Synthesis and Photodynamic Therapy Properties of a Water-Soluble Hypocrellin Modified by Cyclodextrin

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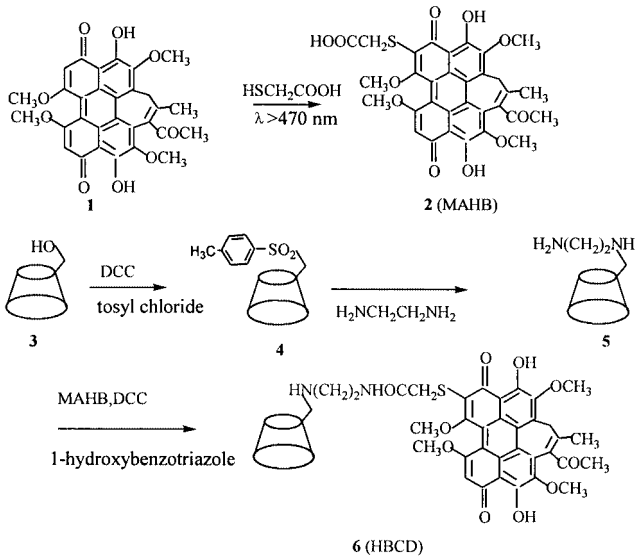
(Received May 15, 2001; CL-010447)

For improving water solubility of hypocrellin B (HB), a cyclodextrin modified hypocrellin B (HBCD) was designed and synthesized. Electron spin resonance (ESR) measurement indicated that this HB derivative remained photodynamically active in terms of type I and type II mechanisms. HBCD is water-soluble and possesses stronger photosensitized damage ability to calf thymus DNA than hypocrellin B.

Hypocrellins, including hypocrellin A (HA) and hypocrellin B (HB), are new photodynamic agents isolated from the fungus of *Hypocrella bambuase*. These lipid–soluble agents exhibit strong absorption in the phototherapeutic window (600–900 nm). They have several advantages over the present used phototherapy agents, e.g. ready preparation and purification, low aggregation tendency, and significantly reduced photosensitivity on normal tissue because of its rapid metabolism.[1] As a result, HA has been successfully employed in the photodynamic therapy (PDT) treatment of certain skin diseases.[2] The poor solubility in water limited their further use in the photodynamic therapy, which is an impetus to design and synthesize water-soluble hypocrellins. In this letter, a covalently linked hypocrellin B of β-cyclodextrin was synthesized for improving water solubility.[3] The amino group was selected as the spacer for superior water solubility as well as for an affinity increase to the negative charged groove of double-strand DNA.[4]

The compound **2** (MAHB) can be prepared in a yield of 12% by photoreaction of mercaptoacetic acid with hypocrellin B (HB) in the presence of O_2.[5] The mechanism for this photoreaction has proven to be a nucleophilic addition of mercaptoacetic acid anion to triplet HB followed by oxidation. The role of O_2 in this reaction, however, is complex. On the one hand, O_2 is indispensable; on the other hand, too high concentration of O_2 will either quench HB triplet or speed up the oxidation of mercaptoacetic acid anion, and consequently reduce the yield of mono- or di- mercaptoacetic acid substituted HB. Therefore it is necessary to optimize the concentration of O_2 for achieving high yield. In our experiments,[6] this factor was controlled carefully, and a collection yield as high as 67% was obtained when O_2 was bubbled into the reaction system at the rate of 5 mL/min and the molar ratio of mercaptoacetic acid to HB was enhanced from 50 to 100 (Scheme 1). This mixture of 5- or 8– monomercaptoacetic acid substituted hypocrellin B (MAHB) is able to meet the PDT requirement because both isomers have the same photochemical and photophysical properties. Compound **4** was prepared by reaction of β-cyclodextrin (β-CD) with tosyl chloride in dry pyridine. A low yield (ca. 31%)[7] was often obtained in this reaction because the β-cyclodextrin was difficult to be dehydrated completely. When we added the equal molar dicyclohexylcarbodiimide (DCC) to serve as dehydrating agent, the yield increased to 42%.

Scheme 1.



1 **2** (MAHB) **3** **4** **5** **6** (HBCD)

Compound **5** was prepared by treating compound **4** with ethylenediamine. The compound **6** (HBCD) was synthesized by amidation of compound **2** with 1.5 equiv of compound **5** in dry DMF at room temperature using dicyclohexylcarbodiimide/1-hydroxybenzotriazole hydrate (DCC/HOBT, 1 equiv/1.4 equiv) as coupling reagents, and was purified on Sephadex C-25 (30% yield).[8] HBCD was characterized by ^{1}H NMR and MALDI-TOF ([M + Na^+]: 1780).

