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Photodynamic properties of supramolecular assembly constructed by magnesium complex of hypocrellin A and fullerene C_{60}

Yunyan Gao,[a] Zhize Ou,*[a] Jingrong Chen,[b] Guoqiang Yang,*[c] Xuesong Wang,*[b] Baowen Zhang,[b] Mimi Jin[a] and Lihua Liu[a]

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A supramolecular assembly, composed of magnesium complex of hypocrellin A (Mg^{2+}–HA) and pristine C_{60} with a molar ratio of 2 : 1, is constructed and characterized. The supramolecular assembly (Mg^{2+}–HA/C_{60}) shows moderate solubility in polar solvents and strong absorption in visible region. EPR studies demonstrate that one-electron transfer from *N*,*N*-diethylaniline (DEA) to excited-state Mg^{2+}–HA induces the generation of the reduced form of Mg^{2+}–HA (Mg^{2+}–$HA^{-\bullet}$ radical), followed by electron transfer from the Mg^{2+}–$HA^{-\bullet}$ radical to C_{60}, forming the corresponding anion radical $C_{60}{}^{-\bullet}$ under anaerobic conditions. In aerobic media, the generation of reactive oxygen species, including superoxide anion radical ($O_2{}^{-\bullet}$) and singlet oxygen ($^1O_2$), by Mg^{2+}–HA/C_{60} photosensitization is observed. Mg^{2+}–HA/C_{60} exhibits much stronger photodamage ability on pBR322 plasma DNA than Mg^{2+}–HA and HA under both aerobic and anaerobic conditions.

Introduction

Fullerene C_{60} has received a great deal of interest owing to its unique photophysical properties and favorable biological activities.[1] However, the application of fullerene C_{60} and its derivatives has been limited, owing to their low solubility in water and other frequently used solvents. To solve this problem, one of the important approaches is the hybridization of fullerene C_{60} with polymeric materials, which may also offer a possibility of tuning the electronic properties of fullerene C_{60}.[2]

Hypocrellins, a type of naturally occurring perylenequinonoid, have received considerable attention because of their photoinduced antitumor and antiviral activity.[3] There is evidence suggesting that DNA as well as protein and membrane is involved in the photodynamic therapy effect of hypocrellins on cells.[4] Structural modification[5] and complexing with metal ions[6] are two approaches adopted to improve the photodynamic efficacy of hypocrellins.

Mg^{2+}–HA, known as a linear polymer, has been shown to exhibit better photodynamic activities and water solubility than parent HA.[6] It can be expected that Mg^{2+}–HA, possessing many perylenequinonoid rings, might show strong π–π stacking interactions with the surface of fullerene C_{60} to form a supramolecular assembly.[7] In the current work, the interaction between Mg^{2+}–HA and pristine fullerene C_{60} is investigated. Photodynamic properties of the Mg^{2+}–HA/C_{60} system and the radical intermediates responsible for electron transfer from *N*,*N*-diethylaniline to Mg^{2+}–HA/C_{60} system are studied in detail.

Experimental

Chemicals

Hypocrellin A (HA) was isolated from the fungus sacs of *Hypocrella bambusae* and recrystallized twice from acetone before use. Diethylformamide (DMF) was dried over K_2CO_3 and distilled in the presence of calcium hydride *in vacuo*. All the other solvents were distilled before use. Fullerene C_{60}, and pBR322 plasma DNA were purchased from Sigma Chemical Company. Dimethyl sulfoxide (DMSO), 2,2,6,6-tetraethyl-4-piperidone (TEMP), 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), 1,4-diazabicyclo[2.2.2]octane (DABCO), and superoxide dimutase (SOD) were purchased from Aldrich Chemical Company.

Preparation of Mg^{2+}–HA and Mg^{2+}–HA/C_{60}

The complex Mg^{2+}–HA was prepared according to literature with minor modification.[6] A solution of magnesium acetate (1 mM) was added dropwise to equal volume of HA (1 mM) in ethanol. The resulting solution was stirred for 2 h at room temperature in the dark. After completion of chelation, the solvent was evaporated. The solid was quickly washed with cold chloroform and 10% aqueous ethanol to remove uncomplexed HA and magnesium acetate. The residue was chromatographed on a calcium hydrogen phosphate column using petroleum ether–ethyl acetate (1 : 1) as eluent, and evaporation of the eluent in vacuum gave the Mg^{2+}–HA chelate.

