J. Opt. A: Pure Appl. Opt. **10** (2008) 104026 (8pp) doi:10.1088/1464-4258/10/10/104026

Photodynamic potentiality of hypocrellin B and its lanthanide complexes

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Received 7 March 2008, accepted for publication 26 June 2008
Published 2 September 2008
Online at stacks.iop.org/JOptA/10/104026

Abstract
The search for new photosensitizers with strong absorption bands, fast elimination from the body and high singlet oxygen generation quantum yield has been the subject of intensive research over the past two decades. Perylenequinonoid pigments, such as hypocrellins and hypericin, stand out among other photosensitizers due to their remarkable properties, which fulfil these requirements. Besides, it was shown that hypocrellin B complexes with metal ions possess even more notable optical and photodynamic properties. In this work, a study of the photosensitizer hypocrellin B and its complexes formed with the lanthanide ions europium, lanthanum and terbium, in ethanol and dimethyl sulfoxide solutions, was carried out by spectroscopic methods, with the purpose of verifying its potentiality for use in photodynamic therapy. The photobleaching of this molecule was also investigated, and our results show that, under experimental conditions, there is a significant decrease in HB emission intensity after about 30 min of exposure to white light. Moreover, lanthanide ions are able to modify hypocrellin B energy levels, as well as its radioactive decay probabilities. Infrared emission revealed the capacity of these complexes for generating singlet oxygen. Hypocrellin B complex with lanthanum in ethanol showed the best results among the studied complexes regarding optical characteristics of ideal photosensitizers, since it induced the larger redshift of about 30 nm, as well as enhanced HB singlet oxygen generation quantum yield of about 32%.

Keywords: hypocrellin B, photodynamic therapy, photosensitizer, photobleaching, singlet oxygen

(Some figures in this article are in colour only in the electronic version)

1. Introduction

At the end of the 1990s, a great drawback in medicine was overcome with the development of Photofrin®, a mixture of hematoporphyrin derivatives which was approved by the FDA/USA for clinical use in photodynamic therapy (PDT) of tumours as a photosensitizer agent [1]. A photosensitizer agent is a chromophore with high molar absorptivity in the visible spectrum range and low toxicity to biological tissues, and when subjected to specific wavelength irradiation it is able to give rise to photochemical reactions with non-absorber molecules present in its environment [1, 2]. Electron transfer (type I) and/or energy transfer (type II) reactions may occur, and reactive oxygen species (ROS) and/or singlet oxygen ($^1\Delta_g$) are then formed. PDT mechanisms are permitted to occur if the photosensitizer reaches its first triplet excited state T_1 after some decay processes (see the Jablonski diagram [6, 7]). These substances are able to induce chain reactions with cell components, as well as to oxidize a large variety of biomolecules, owing to their high transmembrane potential [3]. This way, ROS and $^1\Delta_g$ may cause necrosis (by direct lethal effects or by vasculature damage) or apoptotic processes (programmed cell death) [4, 5].

Singlet oxygen ($^1\Delta_g$), in particular, is formed when there is energy transfer from the photosensitizer's first triplet excited

state (T_1) to oxygen in its ground state ($^3\Sigma_g^-$, also a triplet state); $^1\Delta_g$ corresponds to the lowest excited state of $^3\Sigma_g^-$ (lying only 94 kJ mol^{-1}—or 7900 cm^{-1}—above the triplet ground state) [7, 8]. As the majority of the organic molecules are singlets in their ground state, there is no spin prohibition for these reactions with singlet oxygen. Furthermore, $^1\Delta_g$ have an empty molecular orbital, which gives its high electrophilic character. Hence, this excited oxygen species is expected to be extremely reactive and to interact heavily with a large number of biological substrates [1, 2, 7, 8]. Obviously, the complexity of biological systems affects the *in vivo* response to PDT. Specific enzymes may react with some ROS formed, minimizing the PDT effects; nevertheless, the $^1\Delta_g$ is not directly affected by many enzymes [9]. Therefore, efficiency in PDT treatment is attributed to a higher singlet oxygen generation quantum yield.

Many new photosensitizers, as plant extracts or even complex synthetic macrocycles, are being studied in order to overcome the limitations of Photofrin® (mainly low *in vivo* metabolism, low singlet oxygen generation quantum yield and poor absorption in the therapeutic window). An ideal photosensitizer must induce low systemic toxicity, have long excited triplet state lifetime (which would permit sufficient time for energy transfer reactions to occur) and high absorption coefficient in wavelengths ranging from 600 to 800 nm, a region in which light penetration in tissues reaches its maximum [1, 6, 7]. Furthermore, it must have high selectivity to the injured tissues—it was shown that tumour selectivity rises with the photosensitizer lipophilic character, which is easily understood since neoplastic cells have a particularly large number of membrane receptors for low density lipoproteins, LDL, due to their high demand for cholesterol [10].

