Metal Ions Affect on the Photodynamic Actions of Cyclodextrin-Modified Hypocrellin

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Cu^{2+}, Fe^{2+} and Fe^{3+} could chelate with cyclodextrin-modified hypocrellin (HBCD) efficiently and the UV-visible spectra of the resultant metal complexes red shifted by more than 40 nm. ESR study revealed that the hydroxyl radical was the main product during irradiation of these metal complexes because Cu^{2+}, Fe^{2+} and Fe^{3+} initiated the Fenton reaction. Also, these metal complexes photodamaged the calf thymus DNA in the liposome system 20 folds faster than that in the buffer solution due to the initiation of lipid peroxidation.

Hypocrellins are new photodynamic agents and have been used in the treatment of certain skin disease.[1] Photophysical and photochemical properties, photobiological activities and biomedical application of hypocrellins have been intensively investigated by a broad spectrum of researchers.[2–4] The light-produced active oxygen species and radicals of these sensitizers are regarded as the intermediates in phototherapy. Recently, we reported the synthesis and phototherapeutic behavior of a novel cyclodextrin-modified hypocrellin.[5] The modification of hypocrellin with cyclodextrin significantly enhances the water-solubility of these lipid-soluble perylenequinone derivatives and as the result makes HBCD more photodamaging to calf thymus DNA in buffer solution than mercaptoacetic acid-substituted hypocrellin B or hypocrellin B. However, Hu et al.,[6] Diwu et al.[7] and Ma et al.[8] reported that metal ions exerted remarkable influence on the absorption spectrum and photodynamic actions of hypocrellins. Hence, it is of importance to study the interaction between the metal ions and HBCD and their effects on the photodynamic actions of HBCD before examining the practicability of HBCD in the clinic test. On the other hand, the DNA was wrapped by the nuclear envelope and cell membrane in the body,[9] which consist of polyunsaturated lipid, and it has been reported that the lipid peroxidation of the membrane could induce the DNA damage in biological system.[10] In this letter, the egg phosphatidylcholine (EPC) liposome was employed to investigate the role of lipid peroxidation in the DNA photodamage by HBCD.

The absorption spectrum of HBCD was shown in Figure 1. When the added metal ions chelate with HBCD, the absorption spectrum of HBCD will red shift and the absorption intensity increase. Among the metal ions tested in our experiment, there were only Cu^{2+}, Fe^{2+} and Fe^{3+} making the UV-visible spectrum of HBCD red shifted by more than 40 nm and absorption intensity of HBCD increased obviously (Figure 1), while Ni^{2+}, Co^{2+}, Zn^{2+}, Mg^{2+}, Al^{3+} and Ca^{2+} only caused the absorption spectrum of HBCD red shifted by several nanometers and the absorption intensity increased slightly. This indicated that Cu^{2+}, Fe^{2+} and Fe^{3+} could chelate with HBCD intensely. The composition of the metal complex (Figure 2) was determined by the molar ratio method.[6] In this method, a series of solutions were prepared in which the concentration of HBCD (20 μM) was kept constant and that of the metal ions was varied. The absorbance of the solution was measured at 600 nm and plotted versus the concentration ratio of the metal ion and HBCD (C_M/C_{HBCD}). The obvious extrapolated intersection occurred at the molar ratio of 1 : 1 corresponding to the ratio of

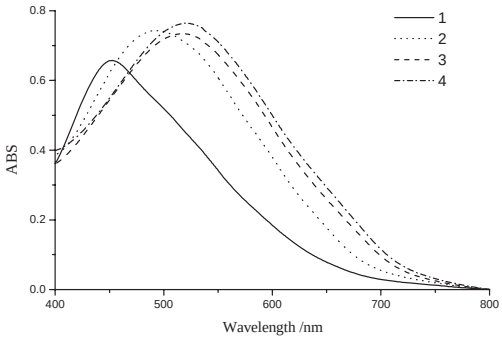

Figure 1. Absorption spectra of HBCD and its complexes (20 μM) in buffer solution (pH = 7), (1) HBCD (2) Cu^{2+}-HBCD, (3) Fe^{2+}-HBCD, (4) Fe^{3+}-HBCD.

