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ESR studies of the photodynamic properties of a long-wavelength and water-soluble hypocrellin B derivative: photogeneration of semiquinone radical anion and activated oxygen

Yu-Zhu Song, Jing-Yi An, Li-Jin Jiang*

Institute of Photographic Chemistry, Chinese Academy of Sciences, Beijing 100101, PR China

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Abstract

15-Deacetyl-13-glycine hypocrellin B (13-Gly-DHB) is a new type of long-wavelength perylenequinone with enhanced water solubility. Its photophysical and photochemical properties were investigated by electron spin resonance (ESR) spectrum associated with spin trapping techniques. When 13-Gly-DHB in DMSO or DMSO-buffer (pH 7.4) were illuminated with visible light >470 nm, semiquinone radical anion, superoxide anion radical, hydroxyl radical and singlet oxygen were formed depending on different conditions. In anaerobic solution, semiquinone radical anion of 13-Gly-DHB was predominantly photoproduced via the self-electron transfer between the excited- and ground-state species. The presence of an electron donor significantly promotes the reduced form of 13-Gly-DHB. In aerobic solution, superoxide anion radical ($O_2^{\bullet -}$) was generated by the 13-Gly-DHB anion radical (13-Gly-DHB$^{\bullet -}$) via electron transfer to oxygen, and this process was significantly enhanced by the presence of electron donors. In aqueous solution, the superoxide radical anion formed can rapidly transform to a hydroxyl radical via the Fenton reaction and via the reaction of the semiquinone radical anion with H_2O_2. Singlet oxygen ($^1O_2$) was also formed in the photosensitization of 13-Gly-DHB in an aerobic solution. These findings suggest that the photodynamic action of 13-Gly-DHB may proceed via both type I and type II mechanisms.

Keywords: Electron spin resonance; Photodynamic action; Semiquinone radical anion; Reactive oxygen species

1. Introduction

Hypocrellin A and B (HA and HB) (Fig. 1) are new types of photosensitive pigments and medicines [1,2], which derive their names from *Hypocrella bambusae* (B. et Br) sacs, growing abundantly in the northwestern region of Yunnan Province in China. These lipid-soluble 4,9-dihydroxy-3,10-perylenequinone derivatives, exhibit several advantages over the presently used haematoporphyrin derivatives (HPD), such as ease of preparation and purification relative to HPD, small aggregation tendency which decreases the efficiency of HPD, strong red light absorptivity and significantly reduced normal tissue photosensitivity because of the rapid metabolism in vivo [3]. In this way, hypocrellins have been successfully employed as antitumour agents in the treatment of various skin diseases [4–6].

Hypocrellins do not, however, display sufficiently strong absorptivity at wavelengths >600 nm and they are insoluble in water. These disadvantages limit their application in photodynamic therapy (PDT). For these reasons, we have recently synthesized a new type of long-wavelength perylenequinone with enhanced water solubility, namely 15-deacetyl-13-glycine substituted hypocrellin B (13-Gly-DHB) (Figs. 1 and 2) in high yield. 13-Gly-DHB displays absorption in a region >500 nm that maybe due to the 13-glycine acting as auxochrome. We have investigated the photodynamic properties of the new compound. Evidence is accumulating that the generation of reactive oxygen species is intimately associated with the photodynamic effect of many sensitizers. A light activated sensitizer can transfer energy from its triplet state by two processes, directly to molecular oxygen with generation of singlet oxygen ($^1O_2$) (type II reaction) or by interaction with solvent or substrate by electron or proton transfer with the generation of radicals (type I reaction). When 13-Gly-DHB was illuminated with visible light, the formation processes of semiquinone radical anion and activated oxygen ($^{\bullet}O_2$, $O_2^{\bullet -}$, $^{\bullet}OH$) were studied by electron spin resonance (ESR) in detail. The formation mechanisms of semiquinone radical anion, superoxide radical anion, hydroxyl radical and singlet oxygen were studied, and the results show that both type I and type II mechanisms

*Corresponding author. Fax: +86-10-64879375; e-mail: jyan@ipc.ac.cn

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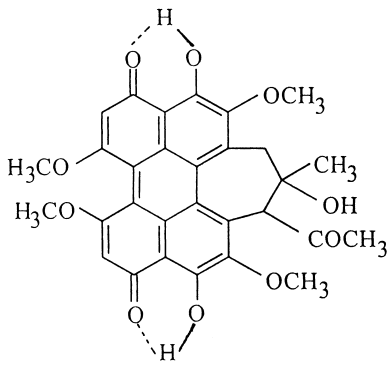
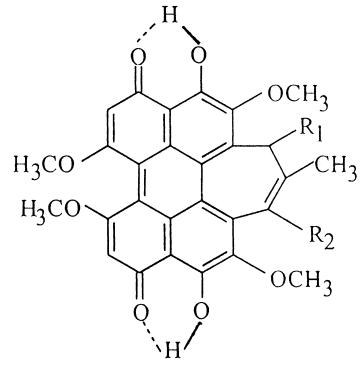


HA



HB (R_1=H, R_2=$COCH_3$)

13-Gly-DHB (R_1=$NHCH_2COOH$, R_2=H)

Fig. 1. Chemical structures of HA, HB, 13-Gly-DHB.

are involved in the photosensitization of 15-deacetyl-13-glycine substituted hypocrellin B.