Also another very important parameter, responsible for making the connection between research centres and medical clinics, is related to photobleaching processes, which are concerned with irreversible changes in the chromophore properties after light exposure. These changes are, in most cases, due to photoreactions of the photosensitizer with reactive oxygen species generated in the substrate. Photobleaching compromises the photosensitizer's performance, leading to the necessity of employing high doses, since the formed products are not able to absorb light at the incident wavelength. Higher doses imply more side effects and make the treatment more expensive. Therefore, an important parameter for new photosensitizers is a low photobleaching quantum yield [11, 12]. However, it must be said photobleaching can lead to some advantages, since it can be well controlled. At last, it can improve the selectivity of the method and decrease the photosensibility after the treatment, due to the degradation of the photosensitizer in the neighbourhood of the tumour or in the skin. Some works suggest that low illumination can in fact bleach Photofrin® in post-therapy patients, minimizing the risks of exposure to sunlight [13].

The ideal photosensitizer has not been discovered yet, but among the new generation studied chromophores, hypocrellins (figure 1) and naturally occurring perylenequinonoid pigments

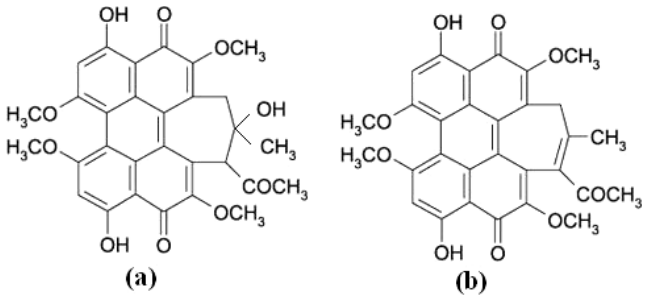

Figure 1. Molecular structure of (a) hypocrellin A ($C_{30}H_{26}O_{10}$) and (b) hypocrellin B ($C_{30}H_{24}O_9$).

extracted from the fungus of *Hypocrella bambusae* stand out. These compounds presents some advantages regarding hematoporphyrin derivatives, such as easy preparation and purification, low aggregation tendency, high singlet oxygen generation quantum yield and fast *in vivo* metabolism [14, 15].

Improvements in water solubility and in absorption intensities in the range 600–800 nm are desired from the point of view of HB clinical applications. Ma *et al* [14] found that complexes of hypocrellin B (HB) formed with aluminium ions are able to fulfil these requirements, as well as to improve the generation of superoxide radicals. However, the singlet oxygen generation quantum yield is drastically reduced, limiting the applicability of this complex. Zhou *et al* [16, 17] showed that the lanthanum complex of hypocrellin A (HA) has good water solubility and a large absorption band, and despite the reduction in type II mechanism observed after addition of aluminium in HB, a improvement in the $^1\Delta_g$ generation is observed as regards pure HA.

The present work was carried out with the objective of investigating the influence of chelation on the lanthanide ions europium (Eu^{3+}), lanthanum (La^{3+}) and terbium (Tb^{3+}) on the optical properties of HB using spectroscopic methods, thus allowing the recognition of the best photosensitizer regarding its optical properties. Absorption and emission wavelengths were determined, and singlet and triplet energy levels were then calculated. HB photobleaching was investigated, and considerations about the rate constants for the complexes were done. Singlet oxygen generation quantum yields were obtained, leading to the conclusion that lanthanide complexes of HB exhibit much higher photodynamic activities than pure HB.

2. Materials and methods

2.1. HB complexes

Hypocrellin B (HB) was purchased from Shaanxi Tianze Bio-Technology Co., Ltd. Methylene blue (MB), lanthanide chlorides ($LaCl_3{\cdot}7H_2O$, $EuCl_3{\cdot}6H_2O$ and $TbCl_3{\cdot}6H_2O$) and the employed solvents, ethanol (CH_3CH_2OH) and dimethyl sulfoxide (DMSO, $(CH_3)_2SO$), were purchased from Sigma-Aldrich Corporation Ltd, with analytical grade. HB was diluted in ethanol and in DMSO as well as the lanthanide chlorides (Ln^{3+}), forming solutions of equal molarity. HB and Ln^{3+} solutions were mixed in 1:1 molar ratio, and stirred

