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Synthesis and characterization of the monomer ruthenium complex of hypocrellin B

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Abstract—We first synthesized and characterized a monomer ruthenium complex of hypocrellin B (HB) by chelation with Ru(bpy)$_2$Cl$_2$ (in which bpy = 2,2′-bipyridine). It possesses the photosensitizing properties and can be applied in photodynamic therapy (PDT). The chemiluminescence assays indicated that the photodamage ability of the complex is stronger than that of HB.

Hypocrellins, including hypocrellin A (HA) and hypocrellin B (HB), as the new efficient phototherapeutic agents, have been receiving intensive interest over the past two decades due to their light-induced antitumor and antiviral activity, most notably against human immunodeficiency virus (HIV).[1,2] Compared to the presently used photodynamic therapeutic agent, *photofrin II*,[3] hypocrellins possess some advantages, such as easy preparation and purification, low aggregation tendency, and rapid metabolism in vivo.[4,5] However, in clinical applications, the water solubility and absorption intensity in the phototherapeutic window (600–900 nm) of the natural hypocrellins need to be improved in order to attain ideal photodynamic efficacy.

Many water-soluble hypocrellin derivatives, such as sulfonated HA,[6] glycosylated HB,[7] cyclodextrin modified HB,[8] and tyrosine-modified HB[9] have been synthesized in recent years. However, chemical modification generally suffered from difficult purification procedures and unsatisfactory property improvements. An alternative approach for improving the water solubility of hypocrellins is to take the advantage of their chelation to metal ions.[10] Comparing with the synthesis of water-soluble hypocrellin derivatives, the preparation of metal complexes of hypocrellins is much easier to operate. Moreover, a remarkable red shift of the absorption band and the resultant increase in absorptivity above 600 nm was always accompanied with the enhancement of water solubility for the metal complexes of hypocrellins.[11,12] However, these complexes possess polymeric structures so that they are difficult to be absorbed by tumor cells.

The ruthenium complexes with aromatic ligands have been applied extensively as the stereoselective probes of the nucleic acid structure and as the potential antitumor agents.[13] In comparison with other metal complexes, these complexes can bind to DNA by intercalation with high binding affinities and significant changes in their photophysical properties can be observed after binding to DNA. In addition, upon irradiation, these complexes can promote DNA cleavage, and they also exhibit obvious enantioselectivity in DNA cleavage.[14]

In the present communication, a mixed-ligand ruthenium complex of hypocrellin B and bipyridine was successfully first synthesized in order to combine the characteristics of both ruthenium complexes and hypocrellin B. Comparing with the previous complexes of hyprocrellins, the complex is the monomer with greatly improved water solubility and stronger absorptivity in longer wavelengths. In addition, the results of

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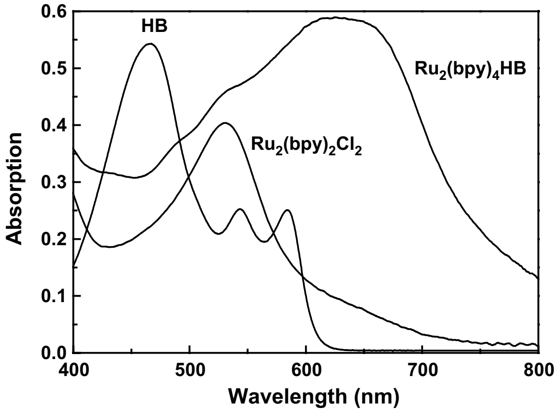


Figure 1. Absorption spectra of HB (50 μM), $Ru(bpy)_2Cl_2$ (50 μM), and $Ru_2(bpy)_4HB$ (50 μM) measured in ethanol solutions.

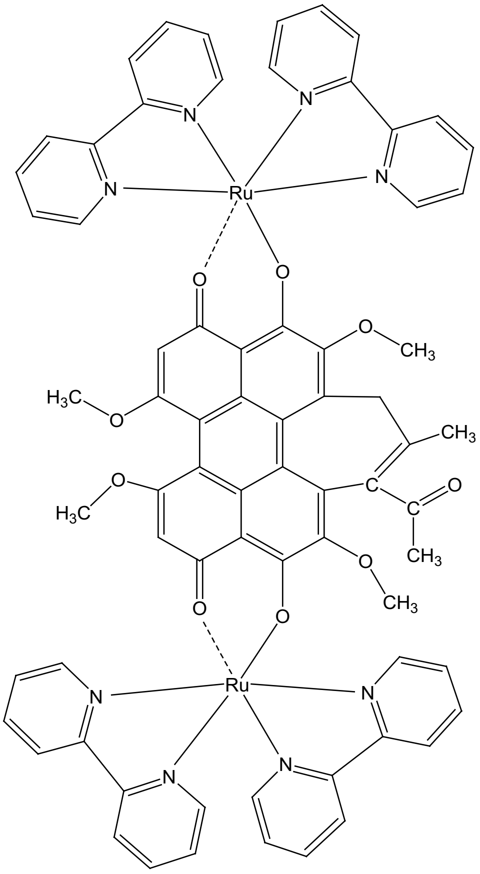
chemiluminescence assays indicated that the photodamage ability of the complex is stronger than that of HB.