2. Materials and methods

HA and HB were extracted from fungus sacs of *Hypocrella bambusae* and were purified by recrystallization from acetone. 13-Gly-DHB (Fig. 1) was synthesized in connection with other work (Yu-zhu Song, Jing-yi An, Li-jin Jiang, unpublished data). 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO), 2,2,6,6-tetramethyl-4-piperidone (TEMP) and diethylenetriaminepentaacetic acid (DTPA) were purchased from Aldrich. Superoxide dismutase (SOD), histidine, catalase, cysteine and reduced glutathione were purchased from Biotech Technology, Chinese Academy of Sciences. 1-Benzyl-1,4-dihydronicotinamide (BNAH) was prepared by the reduction of nicotinamide chloride with dithionite. 1,4-Diazabicyclo[2,2,2] Octane (DABCO) was obtained from Merk. Sodium azide (NaN_3), reduced nicotinamide adenine dinucleotide (NADH), ethylenediaminetetraacetic acid (EDTA), dimethyl sulphoxide (DMSO) and other solvents—all 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. The required high-purity solvents were prepared by further purification of the commercial products, and no impurities were detected by absorption and/or fluorescence spectroscopy.

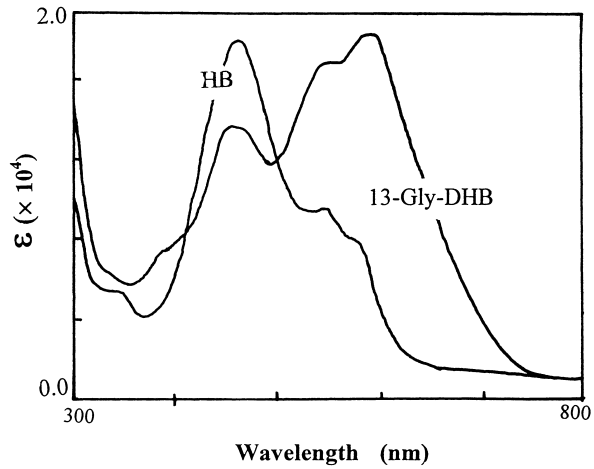


Fig. 2. Absorption spectra of HB (5×10^{-5} M) and 13-Gly-DHB (5×10^{-5} M) in chloroform.

Measurements of the ESR spectra were carried out on a Bruker ESP 300 E spectrometer operating at room temperature (*x* band; microwave frequency, 9.5 GHz). Samples were purged with oxygen or argon for 30 min in the dark according to experimental requirements; thereafter, they (30 μg) were injected quantitatively into specially made quartz capillaries for ESR analysis. A 450-W medium-pressure sodium lamp was used as a light source. A long pass filter was employed to eliminate light of wavelengths <470 nm. In all of these experiments, solutions were irradiated outside the cavity in capillaries and the ESR spectra were recorded within 3 min after exposure. The superoxide radical anions ($O_2^{\bullet -}$), and hydroxyl radicals ($^{\bullet}OH$) produced were identified by means of spin-trapping method with ESR detection. The reaction of $^1O_2$ with TEMP in combination with ESR detection [7] was used to determine the formation of $^1O_2$ by 13-Gly-DHB.

3. Results and discussion

3.1. Photogeneration of semiquinone radical anion and effect of electron donor

Irradiation of 13-Gly-DHB (1 mM) in an argon-gassed dimethylsulphoxide (DMSO) solution for 1 min led to the generation of a strong ESR signal as shown in Fig. 3

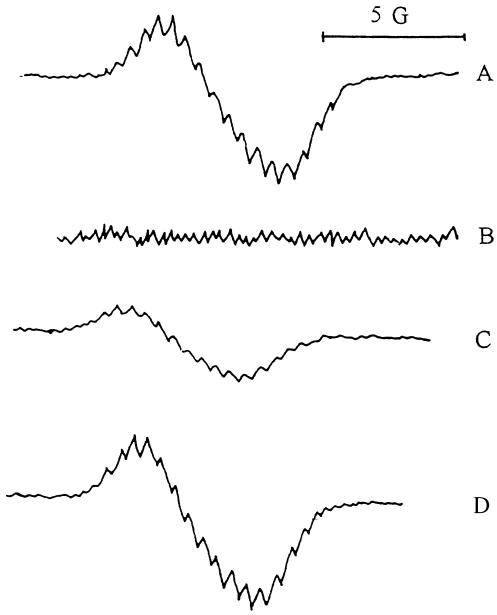


Fig. 3. (A) ESR spectrum from a deaerated DMSO solution of 13-Gly-DHB (1 mM) on irradiation above 470 nm for 1 min; (B) same as spectrum A, but oxygen was bubbled through the solution after illumination; (C) ESR spectrum from a high concentration of 13-Gly-DHB (10 mM) after oxygen was bubbled through the solution on irradiation above 470 nm for 5 min; and (D) same as spectrum A, but in the presence of BNAH (1 mM) and irradiation for 30 s. Instrument setting: microwave power, 8.00 mW; modulation amplitude, 0.102 G; scan range, 50 G; receiver gain, 1×10^5 (spectra A, B, and C); 1×10^4 (spectrum D).

spectrum A, with $g = 2.0038$. The hyperfine structure of this ESR spectrum was very similar to but simpler than that of the semiquinone radical anion of hypocrellin B [8]. The intensity of the signal increased rapidly during photoirradiation and decreased very slowly in the dark. The intensity of the ESR signal of the 13-Gly-DHB radical depended on the presence of oxygen, the concentration of 13-Gly-DHB, illumination time and intensity. When the sample was exposed to oxygen, the ESR signal was quenched (Fig. 3, spectrum B). But if the concentration of 13-Gly-DHB is high enough (10 mM) and the illumination time is long enough (5 min), the semiquinone signal can still be detected even in aerated DMSO solution (Fig. 3, spectrum C). The strong concentration effect indicated that 13-Gly-DHB radical might be generated by self-electron transfer between the ground and excited states according to [9].

$$\text{13-Gly-DHB}^* + \text{13-Gly-DHB} \rightarrow \text{13-Gly-DHB}^{\bullet -} + \text{13-Gly-DHB}^{\bullet +} \quad (1)$$

The ESR spectrum observed may be ascribed to the semiquinone radical anion of 13-Gly-DHB (13-Gly-DHB$^{\bullet -}$) because the radical cation of quinone is very difficult to detect in common organic solvents and water owing to its strong oxidizing ability [10]. In order to identify this deduction, the following experiment was carried out.