Mg^{2+}–HA (2 mM) in *N*,*N*-dimethylformamide was added to five-fold volume of fullerene C_{60} (0.2 mM) xylene solution.

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[a] *Department of Applied Chemistry, School of Science, Northwestern Polytechnic University, Xi'an, 710072, People's Republic of China. E-mail: ouzhize@nwpu.edu.cn*
[b] *Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, 100101, People's Republic of China. E-mail: xswang@mail.ipc.ac.cn*
[c] *CAS Key laboratory of Photochemistry, Institute of Chemistry, Chinese Academy of Sciences, Beijing, 100080, People's Republic of China. E-mail: gqyang@iccas.ac.cn*

The mixture was stirred overnight at room temperature in the dark. The solution was evaporated under high vacuum. The solid was dried overnight *in vacuo* at 100 °C to remove the solvent completely. The obtained Mg^{2+}–HA/C_{60} aggregate was used for UV-Vis absorption spectra, fluorescence spectra and DNA cleavage experiments.

Measurements of spectral and electrochemical properties

The UV-Vis absorption spectra and fluorescence emission spectra were recorded on a Shimadzu UV-2550 spectrophotometer and a Hitachi F-4500 fluorescence spectrometer, respectively.

EPR spectra were obtained using a Bruker ESP-300E spectrometer operating at room temperature, and the operating conditions were as following: microwave bridge, X-band with 100 Hz field modulation; sweep width, 200 G; receiver gain, 1×10^5; microwave power, 5 mW. Samples were injected into the specially made quartz capillaries for EPR analyses, purged with argon, air or oxygen for 30 min in the dark, respectively, according to the experimental requirements, and illuminated directly in the cavity of the EPR spectrometer with a Nd:YAG laser (532 nm, 5–6 ns pulse width, 10 mJ $pulse^{-1}$ energy) except as noted elsewhere. The kinetics of spin adducts were studied by recording the peak heights of EPR every 1 min.

Cyclic voltammetry (CV) experiments were performed on a potentiostat/galvanostat Model 283A (EG&G Princeton Applied Research) in DMSO–toluene (4 : 1, v/v) solution, using two platinum wires as the working and counter electrodes, respectively, and a saturated calomel electrode (SCE) as reference electrode in the presence of 0.1 mM *n*-tetrabutylammonium tetrafluoroborate as the supporting electrolyte.

UV-Vis titrations

The titrations were performed by adding the required volumes of a solution of C_{60} (1 mM) in toluene to 4 ml of the solution of Mg^{2+}–HA (40 μM) and then the solution was diluted to 5 ml. The vertical coordination (ΔA) in differential absorption spectra represents the difference between the absorption of the mixed solution (C_{60} + Mg^{2+}–HA) and that of the neat C_{60} and Mg^{2+}–HA solutions at corresponding concentrations, which can be calculated according to the following equation:

$$\Delta A = A_{\mathrm{mix}} - A_{\mathrm{Mg^{2+}\text{–}HA}} - A_{\mathrm{C_{60}}} \qquad (1)$$

where A_{mix}, $A_{\mathrm{Mg^{2+}\text{–}HA}}$, $A_{\mathrm{C_{60}}}$ is the absorbance of the mixed solution, neat Mg^{2+}–HA and neat C_{60} at corresponding concentrations, respectively.

UV-Vis Job plots of Mg^{2+}–HA with C_{60}

Stock solutions of Mg^{2+}–HA (80 μM) and C_{60} (80 μM) in toluene were prepared. Nine solutions with molar fractions of Mg^{2+}–HA from 0.1 to 0.9 were prepared in 5 ml volumetric flasks by diluting the required amounts of the stock solutions. The total concentration of Mg^{2+}–HA and C_{60} was fixed at 80 μM. $A_{\mathrm{exp}} - A_0$ (Y) represents the difference of the absorbance at 625 nm, and were calculated according to the following equation:

$$A_0 = \varepsilon_{\mathrm{C_{60}}} x C_0 + \varepsilon_{\mathrm{Mg^{2+}\text{–}HA}}(1 - x)C_0 \qquad (2)$$

where χ is the molar fraction of titration reagent C_{60} and C_0 is the total concentration of the solutions. The plotting of Y as a function of χ can be used to draw a curve that is useful to know the stoichiometry of the complex.

DNA cleavage experiments

A supercoiled pBR322 plasmid DNA was diluted with 50 mM Tris-HCl buffer (pH 7.4) to 125 μg ml^{-1}. The DNA solution, hypocrellin solutions, and other reagent solutions were mixed well. The samples were purged with argon or air for 15 min before irradiation according to the experimental requirements.

