The photodynamic property improvement of hypocrellin A by chelation with lanthanum ions

Jia-Hong Zhou, Sheng-Qin Xia, Jing-Rong Chen, Xue-Song Wang* and Bao-Wen Zhang*

Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Beijing, 100101, People's Republic of China

A 1:1 complex of lanthanum ion with hypocrellin A (La^{3+}–HA) possessing high singlet oxygen generation efficiency, large absorbance in the phototherapeutic window, and great water solubility exhibits promising photodynamic properties.

Hypocrellins, including hypocrellin A (HA) (Fig. 1) and hypocrellin B (HB), isolated from the fungus *Hypocrella bambuase*, have been receiving intensive interest over the past two decades in the field of photodynamic therapy (PDT) due to their wide absorption band in the visible region and extremely high singlet oxygen ($^1O_2$) generation ability [1]. From the viewpoint of clinical applications, the water solubility and absorption intensity in the phototherapeutic window (600 ~ 900 nm) of the natural hypocrellins need to be improved for attaining ideal photodynamic efficacy, which gives great impetus to the synthesis of water-soluble hypocrellin derivatives, such as sulfonated HA,[2] glycosylated HB,[3] and cyclodextrin modified HB.[4] An alternative approach to improving the water solubility of hypocrellins is to take advantage of their chelation to metal ions.[5] Compared with the synthesis of water-soluble derivatives, the preparation of metal complexes of hypocrellins is much easier to operate. Moreover, a remarkable red shift of the absorption band and the increase in absorbance above 600 nm are always accompanied by the enhancement of water solubility for the metal complexes of hypocrellins. However, the chelation of hypocrellins to metal ions generally results in a significant reduction of the $^1O_2$ generation quantum yield,[5] which unfortunately offsets the improvement in water solubility and light absorption. So, we focused our attention on the maintenance of $^1O_2$ generation efficiency of hypocrellins upon complexing with metal ions, and report here an HA lanthanum ion (La^{3+}) complex with higher $^1O_2$ generation quantum yield, greater water solubility and longer wavelength absorption than HA. To our best knowledge, this is the first report that a chelate complex of La^{3+}, a lanthanide, presents promising application potential in PDT.

HA has three absorption peaks at 581, 542, and 463 nm, respectively in the visible region from 400 to 700 nm in ethanol. Upon addition of La^{3+} into the solution, remarkable red shifts as large as 40 nm were observed and the three absorption peaks appeared at 626.5, 580, and 492.5 nm, respectively. Such a large red shift reflects a strong coordination interaction between La^{3+} and HA. During the addition of La^{3+}, a set of isobestic points was also observed, indicating the presence of only one form of complex. The ratio of La^{3+} to HA in La^{3+}–HA complex was determined to be 1:1 by molar ratio and continuous variation methods.[6] Additionally, the dissociation constant for the complex was calculated to be 1.21×10^{-7} M. In 1:1 complex of Al^{3+} and HA, a polymeric cationic structure was proposed in which Al^{3+} ions link HA together to form a linear polymer by chelation to the phenolic hydroxyl and quinonoid carbonyl oxygens of HA.[5a] A similar structure maybe accounts for the 1:1 complex between La^{3+} and HA as well. Taking into consideration the high coordination number of La^{3+}, (generally higher than 6[7]), the involvement of solvent molecules in the La^{3+}–HA complex is also possible. So, the structure of La^{3+}–HA complex is still not very clear and further study is underway.

Many efforts have also been made to enhance the long wavelength absorption of other types of phototherapeutic sensitizers. A key example is the work of D. G. Hilmey *et al.*,[8] where porphyrins exhibit good absorption in the range 630–710 nm. Although La^{3+}–HA's absorption band is not yet red enough compared with these porphyrins, the ease of the preparation and the extremely high $^1O_2$ quantum yield (0.9, see below) makes La^{3+}–HA prospective for PDT application.

