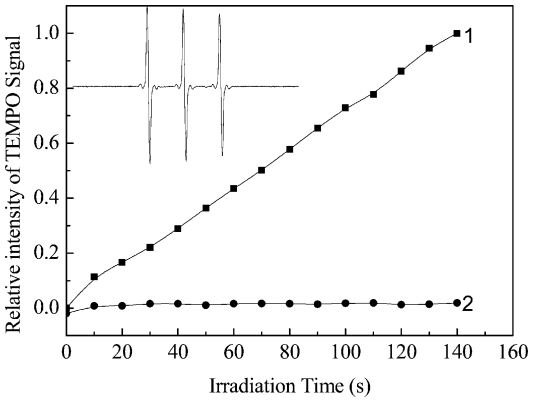
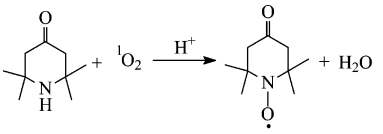


Fig. 5 The EPR signal intensities of the TEMPO radicals formed in oxygenated DMSO solution of HBO_2H (1 mM) and TEMP (30 mM) as a function of irradiation time using 532 laser light (line 1). Line 2, Same as line 1 except that DABCO (10 mM) was added.

Generation of $^1O_2$ by HBO_2H

It was reported that TEMP can react with singlet oxygen to produce TEMPO and could thus be used for detection of singlet oxygen[30] [eqns (9) and (10)]. The TEMPO nitroxide radicals can easily be detected by EPR measurement.

$$^3HBO_2H + O_2 \rightarrow {}^1O_2 + HBO_2H \quad (9)$$



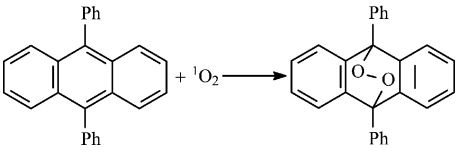
(10)

TEMP TEMPO

When an oxygen-saturated DMSO solution of HBO_2H (1 mM) and TEMP (30 mM) was irradiated at room temperature, an EPR spectrum of triplet peaks with equal intensity, characteristic of a nitroxide radical, was observed (inset of Fig. 5). The hyperfine splitting constant and g factor were identical to those of commercial TEMPO. Control experiments showed that TEMP and HBO_2H and oxygen and light were necessary for observation of the TEMPO signal. These data demonstrate that $^1O_2$ was involved in the formation of TEMPO radicals.

To provide further evidence for the generation of $^1O_2$ in the photosensitization, when a $^1O_2$ scavenger, DABCO, NaN_3 or histidine, was added the TEMPO signal was suppressed (line 2 in Fig. 5). On the other hand, when deuterated DMSO was used as a typical diagnostic for $^1O_2$, the intensity of the TEMPO signals increased by 6–8 times (data not shown), suggesting that TEMPO was derived from the reaction of TEMP with $^1O_2$, which in turn was produced by energy transfer from excited triplet HBO_2H to ground state molecular oxygen [eqns (9) and (10)].[10]

The DPA-bleaching method has been confirmed to be an efficient measurement for the quantum yield of $^1O_2$ generated through photosensitization [eqn. (11)];[10] therefore, it was adopted to determine the quantum yield of singlet oxygen produced by HBO_2H, using the quantum yield of HB (0.76) as the reference.



(11)

Prior to illumination, the optical densities of both HB and HBO_2H at 436 nm were adjusted to be the same. Fig. 6 shows the rates of DPA-bleaching photosensitized by HB (line 1) and HBO_2H (line 2) as a function of irradiation time in $CHCl_3$. The absorption spectra of HBO_2H in the DPA bleaching system at a series of illumination times are shown in the inset of Fig. 6. Control experiments indicated that no DPA-bleaching occurred without photosensitizers, oxygen or irradiation. Addition of DABCO or NaN_3 (10 mM) completely inhibited DPA-bleaching (line 3). These data confirm that the bleaching of DPA resulted from the reaction of DPA with singlet oxygen formed by photosensitization of HBO_2H. The $^1O_2$ quantum yield for HBO_2H was estimated to be 0.63.