Then the samples were subjected to photoirradiation with a 400 W medium pressure sodium lamp (>470 nm) at room temperature. After irradiation, an aliquot (20 μl) of the irradiated solution was mixed with a Bromophenol Blue–glycerol solution (5 μl) and subjected to agarose gel electrophoresis. The gel was stained with ethidium bromide and photographed on the transilluminator for analysis of the ratio of form I (supercoiled) and form II (nicked). The damage is defined as following equation:[8]

$$\text{damage (\%)} = (F_{\mathrm{II}} - F_{\mathrm{II\,0}})/F_{\mathrm{I\,0}} \qquad (3)$$

where F_{II} and $F_{\mathrm{II\,0}}$ are the percentage of form II of DNA with or without photosensitizer and $F_{\mathrm{I\,0}}$ is the percentage of form I of initial DNA.

Results and discussion

Interaction between Mg^{2+}–HA and C_{60}

It has been reported that Mg^{2+} can form a 1 : 1 complex with HA, and the resulting complex Mg^{2+}–HA has a linear polymeric structure (Fig. 1(a)).[6] Mg^{2+}–HA shows strong absorption in the visible region and the UV-Vis absorption spectra of Mg^{2+}–HA can be affected by the polarity of solvent. In toluene, Mg^{2+}–HA exhibits three absorption peaks at 582, 542 and 465 nm (Fig. 1(b)). However, when the polarity of solvent increases, the absorption spectra shift to longer wavelengths with peaks at 624, 580, 498 nm in DMSO, respectively.

The interaction between Mg^{2+}–HA and C_{60} was studied in toluene by titration experiments. In detail, the toluene solution of Mg^{2+}–HA was titrated with variable amounts of C_{60} and changes were routinely monitored by absorption spectroscopy (Fig. 2(a)). Differential absorption spectra (Fig. 2(b)) clearly indicate that a strong interaction exists between Mg^{2+}–HA and C_{60}. The composition of the complex was determined by the continuous variation method.[9] The total concentration of Mg^{2+}–HA and C_{60} was kept constant (80 μM) while the molar fraction of Mg^{2+}–HA and C_{60} was continuously varied. The difference (Y) between the mixed solution and that of the free Mg^{2+}–HA and that of free C_{60} was plotted against the molar fraction of C_{60}. As shown in Fig. 3, the maximum Y occurred at the molar fraction of 0.34, suggesting a 2 : 1 molar ratio of Mg^{2+}–HA and C_{60} in the complex.

Characterization of the supramolecular assembly

The preparation of supramolecular assembly Mg^{2+}–HA/C_{60} (2 : 1) was carried out in DMF–xylene (1 : 5, v/v) mixed solvent and the resulting complex shows moderate solubility

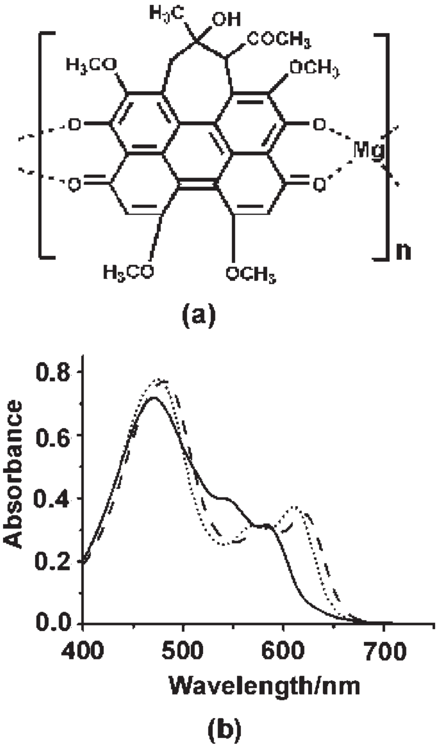

Fig. 1 (a) Chemical structure of Mg^{2+}–HA. (b) Absorption spectra of Mg^{2+}–HA (40 μM) in toluene (solid line), ethanol (dotted line), DMSO (dashed line).

(>10 μM) in polar solvents, such as DMF, ethanol and chloroform. The concentration of Mg^{2+}–HA/C_{60} in DMSO can reach up to 50 μM. It was reported that the solubility of C_{60} is very low in most polar solvents (<2.0 μM).[10] The enhanced solubility of C_{60} can be attributed to the interaction between Mg^{2+}–HA and C_{60}.

