# Using electrostatic interactions to increase the photodamaging ability of hypocrellin B: synthesis and study of 2-(dimethylamino)ethanethiol-modified HB†

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**A new 2-(dimethylamino)ethanethiol-modified HB derivative was synthesized and enhanced photodamaging ability towards DNA was achieved by making use of the electrostatic attraction.**

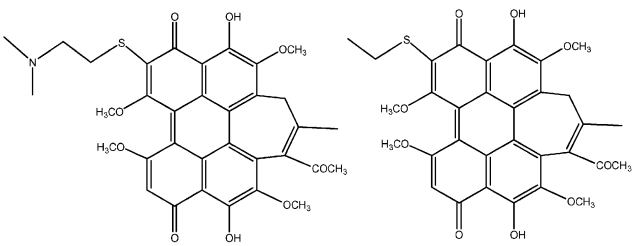
Photodynamic therapy (PDT) has been the subject of intensive interest over the past two decades as a novel cancer therapy.[1] It depends on light, oxygen and photosensitizer to induce tumor and other abnormal cells to die *via* apoptosis and necrosis.[2] The first PDT photosensitizer to receive regulatory approval in several countries was Photofrin. However, the limitations of Photofrin, particularly its cutaneous photosensitivity, have encouraged the development of many new photosensitizers, including porphyrin-like and non-porphyrin organic dye stuffs and inorganic materials.[3]

As a new type of non-porphyrin photosensitizer, hypocrellin A (HA) and hypocrellin B (HB), naturally occurring perylenequinonoid pigments isolated from *Hypocrella bambusae*, possess wide absorption in the visible region and a highly efficient ability to generate reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide anion radical ($O_2^{-\bullet}$) and hydroxyl radical ($^\bullet OH$).[4] Thus, they presented strong photodynamic activity against many tumor cell lines and viruses.[5] Compared with Photofrin, HA or HB is a single substance and not prone to aggregation. A more intriguing merit of HA and HB lies in their much less delayed skin photosensitivity.[6]

Since ROS are generally short-lived, for example the lifetime of $^1O_2$ is only 3.2 μs in aqueous solutions,[7] it is of importance to generate ROS as near as possible to the desired target. Our recent results indicated that any effects that can promote interactions between photosensitizer and DNA, which is a kind of target biomolecule in PDT, can enhance photodynamic activities.[8] Association modes between small molecules and double-stranded DNA include intercalation, electrostatic interaction, and hydrogen bonding.[9] Quaternary ammonium groups (QAG), which are positively charged, can be utilized to improve the interaction of a photosensitizer with negatively-charged DNA.[10] Many data indicated that QAG play a positive role in the binding of some small molecules with DNA.[11] QAG may also improve a drug's water solubility and transport.[11] Though many hypocrellin derivatives had been synthesized to improve the water solubility,[12] no work was reported to utilize QAG to increase water solubility of hypocrellins. These factors intrigued our interest to synthesize hypocrellins bearing QAG. In this letter, we report on an improved DNA photodamage by 2-(dimethylamino)ethanethiol-modified HB (DEHB) and the involved QAG effect through comparison with ethanethiol-modified HB (EHB).

DEHB and EHB (Scheme 1) were synthesized by photoaddition of 2-(dimethylamino)ethanethiol or ethanethiol to HB using a reported method.[13] They were characterized by $^1H$ NMR spectroscopy and MALDI-TOF MS (ESI†). Both DEHB and EHB should be the mixtures of 5- and 8-substituted product with a molar ratio of 1 : 1.[8b] Any attempt to obtain isomerically pure materials was unsuccessful. It should be mentioned that 5- and 8-substituted HB may have different *in vitro* and *in vivo* PDT activities though they are closely analogous in structure.