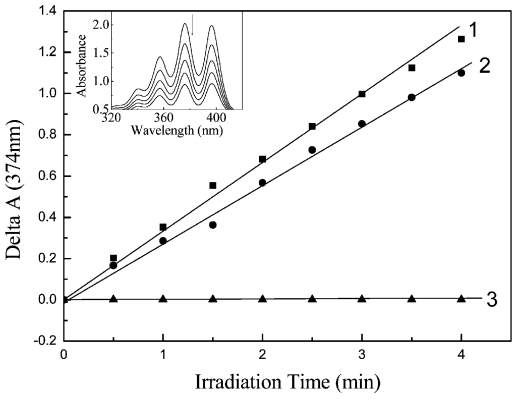


Fig. 6 Line 1: Photosensitized DPA-bleaching by measuring the absorbance decrease (ΔA) at 374 nm as a function of irradiation time in oxygen-saturated $CHCl_3$ solution containing HB and DPA (0.3 mM). Line 2: same as line 1 but HB was replaced by HBO_2H. Line 3: same as line 1 except that DABCO (10 mM) was added.

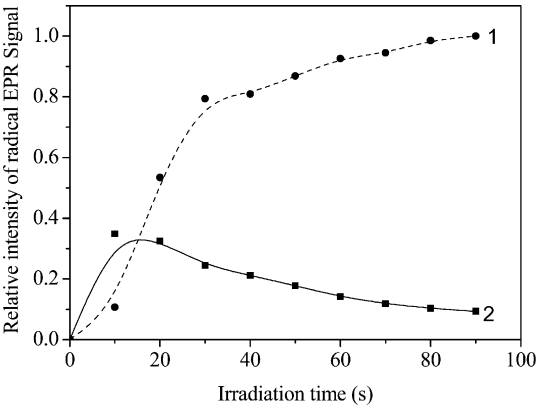


Fig. 7 Line 1: Dependence of the $HBO_2H^{\bullet -}$ signal intensity of a sealed air-saturated system of HBO_2H (1 mM) and TEMP (30 mM) on the irradiation time. Line 2: Dependence of the TEMPO signal intensities on the irradiation time in the same system.

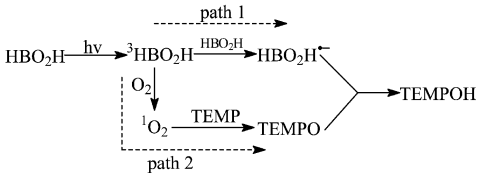
The competition and transformation between TEMPO and $HBO_2H^{\bullet -}$ radicals in an air-saturated system

TEMPO can react with $Sens^{\bullet -}$ *via* eqn. (12) to produce TEMPOH, the non-radical product. In fact, both TEMPO and $Sens^{\bullet -}$ radicals are formed by the same precursor, the excited triplet photosensitizer. The EPR signal intensity of TEMPO is also dependent on the concentration of singlet oxygen. The reactions of $HBO_2H^{\bullet -}$ and TEMPO may be summarized in Scheme 2.

$$Sens^{\bullet -} + TEMPO \rightarrow TEMPOH + Sens^{2-} \qquad (12)$$

Just as shown in Scheme 2, the $HBO_2H^{\bullet -}$ radicals will be generated through path 1 and TEMPO through path 2 from the same species, the excited triplet HBO_2H molecule; therefore, the two reactions will compete for the triplet HBO_2H. In the reacting system, the two reactants, TEMP and HBO_2H, are abundant relative to singlet oxygen so that their concentrations can be taken to be roughly invariable during the reactions. In this case, the concentration of singlet oxygen ($^1O_2$) produced in the system can be directly estimated by the EPR signal intensity of TEMPO, while the rise and fall of the EPR signal intensities of $HBO_2H^{\bullet -}$ and TEMPO reflects consumption of singlet oxygen during the reaction processes.

When a sealed air-saturated DMSO solution of HBO_2H (1 mM) and TEMP (30 mM) was irradiated, the EPR signal of TEMPO increased to its maximum in about 10 s in virtue of the presence of enough oxygen, while the signal of $HBO_2H^{\bullet -}$ also increased slowly within this time scale (Fig. 7) but with a lower rate than that of path 2. Along with the consumption of oxygen in the system, the formation of $^1O_2$ was suppressed while the generation of $HBO_2H^{\bullet -}$ was promoted. Because the reaction of TEMPO with $HBO_2H^{\bullet -}$ was still occurring, there was a relative accumulation of $HBO_2H^{\bullet -}$ and depletion of TEMPO until the singlet oxygen (as well as oxygen) in the system was exhausted.



Scheme 2 The generation and reaction course of $HBO_2H^{\bullet -}$ and TEMPO in a sealed air-saturated system.

