

# Reactivity of the photo excited forms of Hypericin, Hypocrellin A, Hypocrellin B and methylated Hypericin towards molecular oxygen The role of charge transfer interaction

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## Abstract

This work concerns the comparison of four structurally close photosensitizers (PSS) towards their abilities to react with molecular oxygen. Indeed, Hypocrellin's A (HAH<sub>2</sub>) and B (HBH<sub>2</sub>) and Hypericin's (HypH<sub>2</sub>) (native and permethylated forms) present interesting medicinal properties which can be due to their abilities to produce singlet oxygen (type II) or in parallel of superoxide anion (type I). Both mechanism implies reaction between the triplet state of the photosensitizer and O<sub>2</sub>, meanwhile these processes are quite different since only the second one involved electron exchange (redox process). In order to identify the nature of the interaction between these two species, the photosensitizers have been characterized by means of photophysical and electrochemical investigation. Singlet oxygen and triplet yields have been determined in organic medium, in both neutral and basic condition. For Hypocrellin's the yields of singlet oxygen and triplet are almost quantitative whereas for Hypericin these values are lower. The discrepancy towards these yield values between the studied photosensitizers does not appear to be thermodynamically ruled. Indeed, although the excited form of Hypericin's present reducing properties towards molecular oxygen (whereas such redox process is not thermodynamically favored), the electron transfer step has not been displayed since transient absorption experiments at short time do not allow the observation of radical species of Hypericin's. The very poor efficiency of O<sub>2</sub>•<sup>-</sup> generation seems to be linked with the very slow kinetic of electron transfer between the excited form of the photosensitizer and molecular oxygen. As a result, high efficiency of O<sub>2</sub>•<sup>-</sup> production should be obtained for the four studied photosensitizers in the presence of additional electron donor.

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## 1. Introduction

Hypericin (HypH<sub>2</sub>) (Fig. 1) (2,2'-dimethyl-4,4',5,5',7,7'-hexahydroxymesonaphthodianthrone), a natural pigment [1], exhibits light induced anti-tumoral [2] and anti-viral activities [3,4]. It was suggested that these activities may be related, to some extent, either to its ability to produce singlet oxygen (type II mechanism) [2–5] or to allow the formation of the superoxide anion (type I mechanism) [6–8].

Hypocrellin's A (HAH<sub>2</sub>) and B (HBH<sub>2</sub>) (Fig. 1) (4,9-dihydroxyperylene-3,10-quinone) which can be extracted from *Hypocrella bambusa*, a parasitic fungus of *Siramudinaria* [9], and *Skiroia Bambusicola*, respectively, are structurally related to perhydroxylated poly-

cyclic quinones, as Hypericin, and display anti-tumoral and anti-viral activities against several types of viruses including human immunodeficiency virus [9–12]. Their biological properties are combined with photochemical irradiation but, unlike Hypericin, Hypocrellin's absolutely require oxygen for their antiviral activity [13,14]. Thus, although these photosensitizers (PSS) present important structural similarity, their reactivity towards molecular oxygen seems to be quite different. It is crucial to understand the mechanism involving the interaction between the excited form of the sensitizer and O<sub>2</sub>, and the processes which govern the production of either singlet oxygen or the superoxide anion [15]. In this context, we interested to examine the reactivity of the photosensitizer towards molecular oxygen in order to determine the relative contribution of the energy transfer involved in the production of singlet oxygen, in aerobic condition. These results will be compared to that concerning the electron transfer between the excited form of the photosensitizers and molecular oxygen [16,17].

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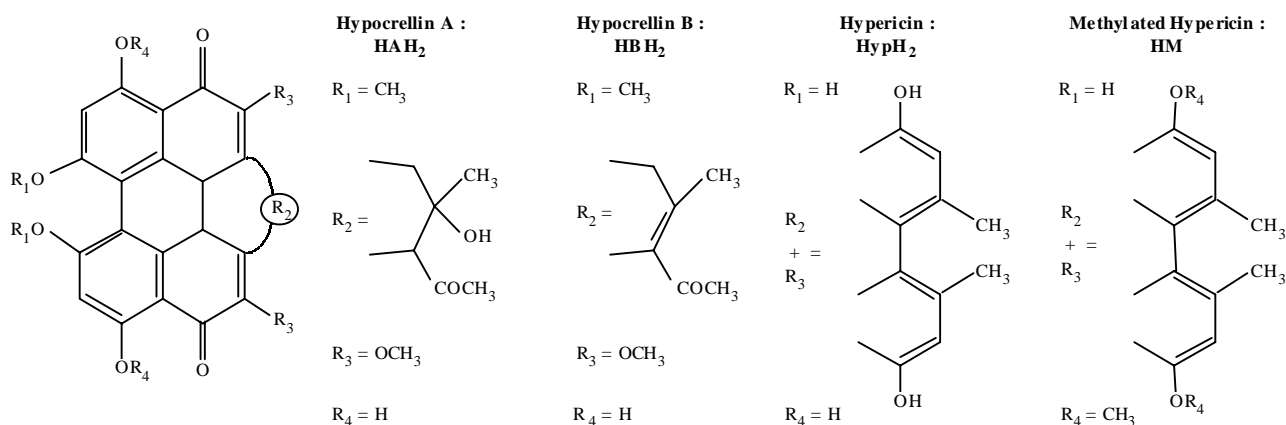


Fig. 1. Structure of the photosensitizers studied.

## 2. Experimental

Hypericin, Hypocrellin's A and B (Molecular Probes), all solvents (Merck) were purchased without further purification. Absorption spectra were recorded on a Perkin-Elmer lambda 17 spectrophotometer. All experiments were carried out with air-saturated solutions at  $20 \pm 1$  °C. Hexamethoxyhypericin (HM) was synthesized by adding 6 molar equivalents of NaH and iodomethane to a solution of Hypericin in dimethylformamide. After the solution was stirred for 5 h at room temperature, the crude mixture was purified by HPLC (column: C18 nucleosil, eluant: water/methanol: 1/10, flow rate: 5.5 ml/min), the products separated were detected at  $\lambda = 300$  nm using an UV-Vis detector. The purity of the expected product was controlled by mass spectroscopy, <sup>1</sup>H NMR, absorption and emission spectrophotometry as previously described [18].