The supramolecular assembly Mg^{2+}–HA/C_{60} was characterized by FT-IR, UV-Vis and fluorescence spectra. The typical FT–IR band of the quinonoid carbonyl group in Mg^{2+}–HA (1596 cm^{-1}) shifts to 1604 cm^{-1} upon complexation with C_{60}, indicating the alteration of the quinonoid carbonyl group upon coordination. Additionally, a broad absorption band around 450–750 cm^{-1} can be attributed to a Mg–O bond.[6] The appearance of the typical FT–IR band at 527 cm^{-1} and the absorption at 331 nm in the UV-Vis spectrum are assigned to the C_{60} unit (Fig. 4(a)). Upon complexation with C_{60}, a remarkable blue shift is observed in the absorption spectra of Mg^{2+}–HA in DMSO, and the color of solution changes from green to red. Moreover, the fluorescence intensity of Mg^{2+}–HA at 607 nm is significantly enhanced after complexing with C_{60} (Fig. 4(b)), which may be attributed to the interactions between perylenequinonoid rings and C_{60}. The nature of the binding of C_{60} to Mg^{2+}–HA may be due to the π–π stacking interactions between the perylenequinonoid rings and C_{60}, as in the case of noncovalent binding of aromatic components with C_{60}.[7]

Electron transfer between Mg^{2+}–HA/C_{60} and *N*,*N*-diethylaniline (DEA)

It has been well established that C_{60} is an excellent electron acceptor in the ground state and can accept, reversibly, up to six electrons.[11] EPR, a powerful technique for investigating photoreaction intermediates, was employed to study the mechanism of electron transfer from donor DEA to Mg^{2+}–HA/C_{60}.

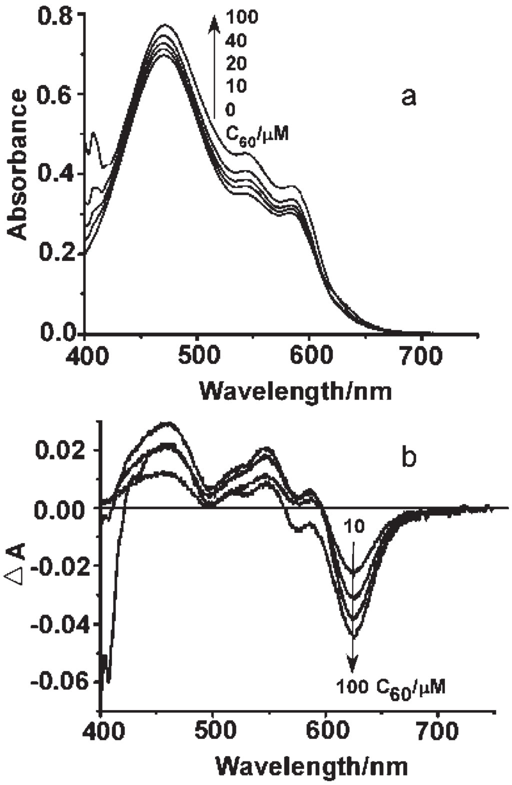

Fig. 2 (a) Absorption and (b) differential absorption spectral changes of Mg^{2+}–HA (40 μM) upon titration with C_{60} in toluene.

Metal ion complexes of HA retain the ability to generate semiquinone anion radical, which originates from the electron transfer from reductant to excited-state hypocrellins.[6] Upon irradiation of an argon-saturated solution of Mg^{2+}–HA (100 μM) in the presence of DEA (1 mM) with a medium-pressure sodium lamp, an EPR signal was observed (Fig. 5(a)). The *g* value (2.002) of this signal correlates well with that of HA semiquinone anion radical.[12] Photoinduced electron transfer from DEA, a typical electron donor, to Mg^{2+}–HA may occur as follows (eqn (4)):

$$Mg^{2+}\text{–HA}^{*} + \text{DEA} \rightarrow Mg^{2+}\text{–HA}^{-\bullet} + \text{DEA}^{+\bullet} \qquad (4)$$

Introduction of a deoxygenated solution of C_{60} to the above irradiated solution results in remarkable quenching of the EPR signal of Mg^{2+}–$HA^{-\bullet}$, and a new EPR signal (g = 2.000) appears simultaneously (Fig. 5(b)). The new signal at g = 2.000 agrees with that reported for $C_{60}^{-\bullet}$.[13] That suggests electron transfer from Mg^{2+}–$HA^{-\bullet}$ to C_{60} occurs (eqn (5)):

$$Mg^{2+}\text{–HA}^{-\bullet} + C_{60} \rightarrow Mg^{2+}\text{–HA} + C_{60}^{-\bullet} \qquad (5)$$