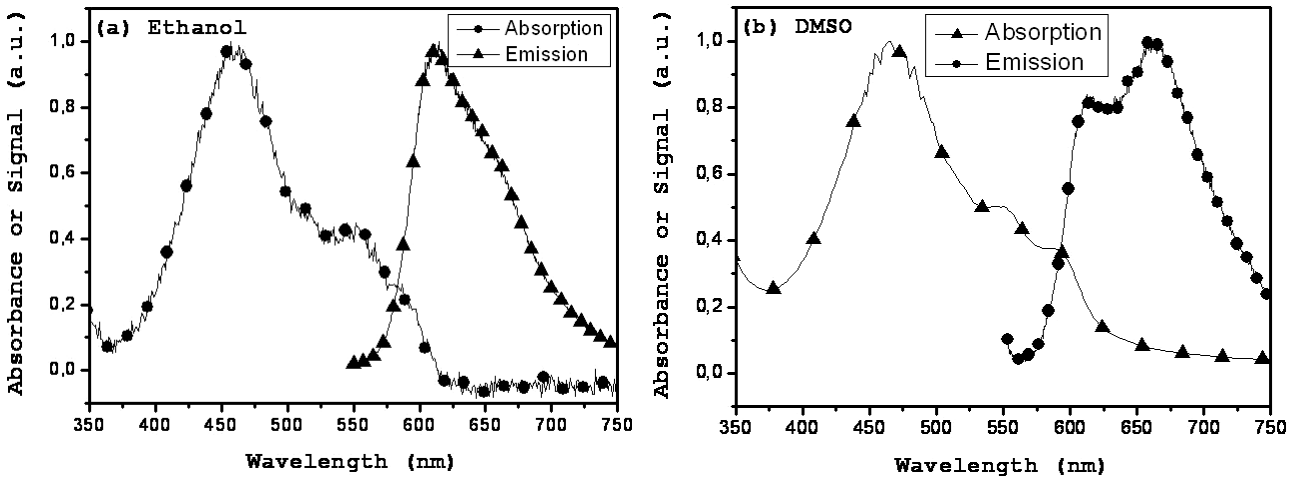

Figure 2. Absorption and emission normalized spectra of HB (a) in ethanol and (b) in DMSO.

for 20 min. Then, an equilibrium mixture of HB complexes and free HB was obtained, called an HB:Ln^{3+} solution. HB concentrations for complexes in ethanol and DMSO were, respectively, 1.89 mM and 0.95 mM.

2.2. Photobleaching

Photobleaching can be identified as losses in absorption or emission intensities of the chromophore after exposure to light. White light from a 300 W xenon lamp was focused on the cuvette for 50 min, and the absorption and emission spectra were measured every 5 min. HB solutions were continuously stirred during this experiment in order to ensure homogeneous bleaching. The samples' temperatures were kept at 18 °C. The samples' emission signals were measured from a 1 mm optical cuvette by a OSM-400-UV/VIS optical spectrometer from Newport (resolution = 0.2 nm), under laser excitation at 532 nm.

2.3. Optical characterization of HB:Ln^{3+}—absorption and emission spectra

Optical absorption in a 1 mm optical path cuvette was carried out in an Olis Cary-17 D spectrophotometer from Varian (resolution = 1 nm) to verify the conformity of the photosensitizer absorption band with the region in which light penetration in tissues is maximum (600–800 nm, the therapeutic window).

For emission experiments in the visible, a lock-in technique was employed. A xenon lamp (300 W) and a 0.25 m Jarrel-Ash monochromator (resolution = 1 nm) were used to select the excitation wavelength. The luminescence from the sample was collected orthogonally from the excitation, and was injected into a 0.5 m Spex monochromator, detected by a Hamamatsu photomultiplier tube and amplified by an EG&G 7220 lock-in. In order to avoid external noise, a Stanford chopper was put on the optical path of excitation and connected to the lock-in. The cuvette optical path was 1 mm. By analysis of emission and absorption spectra it was possible to determine the energies of the first singlet (S_1) and triplet (T_1) excited states for all complexes.

2.4. Singlet oxygen generation quantum yields

Infrared emission around 1270 nm (due to $^1\Delta_g$) was verified with an emission system from Edinburg Instruments. Such a system consisted of a Q-switched Nd:YAG laser (continuum Surelite III), emitting pulses with 5 ns (FWHM) at second harmonic, at 10 Hz repetition rate, a Hamamatsu R5509 photomultiplier tube and a 1 cm optical path cuvette. Lifetime measurements were obtained with a Opotek Nd:YAG + OPO laser excitation, a 0.25 m Kratos monochromator, S-20 photomultiplier tube, a 1300 nm filter and an oscilloscope. The emission around this wavelength is due to the transition from singlet oxygen $^1\Delta_g$ to ground state (triplet) oxygen $^3\Sigma_g^-$ [7]:

$$^1\Delta_g \rightarrow {}^3\Sigma_g^- + h\nu(1270\ \text{nm}) \tag{1}$$

where h is Planck's constant and ν is the frequency of the emitted radiation during the transition. So, $^1\Delta_g$ lifetime (τ_Δ) and singlet oxygen generation quantum yields (Φ_Δ) were determined from these measurements.

Values of Φ_Δ were calculated through the following equation:

$$\Phi_\Delta^S = \frac{I_S}{I_R}\frac{A_R}{A_S}\Phi_\Delta^R \tag{2}$$

where I is the signal intensity at 1270 nm, A is the absorbance in 532 nm and the indexes S means sample and R reference.

3. Results and discussion

3.1. Hypocrellin B energy levels

Figure 2 depicts the absorption and emission spectra of Hypocrellin B (HB) in both solvents. The spectra were normalized to have maximum band equal to unity. It can be seen that HB has a very large absorption band in the visible, with bands at 460, 546 and 584 nm in ethanol, and at 470, 549 and 590 nm in DMSO. Under excitation at 470 nm, HB shows

Table 1. Optical properties for HB complexes.

