Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Photochemistry and Photobiology A: Chemistry 165 (2004) 143–147
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Surface binding and improved photodamage of the lanthanum ion complex of hypocrellin A to calf thymus DNA

Jia-Hong Zhou [a], Sheng-Qin Xia [b], Jing-Rong Chen [b], Xue-Song Wang [b,1], Bao-Wen Zhang [b,*], Hui-Jun Zhang [c], Ping Zou [c], Xi-Cheng Ai [c], Jian-Ping Zhang [c,1]

[a] *Analysis & Testing Center, Nanjing Normal University, Nanjing 210097, PR China*
[b] *Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, 5061 Group, Beijing 100101, PR China*
[c] *Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, PR China*

Received 11 November 2003; received in revised form 27 January 2004; accepted 8 March 2004

Abstract

The surface binding association between a positively charged polymeric complex of hypocrellin A (HA) with La^{3+} (La^{3+}-HA) and calf thymus DNA (CT DNA) was confirmed by ESR and laser flash photolysis techniques along with UV-Vis absorption and fluorescence emission spectra. This surface binding, in cooperation with the longer triplet excited state lifetime of La^{3+}-HA, gave rise to a stronger photodamage of CT DNA both in aerobic and anaerobic conditions by La^{3+}-HA compared with HA.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Surface binding; Hypocrellin A; Lanthanum ion complex; Photodamage of DNA

1. Introduction

Hypocrellins, as new efficient phototherapeutic agents, have been receiving intensive attention over the past two decades [1,2]. Many efforts have been paid to evaluate the abilities of hypocrellins to generate the reactive oxygen species as well as to obtain hypocrellin derivatives with improved water solubility and enhanced light harvesting capability in the phototherapeutic window (600–900 nm) [3–9]. For achieving effective photodynamic treatment, however, it is essential that the photosensitizer recognizes and concentrates exclusively in the targeted substrate [10]. So far, few chemical modifications on natural hypocrellins concern the affinity of hypocrellins to biomaterials, and the investigations on the static interaction or association between hypocrellins and their targeted substrates are lacking [11]. Recently, we prepared a novel complex of HA with La^{3+} (La^{3+}-HA), which has a polymeric structure and exhibits, compared with HA, better water solubility, larger absorbance in phototherapeutic window, longer triplet excited state lifetime, higher $^1O_2$ generation efficiency, and stronger photodamage capability to CT DNA in both aerobic and anaerobic conditions [12]. In that communication, the greatly improved photodamage capability of La^{3+}-HA to CT DNA was partly attributed to the longer triplet excited state lifetime and the higher $^1O_2$ quantum yield of La^{3+}-HA. To better understand the photodynamic mechanism of La^{3+}-HA, a detailed study of the static interaction between La^{3+}-HA and CT DNA has been undertaken. In this contribution, ESR and time-resolved absorption spectrum were employed along with UV-Vis absorption and fluorescence emission to probe the interaction between La^{3+}-HA and CT DNA, and the strong affinity of La^{3+}-HA to CT DNA was proved to contribute to its potent photodamage ability.

2. Materials and methods

2.1. Materials

HA was isolated from the fungus sacs of *Hypocrella bambusae* and recrystallized twice from acetone before use. $LaCl_3{\cdot}7H_2O$, ethidium bromide (EB), guanine, 2′-deoxyguanosine 5′-monophosphate (dGMP) and CT DNA were all purchased from Aldrich, USA. Diethylamine, anhydrous ethanol and other chemicals of analytical grade were obtained from Beijing Chemical Plant. Water was freshly distilled before use.

* Corresponding author. Tel.: +86-10-6488-6984; fax: +86-10-6487-9375.
E-mail address: g203@mail.ipc.ac.cn (B.-W. Zhang).
[1] Co-corresponding authors.

1010-6030/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2004.03.015

All experiments involving CT DNA were performed in buffer solution (10 mM ammonium acetate, 100 mM sodium chloride, pH 7), unless otherwise noted. DNA solutions were prepared by dispersing the desired amount of DNA in buffer solution with stirring overnight below 4 °C. In the experiments where titration with DNA was required, the DNA solution was sonicated at 0 °C for 10 min using a brason probe ultrasonicator. This operation significantly reduced the viscosity of the DNA solutions and permitted more accurate and precise titration. The concentration of CT DNA was expressed as the concentration of nucleotide and was calculated using an average molecular weight of 338 for a nucleotide and an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm.