Determination of the redox potentials was important to prove the existence of electron transfer interactions between the donor and acceptor in the ground state. The first reduction potential of C_{60} (−0.59 V) in DMSO–toluene (4 : 1, v/v) occurs at less negative value compared to the reduction potential of

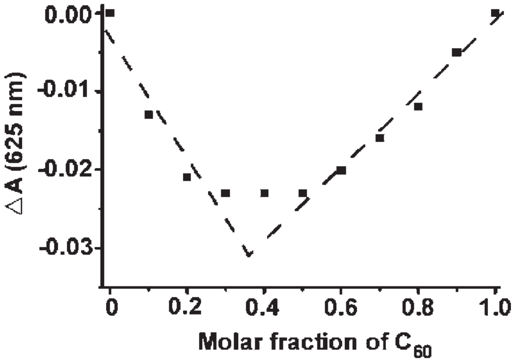

Fig. 3 Job plots for the 2 : 1 complexation of Mg^{2+}–HA with C_{60} in toluene, obtained by plotting the absorbance difference (*Y*) at 625 nm with respect to neat Mg^{2+}–HA and neat C_{60} at the same wavelength as a function of the molar fraction of C_{60} ($[Mg^{2+}$–HA] + $[C_{60}]$ = 80 μM).

Mg^{2+}–HA (−0.69 V) (all potentials are *vs.* SCE). That means C_{60} is a stronger electron acceptor than Mg^{2+}–HA, and the electron transfer from Mg^{2+}–$HA^{-\bullet}$ to C_{60} is a thermodynamically favorable process ($\Delta G = -0.1$ eV), calculated from eqn (6):[14]

$$\Delta G = E_{ox}(\text{donor}) - E_{red}(\text{acceptor}) \qquad (6)$$

After irradiation of Mg^{2+}–HA/C_{60} in the presence of DEA, the resulting EPR spectrum is shown in Fig. 5(c). It consists of two EPR signals with *g* values of 2.002 and 2.000, respectively. These EPR signals are assigned to Mg^{2+}–$HA^{-\bullet}$ and $C_{60}^{-\bullet}$, respectively. A control experiment indicates that Mg^{2+}–HA is essential under our irradiation conditions to generate $C_{60}^{-\bullet}$ (Fig. 5(d)). That is reasonable because C_{60} has weak absorbance in the visible region and can not be effectively excited. The electron transfer from an aniline derivative to C_{60} is thermodynamically inhibited.[15] All the above EPR results indicate that Mg^{2+}–HA can act as a light-harvesting antenna in the supramolecular system and mediate electron transfer from DEA to C_{60}.

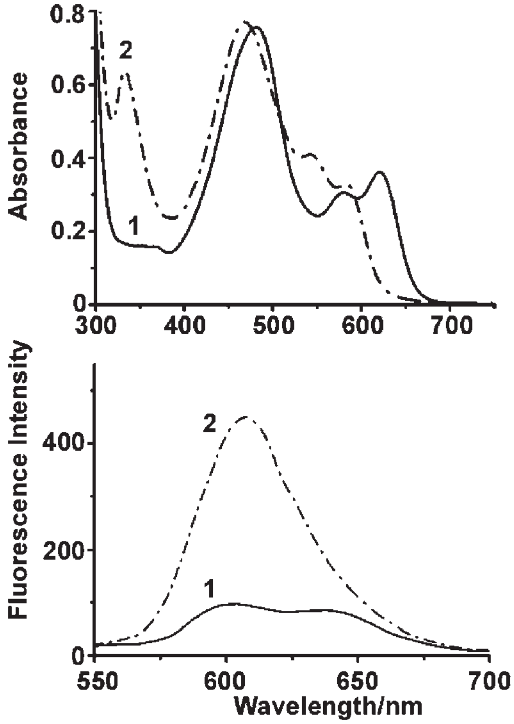

Fig. 4 (a) UV-Vis absorption spectra and (b) fluorescence spectra (λ_{ex} = 470 nm) of 1 (Mg^{2+}–HA, 40 μM) and 2 (Mg^{2+}–HA/C_{60}, 20 μM) in DMSO solution.