### 2.1. Physico-chemical

Several techniques have been employed in order to quantify by means of physico-chemical investigation, the properties of the photosensitizers. This has been performed in organic medium. Electrochemistry was carried out in acetonitrile medium. Determination of quantum yield of <sup>1</sup>O<sub>2</sub> was performed in acetonitrile and ethanolic solution.

### 2.2. Electrochemistry

Cyclic voltammograms (CV) were recorded using a potentiostat EGG 273. Cyclic voltammetry were performed using a platinum disc (5 mm diameter) as the working electrode. An Ag/10 mM Ag<sup>+</sup> + CH<sub>3</sub>CN + tetra-butylammonium perchlorate 0.1 M (TBAP) electrode was used as reference electrode. CH<sub>3</sub>CN (Rathburn) was used as received, nitrogen flushed and the experiments were carried out under an argon atmosphere in a dry glove-box. Dry TBAP, used as the supporting electrolyte in CH<sub>3</sub>CN medium, was purchased from Aldrich, recrystallized from ethyl acetate-cyclohexane

and dried under vacuum at 80 °C for 3 days. Potential have been converted to the NHE scale using Ag/10 mM Ag<sup>+</sup> + CH<sub>3</sub>CN + 0.1 M TBAP = 0.55 V/NHE [19]. The results concerning the electrochemical behavior of Hypericin and Hypocrellin A have been described in previous papers [16,17].

### 2.3. Photophysical

#### 2.3.1. Detection of singlet oxygen

Two methods have been employed for the determination of the production of singlet oxygen.

**2.3.1.1. Method I: Luminescence of singlet oxygen at 1270 nm.** The intensity and decay kinetics of O<sub>2</sub> (<sup>1</sup>Δ<sub>g</sub>) luminescence at 1270 nm were recorded by means of an IR. laser fluorimeter with the resolution time  $\approx 1$  μs, described elsewhere [20]. The solutions were excited by a pulse at  $\lambda = 308$  nm, 10 ns,  $\leq 30$  mJ of an excimer laser (Lamda physik EM6,-100) or by pulses at  $\lambda = 482$  nm and 550 nm,  $\approx 10$  ns,  $\leq 20$  mJ of a dye laser (coumarin 102 and coumarin 153). The intensity of O<sub>2</sub> (<sup>1</sup>Δ<sub>g</sub>) luminescence extrapolated to the center of the laser pulse ( $I_0$ ) was determined on a linear part of the dependence of  $I_0$  on the pulse energy. Neutral filters decreased the energy of the laser pulse. The absorbance of the solutions ranges between  $A = 0.3$  and  $0.5$  at the wavelength excitation ( $\lambda_{ex}$ ), corresponding to the concentration of compound around  $(1-2 \times 10^{-5})$  M. The determination of the quantum yield ( $\phi_{\Delta}$ ) was carried out by comparing the <sup>1</sup>O<sub>2</sub> luminescence intensity of the compounds in a given solvent with that obtained with perinaphtenon, used as standard, which has a quantum yield ( $\phi_{\Delta}^{st}$ ) equal to the unity [21]. The next expression (Eq. (1)) from the work [22] was used for the estimation of the  $\phi_{\Delta}$  values:

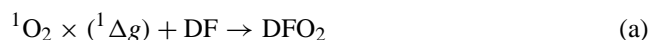
$$\phi_{\Delta} = \phi_{\Delta}^{st} \times \frac{I_0}{I_0^{st}} \times \frac{1-10^{-A}^{st}}{1-10^{-A}} \times \frac{n^2}{n_{st}^2} \times \frac{k_r}{k_r^{st}} \quad (1)$$

where  $n$  is the refractive index of solvent and  $k_r$  is the radiative rate constant of singlet oxygen in the solvent

investigated, the latter strongly depending on the solvent nature, (the parameters with st as exponent are relative to the standard). When the compounds and standard were studied in the same solvent, the last two terms  $((n^2/n_{st}^2) \times (k_r/k_r^{st}))$  in Eq. (1) are equal to the unity. The lifetimes of singlet oxygen determined in ethanol (9.2  $\mu$ s) and in acetonitrile (71.9  $\mu$ s) are in agreement with literature data upon photoexcitation of the sensitizers [23].

**2.3.1.2. Method II: Photooxydation of the diphenyl-1,3-isobenzofuranne.** The diphenyl-1,3-isobenzofuranne (DF) (FLUKA) is a well-known acceptor of singlet oxygen [24] which in presence of  $^1O_2$  leads to its oxidized form DFO<sub>2</sub>. Then, the  $^1O_2$  formation, through irradiation at  $\lambda = 459$  nm, of the solutions of sensitizer have been performed in the presence of DF, the samples received as an average of  $1.7 \times 10^{-4} \text{ J s}^{-1} \text{ cm}^2$  during 2 h of exposure, determined by ferrioxalate actinometry. The concentration of the photosensitizers is close to  $10^{-5}$  M whereas that of DF is  $4 \times 10^{-5}$  M. The photooxydation of the DF is followed by absorption spectroscopy at 410 nm [25]. The results have been corrected according to the energy received by the sample at the wavelength of excitation and compared to that obtained in an ethanolic solution of Hypericin, used as external reference [6].

The deactivation of photogenerated singlet oxygen could follow two different mechanisms, a first one involving DF as a  $^1O_2$  trapping agent (a) whereas the second one corresponds to the thermal deactivation of  $^1O_2$  (b) [6]:



allowing the establishment of Eq. (2)

$$-\frac{1}{\Delta A} = \frac{1}{\epsilon l [DF]_r^\infty} + \frac{\beta}{[DF]_r^\infty} \times \left( \frac{1}{A} \right) \quad (2)$$

with  $\Delta A$  corresponding to the variation of absorbance of the solution at a wavelength specific to DF ( $\lambda = 410$  nm) after the irradiation step (2 min) ( $\epsilon = 25,000 \text{ M}^{-1} \text{ cm}^{-1}$ ),  $l = 1$  cm,  $[DF]_r^\infty$  is the limit number of mole of DF by unity of volume when A tends towards  $\infty$ ,  $\beta = k_\Delta^0/k_r$  and A corresponds to the absorption of DF at 410 nm [6]. All the experiments are carried out in the same conditions and the spectra are recorded after each 2 min irradiation. In these conditions, the straight line obtained for  $1/\Delta A = f(1/A)$  allows to characterize the formation of singlet oxygen, and to determine the parameter  $\beta$ . In our case,  $\beta$  is equal to  $6.7 \times 10^{-5}$ , a value in good accordance with that of the literature which shows that the a mechanism is preponderant compared to the b one [26].