2.2. Preparation of the complex

0.18 g of $LaCl_3$ (0.5 mmol) and 0.27 g of HA (0.5 mmol) were mixed and stirred in ethanol for 10 h at room temperature in the dark. The mixture was then filtered and the filtrate was evaporated to dryness under high vacuum. The residue obtained was redissolved in deionized water and dialyzed against dehydrated ethanol using a spectrapor membrane with a molecular weight cut-off of >3500, so the low molecular weight (<3500) components can diffuse across the spectrapor, and the higher molecular weight materials are left inside the dialysis bag. The remained solution was dried and the desired complex was obtained.

The La^{3+}-HA complex is expected to have a polymeric structure and the molar ratio of La^{3+} to HA in such a polymeric complex was measured to be 1:1 by molar ratio and continuous variation methods [13], from which the dissociation constant of the complex (1.21×10^{-7} M) was also obtained [12]. The La^{3+}-HA has three absorption peaks centered at 626.5, 580, and 492.5 nm respectively, having a remarkable red shift with respect to HA's corresponding absorption peaks at 581, 542, and 463 nm.

2.3. Methods

The absorption and the fluorescence spectra were measured with Shimadzu UV-1601PC UV-Vis and Hitachi F-4500 Fluorescence spectrophotometer, respectively.

The flash photolysis set-up employed the 532 nm second harmonic generation of Nd^{3+}:YAG laser as the excitation source (Quanta Ray DCR) with a pulse width (FWHM) of 8 ns (0.64 mJ/pulse, 10 Hz). The probe beam source was a 500 W cw Xe lamp. The transient signal was detected by a six-stage R456 (Hamamatsu) photo-multiplier tube, amplified by a 300 MHz dc amplifier and was finally fed into a 500 MHz digital oscilloscope (Tektronix) interfaced to a PC by GPIB board for data handling and processing.

Electron spin resonance (ESR) spectra were obtained using a Bruker ESR-300E spectrometer operating at room temperature, and the operating conditions were as follows: microwave bridge: X-band; sweep width: 100 G; modulation amplitude: 1.0 G; modulation frequency, 100 kHz; receiver gain: 1×10^5; microwave power: 5 mW. Samples were injected into the specially made quartz capillaries for ESR analyses, and purged with argon for 30 min in the dark, respectively, according to the experimental requirement and 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/puse energy). To make the production efficiencies of all spin adducts comparable, the concentrations of La^{3+}-HA and HA were adjusted to keep the same optical density at 532 nm.

3. Results and discussion

3.1. Steady-state UV-Vis absorption and fluorescence measurements

To make La^{3+}-HA and CT DNA or other small molecule substrates have sufficient interaction, all spectral measurements were performed after stirring the solutions of La^{3+}-HA and substrates for 12 h in the dark. Thus the solutions containing fixed concentration of La^{3+}-HA and varied concentrations of CT DNA were prepared. A low concentration of La^{3+}-HA (0.01 mM) was used in fluorescence measurements to avoid the inner filter effect, while a high concentration of La^{3+}-HA (0.1 mM) was kept in UV-Vis absorption measurements to facilitate the observation of spectrum changes. With increasing the concentration of CT DNA, the absorption bands of La^{3+}-HA peaking at 626.5, 580, and 492.5 nm respectively decreased in absorbance (Fig. 1), suggesting there is a static interaction between La^{3+}-HA and CT DNA. Though the absorbance decreased, the emission band at 650 nm increased in intensity (Fig. 2). Ethidium bromide (EB) fluorescence displacement experiments provide the further evidence for the static interaction between La^{3+}-HA and CT DNA. EB, a well-known duplex DNA intercalator, undergoes a significant enhancement

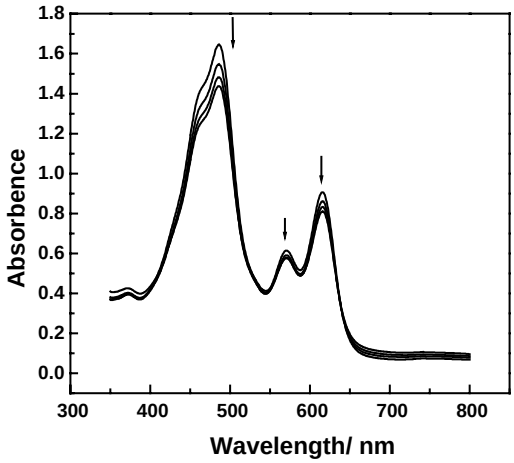

Fig. 1. The changes of the UV-Vis absorption spectra of La^{3+}-HA aqueous buffer solutions (0.1 mM) in the presence of increasing concentrations of CT-DNA (0, 0.04, 0.08, and 0.12 mM, respectively).