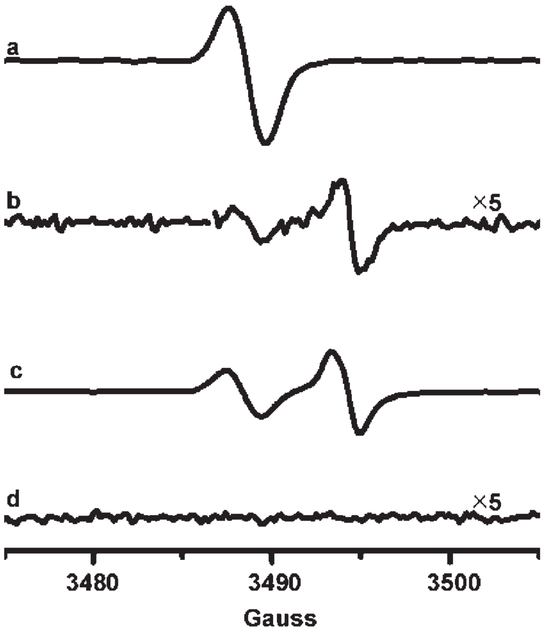

Fig. 5 (a) Photoinduced EPR spectrum of a deoxygenated solution of Mg^{2+}–HA (100 μM) and diethylaniline (1 mM) in DMSO–toluene (4 : 1, v/v), illuminated for 10 min with a medium-pressure sodium lamp (>470 nm) at a distance of 15 cm. (b) Similar to (a), but with the addition of C_{60} (50 μM) after illumination. (c) Similar to (a), but Mg^{2+}–HA/C_{60} (50 μM) instead of Mg^{2+}–HA. (d) Similar to (a) but C_{60} (50 μM) instead of Mg^{2+}–HA.

Generation of superoxide anion radical ($O_2^{-\bullet}$)

The photoinduced EPR signals of Mg^{2+}–$HA^{-\bullet}$ and $C_{60}^{-\bullet}$ disappear when oxygen was bubbled through the argon-saturated DMSO–toluene (4 : 1, v/v) solution containing Mg^{2+}–HA/C_{60} (50 μM) and DEA (1 mM) after irradiation. One possible explanation for this observation is that O_2 can oxidize Mg^{2+}–$HA^{-\bullet}$,[6] and $C_{60}^{-\bullet}$,[16] leading to the formation of $O_2^{-\bullet}$ (eqn (7) and eqn (8)).

$$Mg^{2+}\text{–}HA^{-\bullet} + O_2 \rightarrow Mg^{2+}\text{–}HA + O_2^{-\bullet} \qquad (7)$$

$$C_{60}^{-\bullet} + O_2 \rightarrow C_{60} + O_2^{-\bullet} \qquad (8)$$

The following experiments were carried out to examine this possibility. In air-saturated DMSO-toluene (4 : 1, v/v) solution containing Mg^{2+}–HA/C_{60} (50 μM), DEA (1 mM), laser irradiation at 532 nm results in a new EPR signal in the presence of DMPO, a typical spin trapping agent for superoxide anion radical (Fig. 6, inset). The new EPR signal can be characterized by three hyperfine coupling constants: α^N = 13.0 G, α^H_β = 10.1 G, α^H_γ = 1.5 G, in good agreement with the EPR signal of the DMPO–$O_2^{-\bullet}$ adduct.[17] That the EPR

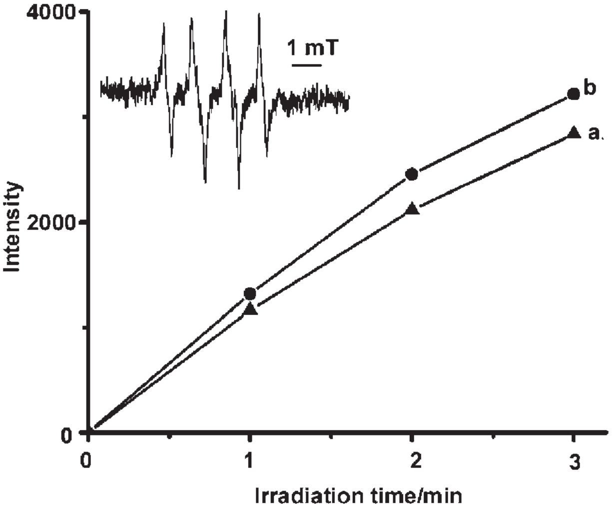

Fig. 6 (a) Dependence of EPR signal intensity of the DMPO–$O_2^{-\bullet}$ adduct on irradiation time for an air-saturated DMSO–toluene solution (4 : 1, v/v) containing Mg^{2+}–HA/C_{60} (50 μM), *N*,*N*-diethylaniline (1 mM) and DMPO (50 mM) irradiated at 532 nm. (b) Same as (a) but Mg^{2+}–HA (100 μM) instead of Mg^{2+}–HA/C_{60}. Inset: EPR spectrum of the DMPO–$O_2^{-\bullet}$ adduct produced in the irradiated solution.