The quantum yield of the formation of singlet oxygen is determined with relation (3):

$$\varphi_\Delta = \varphi_\Delta^{\text{ref}} \times \frac{[DF]_t}{[DF]_t^{\text{ref}}} \times \frac{1-10^{-A}}{1-10^{-A}^{\text{ref}}} \quad (3)$$

where A is the absorption of the analyzed solution at the irradiation wavelength ( $\lambda = 459$  nm),  $[DF]$  and  $[DF]^{\text{ref}}$  correspond to the quantity of DF oxidized by singlet oxygen in presence of the photosensitizer and in presence of the reference (Hypericin in ethanol [6,27]), respectively after the same irradiation time. In these experimental conditions, the  $\varphi_\Delta$  values are close (less than 10% deviation) to that determined using method I. Thus, in neutral condition,  $\varphi_\Delta$  represents the average value determined according to the two methods.

In addition, since these photosensitizers exhibit labile protons (see later), the influence of the basic character of the medium on these experimental parameters was also examined. In ethanolic medium, the formation of the deprotonated forms of the photosensitizer has been obtained by addition of concentrated NaOH in ethanol and the anionic nature of the photosensitizer (mono- or di-deprotonated forms) was checked by UV-Vis investigation according to [16,17,28–30]. The same experimental conditions were employed to measure the  $^1O_2$  production. Meanwhile, since DF oxidation seems to be disrupted in such conditions, only the method I allows the determination of the parameters investigated. This study has not been effectuated in acetonitrile due to the too low solubility of NaOH in this solvent.

### 2.3.2. Triplet–triplet absorption

In order to observe triplet–triplet absorption spectrum of the sensitizers, a dye laser (coumarin 153) coupled with an exiplex laser (Lambda Physic EM6100) has been used. The solutions previously deoxygenated (residual pressure  $10^{-2}$  Pa) were excited by a pulse at  $\lambda = 550$  nm (10 ns, 10 mJ). The detection system of transient absorption has been reported elsewhere [31]. The intensity and decay kinetics of triplet sensitizer are recorded on an oscilloscope Tektronic TDS520A.

## 3. Results and discussion

Since HA, HB, HypH<sub>2</sub>, HM present significant differences of behavior towards their phototherapeutic properties in aerobic condition, we interested to compare  $^1O_2$  and superoxide anion production for these photosensitizers. This has been carried out by the determination of quantum yield of singlet oxygen and triplet formation.

The spectroscopic properties of absorption and emission of the photosensitizers have been investigated in a previous work [17,28,29]. Therefore, these photosensitizers bear labile protons and can undergo deprotonated steps. In the case of Hypericin, four species have been underlined leading to three acid–base couples: HypH<sub>3</sub><sup>+</sup>/HypH<sub>2</sub>, HypH<sub>2</sub>/HypH<sup>−</sup>, HypH<sup>−</sup>/Hyp<sup>2−</sup> with pK<sub>a</sub> values equal to 1.0, 7.2 and 11.5 in a micellar buffered medium [16,28]. In the same experimental conditions, for Hypocrellin's, two acid–base equilibrium have been observed (pK<sub>a</sub> = 8.4 and 11.4) for Hypocrellin A [17], corresponding to the couples

HAH<sub>2</sub>/HAH<sup>-</sup>, HAH<sup>-</sup>/HA<sup>2-</sup> whereas for Hypocrellin B, pK<sub>a</sub> values are equal to 8.3 and 11.7 relative to the HBH<sub>2</sub>/HBH<sup>-</sup>, HBH<sup>-</sup>/HB<sup>2-</sup> couples [29]. On the other hand, since Hexamethoxyhypericin does not exhibit acidic proton, no spectral change versus pH was observed. Thus, for the determination of parameters illustrating the interaction between molecular oxygen and excited state of PSS (i.e. quenching yields), the nature of the basic character of the photosensitizer has been considered.

Two acid–base conditions have been studied: (i) neutral condition (ethanol and acetonitrile media) with PSS under its neutral form, (ii) in basic condition (ethanolic + NaOH medium) for which the sensitizers are mono and/or di-deprotonated.

### 3.1. <sup>1</sup>O<sub>2</sub> formation in neutral condition

Table 1 summarized data ( $\varphi_{\Delta}$  values), illustrating the efficiency of the photosensitizer to produce <sup>1</sup>O<sub>2</sub>. All these values are similar to those determined for parent compounds such as other perylen quinone [32]. The comparison of the  $\varphi_{\Delta}$  values allows to establish some remarks:

- (i)  $\varphi_{\Delta}$  value depends slightly on the nature of the solvent since in most cases (HAH<sub>2</sub>, HBH<sub>2</sub>, HM) the  $\varphi_{\Delta}$  variation according to the solvent is in the same order of magnitude of the experimental error for the determination of the parameter (less than 10%). Moreover, the  $\varphi_{\Delta}$  HAH<sub>2</sub> and HBH<sub>2</sub> values are close to those of literature (e.g. in ethanol 0.94 and 0.86, in benzene 0.84 and 0.74 for HAH<sub>2</sub> and HBH<sub>2</sub> respectively [7,30]). On the other hand, for Hypericin, a larger difference is observed,  $\varphi_{\Delta}$  value in acetonitrile (0.25) being weaker to that determined in ethanol (0.35). This influence of the nature of the solvent for this photosensitizer has been previously observed. It has been suggested that a supplementary deactivation channel can be involved in aprotic solvent (acetonitrile) due to the formation of intramolecular bond between a CO group and the nearest OH function

[27]. This particular behavior is not observed for HM since the methylation of the OH groups precludes the formation of the intramolecular hydrogen bond.