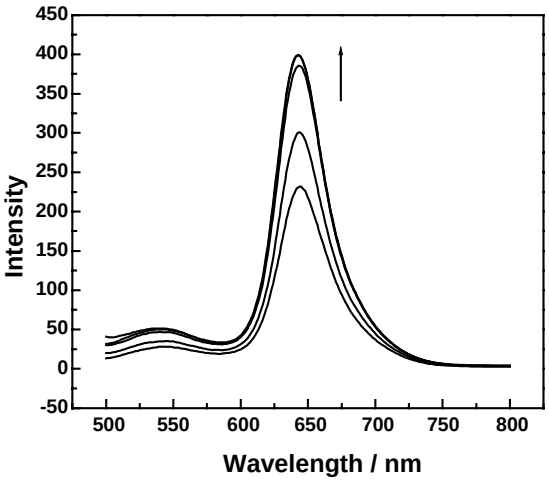

Fig. 2. The changes of the emission spectra of La^{3+}-HA (0.01 mM) in buffer solutions upon addition of CT-DNA (0, 0.04, 0.08, and 0.12 mM, respectively, $\lambda_{ex} = 480$ nm).

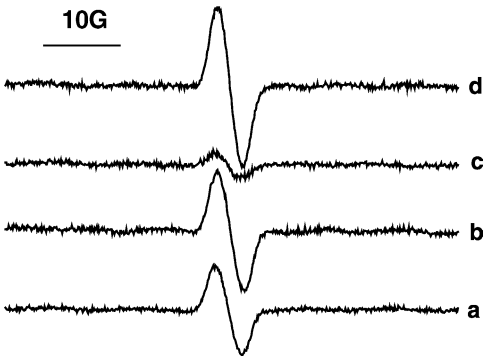

Fig. 4. Spectrum (a) photoinduced ESR spectrum from the deaerated solution of HA (0.1 mM) in buffer (pH 7.0), illuminated with light of 532 nm laser for 2 min. Spectrum (b) similar to the spectrum a but in the presence of CT DNA (1 mM). Spectrum (c) similar to the spectrum a but La^{3+}-HA instead of HA (0.1 mM). Spectrum (d) similar to the Spectrum c but in the presence of CT DNA (1 mM). The relative intensities are 1.0, 1.4, 0.3, 1.9, respectively, for spectra a, b, c and d.

in fluorescence intensity upon intercalating in CT DNA. When the buffer solution of CT DNA-EB was titrated with La^{3+}-HA, the emission intensity of EB decreased remarkably (Fig. 3), indicating some EB molecules were extruded from CT DNA (La^{3+}-HA does not quench the fluorescence of EB in the absence of CT DNA). We can not exclude the possibility that the presence of CT DNA which is negatively charged reduced the repulsion between both positively charged La^{3+}-HA and EB, and as a result La^{3+}-HA may quench directly the fluorescence of EB, supporting also the interaction of La^{3+}-HA with CT DNA. The static interaction revealed by UV-Vis absorption and fluorescence emission spectra can be attributed to the surface binding association of La^{3+}-HA to negatively charged double-strand CT DNA driven by electrostatic stabilization, considering the positively charged polymeric structure of La^{3+}-HA. Surface binding of La^{3+}-HA to CT DNA can prevent its fluorescence emission from being quenched by

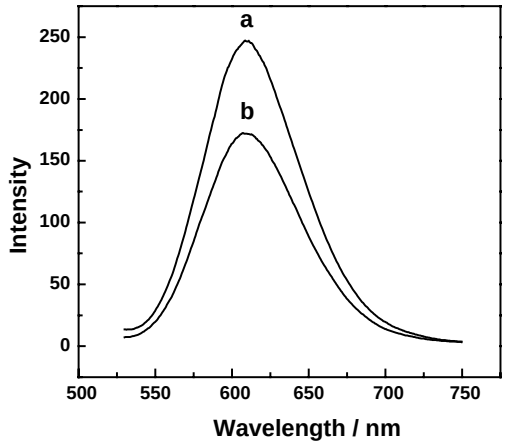

Fig. 3. Emission spectra of CT DNA-EB system (0.08 mM EB, 0.04 mM CT DNA) in the absence (a) and presence (b) of La^{3+}-HA (0.05 mM, $\lambda_{ex} = 510$ nm).

polar solvent molecules and result in the fluorescence increase. Also, the surface binding of La^{3+}-HA may change the space structure of the CT DNA helix, and consequently expel EB from CT DNA.