After coordination reaches equilibration (no changes in absorption spectrum) and removal of organic solvent under vacuum, the resultant complex can be readily re-dissolved in water and the absorbance at 488 nm can reach as high as 2.38, corresponding to a concentration of 85 μM (assuming similar extinction coefficients in aqueous and ethanol solutions for the complex). This proves that the chelation of La^{3+} to HA improves the light absorption capability in the phototherapeutic window as well as the water solubility greatly (HA is insoluble in water).

ESR spin-trapping technique was applied to study the active oxygen generation capability of La^{3+}–HA complex in a qualitative way. Upon irradiation of a nitrogen-saturated DMSO solution of La^{3+}–HA (50 μM) at 532 nm, an ESR signal (Fig. 1, spectrum a) with the same position and line-shape as that of radical anion of HA was observed,[9] but the signal intensity was much lower than that obtained with HA. The formation of radical anions of hypocrellins is believed to be the result of self-electron transfer between excited and ground state hypocrellins. The weaker signal indicates the self-electron transfer is restrained in La^{3+}–HA.

When irradiation was carried out in an oxygen-saturated DMSO solution of La^{3+}–HA in which TEMP (2,2,6,6-tetra-ethyl-4-piperridone) was also present as spin-trapping agent, a characteristic ESR signal of TEMPO (adduct of TEMP with
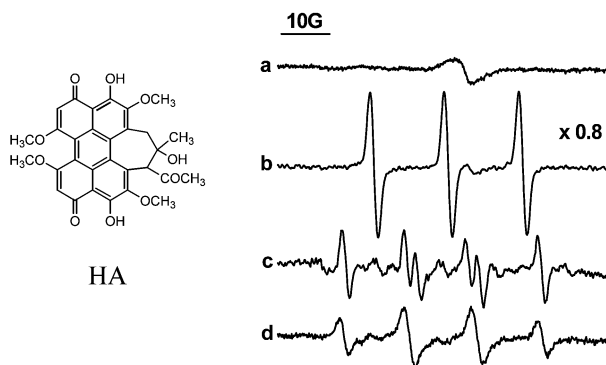

Fig. 1 Spectrum a: photoinduced ESR signal in nitrogen-saturated DMSO solution of La^{3+}–HA (50 μM), illumination was with 532 nm pulsed laser for 2 min. Spectrum b: similar to spectrum a, but in oxygen saturated solution and in the presence of TEMP (20 mM), and irradiated for 40 s. Spectrum c: similar to spectrum a, but in air-saturated solution and in the presence of DMPO (450 mM), and irradiated for 1 min. Spectrum d: similar to spectrum c, but in the presence of a small amount of water. Spectral parameter settings: microwave bridge: X-band; sweep width: 100 G; modulation amplitude: 1.0 G; receiver gain: 1×10^5; microwave power: 5 mW. The chemical structure of HA is also included.

$^1O_2$) was detected (three lines with identical intensity and hyperfine coupling constant of 16.0 G, Fig. 1, spectrum b), and the presence of O_2, La^{3+}–HA, and light are all essential for its observation. The addition of 1,4-diazabicyclo[2,2,2]octane, a specific scavenger of $^1O_2$, suppressed the signal efficiently. Moreover, TEMPO signal produced by La^{3+}–HA was even stronger than that produced by HA, implying the $^1O_2$ generation ability of La^{3+}–HA might be higher than that of HA. This is further confirmed by $^1O_2$ generation quantum yield measurements *via* 9,10-diphenylanthracene (DPA) bleaching method (taking hematoporphyrin as a reference).[10] In DMSO, the $^1O_2$ generation quantum yield of HA is 0.62, while that for La^{3+}–HA is 0.9. High singlet oxygen quantum yields up to 0.58 and 0.69 were also observed in Lu(III) and Gd(III) containing metal-lotexaphyrins, respectively.[11] Generally, for metal–HA complexes, such as Al^{3+}–HA,[5] the $^1O_2$ generation quantum yields are usually reduced greatly with respect to HA. The improved $^1O_2$ generation ability, as well as the large absorbance above 600 nm and great water solubility, suggests La^{3+}–HA is a promising candidate as a PDT sensitizer.