- (ii) As a result, two series of photosensitizers can be distinguishable according to the efficiency of <sup>1</sup>O<sub>2</sub> production. Both Hypocrellin's presents higher  $\varphi_{\Delta}$  value [33] whereas HypH<sub>2</sub> and HM exhibit poorer efficiency towards <sup>1</sup>O<sub>2</sub> production. One can suggest that for Hypocrellin's, the reaction between molecular oxygen and the triplet state of PSS leads mainly to the formation of singlet oxygen. On the other hand, for HypH<sub>2</sub> and HM, this process appears less efficient. This weak  $\varphi_{\Delta}$  value could be due to an additional channel involving the triplet state reactivity of the photosensitizer, such as electron or proton transfer (exclusively for HypH<sub>2</sub>) steps as previously evoked by English et al. [34].
- (iii) Concerning the comparison of HAH<sub>2</sub> and HBH<sub>2</sub>, both Hypocrellin's are efficient photosensitizers since they present a quantum yield of internal system conversion and of singlet oxygen closed to the unity. The weak difference between these two photosensitizers towards the  $\varphi_{\Delta}$  value, can be due to the nature of the linker (R<sub>2</sub>, Fig. 1), moreover these values are in good accordance with that reported by Zang et al. [30] which determined  $\varphi_{\Delta}$  by E.P.R. using 2,2,6,6-tetramethylpiperidine as <sup>1</sup>O<sub>2</sub> trapping agent.

### 3.2. <sup>1</sup>O<sub>2</sub> formation in basic condition

In basic ethanolic solution (by addition of saturated NaOH in ethanol)  $\varphi_{\Delta}$  values have been determined (Table 1). It emerges from these values that although HAH<sub>2</sub>, HBH<sub>2</sub> and HypH<sub>2</sub> exhibit close acid–base properties, the formation of anionic forms of the photosensitizers leads to difference of efficiency towards <sup>1</sup>O<sub>2</sub> production. For Hypericin under its di-deprotonated form (Hyp<sup>2-</sup>), singlet oxygen is entirely inhibited whereas, for HypH<sup>-</sup> and HypH<sub>2</sub> the same experimental parameters are obtained ( $\varphi_{\Delta} = 0.35$ ). On the other hand, for Hypocrellin's the  $\varphi_{\Delta}$  variation versus the strong basic character of the medium is not significant (e.g.  $\varphi_{\Delta}$  HAH<sub>2</sub> = 0.88 compared to  $\varphi_{\Delta}$  HAH<sup>-</sup> and HA<sup>2-</sup> = 0.85). This particular behavior of the dianionic form of Hypericin can be due to the inhibition of formation of the triplet state of the photosensitizer [25,35] in agreement with the absence of triplet spectrum; same experimental observations are done in aqueous micellar medium at pH more than 12 [36]. We previously suggested that this particular behavior is due to a rapid deactivation of the singlet state of Hypericin [2,36]. For HAH<sub>2</sub> and HBH<sub>2</sub>, triplet–triplet absorption is detected in strong basic ethanolic solution (by addition of an excess of NaOH) (Fig. 2) or in micellar aqueous solution for pH ranging from 4 to 12 (data not shown). For HBH<sub>2</sub>, in neutral condition transient absorption spectra close to that described by Wheng et al. [37] are obtained with the specific

Table 1  
Values of the quantum yield of singlet oxygen

	Acidic forms	$\varphi_{\Delta}^a$ ( $\pm 0.05$ )	$\varphi_{\Delta}^b$ ( $\pm 0.05$ )
Perinaphtenone		1.0	1.0
Hypericin	HypH <sub>2</sub> , HypH <sup>-</sup> Hyp <sup>2-</sup>	0.35 ≪0.05	–
Hypocrellin A	HAH <sub>2</sub> HAH <sup>-</sup> , HA <sup>2-</sup>	0.88 0.85	0.79 –
Hypocrellin B	HBH <sub>2</sub> HBH <sup>-</sup> , HB <sup>2-</sup>	0.70 0.66	0.68 –
Methylated Hypericin	HM HM (+NaOH)	0.36 0.41	0.39 –

Values determined according to method I and/or II.

<sup>a</sup> In ethanolic solution.

<sup>b</sup> In acetonitrile solution.