A mixture of $Ru(bpy)_2Cl_2$(1 mM) and HB (0.5 mM) dissolved in 100 ml of ethanol was refluxed at 70 °C for 5 h under the protection of nitrogen. The pH of the solution was adjusted to 11 with NaOH. After completion of chelation, the solution was evaporated to afford a bottle green solid under high vacuum. Then, the product was separated by column chromatography on silica gel using the solution of $CHCl_3$–CH_3OH (1:1, v/v) as eluent, and $Ru_2(bpy)_4HB$ was obtained with 85% yield.

The UV–vis absorption spectra of HB has three absorption peaks at 584, 543, and 467 nm, respectively, and $Ru(bpy)_2Cl_2$ has one absorption peak at 531 nm. In the UV–vis absorption spectrum of $Ru_2(bpy)_4HB$, a new absorption peak appears at 647 nm (Fig. 1). Thus, it could illustrate that the complex has been formed.

The IR measurements indicated that characteristic IR band of the quinonoid carbonyl group in HB (1702 cm^{-1}) shifted bathochromically to 1503 cm^{-1} due to the coordination of the carbonyl oxygen with Ru.[15] In addition, in the region between 120 and 600 cm^{-1}, $Ru_2(bpy)_4HB$ has four characteristic IR bands at 312, 348, 373, and 424 cm^{-1}, respectively. According to the literatures, the bands at 312 and 348 cm^{-1} can be ascribed to the bond of Ru–O and the bands at 373 and 424 cm^{-1} are due to the bond of Ru–N.[16,17] The IR bands of the bond Ru–Cl at 240 and 258 cm^{-1} in the IR spectrum of $Ru(bpy)_2Cl_2$cannot be observed in the IR spectrum of $Ru_2(bpy)_4HB$ because of the formation of the bond of Ru–O.[18] Therefore, the IR results also demonstrated that the $Ru_2(bpy)_4HB$ complex is formed and the complex is a monomer (Scheme 1).

Another evidence of the chelation between HB and $Ru(bpy)_2^{2+}$ is the difference of solubility. HB can dissolve easily in apolar solvent (such as CCl_4), but with difficultly in polar solvent (such as H_2O). After chelating with $Ru(bpy)_2^{2+}$, the product dissolves easily in the polar solvent, but with difficulty in the apolar solvent.



Scheme 1. Structure of $Ru_2(bpy)_4HB$.

The above results clearly demonstrated the formation of $Ru_2(bpy)_4HB$. The above results also illustrated that $Ru_2(bpy)_4HB$ can easily dissolve in the aqueous solution and possess the good light absorption capability in the phototherapeutic window of 600–900 nm. Therefore, $Ru_2(bpy)_4HB$ is more suitable to be studied in PDT than HB. In addition, $Ru_2(bpy)_4HB$ may be absorbed by tumor tissues more easily than other metal–hypocrellin complexes because of its monomer structure.

In Figure 2, curve 'a' is the photoinduced ESR signal of 50 μM $Ru_2(bpy)_4HB$ in the nitrogen-saturated dimethyl sulfoxide (DMSO) solution obtained after the solution was illuminated with 532 nm pulsed laser for 2 min. An ESR signal with a similar position and line shape as that of the radical anion of HA was observed.[8] The formation of radical anions of hypocrellin is believed to be the result of the self-electron transfer between the excited and ground states of hypocrellin in $Ru_2(bpy)_4HB$.