13-Gly-DHB (1 mM) in DMSO was illuminated in the presence of BNAH, a typical reductant, for 1 min, the ESR signal of 13-Gly-DHB was intensified significantly (Fig. 3, spectrum D). Moreover, in the presence of other reductants such as cysteine, reduced glutathione, EDTA and NADH, the ESR signal of 13-Gly-DHB radical was also intensified. These indicate the anionic character of the 13-Gly-DHB radical.

In the presence of electron donor (D), 13-Gly-DHB may be generated by as follows

$$\text{13-Gly-DHB}^* + \text{D} \rightarrow \text{13-Gly-DHB}^{\bullet -} + \text{D}^{\bullet +} \quad (2)$$

It must be emphasized that 13-Gly-DHB$^{\bullet -}$ was also formed under aerobic conditions but is not observed due to the rapid reaction with O_2.

3.2. Spin trapping with DMPO

The ESR signal of the 13-Gly-DHB was quenched significantly by oxygen. When oxygen was bubbled through a solution of the 13-Gly-DHB, the signal of 13-Gly-DHB$^{\bullet -}$ disappeared completely. This observation indicated that the oxidation of the semiquinone by dissolved oxygen took place.

Irradiation of an aerobic 13-Gly-DHB DMSO (1 mM) solution with visible light ($\lambda > 470$ nm) in the presence of DMPO (45 mM) immediately generated an ESR spectrum of the DMPO–superoxide radical adduct. The typical spectrum of the adduct DMPO–$O_2^{\bullet -}$ was presented in Fig. 4, spectrum A. This multiple ESR spectrum is characterized by three coupling constants due to the presence of a nitrogen atom and two hydrogen atom in the β and γ positions. The determined constants $a^N = 12.9$, $a_\beta^H = 10.5$, $a_\gamma^H = 1.5$ G are in good agreement with the literature [11].

No signal of DMPO–$O_2^{\bullet -}$ was observed when any component in the system was missing, i.e. oxygen, DMPO, light and sensitizers were all necessary.

The ESR spectrum could be inhibited by the addition of SOD (Fig. 4, spectrum B), a specific and efficient scavenger for superoxide radical, which can inhibits the $O_2^{\bullet -}$ dependent DMPO–$O_2^{\bullet -}$ adduct formation. This result confirmed the correct assignment of the ESR spectrum to DMPO–$O_2^{\bullet -}$.

When BNAH—a typical electron donor—was added, the signal of the DMPO–$O_2^{\bullet -}$ was enhanced greatly (Fig. 4, spectrum C). Analogous results were obtained when other electron donors, such as reduced glutathione and NADH replaced BNAH.

Illumination of an Ar-saturated DMSO solution containing a low concentration of 13-Gly-DHB (20 μM) with visible light in either the presence or absence of electron donor, no signal of 13-Gly-DHB$^{\bullet -}$ was observed, owing to the fact that self-electron transfer between the ground and excited states of 13-Gly-DHB$^{\bullet -}$ was difficult at low concentrations. Repeating this experiment with a high concentration of 13-Gly-DHB, the ESR spectra of 13-Gly-DHB$^{\bullet -}$ and DMPO–O_2^- (after oxygen were bubbled) were obtained immediately, because the addition of an electron donor promotes both, the formation of 13-Gly-DHB$^{\bullet -}$ and the