3.2. ESR experiments

ESR experiments corroborate that the electrostatic interaction plays an important role in the surface binding of La^{3+}-HA to CT DNA. Irradiation of nitrogen-saturated buffer solution of HA or La^{3+}-HA with 532 nm laser can produce a characteristic ESR signal of the semiquinone anion radical of HA or La^{3+}-HA (Fig. 4) [12], which originates from the disproportionation between an excited hypocrellin and a ground state hypocrellin. In the presence of the same amount of CT DNA, the semiquinone anion radical signals were intensified by 1.4-fold in HA solution and by 6.4-fold in La^{3+}-HA solution (Fig. 4), respectively. The increased signal intensity can be ascribed to the electron donating property of CT DNA, of which the bases, particularly guanine, may efficiently donate an electron to the triplet excited state of HA or La^{3+}-HA (see flash photolysis experiments) according to the Rehm–Weller equation [14] ($\Delta G < 0$ taking E_{ox} (guanine) = 1.4 V versus NHE [15,16], E_{red} (HA) = −0.2 V, E_{red} (La^{3+}-HA) = −0.43 V versus NHE, and $E_{0,0}$ ($^3La^{3+}$-HA*) ≈ $E_{0,0}$ (^{3}HA*) = 1.84 eV [17]). Based on the Rehm–Weller equation the electron transfer from CT DNA to excited singlet state La^{3+}-HA is also thermodynamic allowed, but the surface binding of La^{3+}-HA gave rise to fluorescence enhancement rather than quenching, implying that the electron transfer rate is not fast enough to compete with the intrinsic monomolecular decay rate of La^{3+}-HA singlet excited state. The more significant ESR signal intensity enhancement effect occurred in the case of La^{3+}-HA may arise from either the association of La^{3+}-HA with CT DNA favored by electrostatic interaction between them or

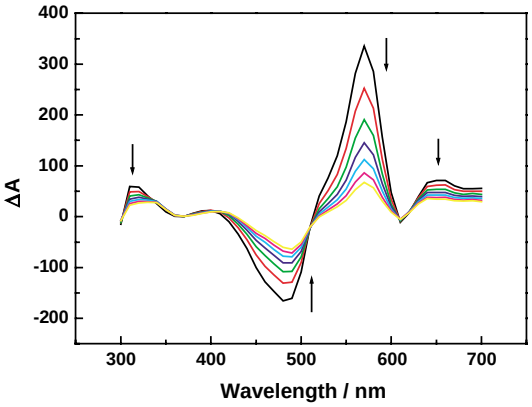

Fig. 5. Time-resolved spectra of the transients formed by flash photolysis of La^{3+}-HA (0.05 mM) in deaerated aqueous buffer solution at 200 ns, 2.6, 3.2, 3.7, 4.3, 4.9, 5.4 μs after the laser flash (532 nm). Arrows indicate direction of changes.

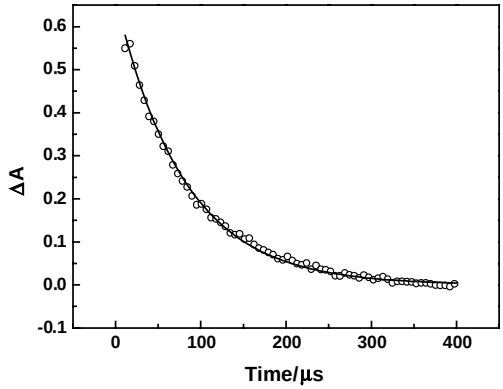

Fig. 6. Time-resolved transient absorption traces of La^{3+}-HA (0.05 mM) in deaerated aqueous buffer solution probed at 570 nm. Open circles: experimental data; solid line: mono-exponential fit with the time constant of 80 μs.

the longer triplet state lifetime of La^{3+}-HA (80 μs in aqueous buffer solution) with respect to that of HA (0.1 μs in aqueous buffer solution). To clarify which factor is the predominant one, a neutral electron donor, diethylamine, was used to replace CT DNA in ESR experiments. Upon addition of the same amounts of diethylamine, the semiquinone anion radical signal intensities of HA and La^{3+}-HA were increased by 3.8-fold and 2.7-fold, respectively. This indicates that the electrostatic interaction indeed takes an important effect in the surface binding of La^{3+}-HA to CT DNA, which in turn promotes the formation of semiquinone anion radicals via the electron transfer between triplet La^{3+}-HA and CT DNA.

