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A tyrosine-modified hypocrellin B with affinity for and photodamaging ability towards calf thymus DNA†

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An enhanced photodamaging ability towards CT-DNA was achieved in a tyrosine-modified hypocrellin B by improving the affinity of the sensitizer to DNA.

Hypocrellins, including hypocrellin A (HA) and hypocrellin B (HB), have been intensively studied over the past two decades in the field of photodynamic therapy (PDT) due to their wide absorption band in the visible region and high efficiency in reactive oxygen species generation.[1] Recent investigations demonstrated the photodynamic damage by hypocrellins and their derivatives to viruses, tumor cells, pBR332 DNA and extracted cellular DNA.[2–4] The photodynamic damaging ability of a sensitizer towards its biomaterial substrate not only depends on the reactive oxygen generation efficiency of the sensitizer, but also, maybe more importantly, relies on the affinity of the sensitizer to its targeted substrate.[5] So far, nearly all chemical modifications on native hypocrellins have been to improve the water-solubility of hypocrellins and to extend their absorption band to longer wavelength.[6,7] As far as we know, however, few efforts[6a] have been made to enhance their affinity to biomaterials for improving their photodamaging ability. Tyrosine plays key roles in the activities of biological systems such as ribonucleotide reductase,[8] prostaglandin H synthase,[9] photosystem II[10] and many other biologically relevant systems. What aroused our interest is that tyrosine can bind to duplex DNA *via* an intercalation mechanism,[11] in which tyrosine or other aromatic amino acids, *e.g.* tryptophan and phenylalanine, may behave as "book marks" and thus "anchor" the proteins bearing aromatic amino acids to double strand DNA. Hypocrellins having such "book marks" as functional groups are attractive and herein a tyrosine-modified HB (TYHB) is demonstrated to have a unique affinity to and an improved photodamaging ability towards calf thymus DNA (CT-DNA) with respect to HB.

TYHB, a mixture of 5- and 8-substituted HB with a molar ratio of 1 : 1 (determined from integrated strength in ^{1}H NMR), was synthesized by the photoaddition of mercaptoacetic acid to HB,[12] followed by an amidation reaction with tyrosine methyl ester hydrochloride (see ESI for synthesis and ^{1}H NMR data), and was characterized by ^{1}H NMR and MALDI-TOF ([M + H$^+$] 796). The experiments, including recrystallization, precipitation, and column chromatography, failed to yield isomerically pure materials and the mixture of them was used throughout the study. But it should be borne in mind that 5- and 8-TYHB may have different *in vitro* and *in vivo* PDT activity though they are closely analogous in structure. The effort of synthesizing 5,8-di(tyrosine) substituted HB is underway to avoid the ambiguity occurring in monosubstituted TYHB.

The ESR technique was applied to evaluate the reactive oxygen species (*e.g.*, $^1O_2$, $O_2^{-\cdot}$, $OH^{\cdot}$) generation ability of TYHB. Similarly to HB or MAHB (5- and 8-mercaptoacetic acid substituted HB), irradiation of an argon-gassed DMSO solution of TYHB (50 μM) with a 532 nm pulsed laser can generate an ESR signal of the semiquinone anion radicals of TYHB (TYHB$^{-\cdot}$), which has the same position and line shape as, but much higher intensity than, that of HB$^{-\cdot}$ or MAHB$^{-\cdot}$ (see ESI). While the HB$^{-\cdot}$ (or MAHB$^{-\cdot}$) originates from the disproportionation between an excited HB (or MAHB) and a ground state HB (or MAHB), the TYHB$^{-\cdot}$ may be generated *via* the photoinduced intra- and/or inter-molecular electron transfer between the tyrosine moiety and the excited HB moiety other than disproportionation of HB moieties. In TYHB the electron transfer between tyrosine and excited HB is thermodynamically allowed by a negative free energy change ΔG of −87.1 kJ mol^{-1} calculated in terms of the Weller equation[13] by using the oxidation potential of tyrosine (0.93 V *vs.* NHE[14]), the reduction potential of HB (−0.2 V *vs.* NHE[15]) and the excited state energy of HB (2.03 eV[16]).

When irradiation was carried out in an oxygen-saturated DMSO solution of TYHB with 2,2,6,6-tetramethyl-4-piperidone (TEMP) or 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO) present as spin-trapping agent, characteristic ESR signals of TEMPO (adduct of TEMP with $^1O_2$), DMPO–$O_2^{-\cdot}$, and DMPO–$OH^{\cdot}$ (in the presence of water) could be readily detected (see ESI). Estimated from the ESR signal intensities, the $^1O_2$ and $O_2^{-\cdot}$ generation efficiencies of TYHB are around 28% and six-fold that of HB, respectively. The photobleaching of 9,10-diphenylanthracene by $^1O_2$[17] and the reduction of cytochrome c by $O_2^{-\cdot}$[18] were also utilized for the measurement of $^1O_2$ and $O_2^{-\cdot}$, and the result is consistent with that of the spin trapping method. The photoinduced electron transfer between the tyrosine moiety and the HB moiety quenches the triplet HB and produces semiquinone anion radicals of TYHB, and therefore accounts for the weakened $^1O_2$ and enhanced $O_2^{-\cdot}$ generation efficiencies of TYHB, because $^1O_2$ and $O_2^{-\cdot}$ occur *via* energy transfer from triplet HB and electron transfer from TYHB$^{-\cdot}$, respectively. All findings in the ESR experiments indicate that TYHB is photodynamically active in terms of type I and type II mechanisms.