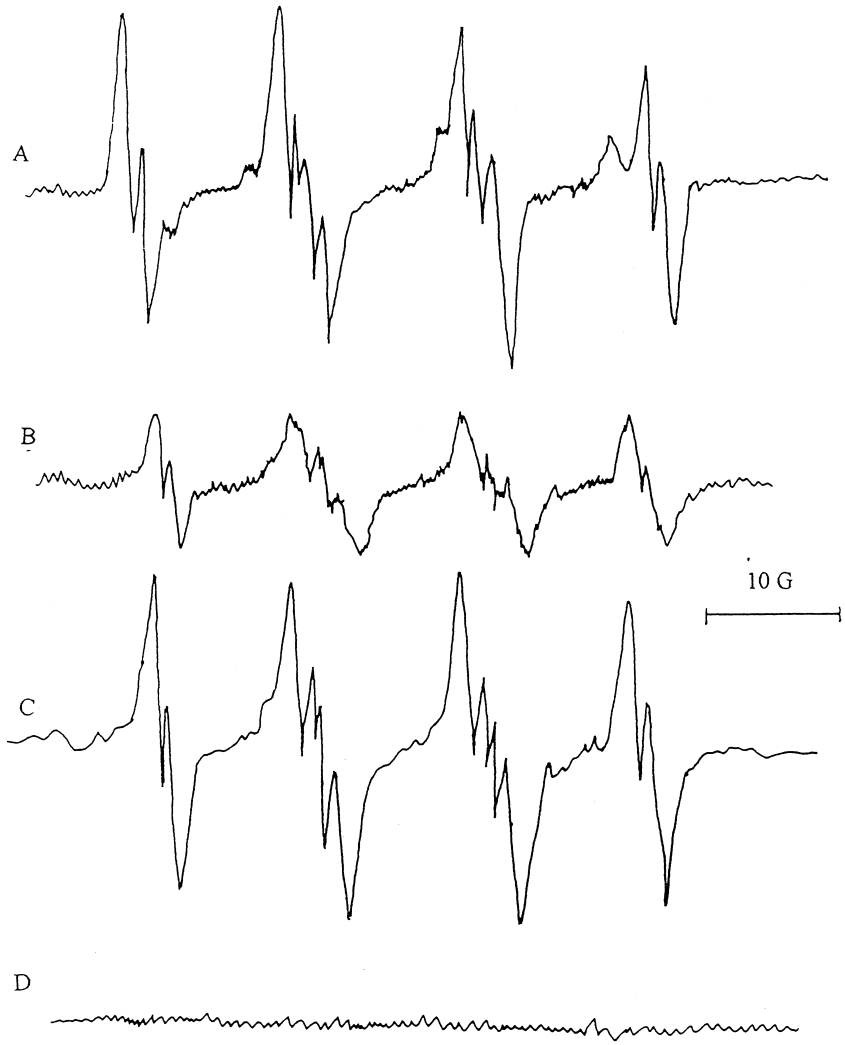


Fig. 4. (A) ESR spectrum of DMPO-superoxide radical adduct produced on irradiation of an oxygenated DMSO solution of 13-Gly-DHB (1 mM) and DMPO (45 mM); (B) same as spectrum A, but in the presence of SOD (25 μg ml^{-1}); (C) same as spectrum A, but in the presence of BNAH; and (D) same as spectrum A, but argon was bubbled through the solution. Instrument setting: microwave power, 5.05 mW; modulation amplitude, 1.835 G; scan range, 100 G; receiver gain, 2×10^5.

formation of DMPO–$O_2^{\bullet -}$. This consistent environmental effect suggested that 13-Gly-$DHB^{\bullet -}$ is the precursor for the formation of $O_2^{\bullet -}$ by 13-Gly-DHB. The following process could rationalize the generation of superoxide:

$$13\text{-Gly-}DHB^{\bullet -} + O_2 \rightarrow 13\text{-Gly-DHB} + O_2^{\bullet -} \quad (3)$$

It is very difficult to detect superoxide radical in water even when high concentrations of DMPO (0.4 M) and 13-Gly-DHB (10 mM) were used, and catalase (30 μg ml^{-1}) was added to minimize the signal from DMPO–OH.

Irradiation of an aerated solution (phosphate buffer: DMSO 1 : 1 v/v; pH 7.4) containing 13-Gly-DHB (0.5 mM) and DMPO (45 mM) led to the formation of a large amount of DMPO-($^{\bullet}$OH adducts. A four-line ESR spectrum with hyperfine splittings ($a^N = a^H = 14.9$ G) characteristic of the DMPO–$^{\bullet}$OH spin adduct was recorded (Fig. 5, spectrum A). The values of coupling constants were in good agreement with those found in the literature [12–14]. The signal intensity increased with the concentration of 13-Gly-DHB (Fig. 6) and the irradiation time (Fig. 7). Control experiments ensured that no signal was obtained without light, oxygen, 13-Gly-DHB or DMPO. On the other hand, the singlet-oxygen-mediated oxidation of DMPO to radical species has been reported in an aqueous solution [14]. To check this mechanism, histidine and DABCO were used as $^1O_2$ quenchers in competition experiments. The ESR signal intensity of the DMPO–$^{\bullet}$OH adducts does not decrease, indicating that $^1O_2$ is not involved in the formation of DMPO–$^{\bullet}$OH.

When ethanol was introduced into the system as $^{\bullet}$OH inhibitor, the ESR spectrum of the DMPO–$CH_3^{\bullet}CHOH$ was observed ($a^N = 15.8$, $a^H = 23.0$ G) (Fig. 5, spectrum B). This competitive scavenging result clearly indicated the formation and the trapping of freely diffusing $^{\bullet}$OH during the irradiation of 13-Gly-DHB in the presence of DMPO.