3.3. Laser flash photolysis experiments

Time-resolved absorption spectra give further support to the conclusion mentioned above. Due to the lowest oxidation potential among the four bases and their corresponding deoxyribonucleotides, guanine and dGMP were selected to investigate their electron transfer quenching to triplet excited state of La^{3+}-HA. Fig. 5 shows the difference absorption spectra of La^{3+}-HA in deaerated aqueous buffer solution at room temperature after pulsed excitation at 532 nm. There are three positive absorption bands with maxima at 310, 570 and 660 nm, respectively, and a ground state bleaching band with maximum at 490 nm. The three positive bands can be attributed to the triplet–triplet (T–T) absorption of La^{3+}-HA based on the efficient quenching of them by O_2, which is similar to the reported T–T absorption of HA [17,18]. The triplet state lifetime of La^{3+}-HA is determined to be 80 μs by monitoring the transient absorption at 570 nm which decays mono-exponentially (Fig. 6). In the presence of guanine or dGMP (20 mM) the absorbance decay can be well fitted by using bi-exponential function, $\tau_1 = 0.2\,\mu s$ ($A_1 = 99.8\%$), $\tau_2 = 17\,\mu s$ ($A_2 = 0.2\%$) in the case of guanine, $\tau_1 = 11.3\,ns$ ($A_1 = 98.7\%$), $\tau_2 = 233\,ns$ ($A_2 = 1.3\%$) in the case of dGMP were obtained, respectively. Based on the main decay component, namely the short time constant, the apparent bimolecular quenching constants of triplet La^{3+}-HA by guanine or dGMP are calculated to be 4.98×10^8 and $8.85 \times 10^9\,M^{-1}\,s^{-1}$, respectively. The one order of magnitude larger biomolecular quenching constant of dGMP than that of guanine is consistent with the strong association of negatively charged dGMP and positively charged La^{3+}-HA by electrostatic interaction. This further demonstrates that La^{3+}-HA can efficiently associate with negatively charged double helix of CT DNA.

3.4. The influence of surface binding on the photodamage of CT DNA by La^{3+}-HA

Our previous study has shown that La^{3+}-HA exhibits stronger photodamage capability to CT DNA than its counter part HA in both aerobic and anaerobic conditions [12]. Because La^{3+}-HA can generate efficiently reactive oxygen species such as $^1O_2$, superoxide anion radical ($O_2^{\bullet -}$) and hydroxyl radical ($OH^{\bullet}$) as well as semiquinone anion radical [12], both type I and type II mechanisms may take effects in the photodamage of CT DNA by La^{3+}-HA. In type I mechanism, the surface binding of La^{3+}-HA to CT DNA will favor the photoinduced electron transfer between them, generating more sensitizer anion radicals which under physiological conditions, can react with O_2 to form $O_2^{\bullet -}$ and other reactive oxygen species (e.g. H_2O_2 and $OH^{\bullet}$), and as a result improve photodamage efficiency, particularly in anaerobic environments where the photodamage mainly originates from the oxidized CT DNA itself. In type II mechanism, the photodamage of CT DNA is achieved by the attack of $^1O_2$, generated via energy transfer from triplet La^{3+}-HA to ground state O_2. Owing to the very short lifetime of $^1O_2$ in aqueous solution, the close proximity between $^1O_2$ and CT DNA as the result of the surface binding of La^{3+}-HA to CT DNA will guarantee more $^1O_2$ to react with CT DNA before its deactivation to the ground state O_2.

4. Conclusion

In summary, the spectral experiments including UV-Vis absorption, fluorescence emission, ESR, and time-resolved absorption reveal that there is a surface binding interaction between La^{3+}-HA and CT DNA, and the association between them is driven mainly by electrostatic interaction. Such a static interaction, in cooperation with the longer triplet excited state lifetime and higher $^1O_2$ generation efficiency of La^{3+}-HA, bring about a stronger photodamage to CT DNA. This work indicates that it is important to study the static interaction of a photosensitizer and its substrate for gaining a deep insight into the photodynamic mechanism. These findings encourage us to make chemical modifications on hypocrellin A or hypocrellin B by taking more concerns on improving their affinity to the biomaterials.