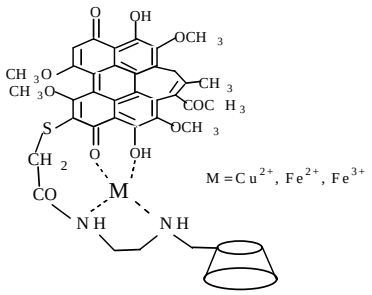

Figure 2. Structure of the metal ion chelated HBCD.

HBCD to metal ion in the complex. The molar ratio method was also used to determine the dissociation constant of a complex.[6] The dissociation constant of Cu^{2+}-HBCD, Fe^{2+}-HBCD and Fe^{3+}-HBCD was 4.32×10^{-8} M, 6.39×10^{-9} M, 9.22×10^{-10} M respectively, and that means Cu^{2+}, Fe^{2+} and Fe^{3+} formed steady complex with HBCD.

ESR trapping method was used to characterize the intermediates involved in the phototherapic behavior of HBCD and its metal complexes. Irradiation of oxygen-saturated DMSO solution of HBCD (0.1 mM) generated ESR signal of singlet oxygen and superoxide anion radical, while hydroxyl radical was detected also when HBCD was irradiated in oxygen-saturated buffer solution (pH = 7). When Cu^{2+}, Fe^{2+} and Fe^{3+} ions were present and consequently metal ion-HBCD complexes were formed, the ESR signal of singlet oxygen was suppressed remarkably and meanwhile the ESR signal of hydroxyl radical was enhanced greatly, implying that a different phototherapeutic mechanism may be undergone for Cu^{2+}, Fe^{2+} and Fe^{3}+-HBCD complexes compared with that of HBCD itself. Cu^{2+}, Fe^{2+} and Fe^{3+} ions can catalyze the Fenton reaction by which the superoxide anion radical could transform to hydroxyl radical and resultantly make the hydroxyl radical to be the main active intermediate responsible for the phototherapeutic behavior of these metal complexes. Moreover, the chelation of Cu^{2+}, Fe^{2+} and Fe^{3+} with HB the CD lowered triplet energy of HBCD and decreased the energy transfer efficiency between sensitizers and ground state oxygen and as the result the formation

of singlet oxygen was restrained.[11]

The DNA is a phototherapy target of hypocrellin under the isolated and cellular condition.[12–14] When the DNA was destroyed, the number of available binding site for the intercalator ethidium bromide (EB) reduced[15] and consequently the fluorescence quantum yield of EB decreased dramatically. This property was employed in this work to monitor the photodamage of calf thymus DNA (CT DNA) by HBCD and its metal complexes. HBCD presented much higher efficiency to damage DNA than its metal complexes in the buffer solution (Table 1). The addition of NaN_3, SOD and sodium benzoate decreased the cleavage efficiency of HBCD, indicating that singlet oxygen, superoxide anion radical and hydroxyl radical were all included in the cleavage of CT DNA. Because the strong hydrophilic, short-lived hydroxyl radical was the major reactive oxygen species in the case of HBCD metal complexes, the photodamage efficiency of these metal complexes was lower than that of HBCD.

However, when photodamage experiment performed in the EPC liposome, the photocleavage efficiency of HBCD was enhanced about 10 folds of that in the buffer solution and the

Table 1. Photocleavage of CT DNA by HBCD and its metal complexes (1 μM) detected by reduced binding site remaining (BSR) of ethydium bromide under aerobic conditions [CT DNA] = 40 μM, [EB] = 80 μM.