	Ethanol solutions				DMSO solutions			
	HB	HB:La^{3+}	HB:Eu^{3+}	HB:Tb^{3+}	HB	HB:La^{3+}	HB:Eu^{3+}	HB:Tb^{3+}
λ_1^A (nm)[a]	460	484	484	466	470	470	470	473
λ_2^A (nm)[a]	546	571	571	566	549	552	552	563
λ_3^A (nm)[a]	584	614	614	600	590	598	598	608
λ_{max}^E (nm)	612	650	654	648	662.5	660	658.5	663
I_{470}^{norm} (au)	1.23×10^{-3}	0.12×10^{-3}	0.02×10^{-3}	0.36×10^{-3}	23.09	32.24	76.95	17.24
S_1 (cm^{-1})	17 123	16 025	15 948	16 722	16 778	16 233	16 207	15 673
T_1 (cm^{-1})	16 366	15 384	15 267	15 432	16 286	15 174	15 174	15 082
ΔE (cm^{-1})[b]	757	641	681	1 290	492	1 059	1 033	591
τ_Δ (μs)	21.88	25.56	32.79	15.61	0.98	0.98	0.97	1.10
$\Phi\Delta$	0.47	0.62	0.03	0.42	0.76 [14]	0.33	0.30	0.14

[a] $\lambda_x^A = x$th absorption wavelength.
[b] $\Delta E = S_1 - T_1$.

red luminescence with bands at about 612 and 662 nm in both solvents. No mirror image between these spectra is observed, which means that no vibrational structure can be seen. This is due to the fact that HB has a long carbon chain and hence there is superposition of each single vibrational band, which leads to such broad absorption and emission bands.

The stronger absorption bands at 460 (ethanol) and 470 nm (DMSO) may be assigned to a $\pi \rightarrow \pi^*$ transition, which is more probable due to the fact that these orbitals are placed in the same spatial region. The other absorption bands are due to intramolecular proton transfer processes. Similarly, the shorter wavelength emission band is due to a $\pi \rightarrow \pi^*$ transition, and the other, to intramolecular proton transfer [15].

The first singlet (S_1) and first triplet (T_1) excited state energy levels were calculated from both absorption and emission spectra and are shown in table 1. These spectra were plotted with the same maximum value (normalized as in figure 2). The wavelength at which the two curves intercept represents, with good accuracy, the photon emitted in the transition from S_1 to S_0—since the higher absorption wavelength represents lower energy emitted and, through Kasha's rule [18] (vibrational relaxation is much faster than photon emission), emissions starts on S_1, so the S_1 energy may be calculated from this point—and the lower emitted wavelength represents T_1.

It is well known that photosensitizers in which T_1 is higher than 7900 cm^{-1} s are generally good at generating $^1\Delta_g$, since this value corresponds to the excitation energy for singlet oxygen [7, 8]. Our results show all samples have $T_1 >$ 7900 cm^{-1} (see table 1), which indicates their potentiality for use in PDT.

3.2. *Photobleaching*

Figure 3 shows the absorption spectra obtained for hypocrellin B (HB) (a) in ethanol and (b) in DMSO 70 μM solutions, for different time intervals after illumination with the xenon lamp.

It can be seen from figure 3 that HB in ethanol suffers photobleaching, since a continuous decrease in the absorption spectrum can be observed after exposure to white light. In ethanol, one can note a decrease in the ratio A_{460}/A_{590} (where A means absorbance) due to illumination; in DMSO, a slight displacement in the absorption bands can be observed after illumination. These changes can be due to the photoproducts generated by the reaction of HB with singlet oxygen or another reactive oxygen species.

The emission spectra were measured, under excitation at 532 nm, also in time intervals of 5 min after irradiation with the xenon lamp. Figure 4 shows the maximum signal intensities as a function of the exposure time.

Analysing the data shown in figure 4, the remarkable reduction of the luminescence intensity of the chromophores after light exposure can be attributed to photobleaching. This reduction took place suddenly after a period of time of about 30 min for the two samples. These changes in emission spectra were even visible to the naked eye: HB red luminescence had its intensity reduced after illumination.

Approximately 20 min after white light irradiation of HB, precipitates were formed. The authors propose that this indicates a great change in the molecule and the formation of an insoluble photoproduct (not identified). As time passed and it reached 30 min after irradiation, more insoluble photoproducts were formed and then the HB emission in its ground state (that did not photobleach) was scattered by the particles that were in the solution. Furthermore, the number of HB molecules that have not suffered photobleaching have continuously fallen. This is a possible mechanism to explain the signal decrease.

Additional studies must be done to determine the mechanisms responsible for the HB photobleaching, using known quenchers for singlet oxygen and free radicals. It is important to emphasize that in the two solvents HB showed similar behaviour. This was expected, since ethanol and DMSO are both polar solvents.