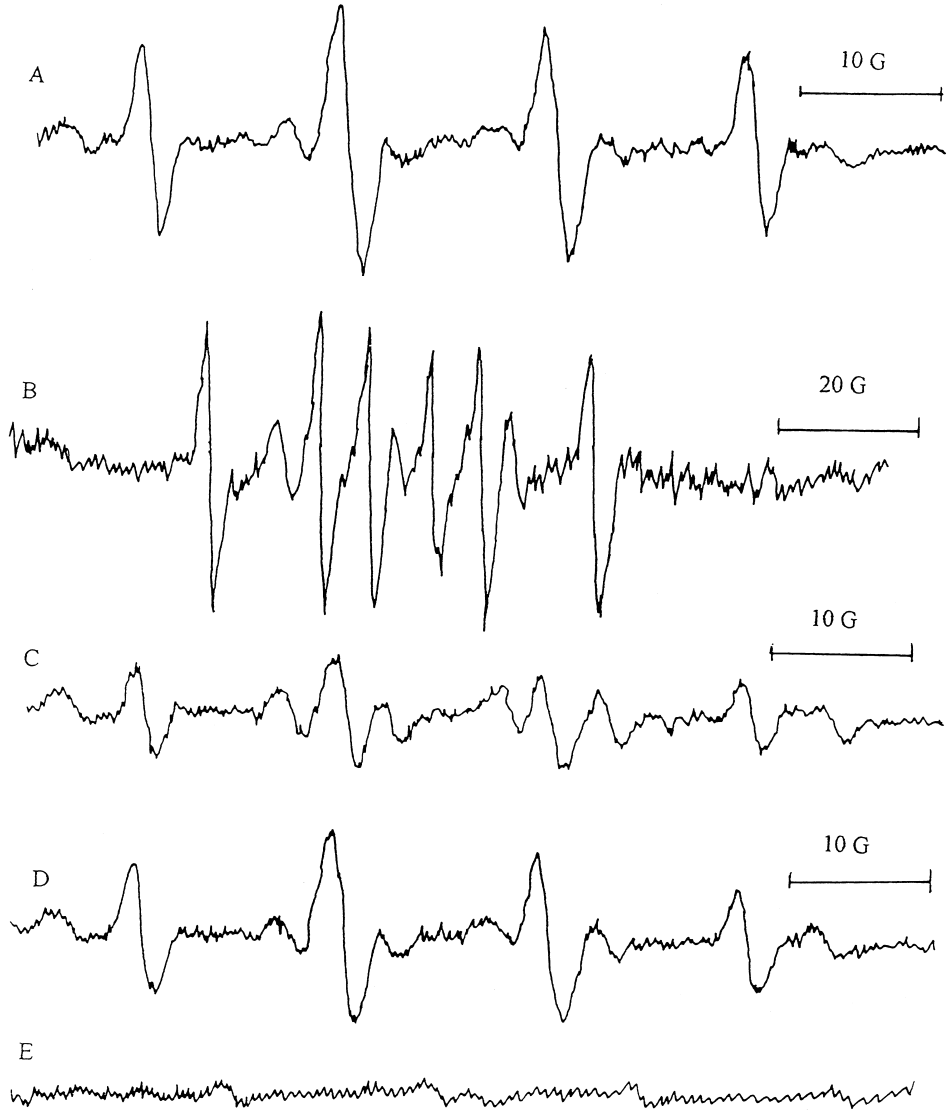


Fig. 5. (A) ESR spectrum produced from the irradiation of an oxygenated solution (DMSO–H_2O) (1 : 1 v/v) containing 13-Gly-DHB (0.5 mM) and DMPO (45 mM) above 470 nm for 2 min; (B) same as spectrum A, but in the presence of CH_3CH_2OH; (C) same as spectrum A, but in the presence of SOD (25 μg ml^{-1}); (D) same as spectrum A, but in the presence of catalase (30 μg ml^{-1}); and (E) same as spectrum A, but with argon bubbled through the solution. Instrument setting: microwave power, 5.05 mW; modulation amplitude, 1.835 G; scan range, spectrum A, C, and D: 100 G; spectrum B: 200 G; receiver gain, 2×10^5.

We have demonstrated that $O_2^{\bullet -}$ can be generated from 13-Gly-DHB$^{\bullet -}$ on photoexcitation in the presence of oxygen. In aqueous solution, the $O_2^{\bullet -}$ formed after irradiation can then undergo rapidly dismutation to H_2O_2 and O_2:

$$O_2^{\bullet -} + H^+ \rightleftharpoons HO_2^{\bullet} \quad (4)$$

$$H_2O + HO_2^{\bullet} + O_2^{\bullet -} \rightarrow H_2O_2 + O_2 + OH^- \quad (5)$$

It is also known that DMPO–$O_2^{\bullet -}$ decomposes to form DMPO–$^{\bullet}$OH in aqueous solutions. In order to determine whether the DMPO–$^{\bullet}$OH adducts originates from DMPO–$O_2^{\bullet -}$ or from the direct addition of hydroxyl radical to DMPO, the following experiments were carried out.

1. The addition of SOD (25 μg ml^{-1}) to an oxygen-saturated 13-Gly-DHB aqueous solution (0.5 mM) containing DMPO (45 mM) prior to illumination decreased the amount of DMPO–$^{\bullet}$OH signal intensity to 50% of its value without SOD (Fig. 5, spectrum C), whereas thermally denatured SOD had no effect on the ESR spectrum, indicating that the superoxide radical anion was involved in the formation of $^{\bullet}$OH by 13-Gly-DHB.
2. When catalase was introduced in the system before irradiation, the signal intensity of DMPO–$^{\bullet}$OH decreased strongly with increasing amount of catalase and 30 μg ml^{-1} catalase can inhibit 40% ESR signal intensity of DMPO–$^{\bullet}$OH (Fig. 5, spectrum D), but the heat inactivated catalase without effect. These demonstrated that H_2O_2 was involved in the formation of one part of the observed $^{\bullet}$OH.

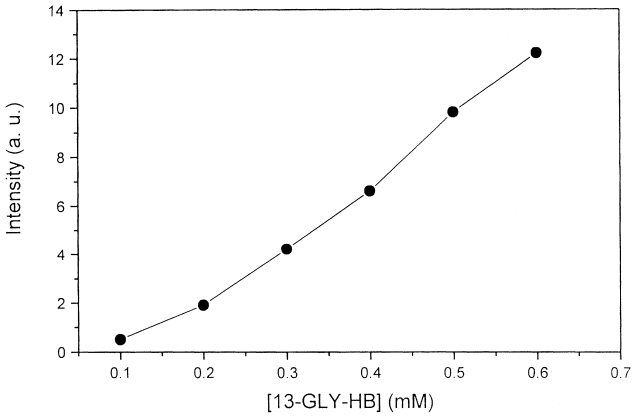


Fig. 6. Plot of the effect of various concentrations of 13-Gly-DHB on the intensity of ESR signal of the DMPO–$^{\bullet}$OH adducts.