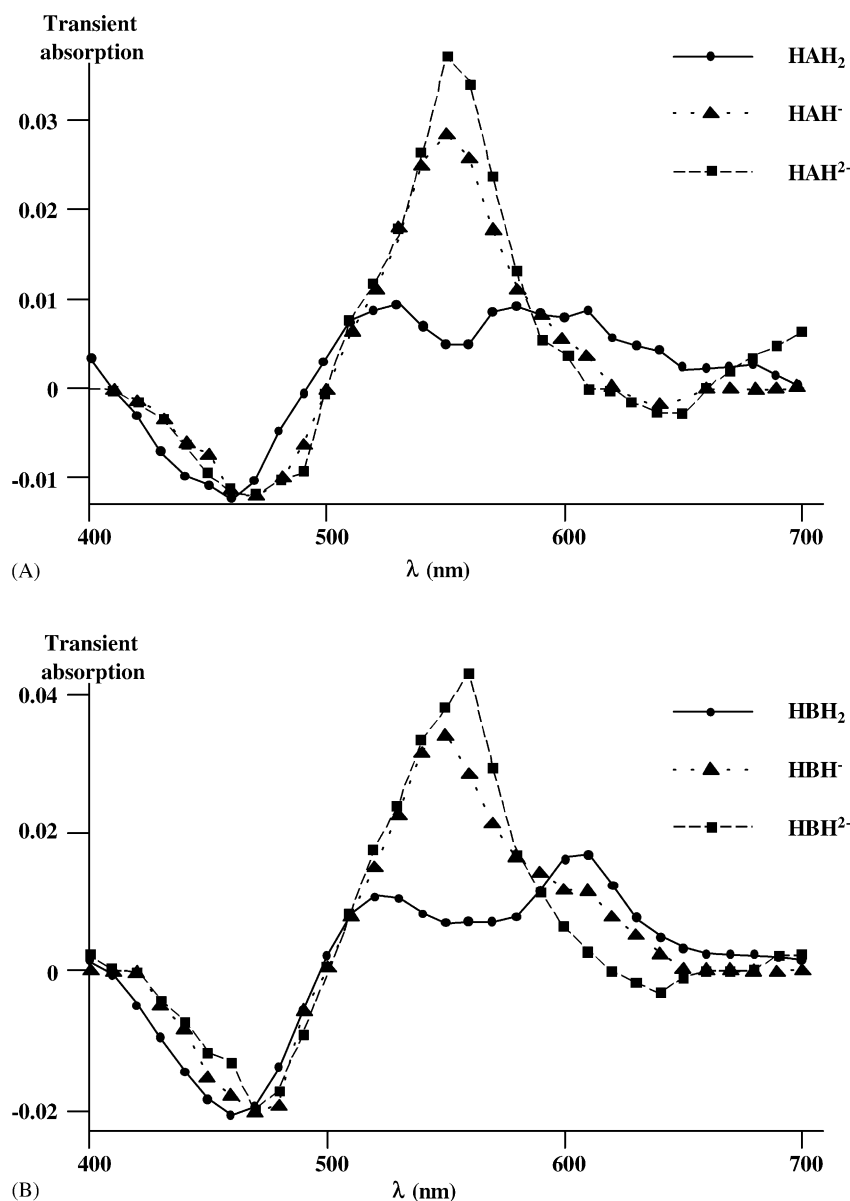


Fig. 2. (A) Transient difference absorption spectra of  $\text{HAH}_2$ ,  $\text{HAH}^-$  and  $\text{HA}^{2-}$ ; (B) transient difference absorption spectra of  $\text{HBH}_2$ ,  $\text{HBH}^-$  and  $\text{HB}^{2-}$ . Spectra recorded at delay time  $10 \mu\text{s}$ , from a deaerated ethanolic solution of photosensitizer ( $1.2 \times 10^{-5} \text{ M}$ ); mono- and di-anionic forms of PSS are produced by addition of one and two molecular equivalent of  $\text{NaOH}$ .

bands at 400, 530, 570 and 620 nm. Addition of  $\text{NaOH}$  in the ethanolic solution to produce  $\text{HBH}^-$  leads to the persistence of triplet–triplet absorption with some slight modifications (compared to the spectra in neutral condition), such as, the increase in magnitude of the band at 520 nm along with the decrease of that at 620 nm. In more basic condition ( $\text{HB}^{2-}$  form), the band at 620 nm becomes more intense (Fig. 2B). Concerning  $\text{HAH}_2$ , as illustrated by Fig. 2A, close modification are observed towards the basic character of the medium with a persistence of triplet–triplet absorption along with the anionic form of the photosensitizer. Thus, taken into account that the  $^1\text{O}_2$  formation requires the reactivity of the triplet state of PSS, singlet oxygen production should be precluded only for the di-deprotonated form of  $\text{HypH}_2$ . Concerning

$\text{HM}$ , since no labile proton is present in this molecule, the formation of the triplet state (Fig. 3) is not modified by addition of base, and thus  $\varphi_\Delta$  is independent of the acid–base character of the medium.

### 3.3. Comparison of triplet and $^1\text{O}_2$ yields

This study has been extended to the analysis of the mechanism of singlet oxygen formation. Since this process is effectuated by energy transfer from the triplet state of the photosensitizer and molecular oxygen, we have compared the yield of the triplet state of PSS ( $\varphi_T$ ) with  $\varphi_\Delta$  value, previously measured, by means of the determination of  $S_\Delta = \varphi_\Delta / \varphi_T$  (Table 2). Indeed, the value of the ratio  $\varphi_\Delta / \varphi_T$  should

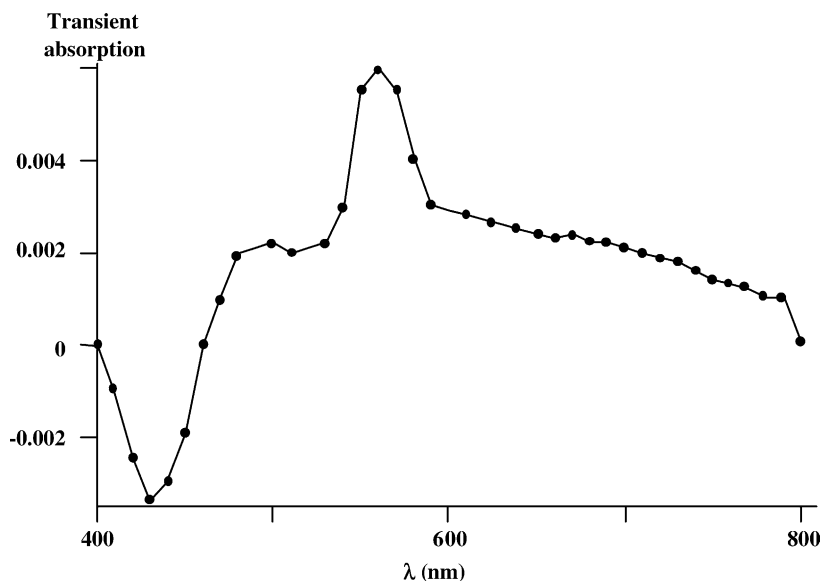


Fig. 3. Transient difference absorption spectra of HM recorded at delay time 100  $\mu$ s, from a deaerated ethanolic solution of HM ( $1.0 \times 10^{-5}$  M).

give us some information concerning the deactivation channel of the excited triplet state of the photosensitizers and about the process which occurs between molecular oxygen and the compounds. When the excited species does follow only one channel in air-saturated solvent (i.e. production of  $^1\text{O}_2$ ), the  $S_\Delta$  should be close to the unity while when several processes are competitive this ratio should be weaker.

One can remark that, as expected, in accordance with the persistence of triplet–triplet spectra,  $\varphi_T$  does not vary upon addition of base for no- and mono-deprotonated forms of PSS. As previously specified, significant  $\varphi_T$  variation is observed only in the case of the di-deprotonated form of HypH<sub>2</sub>.

In neutral condition, considering PSS under its native form (no-deprotonated), one can notice that the nature of the solvent has an influence on the  $\varphi_T$  value particularly for HypH<sub>2</sub>. Indeed,  $\varphi_T$  values for Hypocrellin's slightly depend on the protic character of the two solvents used (0.83 for HAH<sub>2</sub> and

0.70 for HBH<sub>2</sub> in ethanol and acetonitrile), whereas Hypericin presents two different values (0.71 in ethanol, 0.51 in acetonitrile for HypH<sub>2</sub> [27]). Moreover, considering the  $S_\Delta$  parameter, a discrepancy between HypH<sub>2</sub>, HM and HAH<sub>2</sub>, HBH<sub>2</sub> is observed. Concerning Hypocrellin's this value is close to the unity showing that singlet oxygen formation is very efficient. These results are consistent with those obtained by Diwu and Lown [38] which observed  $^1\text{O}_2$  production with high yield for both compounds, in ethanolic medium using 2,2,6,6-tetramethylpiperidine as singlet oxygen scavenger. For Hypericin's,  $S_\Delta$  values are significantly lower. This difference of behavior between these two families of photosensitizer could be explained. Indeed, contrary to HAH<sub>2</sub> and HBH<sub>2</sub>, the triplet excited state of Hypericin's can follow another additional deactivation channel, since, for HM and HypH<sub>2</sub>, it has been reported that irradiation can lead to proton [27,39] or electron transfer [40] without omitting the other possible deactivation way of the triplet state of the excited sensitizer.