The obtained results confirm that the action of light (and consequently of the reactive species photochemically formed in the medium) in HB in both solvents is prejudicial for PDT, because the photosensitizer-bleached molecules would not be able to absorb light and therefore transfer energy or electrons to oxygen. Consequently, the generation of reactive oxygen species via type I and II mechanisms would be compromised,

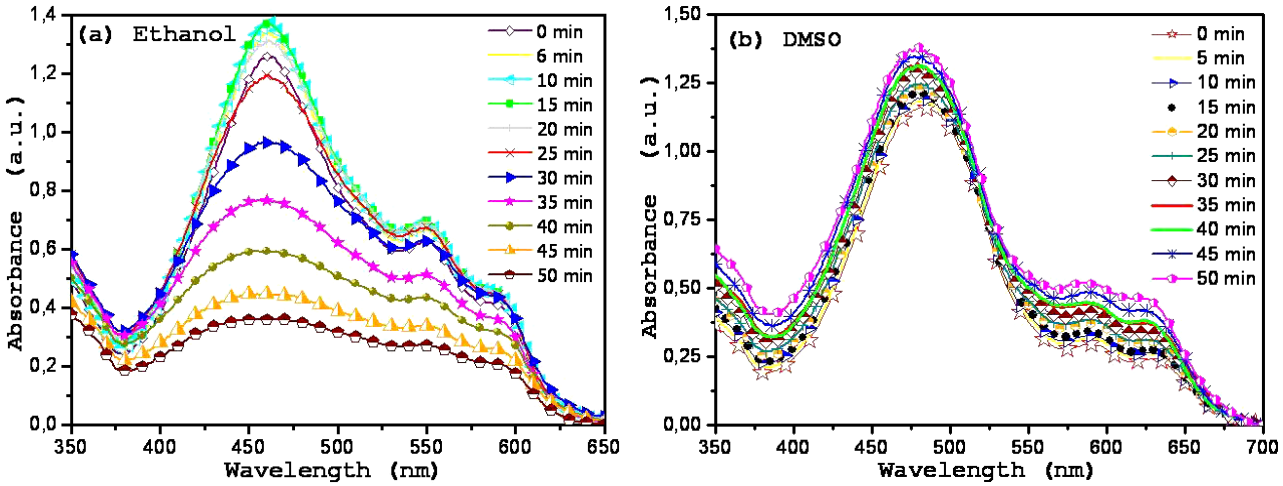

Figure 3. Absorption spectra of (a) HB ethanol (70 μM) and (b) HB DMSO (70 μM).

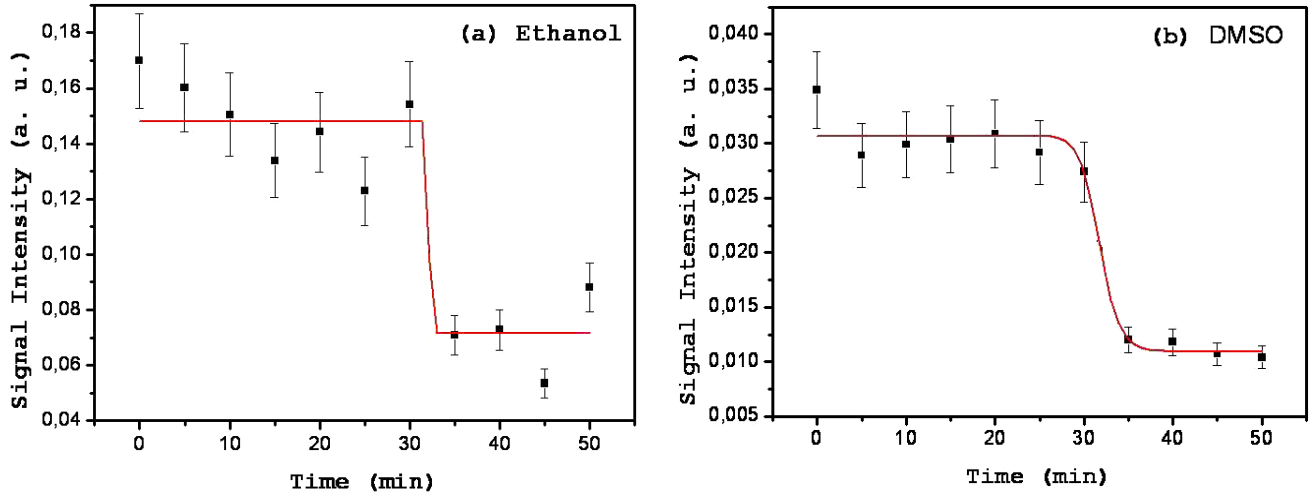

Figure 4. Changes in luminescence intensity of (a) HB in ethanol and (b) HB in DMSO according to the exposition time.

leading eventually to incomplete destruction of the target tissue.

Nevertheless, it must be said that photobleaching, if correctly controlled, can be useful for photosensitizer elimination from the body, diminishing photosensitivity after the treatment. It can also enhance selectivity of the method, since the photosensitizer in the neighbourhood of the target tissue will rapidly lose its action, and healthy tissues will not suffer the effects of PDT.

3.3. Hypocrellin B complexes with lanthanide ions

Figure 5 shows the optical absorption spectra for HB:Ln^{3+} solutions in ethanol and in DMSO. It can be seen that all complexes present a very large absorption band in the visible, ranging from about 400 to 650 nm. These spectra show that, when the mixture of HB complexes and free HB reaches equilibrium, most of the molecules are complexes, instead of pure HB, since HB absorption bands are almost absent in the lanthanide solutions. Such affirmation cannot be done for HB:Tb^{3+} in ethanol and HB:La^{3+} and HB:Eu^{3+} in DMSO; so, in these solutions, HB and lanthanide ions have not complexed efficiently.