The spin-trapping ESR technique was applied to evaluate the ROS generation ability (Table 1) using 2,2,6,6-tetramethyl-4-piperidone (TEMP) and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) as spin-trapping agents for $^1O_2$ and $O_2^{-\bullet}$, respectively. Upon irradiation of an oxygen-saturated DMSO solution of DEHB, EHB, or HB with a 532 nm pulse laser in the presence of TEMP, characteristic ESR signals of TEMPO (adduct of TEMP with $^1O_2$) were detected (Fig. 1S†). The relative $^1O_2$ quantum yields of DEHB and EHB are 0.3 and 0.3, assuming that of HB to be 1.0. The photobleaching method of 9,10-diphenylanthracene (DPA) was also used to estimate the relative $^1O_2$ quantum yields[14] and the results are




**DEHB** **EHB**

**Scheme 1** Chemical structures of DEHB and EHB.

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**Table 1** Relative quantum yields of $^1O_2$, $O_2^{-\bullet}$ and semiquinone anion radical for DEHB, EHB and HB[a]

| | $\Phi_{singlet\ oxygen}$[b] | | $\Phi_{superoxide}$[c] | | $\Phi_{semiquinone}$[d] | |
|---|---|---|---|---|---|---|
| | DMSO | DMSO–buffer[e] | DMSO | DMSO–buffer[e] | DMSO | DMSO–buffer[e] |
| DEHB | 0.3 | 0.2 | 2.0 | 1.8 | 2.0 | 1.6 |
| EHB | 0.3 | 0.2 | 2.9 | 2.3 | 2.5 | 1.9 |
| HB | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |

[a] Estimated by comparing the ESR signal intensities under normalized absorbance at 532 nm, assuming the corresponding quantum yield of HB to be unity. [b] In oxygen-saturated solutions. [c] In air-saturated solutions. [d] In argon-saturated solutions. [e] Volume ratio of 1 : 2.



in agreement with the spin-trapping method (Fig. 2S†). In aqueous solutions (DMSO–phosphate buffer with volume ratio of 1 : 2) the TEMPO signals were also detected upon irradiation, but the signal intensities were lower than those in DMSO, probably due to the shorter lifetime of $^1O_2$ in aqueous solutions. Still assuming the $^1O_2$ quantum yield of HB to be 1.0 in DMSO–buffer, the relative $^1O_2$ quantum yields of DEHB and EHB are both estimated to be 0.2 in the same solvent system with similar trends as in DMSO (Table 1). In contrast, DEHB and EHB generate $O_2^{-\bullet}$ and semiquinone anion radical more efficiently than HB in both DMSO and DMSO–buffer solutions (Table 1, Fig. 3S and Fig. 4S†). The semiquinone anion radical of hypocrellins is believed to be the result of self-electron transfer between excited and ground states, which is in turn the precursor for $O_2^{-\bullet}$.[15] In DMSO–buffer solutions the ratio of the semiquinone anion radical signal intensities of the examined hypocrellins is 1.9 (EHB) : 1.6 (DEHB) : 1.0 (HB). In the presence of calf thymus DNA (CT DNA, 200 μM), semiquinone anion radical signals were intensified and the ratio changed to 2.8 (EHB) : 2.6 (DEHB) : 1.0 (HB), because the guanine bases can donate electrons more efficiently to excited hypocrellins.[16] The more significant enhancement in the case of DEHB may be attributed to the electrostatic interaction aroused by the protonated dimethylamine group, *i.e.* a QAG effect (the p$K_a$ of trimethylamine, structurally similar to the end group of DEHB, is 9.80[17]).

An ethidium bromide (EB) assay[18] was adopted to compare the photodamage abilities of DEHB, EHB, and HB. Upon intercalation into DNA, the fluorescence intensity of EB increases significantly. Any process that leads to DNA damage will decrease the binding site number of EB and the fluorescence intensity. The remaining binding site percentages as the function of irradiation time are collected in Table 2. Obviously, DEHB is the most potent photodynamic agent among the three hypocrellins, and a QAG effect should be involved as soon as compared with EHB (ROS generation abilities are similar for DEHB and EHB).

Besides the fact that CT DNA promoted DEHB to generate semiquinone anion radical more efficiently, the melting temperature ($T_m$) measurements of CT DNA in the presence of the examined hypocrellins provide more evidence of the higher affinity of DEHB towards DNA (Table 3). The melting temperature of CT DNA increased from 63.2 to 69.3 °C (6.1 °C increase) in the presence of DEHB, while only a 0.8 °C increase was observed in the case of EHB, implying a stronger interaction between DEHB and CT DNA. Moreover, increasing the ionic strength of the solution weakened the influence of DEHB on $T_m$, suggesting an electrostatic character of the interaction between DEHB and CT DNA.