(i) Considering the other possible deactivation way of the triplet state of the excited sensitizer, the low  $S_\Delta$  value for Hypericin cannot be explained in air-saturated solvent by such additive phenomenon. Indeed, in the absence of O<sub>2</sub>, triplet state of PSS is well observed ( $\tau_T = 100 \mu$ s for HypH<sub>2</sub>) whereas in presence of molecular oxygen, this excited species is not detected in our experimental conditions ( $\tau_T < 1 \mu$ s for both HypH<sub>2</sub> and HM). The very rapid deactivation of PSS\* in the presence of O<sub>2</sub> demonstrates the strong interaction between these two compounds. Meanwhile,  $kq$  value was not accurately accessible taken into account the quasi-absence of triplet–triplet spectra in aerobic condition ( $kq > 10^9 \text{ M}^{-1} \text{ s}^{-1}$ ). In the presence of molecular oxygen, the triplet state of the photosensi-

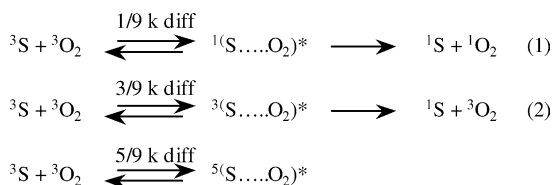
Table 2

Value of  $S_\Delta = \varphi_T/\varphi_\Delta$

		$\varphi_T (\pm 0.05)$	$\varphi_\Delta (\pm 0.05)$	$S_\Delta (\pm 0.1)$
Hypericine	HypH <sub>2</sub>	0.71 <sup>a</sup>	0.35 <sup>a</sup>	0.49 <sup>a</sup>
	HypH <sup>-</sup>	0.51 <sup>b</sup>	0.25 <sup>b</sup>	0.49 <sup>b</sup>
	Hyp <sup>2-</sup>	<0.05 <sup>a</sup>	<0.05 <sup>a</sup>	–
Hypocrelline A	HAH <sub>2</sub>	0.83 <sup>a,b</sup>	0.92 <sup>a,b</sup>	1.10
	HAH <sup>-</sup> , HA <sup>2-</sup>	0.83 <sup>a</sup>	0.85 <sup>a</sup>	1.02 <sup>a</sup>
Hypocrelline B	HBH <sub>2</sub>	0.70 <sup>a,b</sup>	0.69 <sup>a,b</sup>	0.98 <sup>a,b</sup>
	HBH <sup>-</sup> , HB <sup>2-</sup>	0.66 <sup>a</sup>	0.66 <sup>a</sup>	1.00 <sup>a</sup>
Methylated Hypericin	HM	0.96 <sup>a,b</sup>	0.36 <sup>a,b</sup>	0.37 <sup>a,b</sup>
	HM (+NaOH)	0.96 <sup>a</sup>	0.41 <sup>a</sup>	0.43 <sup>a</sup>

<sup>a</sup> In ethanolic solution.

<sup>b</sup> In acetonitrile solution.

Scheme 1.  ${}^1\text{O}_2^*$  formation with PSS\*.

tizer forms a complex in three state of spin (Scheme 1). In this process, Redmond and Braslavsky [41] demonstrated that the reaction (1) leading to singlet oxygen is more rapid than the other way of deactivation. Thus, in these experimental conditions, the other deactivation way of the triplet state should be considered as a minor competitive process.

- (ii) Concerning the photochemically induced proton transfer, HM and HypH<sub>2</sub> do not present important differences (versus  $\varphi_T$  and  $S_\Delta$ ) (e.g.  $S_\Delta$  HM = 0.37 and  $S_\Delta$  HypH<sub>2</sub> = 0.49 in acetonitrile), when, HypH<sub>2</sub> bears acidic proton, and deprotonation of HM is chemically precluded. Thus, although photoinduced proton transfer process has been previously proposed [36], it appears that such phenomenon should not be preponderant in our experimental conditions since  $\varphi_T$  and  $S_\Delta$  are poorly connected with the acidic character of the photosensitizer.

Consequently, one can suggest that the particular behavior of HypH<sub>2</sub> and HM could be due to electron transfer between the excited form of the photosensitizer (PSS\*) and molecular oxygen as previously evoked [42,43]. This phenomenon can be decomposed in different steps. In a first time, the species diffuse allowing the formation of a contact complex  ${}^{1,3}(\text{PSS}^* \cdots \text{O}_2)$ . Then, an intramolecular electron transfer can occur in the excited complex to lead to an ion pair  ${}^{1,3}(\text{PSS}^{\bullet+} \cdots \text{O}_2^{\bullet-})$ . Finally, the complex can evolve to resituate the initial form  ${}^{1,3}(\text{PSS}^* \cdots \text{O}_2)$  and/or to lead by overall redox process to the separated radical ions PSS<sup>•+</sup> and O<sub>2</sub><sup>•-</sup> whose formation is thermodynamically ruled [42,43]. Thus, for a favored electron transfer, the redox potential of O<sub>2</sub>/O<sub>2</sub><sup>•-</sup> couple should be higher than that of the oxidized PSS/PSS\* redox system, O<sub>2</sub><sup>•-</sup> formation paralleling the  $\Delta E^\circ$  values. Moreover, taken into account that the limiting step of this overall redox step is the charge separation process to lead to the pair ion, the energy of  ${}^{1,3}(\text{PSS}^{\bullet+} \cdots \text{O}_2^{\bullet-})$  should be weaker to that of  ${}^{1,3}(\text{PSS}^* \cdots \text{O}_2)$ . This energy can be estimated as follows. The energy of the charge transfer complex  ${}^{1,3}(\text{S}^{\bullet+} \cdots \text{O}_2^{\bullet-})$  compared to the initial molecule can be calculated by the relation extracted from the literature [11] (Eq. (4)):

$$E_{\text{CT}} = E_{\text{S}}^{\text{ox}} - E_{\text{O}_2}^{\text{red}} - \Delta \quad (4)$$

where  $E_{\text{S}}^{\text{ox}}$ ,  $E_{\text{O}_2}^{\text{red}}$  and  $\Delta$  correspond to the oxidation potential of the sensitizer, the  $E^\circ$  value of O<sub>2</sub>/O<sub>2</sub><sup>•-</sup> redox system and the energy of coulombic interaction, respectively. In ad-