To study the PDT properties of TYHB, calf thymus DNA (CT-DNA) in air-saturated buffer solution was used as phototherapeutic target and an ethidium bromide (EB) assay was adopted to monitor the photodamage process of CT-DNA.[19] We compared the PDT properties of HB, MAHB and TYHB in both aerobic and anaerobic conditions and it was found TYHB caused remarkably enhanced photodamage on CT-DNA in the aerobic case (Table 1).

The enhanced affinity ability of sensitizer to CT-DNA may make a contribution to the improvement of TYHB in photodamaging ability towards DNA. Upon addition of increasing amounts of CT-DNA to a series of aqueous solutions containing a fixed concentration of TYHB (30 μM), notable changes in the UV-visible absorption of TYHB were observed after mixing for 12 hours in the dark (Fig. 1). The original absorption band at 502 nm (line a) shifts to longer wavelength (512.5 nm), and the absorbance at λ_{max} decreases by 6.2% (hypochromism) (line b). Under the same conditions, HB and MAHB show no such changes. The bathochromic shift and hypochromism of the TYHB absorption spectrum upon addition of CT-DNA are obviously caused by an interaction between the HB moiety and CT-DNA, which implies that the tyrosine group plays an important role in this interaction. A reasonable explanation may be that the tyrosine moiety serves as an "anchor" to tether the

† Electronic supplementary information (ESI) available: synthesis and ESR spectra of TYHB. See http://www.rsc.org/suppdata/cc/b3/b309024h/

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HB moiety to CT-DNA by its intercalating ability, and thus enhances the interaction of the HB moiety in TYHB with CT-DNA. As a result, more reactive oxygen species will be generated nearby the CT-DNA in the case of TYHB as sensitizer, and improved photodamage of CT-DNA can be observed. The association of TYHB with CT-DNA was also confirmed by melting temperature (T_m) experiments. The T_m of 40 μM of CT-DNA in 10 mM sodium phosphate buffer (pH = 7.4) decreased from 60.1 to 49.2 °C upon adding 10 μM of TYHB. Additionally, upon photoinduced intramolecular electron transfer to the excited HB moiety, tyrosine cation radical or its deprotonated form of tyrosine phenoxyl radical bound on CT-DNA may directly damage its host.[20] Moreover, the shift in photodynamic mechanism from type II to type I, as the result of the weakened $^1O_2$ and enhanced O_2^- generation efficiencies of TYHB, may also have some effects on the improved photodynamic capability of TYHB.

In summary, a new hypocrellin B derivative was synthesized by modification with tyrosine, which exhibits a better affinity to DNA and enhanced photodamage on CT-DNA than HB. These findings encourage us to prepare hypocrellin derivatives by focusing more attention on improving their affinity to the biomaterials.

Table 1 Photocleavage of CT-DNA by TYHB, MAHB and HB detected by remaining binding site (BSR%) of ethidium bromide to the damaged CT-DNA under different conditions. [CT-DNA] = 40 μM, [EB] = 80 μM, [TYHB] = 10 μM, [MAHB] = 10 μM, [HB] = 10 μM

	% of remaining binding sites at varied irradiation time/min				
Sample	10	20	30	40	50
Control experiment[a]	99.89	99.75	99.61	99.53	99.38
TYHB + N_2	95.23	93.28	90.29	87.61	83.63
MAHB + N_2	98.04	94.10	91.89	89.32	86.04
HB + N_2	99.31	97.40	93.12	89.49	88.25
TYHB + O_2	70.32	51.10	44.82	35.05	23.78
MAHB + O_2	83.77	77.61	69.41	60.30	51.83
HB + O_2	90.83	80.50	75.92	63.26	56.08

[a] In the absence of TYHB, MAHB, HB, and oxygen.

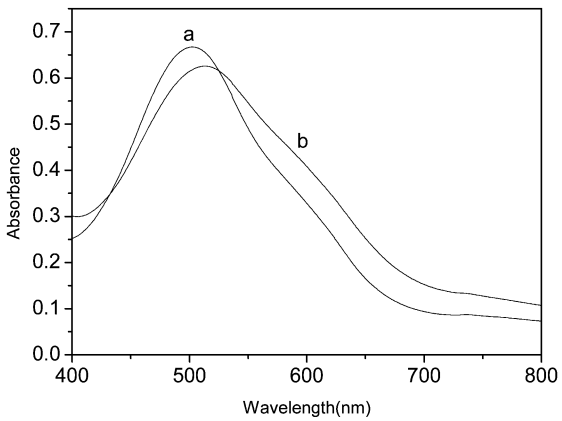

Fig. 1 Absorption spectra of TYHB (10 μM) in the absence (a) and presence (b) of CT-DNA (50 μg mL^{-1}).

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