In ethanol, lanthanum and europium solutions of HB have remarkably modified the HB optical properties. These complexes were able to enhance absorbance in wavelengths longer than 600 nm by a factor of 10^2, as well as induce a redshift of 30 nm in the higher absorption band. In DMSO, just the terbium solution of HB has induced a significant change in HB optical properties, inducing a redshift of 18 nm (table 1).

Figure 6 depicts the sample's luminescence spectra under excitation at 470 nm, and table 1 shows the wavelengths ($\lambda^{E}_{\max}$) which correspond to maximum signal intensities (I^{norm}_{470}). It can be seen that all solutions show a large emission band, ranging from about 550 to 750 nm. Regarding the complexation of the HB molecule by lanthanide ions, there are modifications in luminescence bands and in the radioactive decay rate constants for all lanthanides used. Finally, the HB shorter wavelength luminescence band seems to be suppressed when lanthanide ions are added to the solution. As is well known, aggregates luminesce at longer wavelengths than monomers; so, the

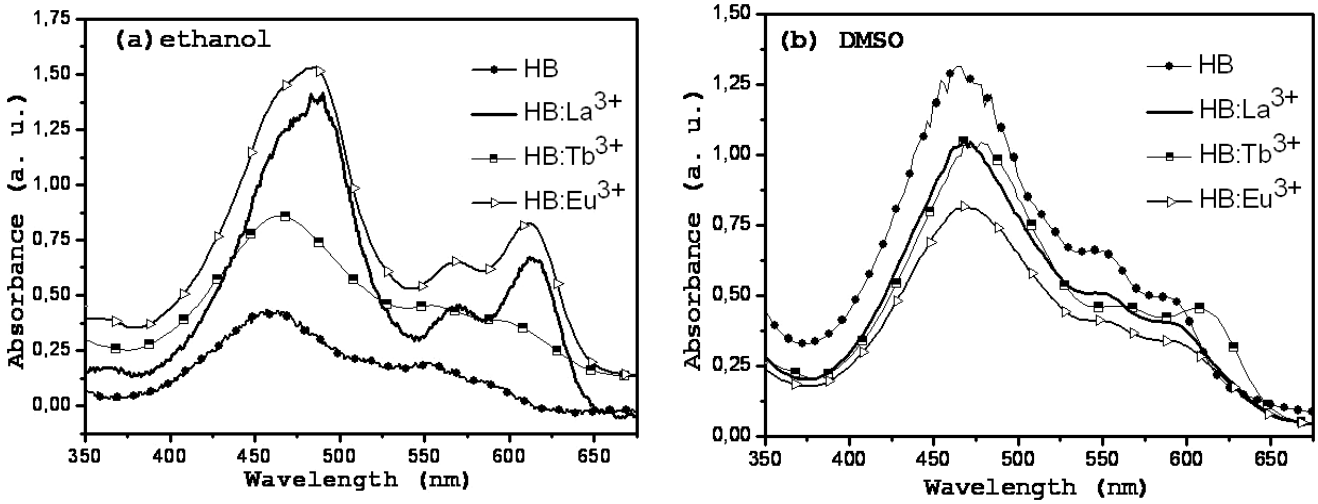

Figure 5. HB complexes' optical absorption spectra (a) in ethanol and (b) in DMSO.

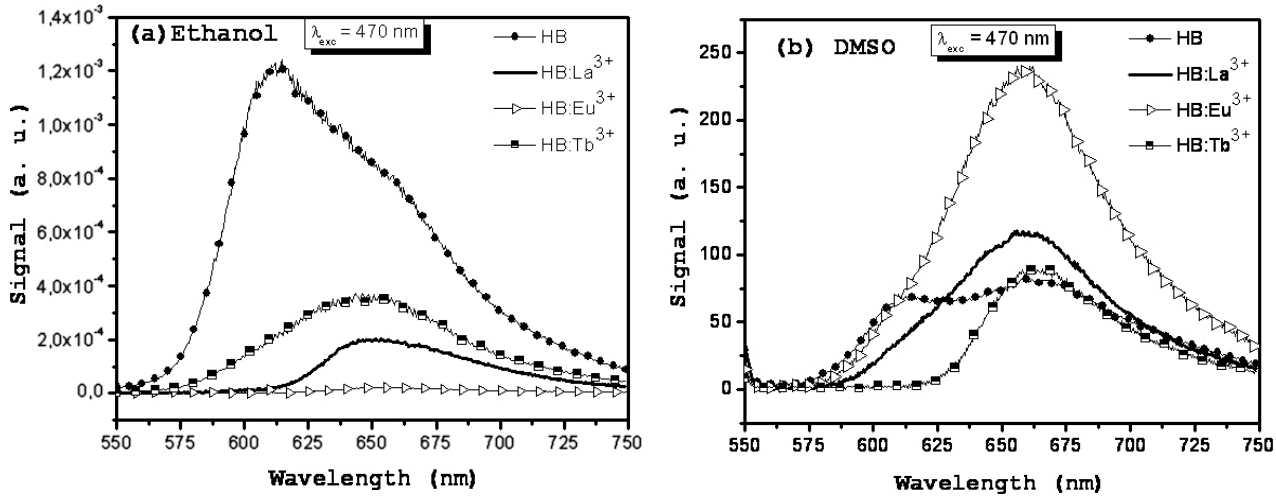

Figure 6. Emission spectra of HB complexes (a) in ethanol and (b) in DMSO, under excitation at 470 nm.