The generation of $^1O_2$ comes from the energy transfer of the excited triplet state of sensitizers to ground state oxygen, and its efficiency depends on the photophysical properties of sensitizers, including the intersystem crossing quantum yield, the triplet-state lifetime, and the triplet-state energy level. Our preliminary study on the transient absorption by way of nanosecond flash photolysis shows the triplet-state lifetime of La^{3+}–HA (179 µs) is far longer than that of HA (13 µs), which is probably responsible for the increased $^1O_2$ generation efficiency for La^{3+}–HA. It has been reported that hypocrellins in their triplet multiplicity can interact with bio-macromolecules directly under anaerobic conditions[12] and give rise to photodamage of the bio-macromolecules. In this regard, such a long-lived triplet state for La^{3+}–HA is also beneficial to the phototherapeutic process carried out in oxygen-deficient cases.

If 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) was used as spin-trapping agent, a typical ESR signal attributed to the adduct of superoxide anion radical with DMPO (DMPO-$O_2^{-\cdot}$) appeared upon irradiating air-saturated DMSO solution of La^{3+}–HA. This signal can be characterized by three hyperfine coupling constants: α^N = 13.0 G, α_β^H = 10.1 G, and α_γ^H = 1.5 G (Fig. 1, spectrum c). An efficient quenching by superoxide dismutase, a scavenger of $O_2^{-\cdot}$, supports the assignment of this signal. ESR signal intensity produced with La^{3+}–HA is about the half of that with HA. This is in good agreement with the observation that the ESR signal of La^{3+}–HA radical anion is much weaker than HA$^{-\cdot}$ (*vide ante*), because $O_2^{-\cdot}$ originates mainly, in the above systems, from the electron transfer between radical anions of hypocrellins and ground state oxygen. The addition of an electron donor such as *N*-methylaniline intensified DMPO-$O_2^{-\cdot}$ signals generated by La^{3+}–HA or HA, obviously due to a favourable electron transfer process between excited La^{3+}–HA or HA and the electron donor, resulting in the production of the sensitizer radical anions. If water is present in air-saturated DMSO solution of La^{3+}–HA and DMPO, irradiation gave a four-line signal with intensity ratio of 1:2:2:1 and hyperfine coupling constant of 14.9 G (Fig. 1, spectrum d), which can be assigned to the adduct of a hydroxyl radical with DMPO (DMPO-OH$\cdot$). In aqueous solution $O_2^{-\cdot}$ undergoes rapid dismutation to form H_2O_2 and though which transforms to OH$\cdot$ further.[4b]

The active oxygen generation ability, particularly the stronger $^1O_2$ generation quantum yield indicates La^{3+}–HA is photodynamic active in terms of type I and type II mechanisms. To study the PDT properties of La^{3+}–HA, calf thymus DNA (CT DNA) in air-saturated buffer solution was used as phototherapeutic target and the ethidium bromide (EB) assay was adopted to follow the photodamage process of CT DNA.[13]

Table 1 Photocleavage of CT DNA by La^{3+}–HA or HA detected by remaining binding site (BSR%) of ethidium bromide to the damaged CT DNA under different conditions. [CT DNA] = 40 µM, [EB] = 80 µM, [La^{3+}–HA] = 5.6 µM, [HA] = 5.6 µM

| | Irradiation time / min | | | | |
Sample	10	20	30	40	50
Control experiment[a]	99.92	99.78	99.65	99.45	99.28
La^{3+}–HA + N_2	96.81	93.01	90.62	87.10	82.42
HA + N_2	99.62	98.29	95.55	92.57	90.07
La^{3+}–HA + O_2	93.59	86.17	78.65	72.00	66.30
HA + O_2	95.63	92.02	87.72	84.66	79.09

[a] In the absence of La^{3+}–HA or HA, and oxygen.

33.70% binding sites were destroyed during a 50 min irradiation with light above 463 nm in EB-CT DNA buffer solution containing 5.6 µM of La^{3+}–HA, while only 20.91% binding sites were damaged with the same concentration of HA as sensitizer under aerobic conditions (Table 1).