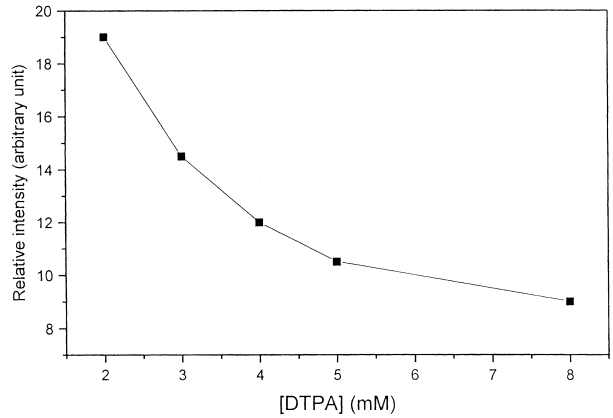


Fig. 8. Plot of the effect of various concentrations of DTPA on the intensity of ESR signal of the DMPO–$^{\bullet}$OH adducts.

3. Since transition-metal ions may be present in the DMSO–H_2O solution in trace amounts as impurities, DTPA was added to chelate them. The ESR signal intensity of DMPO–$^{\bullet}$OH declined with increasing amount of DTPA (Fig. 8).

The parallel observed effect of SOD, catalase and DTPA (Table 1) suggested that $^{\bullet}$OH was generated via the Habar–Weiss type reaction catalyzed by iron:

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2 \quad (6)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH \quad (7)$$

and the reaction

$$Fe^{3+} + \text{13-Gly-DHB}^{\bullet -} \rightarrow Fe^{2+} + \text{13-Gly-DHB} \quad (8)$$

might also take place in the system.

Since the formation of $^{\bullet}$OH could not be completely suppressed by DTPA even though a large amount of DTPA was added to the system (8 mM), it was expected that still another pathway was involved in the formation of $^{\bullet}$OH from 13-Gly-DHB on photosensitization. Irradiation of an argon-saturated aqueous solution containing 13-Gly-DHB (1 mM), H_2O_2 (0.1 mM), DTPA (5 mM) and DMPO (45 mM) with light of wavelength >470 nm, also generated an ESR signal of the DMPO–$^{\bullet}$OH adducts. Accompanied by a strong signal of 13-Gly-DHB$^{\bullet -}$ at the high field region (Fig. 9), this suggested that the reaction

$$H_2O_2 + \text{13-Gly-DHB}^{\bullet -} \rightleftharpoons {}^{\bullet}OH + OH^- + \text{13-Gly-DHB} \quad (9)$$

was also involved in the formation of $^{\bullet}$OH from 13-Gly-DHB on photosensitization. When 13-Gly-DHB was omitted, no ESR signal was observed. When H_2O_2 was omitted, only the signal of 13-Gly-DHB$^{\bullet -}$ could be observed. When DTPA was omitted, the ESR signal intensity of DMPO–$^{\bullet}$OH increased. The result indicated that DTPA suppressed the catalytic activity of transition metal (present as impurities) so that the catalyzed Fenton reaction could not proceed.

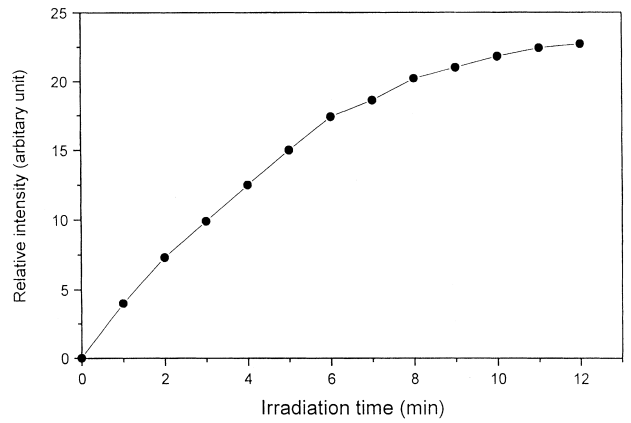


Fig. 7. Plot of the effect of irradiation time of 13-Gly-DHB (0.5 mM) on the intensity of ESR signal of the DMPO–$^{\bullet}$OH adduct.

3.3. Formation of $^1O_2$ by 13-Gly-DHB

Lion et al [15] reported that TEMPO a nitroxide radical detectable by ESR spectroscopy was generated from TEMP and singlet oxygen, as shown in Eq. (10).

Table 1
Ralative intensity of the DMPO–OH signal intensity during 13-Gly-DHB irradiation in the presence of inhibitors

Compound	Concentration	DMPO–OH reduction (%)
SOD (μg ml^{-1})	25	50
Heat-inactived SOD (μg ml^{-1})	25	0
Catalase (μg ml^{-1})	30	40
Heat-inactived catalase (μg ml^{-1})	30	0
DTPA (mM)	8	80
Histidine (mM)	5	0
DABCO (mM)	5	0

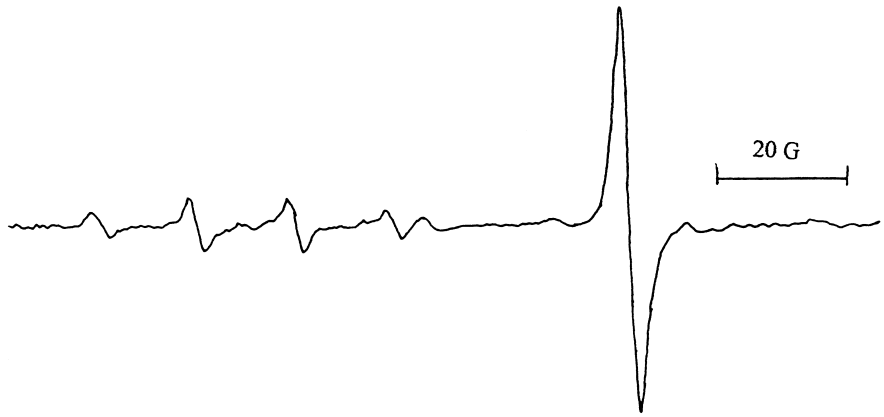
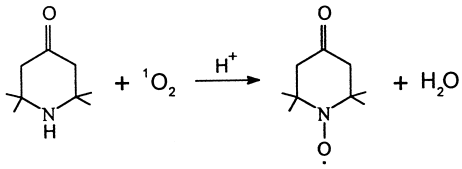