structures of lanthanide complexes of HB can be said to be polymeric, since HB emission peak at about 600 nm does not appear in the emission spectra of the lanthanide solutions.

The energy levels of HB complexes were calculated in the same way as done for pure HB (table 1), and once more it the potentiality of these complexes was verified, since the values of T_1 are higher than 7900 cm^{-1}. Another interesting result extracted from the calculated energy levels is concerned with the gap ΔE between S_1 and T_1. The smaller this gap, the more probable becomes the transition from S_1 to T_1 by phonon emission, which would eventually lead to a higher Φ_Δ, since $^1\Delta_g$ is generated by reactions induced by the photosensitizer in this state. Table 1 shows the calculated values of ΔE for HB and its complexes, and furthermore these values will be related to Φ_Δ.

3.4. Quantum yields and lifetimes of $^1\Delta_g$ and rate constants

Samples' emissions in the infrared range (1230 up to 1320 nm), under excitation at 532 nm were measured (figure 7(a)) with the objective of determining the singlet oxygen generation quantum yields of the samples in ethanol. Methylene blue (MB) was employed as reference ($\Phi_\Delta^{MB} = 0.52$ [19]).

It is important to emphasize that the reference MB solution was also made in ethanol, as for the samples analysed through this method, because singlet oxygen efficiency of luminescence may vary with the environment.

It may be seen in figure 7(a) and table 1 that only the lanthanum addition to the HB in ethanol increases the value Φ_Δ from 0.47 to 0.62. Europium addition was responsible for quenching the singlet oxygen generation from 0.47 to 0.03, while terbium addition did not induce remarkable changes in it (from 0.47 to 0.42). The values measured for Φ_Δ can be related to the emission spectra and energy levels. For example, in figure 6(a) the quenching of the HB luminescence after lanthanum addition leads to a higher value for the non-radioactive decay rate constant, k_{nr}, for this complex. It also indicates there are other decay processes competing with luminescence, and one of them could be an intersystem crossing—which would lead to a higher triplet state yield, Φ_T.

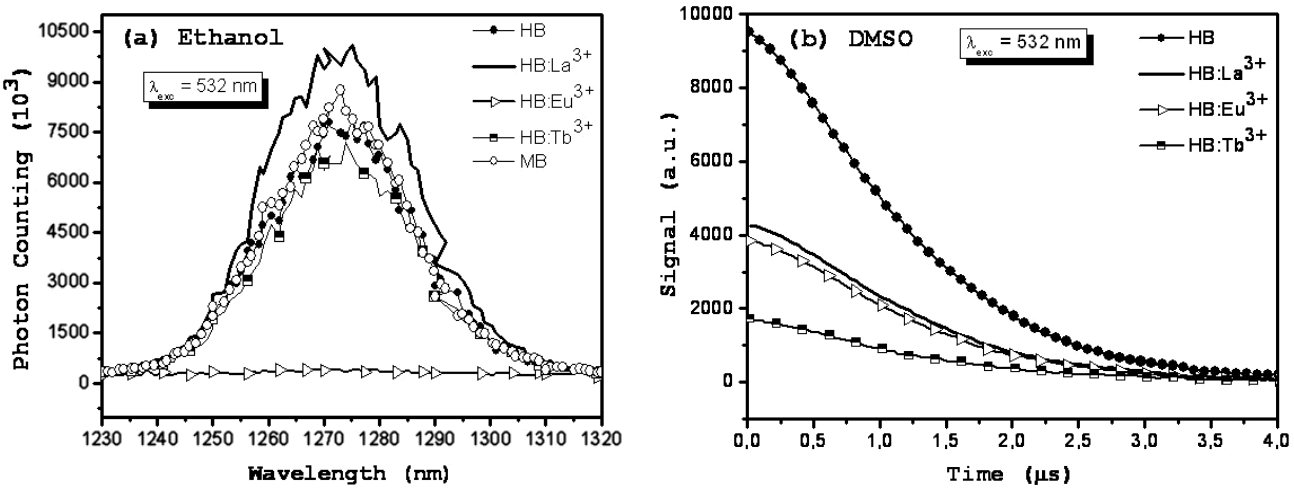

Figure 7. (a) Luminescence spectra in the infrared of samples in ethanol under excitation at 532 nm. (b) Luminescence decays of samples in DMSO under excitation at 532 nm (emission at 1300 nm).