Sample \ Irradiation Time/min, BSR/%	10	20	30
Control experiment[a]	100	100	100
Control experiment[b]	97.6	95.6	94.1
Control experiment[c]	100	100	100
HBCD	64.6	48	40.6
Cu^{2+} - HBCD	87.9	78.5	73.7
Fe^{2+} - HBCD	87.5	82.5	78.7
Fe^{3+} - HBCD	85.2	78.3	73.9
HBCD + SOD[d]	80.3	68.8	59.5
HBCD + NaN_3[e]	73.6	61.4	55.9
HBCD + $C_6H_5CO_2Na$[f]	76.2	95.6	53.7

[a] in the absence of HBCD or its metal complexes and without light irradiation. [b]in the absence of HBCD or its metal complexes. [c]in the presence of HBCD but without light irradiation. [d] [SOD]= 40 μg/ml. e [e][NaN_3]= 1 mM. [f] [$C_6H_5CO_2Na$]=10 mM.

photocleavage efficiency metal complexes of HBCD was enhanced about 20 folds than that of in the buffer solution (Table 2). It has been proposed that in complex biological systems active oxygen species, such as superoxide anion radical, singlet oxygen and hydroxyl radical, can cause DNA damage indirectly by initiating lipid peroxidation,[16] since the polyunsaturated chain of EPC was especially susceptible to free radical initiated oxidation. The lipid radicals had been confirmed to cause DNA damage model system and prokaryotes.[17,18] In our experiment, the significant photodamage of CT DNA in the presence of EPC liposome is likely to be the result of occurrence of liposome peroxidation and formation of lipid radicals induced by active oxygen species, because the lipid radicals are more long-lived and have much higher photodamage efficiency to CT DNA which renders them more toxic to CT DNA than active oxygen species. The addition of NaN_3, SOD and sodium benzoate decreased the photocleavage efficiency of HBCD and its metal complexes (Table 2). That means the lipid radicals were generated from the superoxide anion radical, singlet oxygen and hydroxyl radical. More efficient photodamage obtained in the case of Cu^{2+}, Fe^{2+} and Fe^{3+}-HBCD complexes suggested that the

Table 2. Photocleavage of CT DNA by HBCD and its metal complexes (10 μM) detected by reduced binding site remaining (BSR) of ethydium bromide in EPC liposome [CT DNA] = 40 μM, [EB] = 80 μM [EPC] = 2 mg/ml.

Sample \ Irradiation Time /min, BSR /%	1	2	3
Control experiment[a]	98.4	96.2	95.2
HBCD	61.9	51.2	45.9
Cu^{2+} - HBCD	55.3	47.6	40.8
Fe^{2+} - HBCD	60.5	49.6	44.6
Fe^{3+} - HBCD	61.2	48.6	42.3
HBCD + SOD[b]	80.6	70.3	62.5
HBCD + NaN_3[c]	70.5	62.8	54.8
HBCD + $C_6H_5CO_2Na$[d]	71.7	65.2	57.5
Cu^{2+}-HBCD+ SOD[b]	69.2	64.0	59.3
Cu^{2+}-HBCD + NaN_3[c]	62.6	55.3	51.2
Cu^{2+}-HBCD+ $C_6H_5CO_2Na$[d]	72.9	68.2	65.6

[a] in the absence of HBCD or its metal complexes. [b] [SOD]= 40 μ g/ml. [c] [NaN_3]= 1 mM. [d] [$C_6H_5CO_2Na$]=10 mM.

present of Cu^{2+}, Fe^{2+} and Fe^{3+} ions could accelerate the lipid peroxidation reaction efficiently.

In summary, the chelation of HBCD with Cu^{2+}, Fe^{2+} and Fe^{3+} can make the UV-visible spectrum of HBCD red shifted. Especially Fe^{3+} and Fe^{2+} make the UV-visible spectrum of HBCD red shifted by 70 nm and enhanced the absorbance of HBCD in the phototherapic window (600–900 nm) significantly. The photodamage efficiency of HBCD and its metal complexes to CT DNA increased significantly in the EPC liposome due to the initiation of lipid peroxidation reaction. It indicates that HBCD would be a potent and promising phototherapeutic agent in the clinic test.

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