In summary, a new QAG-modified HB derivative was synthesized and enhanced photodamaging ability towards DNA was achieved by making use of the electrostatic attraction between positively charged QAG and negatively charged DNA.

**Table 2** Photodamage of CT DNA by DEHB, EHB, and HB detected by the remaining binding site (BSR%) of ethidium bromide in the damaged CT DNA[a]

| | % of remaining binding sites at varied irradiation time $t$/min[c] | | | | |
|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 |
| Control experiment[b] | 99.8 | 99.7 | 99.6 | 99.5 | 99.4 |
| DEHB | 71.8 | 50.5 | 28.1 | 7.5 | 0.0 |
| EHB | 87.8 | 73.2 | 58.8 | 44.5 | 29.6 |
| HB | 93.1 | 77.5 | 62.2 | 47.1 | 31.3 |

[a] Air-saturated buffer solutions containing CT DNA (100 μM), EB (50 μM), and photosensitizer (12.5 μM) irradiated with light above 470 nm from a medium pressure sodium lamp (2.2 mW cm$^{-2}$). [b] Similar to *a* but without the presence of photosensitizer and oxygen. [c] %binding site remaining $= 100 \times \left(1 - \frac{I_0 - I_t}{I_0 - I_{buffer}}\right)$ where $I_0$, $I_t$, and $I_{buffer}$ denote the integrated fluorescence intensities before irradiation, after $t$ min of irradiation, and of DNA-free buffer, respectively.

## Experimental

### ESR measurements

ESR spectra were obtained using a Bruker ESP-300E spectrometer operating at room temperature, and the operating conditions were as follows: microwave bridge: X-band with

**Table 3** The effects of DEHB and EHB on the melting temperature of CT DNA

| | $T_m$/°C | $\Delta T_m$/°C |
|---|---|---|
| CT DNA[a] | 63.2 | 0 |
| CT DNA + EHB[b] | 64.0 | 0.8 |
| CT DNA + DEHB[b] | 69.3 | 6.1 |
| CT DNA[c] | 63.6 | 0 |
| CT DNA + EHB[d] | 63.9 | 0.3 |
| CT DNA + DEHB[d] | 67.4 | 3.8 |

[a] Buffer solution (pH = 7.0) of CT DNA (100 μM). [b] Similar to *a* but in the presence of photosensitizer (20 μM). [c] Similar to *a* but in the presence of sodium chloride (300 μM). [d] Similar to *b* but in the presence of sodium chloride (300 μM).

100 Hz field modulation; sweep width: 100 G; modulation amplitude: 1.0 G; modulation frequency: 100 kHz; receiver gain: $1 \times 10^5$; microwave power: 5 mW. Samples were injected into the specially made quartz capillaries, purged with argon, air, or oxygen for 30 min in the dark, respectively, and then illuminated directly in the cavity of the ESR spectrometer with a Nd: YAG laser (532 nm, 5–6 ns of pulse width, repetition frequency: 10 Hz, 10 mJ per pulse).

The superoxide anion radical ($O_2^{-\bullet}$) and singlet oxygen ($^1O_2$) were detected by a spin-trapping technique using DMPO and TEMP as spin-trapping agents, respectively. The relative quantum yields were estimated by comparing the ESR signal intensities at a normalized sample absorbance at 532 nm, assuming that of HB to be unity.

### Melting temperature ($T_m$) determination of DNA samples

The effects of a photosensitizer on the melting temperature of CT DNA were measured by following the changes in absorption at 260 nm as a function of temperature. All the experiments were carried out by heating the CT DNA solutions from 25 °C to 85 °C at a rate of 1 °C $min^{-1}$ in the absence or presence of the photosensitizer.

### Ethidium bromide (EB) assay for DNA cleavage

10 ml of EB–DNA buffer solution (50 μM of EB and 100 μM of CT DNA, 3% DMSO) containing 12.5 μM of HB-based photosensitizer was prepared. Then the solution was divided into 5 aliquots and irradiated in a 'merry-go-round' apparatus with a medium pressure sodium lamp ($h\nu > 470$ nm). The incident light intensity was measured to be 2.2 mW $cm^{-2}$ by a 70 260 radiant power meter with thermopile probe of 70 262 (Oriel Instruments). The aliquots were removed at various times and their fluorescence emissions in the range of 525–800 nm were measured by exciting at 510 nm.

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