References

[1] J.B. Hudson, J. Zhou, L. Harris, L. Yip, G.H.N. Towers, Hypocrellin from hypocrella bamboosae, is phototoxic to human immuneodeficiency virus, Photochem. Photobiol. 60 (1994) 253–255.
[2] J. Hirayama, K. Ikebuchi, H. Abe, K.W. Kwon, Y. Ohnishi, M. Horiuchi, M. Shinagawa, K. Ikuta, N. Kamo, S. Sekiquchi, Photoinactivation of virus infectivity by hypocrellin A, Photochem. Photobiol. 66 (1997) 697–700.
[3] Y.Z. Hu, J.Y. An, L.J. Jiang, Studies of the sulfonation of hypocrellin A and the photodynamic actions of the product, J. Photochem. Photobiol. B: Biol. 17 (1993) 195–201.
[4] Y.Y. He, J.Y. An, L.J. Jiang, Synthesis and ESR investigation of hypocrellin glycoside, Tetrahedron Lett. 39 (1998) 5069–5072.
[5] Z.Z. Ou, J.R. Chen, X.S. Wang, B.W. Zhang, Y. Cao, Synthesis and photodynamic therapy properties of a water-soluble hypocrellin modified by cyclodextrin, Chem. Lett. (2001) 838–839.
[6] Z.Z. Ou, J.R. Chen, X.S. Wang, B.W. Zhang, Y. Cao, Synthesis of a water-soluble cyclodextrin modified hypocrellin and ESR study of its photodynamic therapy properties, N. J. Chem. 26 (2002) 1130–1136.
[7] J.H. Ma, J.Q. Zhao, L.J. Jiang, The aluminium complex of hypocrellin B as a PDT photosensitizer, N. J. Chem. 25 (2001) 847–854.
[8] Z.Z. Ou, J.R. Chen, X.S. Wang, B.W. Zhang, Y. Cao, Metal ions effect on the photodynamic actions of cyclodextrin modified hypocrellin, Chem. Lett. (2002) 206–207.
[9] Y.Z. Hu, J.Y. An, L.J. Jiang, Studies on the chelation of hypocrellin A with aluminium ion and the photodynamic action of the resulting complexes, J. Photochem. Photobiol. B: Biol. 22 (1994) 219–227.
[10] A. Harriman, CRC handbook of Organic Photochemistry and Photobiology, CRC Press, 1992, pp. 1374.
[11] J.H. Zhou, S.Q. Xia, X.S. Wang, B.W. Zhang, Y. Cao, Interaction studies of hypocrellin B or mono-cysteine substituted hypocrellin B with CT-DNA by spectral methods, Chin. Chem. Lett. 14 (2003) 1054–1057.
[12] J.H. Zhou, S.Q. Xia, X.S. Wang, B.W. Zhang, The photodynamic property improvement of hypocrellin A by chelation with lanthanum ions, Chem. Commun. (2003) 1372–1373.
[13] A.E. Harvey Jr., D.L. Manning, Spectrophotometric methods of establishing empirical formulas of colored complexes in solution, J. Am. Chem. Soc. 72 (1950) 4488–4493.
[14] D. Rehm, A. Weller, Kinetics of flurescence quenching by electron and H-atom transfer, Isr. J. Chem. 8 (1970) 259–275.
[15] X.G. Qu, C.Z. Wan, H.-C. Becker, D.P. Zhong, A.H. Zewail, The anticancer drug-DNA complex: femtosecond primary dynamics for anthracycline antibiotics function, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 14212–14217.
[16] A.M.S. Claus, S. Andreas, H.M.S. Markus, Nucleobase-specific quenching of fluorescent dyes. 1. Nucleobase one-electron redox potentials and their correlation with static and dynamic quenching efficiencies, J. Phys. Chem. 100 (1996) 5541–5553.
[17] Y.Z. Hu, J.Y. An, Q. Li, L.J. Jiang, Studies of the triplet state properties of hypocrellin A by nanosecond flash photolysis, J. Photochem. Photobiol. A: Chem. 78 (1994) 247–251.
[18] M. Weng, M.H. Zhang, T. Shen, Study on the singlet and triplet state electron transfer between hypocrellin A and aromatic amines in solvents of various polarity, Chin. Chem. Lett. 7 (1996) 924–927.