Fig. 9. ESR spectrum of DMPO–$^{\cdot}$OH adduct produced on irradiation of an Ar-saturated aqueous solution containing 13-Gly-DHB (1 mM), H_2O_2 (0.1 mM), DTPA (5 mM) and DMPO (45 mM) for 30 s. Instrument setting: microwave power, 8.00 mW; modulation amplitude, 2.909 G; scan range, 200 G; receiver gain, 1×10^5.



(10)

When an oxygenated ethanol-water (1 : 1 v/v) solution containing 13-Gly-DHB (5×10^{-3} M) and TEMP (0.5 M) was illuminated at room temperature, a typical three-line ESR spectrum of TEMPO (Fig. 10, spectrum A) with equal intensities ($a^N = 16.3$ G, $g = 2.0056$) was recorded. Photosensitizers, oxygen and light are necessary to form TEMPO. These data demonstrated that the formation of the nitroxide radical is a photodynamic process. To provide further evidence to support the involvement of $^1O_2$ in the 13-amino-acid substituted hypocrellin B derivatives in the photosen-

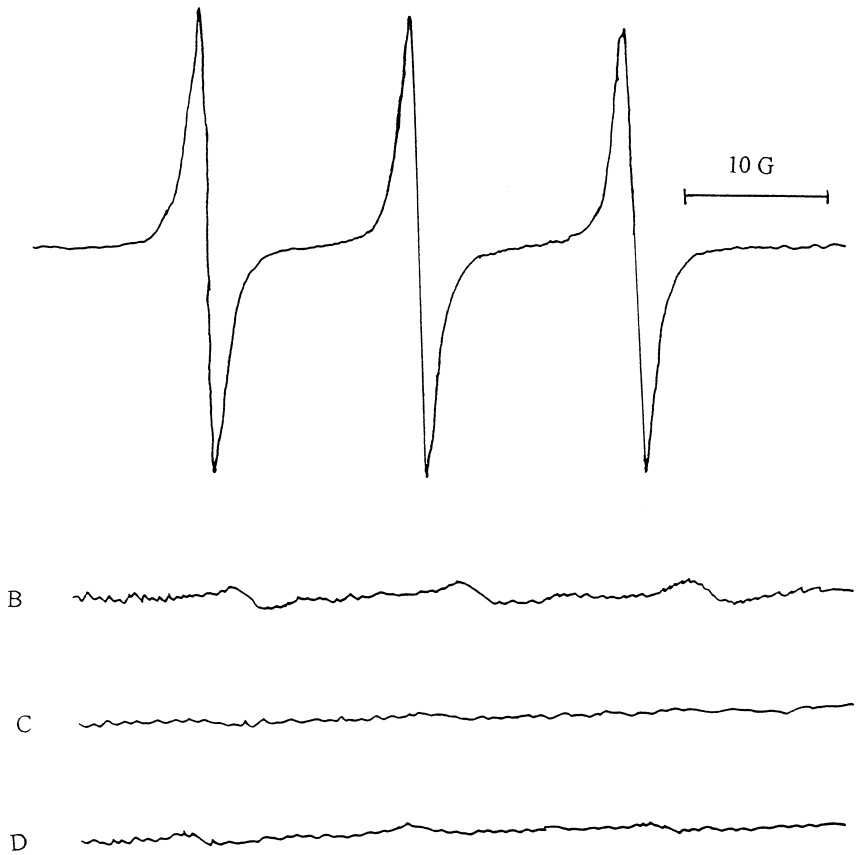


Fig. 10. (A) ESR spectrum produced by irradiation of an oxygenated ethanol–water (1 : 1 v/v) solution containing 13-Gly-DHB (5×10^{-3} M) and TEMP (0.5 M). (B, C, D) same as spectrum A, but in the presence of DABCO, NaN_3 or histidine, respectively. Instrument setting: microwave power, 3.19 mW; modulation amplitude, 1.032 G; scan range, 100 G; receiver gain, 2×10^3.

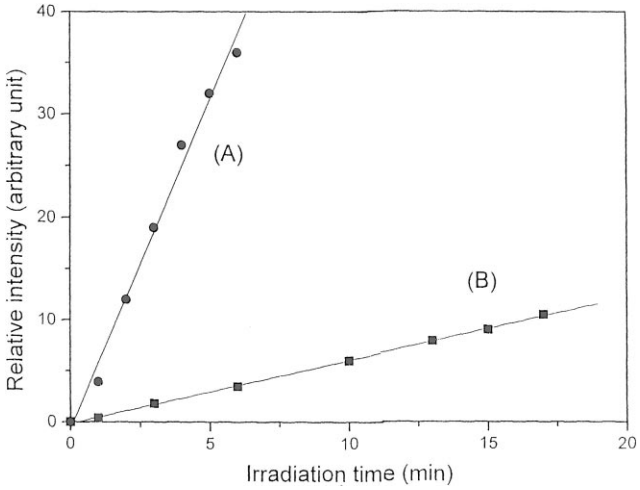


Fig. 11. The effect of solvent deuteration on the ESR signal intensity of the TEMPO radical generated during the photosensitization of 13-Gly-DHB (0.2 mM) in the presence of TEMP (25 mM): (A) CD_3CD_2OD–D_2O (1 : 1 v/v), and (B) CH_3CH_2OH–H_2O (1 : 1 v/v). Instrument setting as in Fig. 10.

sitization process, the following experiments were carried out.