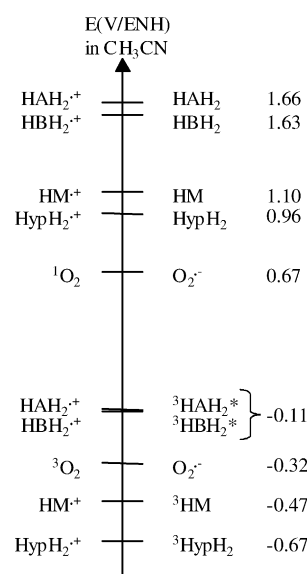
dition, the difference of energy between the triplet state of the compound and the complex of the charge transfer can be calculated considering the entropic variation as negligible [11], whereas the coulombic interaction  $\Delta$ , is estimated equal to 0.15 eV [43] (Eq. (5)).

$$\Delta H = \Delta G = E_{\text{S}}^{\text{ox}} - E_{\text{O}_2}^{\text{red}} - E_{\text{T}} - \Delta = E_{\text{CT}} - E_{\text{T}} \quad (5)$$

where  $E_{\text{T}}$  corresponds to the energy of the triplet state of the photosensitizer.  $E_{\text{S}}^{\text{ox}} - E_{\text{T}}$  is equal to the  $E_{1/2}$  of the redox couple PSS\*/PSS<sup>•+</sup> in the triplet state established by the Rehm-Weller relation.

The oxidation potentials of HypH<sub>2</sub> and HAH<sub>2</sub> are extracted from the literature [16,17]. The redox potential of HBH<sub>2</sub> and HM in acetonitrile medium have been determined by cyclic voltammetry (CV) [29]. For both HBH<sub>2</sub> and HM, CV performed in CH<sub>3</sub>CN + 0.1 M TBAP shares the same shape in the anodic region by the observation of an irreversible peak at E<sub>pa</sub> 1.63 V (Fig. 4) and 1.10 V/NHE for HBH<sub>2</sub> and HM, respectively. In the cathodic region, the first one reversible one-electron reduction appears at  $E^{1/2} = -0.27$  V for HBH<sub>2</sub> (Fig. 4) whereas for HM this reduction is observed at  $E^{1/2} = -1.12$  V. The comparison of the electrochemical behavior of HM and HypH<sub>2</sub> shows, as expected, that the presence of six methyl donor groups induced an important shift towards the more negative value of both reduction and oxidation process for HM. It should be noticed that for the four PSS's, addition of base in the medium (collidine) does not induced modification of E<sub>pa</sub> and  $E^{1/2}$  potential values. The values are summarized in Scheme 2 including the reduction potential of O<sub>2</sub> (−0.32 V/ENH in acetonitrile [40,42]).

In the diagram of energy corresponding to the complex of contact and to that of charge transfer (Scheme 3), the values of energy are referred to the ground state  ${}^3(\text{PSS} \cdots \text{O}_2)$

Scheme 2. Oxidation potentials in the ground and excited states of the compounds and reduction potential of  ${}^3\text{O}_2$  and  ${}^1\text{O}_2^*$ .

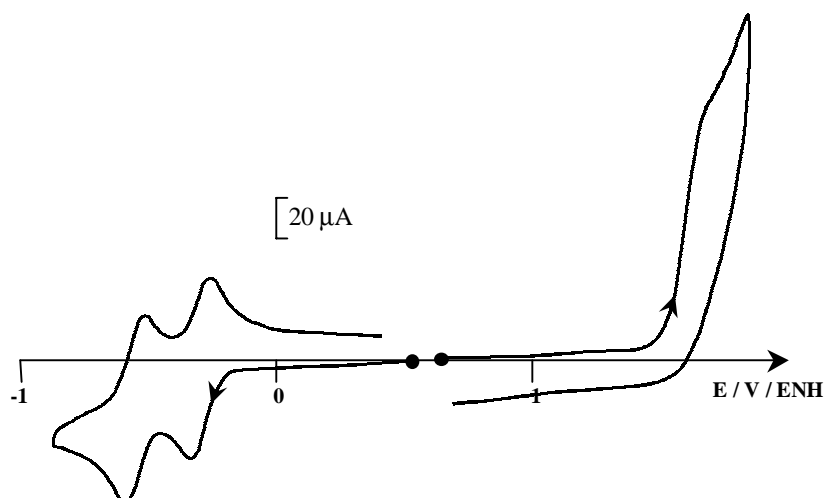
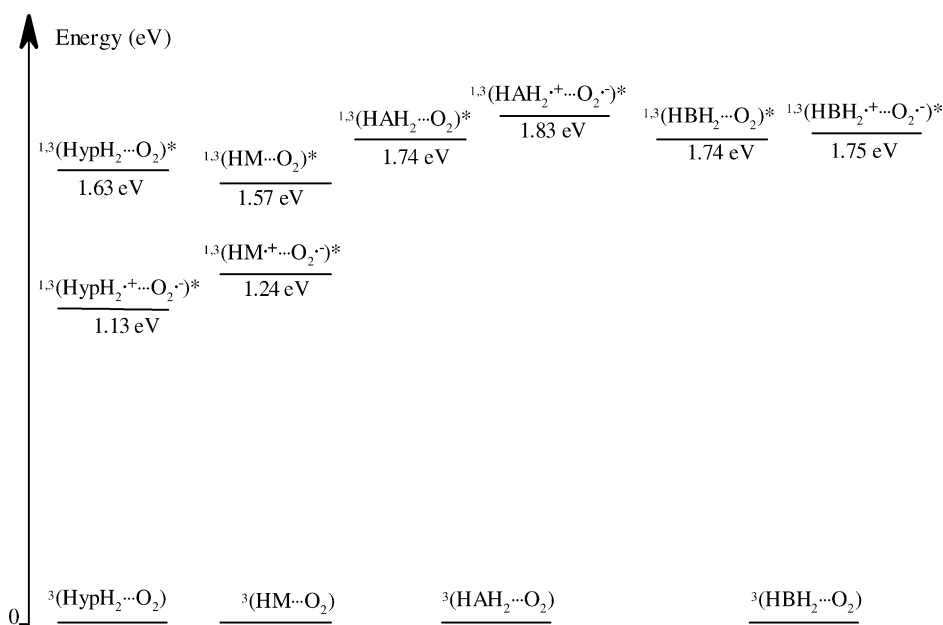


Fig. 4. Cyclic voltammetry of HBH<sub>2</sub> (10<sup>-4</sup> M) in CH<sub>3</sub>CN + 0.1 M TBAP on a platinum electrode (diameter 5 mm), scan rate 100 mV/s.