spectrum can be suppressed by addition of SOD, a typical $O_2^{-\bullet}$ scavenger, confirms the assignment further. The DMPO–$O_2^{-\bullet}$ signal intensity generated in solution containing Mg^{2+}–HA/C_{60} and DEA is close to that in a solution containing Mg^{2+}–HA and DEA (Fig. 6).

Generation of singlet oxygen ($^1O_2$)

It has been reported that $^1O_2$ is involved in many photooxidation reactions sensitized by hypocrellins.[18] The EPR spin trapping technique using 2,2,6,6-tetraethyl-4-piperridone (TEMP) as spin trapping agent was employed to detect the generation of $^1O_2$.[19] TEMP can easily react with $^1O_2$ to yield a nitroxide, TEMPO, which can be detected by EPR. Irradiation of an air-saturated DMSO–toluene (4 : 1, v/v) solution containing Mg^{2+}–HA/C_{60} (50 μM) and TEMP (50 mM) with a 532 nm laser leads to a three-line EPR signal (Fig. 7, inset). The coupling constants α_N = 16.0 G and g = 2.0056 were in good agreement with those of TEMPO in literature.[19] Control experiments confirm that oxygen, Mg^{2+}–HA/C_{60}, and light were all necessary for the EPR signal generation. Addition of DABCO, a singlet oxygen scavenger, suppresses the EPR signal effectively.

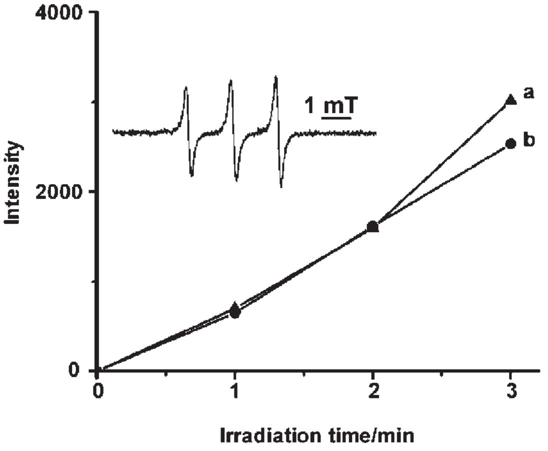

Fig. 7 (a) Dependence of EPR signal intensity of TEMPO on irradiation time for an oxygen-saturated DMSO–toluene solution (4 : 1, v/v) containing Mg^{2+}–HA/C_{60} (50 μM) and TEMP (50 mM) irradiated at 532 nm. (b) Same as (a) but Mg^{2+}–HA (100 μM) instead of Mg^{2+}–HA/C_{60}. Inset: EPR spectrum of TEMPO produced in irradiated solution.

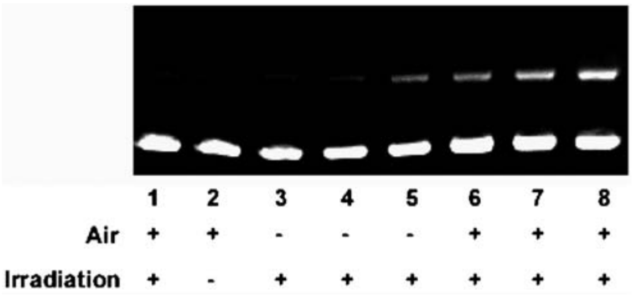

Fig. 8 Agarose gel electrophoretic patterns of plasma DNA. The reaction samples contained 1.0 μg of pBR322 plasmid. Lane 1: no reagent in 50 mM Tris-HCl (pH 7.4). Lanes 2, 5 and 8: 2.5 μM Mg^{2+}–HA/C_{60}. Lanes 3 and 6: 5 μM HA. Lanes 4 and 7: 5 μM Mg^{2+}–HA. Lanes 3–5: samples were purged with argon for 15 min before irradiation. Lanes 6–8: samples were purged with air for 15 min before irradiation.