1. The effect of deuterium on yield of TEMPO was studied. When the solvent ethanol–H_2O was replaced by fully deuterated ethanol–D_2O as solvent, the intensity of the ESR signal increased by about 10 times (Fig. 11)[16,17].
2. NaN_3, histidine, DABCO, are known to quench $^1O_2$ [18,19]. In the presence of these $^1O_2$ scavengers, the ESR signals were suppressed (Fig. 10).

These two methods are powerful tools for diagnosing $^1O_2$. The results suggest that TEMPO is derived from the reaction of TEMP with $^1O_2$ generated by the irradiation of 13-Gly-DHB.

4. Conclusion

The results of this work provide evidence for the generation of the following active agents in the 15-deacetyl-13-glycine substituted hypocrellin B (13-Gly-DHB) in the photosensitization processes:(a) 13-Gly-DHB$^{\bullet -}$ under anaerobic conditions is the precursor of $O_2^{\bullet -}$;(b) $O_2^{\bullet -}$ can be converted to OH via a Fenton reaction; and(c) $^1O_2$.

It is inferred that both, electron transfer (type I) and $^1O_2$ (type II) paths are involved, to different extents, in the 15-deacetyl-13-glycine substitution of hypocrellin B of photosensitization.

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References

[1] L.J. Jiang, The structures, properties, photochemical reaction and reaction mechanisms of hypocrellins (I), Kexue Tongbao 21 (1990) 1608–1616.
[2] L.J. Jiang, The structures, properties, photochemical reaction and reaction mechanisms of hypocrellins (II), Kexue Tongbao 22 (1990) 1681–1690.
[3] Z.J. Diwu, J.W. Lown, Hypocerllins and their use in photosensitization, Photochem. Photobiol. 52 (1990) 609–616.
[4] N.W. Fu, Y.X. Chu, J.Y. An, Photodynamic action of hypocrellin A on hepatoma cell mitochondira and microsomes, Acta Pharm. Sin. 10 (1989) 371–373.
[5] J.B. Wang, J.N. Bao, Clinical analysis and observation of hypocrellin photochemistry in treatment of Lichen amyloidosis in 37 patients, J. Chin. Acad. Med. 7 (1985) 349–351.
[6] R.Y. Liang, G.D. Mei, W.Y. Zhou, 62 cases with hypertrophic scars treated with hypocrellin photochemotherapy, Chin. J. Denna 15 (1982) 87–88.
[7] J. Moan, E. Wold, Detection of singlet oxygen production by ESR, Nature 279 (1979) 450–451.
[8] Y.Z. Hu, J.Y. An, L.J. Jiang, Studies of the photoinduced sulfonation of hypocrellins, J. Photochem. Photobiol. A: Chem. 70 (1993) 301–308.
[9] Y.Z. Hu, J.Y. An, L.J. Jiang, Studies of the sulfonation of hypocrellin A and the photodynamic actions of the product, J. Photochem. Photobiol. B: Biol. 17 (1993) 195–201.
[10] J. Mayer, R. Krasluklanls, Absorption spectra of the radical ions of quinones: a pulse radiolysis study, J. Chem. Soc. Faraday Trans. 87 (1991) 2943–2947.
[11] J.R. Harbour, M.L. Hair, Detection of superoxide ions in nonaqueous media. Generation by photolysis of pigment, J. Phys. Chem. 82 (1978) 1397–1399.
[12] E. Finkelstein, E.G.M. Rosen, E.J. Rauckman, Spin trapping, kinetics of the reaction of superoxide and hydroxyl radicals with nitrones, J. Am. Chem. Soc. 102 (1980) 4994–4999.
[13] K. Lang, M. Wangerova, P. Stopka, W. Dameran, Reduction of dioxygen to superoxide photosensitized by anthraquinone-2-sulphonate, J. Photochem. Photobiol. A: Chem. 67 (1992) 187–195.
[14] J.B. Feix, B. Kalyanaraman, Production of singlet oxygen derived hydroxyl radical adducts during merocyanine-540-mediated photosensitization: analysis by ESR-spin trapping and HPLC with electrochemical detection, Arch. Biophys. 291 (1991) 43–51.
[15] Y. Lion, M. Delmelle, A. Van de Vorst, New method of detecting singlet oxygen production, Nature 263 (1976) 442–443.
[16] B.M. Monroe, in A.A. Frimer (Ed.), Singlet O_2, vol. I, CRC press, Boca Raton, FL, 1985, pp. 177–188.
[17] N.H. Wang, Z.Y. Zhang, Relationship between photosensitizing activities and chemical structure of hypocrellins A and B, J. Photochem. Photobiol, B: Biol. 4 (1992) 207–217.
[18] R.D. Hall, C.F. Chignell, Steady-state near-infrared detection of singlet molecular oxygen: a Stern–Volmer quenching experiment with sodium azide, Photochem. Photobiol. 45 (1987) 459–464.
[19] R.C. Steaight, J.D. Spikes, in A.A. Frimer (Ed.), Singlet O_2, vol. IV, CRC Press, Boca Raton, FL, 1985, p. 108.