(assimilated to the origin of the diagram). The energy of the exciplex  $^{1,3}(\text{PSS} \cdots \text{O}_2)^*$  is assimilated to the energy of the sensitizer according to Garner et al. [42] and the energy of the complex of the charge transfer  $^{1,3}(\text{PSS}^{\bullet+} \cdots \text{O}_2^{\bullet-})$  is calculated using the relation (4). As an evidence on this diagram, the formation of a charge transfer complex is thermodynamically feasible for HypH<sub>2</sub> and HM ( $\Delta G < 0$ ). On the other hand, for HAH<sub>2</sub> and HBH<sub>2</sub>, the formation of this complex is endothermic ( $\Delta G \geq 0$ ) showing that  $\text{O}_2^{\bullet-}$  formation should not be observed considering thermal data, for HAH<sub>2</sub> and HBH<sub>2</sub>, whereas the formation of the superoxide anion should be detected for HypH<sub>2</sub> and HM. In the same way, considering the overall redox process,  $E^\circ$  of  $\text{O}_2/\text{O}_2^{\bullet-}$  redox couple has to be higher to that involving oxidation of

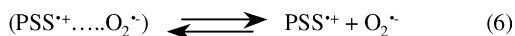
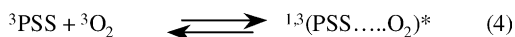
PSS\* (i.e.  $E^\circ \text{PSS}^{\bullet+}/\text{PSS}^*$ ), to afford to the formation of superoxide anion. As illustrated in Scheme 2, such process is authorized for HypH<sub>2</sub> and HM whereas for HAH<sub>2</sub> and HBH<sub>2</sub>,  $\text{O}_2^{\bullet-}$  formation should not be observed.

This thermodynamic aspect could give the explanation of the weak  $S_\Delta$  value obtained in the case of HypH<sub>2</sub> and HM since the possible deactivation channel of the triplet state in aerobic condition can take its origin from redox phenomenon to give  $\text{O}_2^{\bullet-}$  and oxidized PSS. In this context, transient absorption experiments have carried out in order to observe the radical cation of PSS issued from this redox process. For HM and HypH<sub>2</sub> transient absorption experiments do not allow the observation of such typical species. Although, the radical cation of PSS appears to be poorly stable



Scheme 3. Energy diagram corresponding to the complexes of contact and to the complexes of charge transfer.



Scheme 4. Electron transfer between PSS\* and O<sub>2</sub>.

as illustrated by the high irreversibility of the redox process (e.g. CV), and difficult to detect in organic solvents owing to its strong oxidizing character, at short time experiment this radical species should be detected. Indeed, using transient absorption experiments, in particular conditions (i.e. on TiO<sub>2</sub>) Wu et al. [44] has identified the formation of PSS<sup>•+</sup>, whereas in most common conditions (in DMSO medium), we showed that using quinone derivatives acting as electron acceptor, irradiation affords the unambiguously observation of PSS<sup>•+</sup> detected at 710 nm [40]. High rate electron transfer has been measured for electron acceptors having  $E_{1/2}$  value ranging from 0.33 V to -0.50 V/ENH ( $kq > 10^7 \text{ M}^{-1} \text{ s}^{-1}$ ) although the back electron transfer between PSS<sup>•+</sup> and the reduced form of the electron acceptor, to lead to the starting compound, is thermodynamically favored. It should be noticed that for 1,4-dihydroxyanthraquinone whose  $E_{1/2}$  value is equal to that of O<sub>2</sub>/O<sub>2</sub><sup>•-</sup> redox system  $kq$  has been estimated to  $1.8 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$  showing that, using such oxidant, the electron transfer is exothermic. In this context, the absence of observation of PSS<sup>•+</sup> in our experimental conditions could be due to the kinetic forbiddenness of the process. Although HypH<sub>2</sub><sup>\*</sup> (and HM<sup>\*</sup>) strongly interacts with O<sub>2</sub> (Scheme 4, step 4), to lead to a complex of contact (displayed by the strong decrease of  $\tau_T$ ), it appears that the exothermic intramolecular electron transfer is rather slow. One can suggest that the charge separation process (step 6) is kinetically unfavored and does not allow the production of radical species, this intermediate complex quickly evolving to reconstitute the starting compounds at the ground state (equilibriums 4 and 5), presumably contributing to the low  $S_{\Delta}$  value.

Thus, in our experimental conditions O<sub>2</sub><sup>•-</sup> formation should not take its origin from a photoredox process involving PSS\* and O<sub>2</sub> particularly for HypH<sub>2</sub> and HM. Therefore, as previously displayed in numerous papers the presence of additive substrate (i.e. electron donor) should be required to produce superoxide anion with high efficiency.

#### 4. Conclusion

Although the series of photosensitizers studied presents important structural similitude, it has been demonstrated that under aerobic conditions, the excited forms of these photosensitizers can evolve following different processes. In the case of Hypocrellin's, the energy transfer between molecular oxygen and excited species leads only to the

formation of singlet oxygen with high yield. On the other hand, in the case of Hypericin's, two competitive bimolecular processes should be considered. Indeed, the quenching of the triplet state of Hypericin's by molecular oxygen could lead to both <sup>1</sup>O<sub>2</sub> and O<sub>2</sub><sup>•-</sup>. Considering the redox process which is thermodynamically ruled the formation of superoxide anion should be accompanied with the formation of an electron oxidized PSS<sup>•+</sup>. Since the radical cation has not been detected, one can suggest that this exothermic process is inhibited by kinetic consideration leading to the restitution of the initial compound at the ground state. Thus, O<sub>2</sub>/PSS\* interaction leads essentially by energy transfer to singlet oxygen or to the starting material by an intersystem conversion. Hypocrellin's are better photosensitizer of singlet oxygen than Hypericin's. Concerning the formation of superoxide anion often observed through irradiation, other redox processes should be taken into account allowing the formation of the intermediate reduced PSS<sup>•-</sup> which in the presence of molecular oxygen can act as reducing agent. Thus, the physico-chemical properties of the photosensitizer are strongly dependent with the nature of the environment.

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