Irradiation of argon-gassed DMSO-buffer solution (1/1,v/v, pH = 7) of HBCD (0.1 mM) generated an ESR spectrum ascribed to the semiquinone anion radical of HBCD ($HBCD^{\cdot-}$) (Figure 1, spectrum A). It has the same position as that of $HB^{\cdot-}$. The $HBCD^{\cdot-}$ was produced by self-electron transfer between a ground and an excited HBCD.[9] When the oxygen was bubbled through the HBCD solution in the presence of 5,5-dimethyl-1-1-pyrroline *N*-oxide (DMPO) and the HBCD solution was irradiated for 3 min, the ESR signal of DMPO-superoxide anion radical adduct was observed (Figure 1, spectrum B). Superoxide anion radical was generated via electron transfer from $HBCD^{\cdot-}$ to oxygen. When the oxygen saturated DMSO-buffer solution (1/1, v/v pH = 7) of HBCD (0.1 mM) was irradiated for 40 s while 2,2,6,6-tetramethylpiperridine (TEMP) was used as a spin trap, an ESR signal of 2,2,6,6-tetramethyl-1-piperridinyloxy radical (TEMPO) was observed which could be generated from the reacting of TEMP with singlet oxygen. The singlet oxygen was formed via the energy transfer from triplet HBCD to oxygen. All finds above indicates that HBCD was photodynamic active in terms of type I and type II mechanism. It has been reported that hypocrellins and their derivatives

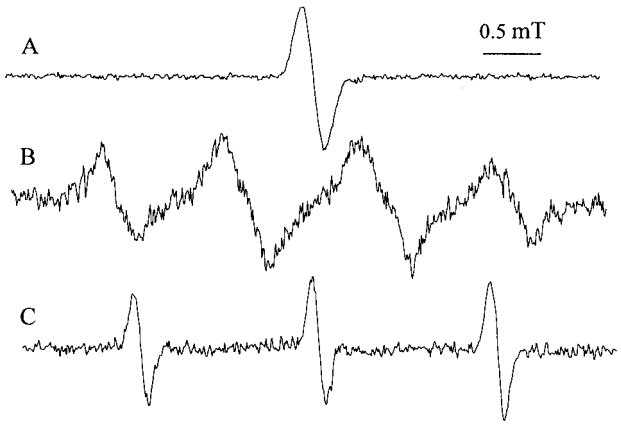


Figure 1. Spectrum A: photoinduced ESR spectrum from the deaerated DMSO-buffer (1/1, v/v, pH=7) solution of HBCD (0.1 M), illumination was with 532 nm pulsed laser for 3 min. Spectrum B: similar to spectrum A but in the oxygen saturated solution and in the presence of DMPO (450 mM) irradiated for 3 min. Spectrum C: similar to spectrum A but in the oxygen saturated solution in the presence of TEMP (20 mM) irradiated for 40 s.

exhibited photosensitized cleavage and damage to isolated and cellular DNA, whether under aerobic or anaerobic condition.[10] Herein, calf thymus DNA was used as phototherapeutic target to examine the photoinduced damage ability of HBCD according to a literature procedure.[10] We compared the capability of photosensitized damage to calf thymus DNA with HB and MAHB under the same condition. The binding site remaining was 62.5%, 59.1%, 35.6%, respectively, when HB, MAHB, HBCD was added into calf thymus DNA solution separately, and irradiation was carried out for 30 min with a medium-pressure sodium lamp ($\lambda > 470$ nm) under aerobic condition. Clearly, HBCD exhibited stronger damage to calf thymus DNA than HB and MAHB under aerobic condition.

In conclusion, a water-soluble derivative of hypocrellin B was synthesized by modification with β-cyclodextrin, which enhanced the PDT ability of hypocrellin B due to the affinity increase of HBCD to the negatively groove of double-strand DNA. Further photophysical, photochemical and phototherapy study on this promising phototherapy agent HBCD is under way.

This research was supported by NNSFC (No. 29971031, 19733100, 20073050).

References and Notes

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6 Synthesis of **2**, 500 mL of methanol-buffer (1:3 vol/vol, pH = 11) solution of a mixture of HB (2.8 mM) and mercaptoacetic acid (0.2 M) was put into a three necked round bottomed flask. The reaction mixture was bubbled with air (5 mL/min) and irradiated (> 470 nm) for 10 h. The mixture was separated by TLC as ref 7. Mono-substituted HB (0.63 g) was obtained (yield: 67%). ^{1}H NMR(400 MHz, $CDCl_3$, δ): 15.97 (s, 2H, exchangeable with D_2O, phenolic OH), 6.56 (s,1H), 3.75–4.2 (s,14H), 2.38 (s,3H), 1.93 (s,3H). FAB-MS ([M-H]$^-$): 617.
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8 Synthesis of **6**: compound **2** (10 mmol) and compound **5** (15 mmol) was dissolved in dry DMF (20 ml), and then DCC (10 mmol), HOBT (14 mmol) was added. After stirring 48 h at room temperature, 200 mL of acetone was poured into the reaction mixture. The final product, **6** (HBCD), was purified on Sephadex C-25 (30% yield). MALDI-TOF: 1780. ^{1}H NMR (400 MHz, DMSO-d_6 δ): 8.04 (s,1H), 6.42 (s,1H), 5.74 (s,14H), 4.81 (s,7H), 4.55 (s,6H), 3.0–4.1 (62H), 2.77 (s,1H), 2.07 (s,3H), 1.89 (s,3H).
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