All these findings confirm the ability of Mg^{2+}–HA/C_{60} to generate $^1O_2$, *via* energy transfer from the triplet of Mg^{2+}–HA and C_{60} to the ground-state oxygen upon irradiation (eqn (9) and eqn (10)).

$$^3Mg^{2+}\text{–HA} + O_2 \rightarrow Mg^{2+}\text{–HA} + {}^1O_2 \qquad (9)$$

$$^3C_{60} + O_2 \rightarrow C_{60} + {}^1O_2 \qquad (10)$$

The TEMPO signal intensity of Mg^{2+}–HA/C_{60} is almost the same as that of Mg^{2+}–HA (Fig. 7), because Mg^{2+}–HA shows much stronger absorbance than the C_{60} unit in Mg^{2+}–HA/C_{60} and most of $^1O_2$ is generated *via* energy transfer from the triplet of Mg^{2+}–HA to the ground state oxygen (eqn (9)).

Photoinduced damage to DNA

Both fullerene and hypocrellin have potential applications in biological and medical fields. PBR322 plasma was chosen as a substrate to investigate the DNA cleavage ability of Mg^{2+}–HA/C_{60}. In the dark, DNA was not cleaved in the presence of Mg^{2+}–HA/C_{60}, Mg^{2+}–HA or HA (Fig. 8, Lane 2, Mg^{2+}–HA/C_{60} as example) while under visible light irradiation (>470 nm), DNA-cleavage activity of all of these three reagents was observed in argon-saturated solution (Fig. 8, lanes 3–5). Based on the percentage conversion from form I to form II for these photosensitizers, DNA–nicking efficiency roughly follows the trends: Mg^{2+}–HA/C_{60} (13.5%) > Mg^{2+}–HA (8.0%) > HA (7.2%). Under anaerobic conditions, photocleavage of DNA by Mg^{2+}–HA, or HA was presumably due to the electron transfer from DNA to the excited-state Mg^{2+}–HA or HA (eqn (11), Mg^{2+}–HA as example).

$$Mg^{2+}\text{–HA}^* + \text{DNA} \rightarrow Mg^{2+}\text{–HA}^{-\bullet} + \text{DNA}^{+\bullet} \qquad (11)$$

For the Mg^{2+}–HA/C_{60} system, electron transfer first from DNA to the excited-state Mg^{2+}–HA (eqn (11)) may occur and then further from Mg^{2+}–$HA^{-\bullet}$ to C_{60} (eqn (5)). Fullerene C_{60}, as an excellent three-dimensional electron acceptor, possesses small reorganization energy in electron transfer, which leads to remarkable acceleration of charge separation and deceleration of charge recombination.[20] As a result, the supramolecular assembly Mg^{2+}–HA/C_{60} presents much stronger photocleavage ability on DNA than HA and Mg^{2+}–HA under anaerobic conditions.

In the presence of oxygen, photocleavage efficiency of pBR 322 DNA by Mg^{2+}–HA/C_{60}, Mg^{2+}–HA and HA all increased (Fig. 8, lanes 6–8) following the trends: Mg^{2+}–HA/C_{60} (27.0%) > Mg^{2+}–HA (20.9%) > HA (14.7%). The effect of oxygen on the enhancement of DNA cleaving ability of Mg^{2+}–HA/C_{60}, Mg^{2+}–HA or HA may be because of the involvement of reactive oxygen species ($O_2^{-\bullet}$, $^1O_2$). Due to the very low solubility of C_{60} in water ($\sim 10^{-24}$ M),[21] the photocleavage of DNA by pristine C_{60} was not carried out in this work.

Conclusions

In summary, a supramolecular system consisting of Mg^{2+}–HA and C_{60} has been constructed and characterized. EPR studies demonstrate that both Mg^{2+}–$HA^{-\bullet}$ and $C_{60}^{-\bullet}$ can be generated by photoinduced electron transfer from DEA to Mg^{2+}–HA then further from Mg^{2+}–$HA^{-\bullet}$ to C_{60}. Mg^{2+}–HA functions as a light-harvesting antenna in the supramolecular system. Mg^{2+}–HA/C_{60} preserves the photodynamic properties and can generate reactive oxygen species such as oxygen anion radicals and singlet oxygen. Under anaerobic conditions, Mg^{2+}–HA/C_{60} displays much stronger photodamage ability on DNA than Mg^{2+}–HA and HA. Photocleavage efficiency of Mg^{2+}–HA/C_{60} increased under aerobic conditions since the reactive oxygen species inflict damage on DNA.

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