When irradiation was carried out in an oxygen-saturated DMSO solution of $Ru_2(bpy)_4HB$ in the presence of 20 mM 2,2,6,6-tetraethyl-4-piperridone (TEMP) as a spin-trapping agent, three lines with identical intensity and hyperfine coupling constant of 16G were observed (Fig. 2, curve b). It is a characteristic ESR signal of TEMPO (adduct of TEMP with $^1O_2$).[19] The presence of O_2, $Ru_2(bpy)_4HB$, and light is essential for the characteristic ESR signal of TEMPO. If 1,4-diazabicy-

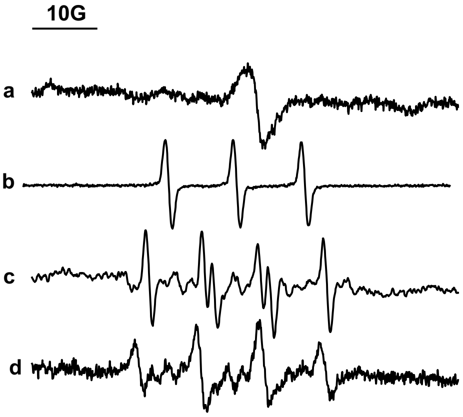


Figure 2. Spectrum a: photoinduced ESR signal in nitrogen-saturated DMSO solution of $Ru_2(bpy)_4HB$ (50 μM), illumination was with 532 nm pulsed laser for 2 min. Spectrum b: similar to spectrum a but in oxygen saturated solution and in the presence of TEMP (20 mM), irradiated for 40 s. Spectrum c: similar to spectrum a, but in air-saturated solution and in the presence of DMPO (450 mM), 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: 100G; modulation amplitude: 1.0 G; receiver gain: 1×10^5; microwave power: 5 mW.

clo[2,2,2]octane, a specific scavenger of $^1O_2$, was added, the signal was suppressed efficiently.

If 5,5-dimethyl-1-pyrrloine-N-oxide (DMPO) was used as a 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 $Ru_2(bpy)_4HB$. This signal can be characterized with three hyperfine coupling constants: $\alpha^N = 13$ G, $\alpha_\beta{}^H = 10$ G, and $\alpha_\gamma{}^H = 1.5$ G (Fig. 2, spectrum c).[20] An efficient quenching by superoxide dismutase, a scavenger of $O_2^{-\cdot}$, supports the assignment of this signal. If water is present in air-saturated DMSO solution of $Ru_2(bpy)_4HB$ and DMPO, the irradiation will give a four-line signal with intensity ratio of 1:2:2:1 and hyperfine coupling constant of 15 G (Fig. 2, spectrum d),[21] which can be assigned to the adduct of hydroxyl radical with DMPO (DMPO–$OH^\cdot$). In aqueous solution, $O_2^{-\cdot}$ undergoes rapid dismutation to form H_2O_2 and then transfers to $OH^\cdot$.

The results of ESR spectroscopy demonstrated that $Ru_2(bpy)_4HB$ possess the photosensitizing properties and can be applied in PDT.

DNA has weak chemiluminescence; if damaged, the chemiluminescence is intensified greatly.[22] In order to examine the photodynamic capacity of $Ru_2(bpy)_4HB$, the chemiluminescence assay was utilized to characterize the calf thymus DNA (CT DNA) damage photosensitized by $Ru_2(bpy)_4HB$.

Figure 3 shows the relationship between the relative intensity of chemiluminescence and time of the CT DNA solution without and with HB or $Ru_2(bpy)_4HB$. It can be clearly observed that the increase in the relative intensity of chemiluminescence with time for the CT DNA solution with $Ru_2(bpy)_4HB$ is quicker than that for the the CT-DNA solution with HB. It indicated that the photodamage of CT DNA induced by $Ru_2(bpy)_4HB$ is about two times stronger than that induced by HB under the same experimental conditions.

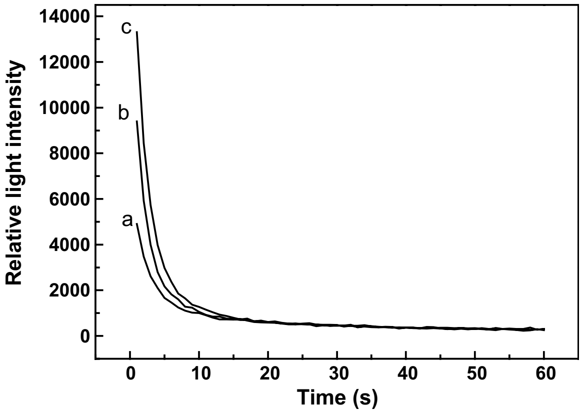


Figure 3. The chemiluminescent assay for (a) 50 μg/ml CT DNA solution, (b) 50 μg/ml CT DNA solution with 50 μM HB and (c) 50 μg/ml CT DNA solution with 50 μM $Ru_2(bpy)_4HB$.

In summary, the monomer ruthenium complex of hypocrellin B and bipyridine was first synthesized. It exhibits large absorbance in phototherapeutic window, great water solubility, and higher photodamage ability than HB, indicating $Ru_2(bpy)_4HB$ would be one of the best candidates in the field of PDT.

Acknowledgments

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