Many cancerous cells grow in anaerobic conditions, therefore the photosensitized cleavage and damage to CT DNA by La^{3+}–HA and HA were also investigated in the absence of oxygen. In such cases, the photodamage capability of La^{3+}–HA (17.58% binding sites destroyed) is almost twice that of HA (9.03% binding sites destroyed) (Table 1). This kind of PDT effect initiated *via* the electron transfer process from CT DNA to the triplet La^{3+}–HA or HA, and the longer lifetime of triplet La^{3+}–HA might be responsible for its stronger PDT behaviour under anaerobic condition.[14]

In summary, La^{3+}–HA exhibits large absorbance in the phototherapeutic window, great water solubility and extremely high $^1O_2$ generation quantum yield, which indicate La^{3+}–HA would be a promising photosensitizer candidate for PDT application.

Notes and references

1 G. G. Miller, K. Brown, M. Ballangrud, O. Barajas, Z. Xiao, J. Tulip, J. W. Lown, J. M. Leithoff, M. J. Allalunis, R. D. Methta and R. B. Moore, *Photochem. Photobiol.*, 1997, **65**, 714.

2 Y. Z. Hu, J. Y. An and L. J. Jiang, *J. Photochem. Photobiol., B: Biol.*, 1993, **17**, 195.

3 Y. Y. He, J. Y. An and L. J. Jiang, *Tetrahedron Lett.*, 1998, **39**, 5069.

4 (*a*) Z. Z. Ou, J. R. Chen, X. S. Wang, B. W. Zhang and Y. Cao, *Chem. Lett.*, 2001, 838; (*b*) Z. Z. Ou, J. R. Chen, X. S. Wang, B. W. Zhang and Y. Cao, *New J. Chem.*, 2002, **26**, 1130.

5 (*a*) Y. Z. Hu, J. Y. An and L. J. Jiang, *J. Photochem. Photobiol., B: Biol.*, 1994, **22**, 219; (*b*) J. H. Ma, J. Q. Zhao and L. J. Jiang, *New J. Chem.*, 2001, **25**, 847; (*c*) Z. Z. Ou, J. R. Chen, X. S. Wang, B. W. Zhang and Y. Cao, *Chem. Lett.*, 2002, 206.

6 A. E. Harvey Jr. and D. L. Manning, *J. Am. Chem. Soc.*, 1950, **72**, 4488.

7 K. A. Gschneidner Jr, *Handbook on the physics and chemistry of Rare Earths*, North Holland Publishing Company, 1978.

8 D. G. Hilmey, M. Abe, M. I. Nelen, C. E. Stilts, G. A. Baker, S. N. Baker, F. V. Bright, S. R. Davies, S. O. Gollnick, A. R. Oseroff, S. L. Gibson, R. Hilf and M. R. Detty, *J. Med. Chem.*, 2002, **45**, 449.

9 L. Y. Zhang, B. R. Misra and H. P. Misra, *Photochem. Photobiol.*, 1992, **56**, 453.

10 Z. J. Diwu and J. W. Lown, *J.Photochem. Photobiol. A: Chem.*, 1992, **64**, 273.

11 L. I. Grossweiner, M. D. Bilgin, P. Berdusis and T. D. Mody, *Photochem. Photobiol.*, 1999, **70**(2), 138.

12 Y. Y. He and L. J. Jiang, *Biochem. Biophys. Acta*, 2000, **1523**, 29.

13 (*a*) W. A. Pruetz, *Radiat. Environ. Biophys.*, 1984, **23**, 1; (*b*) W. A. Pruetz, *Radiat. Environ. Biophys.*, 1984, **23**, 7; (*c*) H. C. Birnboim and J. J. Jevcak, *Cancer Res.*, 1981, **41**, 889.

14 X. G. Qu, C. Z. Wan, H.-C. Becker, D. P. Zhong and A. H. Zewail, *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 14212.