Conclusions

The spectrophotometric and EPR investigations demonstrate that HBO_2H can be photosensitized to produce semiquinone anion radicals ($HBO_2H^{\bullet -}$), superoxide anion radical ($O_2^{\bullet -}$) and siglet oxygen ($^1O_2$) with a quantum yield of 0.63. The competition and transformation between $^1O_2$ and semiquinone anion radical during irradiation on a sealed system depends on the content of oxygen. Therefore, it can be concluded that 14-carboxyl Hypocrellin B (HBO_2H) is a novel, potential phototherapeutic agent that can effectuate the photodynamic action not only by the Type I (electron transfer) but also by the Type II (energy transfer) mechanisms, whose relative importance depends on the oxygen content in the target tissues. Furthermore, due to its better water-solubility than the parent HB, this derivative may be a better choice for photodynamic therapy, especially for that of vas capillary diseases.

Acknowledgements

This Project was supported by NSFC No 20273079.

References

1 B. W. Herderson and T. J. Dougherty, *Photochem. Photobiol.*, 1992, **55**, 145.
2 J. Moan and K. Berg, *Photochem. Photobiol.*, 1992, **55**, 931.
3 D. Kessel, *Oncol. Res.*, 1992, **4**, 219.
4 C. J. Gomer, *Photochem. Photobiol.*, 1991, **54**, 1093.
5 L. I. Grossweiner, *The Science of Phototherapy*, CRC Press Inc., Boca Raton, FL, USA, 1994, ch. 8.
6 J. Y. An, Y. Z. Hu and L. J. Jiang, *J. Photochem. Photobiol., B.*, 1996, **33**, 261.
7 Y. Z. Hu, J. Y. An, L. J. Jiang and D. W. Chen, *J. Photochem. Photobiol., A*, 1995, **89**, 45.
8 Z. J. Diwu and J. W. Lown, *J. Photochem. Photobiol., A*, 1990, **52**, 609.
9 Y. Z. Song, J. Y. An and L. J. Jiang, *Biochim. Biophys. Acta*, 1999, **1472**, 307.
10 Z. J. Diwu and J. W. Lown, *J. Photochem. Photobiol., A*, 1992, **64**, 273.
11 J. B. Hudson, J. Zhou, J. Chen, L. Harris, L. Yip and G. H. N. Towers, *Photchem. Photobiol.*, 1994, **60**, 253.
12 M. J. Fehr, S. L. Carpenter, Y. Wannemuehler and J. W. Petrich, *Biochemistry*, 1995, **34**, 15845.
13 J. Hirayama, K. Ikebuchi, H. Abe, K. W. Kwon, Y. Ohnishi, M. Horiuchi, M. Shinagawa, K. Ikuta, N. Kamo and S. Sekiguchi, *Photochem. Photobiol.*, 1997, **66**, 697.
14 Y. Y. He and L. J. Jiang, *Free Radical Biol. Med.*, 2000, **28**, 1642.
15 Y. Y. He, J. Y. An and L. J. Jiang, *Free Radical Biol. Med.*, 1999, **26**, 1146.

16 W. Zou, J. Y. An and L. J. Jiang, *J. Photochem. Photobiol., B*, 1996, **33**, 73.
17 J. H. Ma and L. J. Jiang, *Free Radical Res.*, 2001, **35**, 767.
18 Y. Y He, J. Y. An and L. J. Jiang, *Free Radical Biol. Med.*, 1999, **27**, 203.
19 Y. Z. Hu, J. Y. An and L. J. Jiang, *J. Photochem. Photobiol., B*, 1993, **17**, 195.
20 J. Zhang, E. H. Cao, J. F. Li, T. C. Zhang and W. J. Ma, *J. Photochem. Photobiol., B*, 1998, **43**, 106.
21 Z. J. Diwu, *Photochem. Photobiol.*, 1995, **61**, 529.
22 K. Reszka, J. A. Hartley and J. W. Lown, *Biophys. Chem.*, 1990, **35**, 313.
23 Y. Y. He, H. Y. Liu, J. Y. An, R. Han and L. J. Jiang, *Dyes Pigm.*, 2000, **44**, 63.
24 Y. Y. He, J. Y. An, W. Zou and L. J. Jiang, *J. Photochem. Photobiol., B*, 1998, **44**, 45.
25 K. H. Zhao and L. J. Jiang, *Chin. J. Org. Chem.*, 1990, **10**, 339.
26 J. Mayer and R. Krasluklanis, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 2943.
27 J. R. Harbour and M. L. Hair, *J. Phys. Chem.*, 1978, **82**, 1397.
28 K. Reszka, P. Koldiziejczyk, P. G. Tsoungas and J. W. Lown, *Photochem. Photobiol.*, 1988, **47**, 625.
29 C. Hadjur, G. Wagnieres, P. Monnier and H. van de Bergh, *Photochem. Photobiol.*, 1997, **65**, 818.
30 Y. Lion, M. Delmelle and A. Van de Vorst, *Nature*, 1976, **263**, 442.