Furthermore, this complex exhibited a decrease in the gap between S_1 and T_1 (ΔE) when compared to pure HB, could favour non-radiative transitions and also lead to a higher Φ_T value. This could explain the high value of Φ_Δ for HB:La^{3+} in ethanol.

Although analogous considerations could be done for the europium complex, there was suppression of Φ_Δ. This indicates that the probability of non-radiative decay through internal conversion (IC) is much higher than the probability of the molecule to suffer an intersystem crossing (and thus generate $^1\Delta_g$) in the europium complex. For terbium complexes, there is suppression in luminescence and increase in ΔE. The decrease of Φ_Δ suggests that the luminescence quenching led to a higher non-radiative decay probability, through IC.

Figure 7(b) shows that the $^1\Delta_g$ lifetime (τ_Δ) is strongly influenced by the solvent employed (table 1). These results were expected, once this dependence of singlet oxygen lifetime on the nature of the solvent is well known [7]. Our measurements show that τ_Δ in ethanol is about 20 times greater than in DMSO. As a large value of τ_Δ may imply a better treatment with PDT (since $^1\Delta_g$ would have sufficient time to reach a wider area in the target tissue before losing energy by emission of photons), it can be inferred that ethanol complexes could induce photodamage in a wider area than in DMSO.

The bands of the exponential decays at 1300 nm were also used for the calculation of Φ_Δ for samples in DMSO (since this wavelength is inserted into the $^1\Delta_g$ emission band), as can be seen in table 1. Calculations of Φ_Δ (equations (1)) for these solutions were done with HB DMSO as standard ($\Phi_\Delta^{\text{HB DMSO}} = 0.76$ [14]).

In DMSO, all complexes decreased the value of Φ_Δ relative to HB. Relating this result to the previous ones, it can be noted that HB:La^{3+} in DMSO induced the enhancement in luminescence (which means shorter k_{nr} and, thus, shorter Φ_T than neat HB) (figure 6(b)), as well as in the energy gap between S_1 and T_1 (which reduces the probability of intersystem crossing to occur) (table 1), and indeed there was a reduction in Φ_Δ. Similar reasoning may be done for the europium addition to HB DMSO. For terbium complexes, such reduction in Φ_Δ was also expected, although less remarkable than for that caused by lanthanum and europium addition, since the increase in the energy gap in this case has a smaller value. Nevertheless, the quenching in Φ_Δ is larger, and one could suggest the energy transfer from the photosensitizer to the environment is more efficient than to molecular oxygen.

Table 1 summarizes all the measurements done.

4. Conclusions

Optical absorption, visible and infrared emission and luminescence decay measurements were done in this work with the purpose of characterizing the complexes of hypocrellin B (HB) formed with lanthanum, europium and terbium. To the best of our knowledge, it is the first time that HB complexes with lanthanide ions are reported. The complexes were prepared in a very simple and inexpensive way, with no need of methods of isolation and purification. The prepared solutions, in a general way, have been able to improve the HB optical properties. In this way, a simple, inexpensive and fast method for HB complexation was created, presenting very satisfactory results.

HB photobleaching was investigated and it was seen that HB suffers photobleaching under our experimental conditions after about 30 min of irradiation with white light. It could be verified that the luminescence measurements are more sensitive than absorption ones for photobleaching detection, since in DMSO absorption spectra have not revealed significant changes in the sample. HB interacts with reactive oxygen species generated in the medium by illumination and insoluble photoproducts are formed. This fact leads to an abrupt decrease in the luminescence signal measured after 30 min of illumination.

Regarding the optical properties, in general lanthanide ions can improve HB performance in PDT, since for all studied complexes absorption redshifts were noted, as well as changes

in the HB emission spectrum. Since aggregates emit in longer wavelengths than monomers, one can say the structure of the lanthanide complexes of HB is polymeric.

Furthermore, the samples' emission around 1270 nm revealed that all of them, except HB:Eu^{3+} in ethanol, are able to generate singlet oxygen. Only the lanthanum complex of HB is able to improve the value of this parameter. Singlet oxygen lifetimes for the samples revealed that oxygen rapidly returns to its ground state when generated in DMSO solutions. Because of this, one could say that ethanol solutions, with larger values for this parameter, may cover a wider area during PDT, and thus, from this point of view, they are more efficient photosensitizers.

In particular, HB:La^{3+} in ethanol was responsible for a remarkable redshift of 30 nm (from 584 to 614 nm) in the HB absorption band, as revealed by absorption spectroscopy. Besides, only HB:La^{3+} in ethanol provided an enhancement of the singlet oxygen generation quantum yield of HB, from 0.47 to 0.62 (32%). For a photosensitizer to be considered for clinical use, several complementary studies, as *in vitro* and *in vivo* ones, must be carried out, for considering its interaction with biological tissues and how this interaction will influence its behaviour. From the point of view of optical properties, HB:La^{3+} is the best between the studied solutions for use as a photosensitizer.

Acknowledgments

The authors would like to thank Dr Frank Herbert Quina, from Instituto de Química da Universidade de São Paulo, for his valuable contributions about the obtained spectra and by his attention and time dispensed, and CAPES—Coordenação de Pessoal de Ensino Superior—for the scholarship.

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