

Theoretical study of hypericin

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Abstract

Electronic properties and phototoxic reactions of the hypericin molecule are explored by the use of density functional theory, integral equation formalism polarised continuum model (to include solvent effects) and a time-dependent formalism for the study of singlet and triplet excited states. Our structural and vibrational data are in very good agreement with previous calculated and experimental results. The results suggest a hypericin (triplet state) reduction and subsequent electron transfer from the drug to molecular oxygen in aqueous solution. The most probable source of singlet molecular oxygen is a transfer of triplet excitation energy from hypericin to molecular oxygen. Direct radiation induced electron transfer between hypericin and oxygen is also explored, as well as the effect of triplet excitation on hypericin auto ionization reactions.

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1. Introduction

The hypericin molecule (Fig. 1) is a natural photoactive pigment which plays a role as an effective photoreceptor [1] in some plants of the *Hypericum* species (the most common of which is Saint John's Wort) and some insect species. The molecule can also be synthesized [2–4] for example by the condensation of two derivatives of anthraquinone [5–7].

Hypericin has long been used in traditional medicine, for example in the treatment of depression and wound healing. The photodynamic effects of *Hypericum* were reported in the beginning of the 20th century. Ingestion of hypericin containing plants by grazing animals has been shown to cause “hypericium syndrome”, a condition of severe sensitivity to light. This photosensitising effect causes severe skin irritation, elevated body temperatures and in extreme cases death of the animal [8].

Recent interest in the hypericin molecule is a result of the discovery of its pharmaceutical potential as antiviral, antiretroviral, antibacterial, antipsoriatic, antidepressant and antitumor agent [9,10]. Hypericin displays light-induced activity in vitro [11–14] and in vivo against several types of viruses (especially enveloped viruses like human immunodeficiency virus, HIV) and its anticancer activity has been demonstrated both on tumour cell lines [15–18] and in vivo [19,20]. The compound can also exhibit anticancer activity in the dark [12,21], which however is enhanced more than hundred fold in the presence of light [16,22].

Several mechanisms have been proposed for the photodynamic activity following irradiation of hypericin, including oxygen-dependent type I and type II reactions (resulting in formation of superoxide radicals and singlet oxygen, respectively), and photoinduced excited state proton or hydrogen atom transfer [23–26].

Although the antiviral and antitumor activities of hypericin and its mode of action have been extensively studied, the mechanism by which it acts still remains largely unresolved. The singlet oxygen quantum yield from hypericin is

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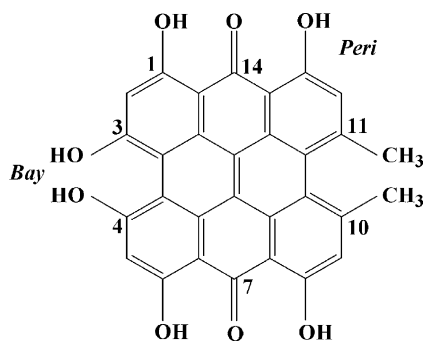


Fig. 1. Hypericin molecule.

lower [27] than the value (0.73) in organic solution initially proposed by Jardon and co-workers [28]. Superoxide anion, the hypericin radical anion ($\text{Hyp}^{\bullet-}$) and/or the radical cation ($\text{Hyp}^{\bullet+}$) have been proposed as direct or indirect candidates responsible for the photobiological activity of hypericin, and electron-transfer quenching of the fluorescent singlet state has been observed.

The energy of the triplet state of hypericin (^3Hyp) has been estimated in condensed media from the position of the 0–0 vibronic band of the phosphorescence spectrum in ethanol to be $13,190\text{ cm}^{-1}$ at 1.2 K [29] and $13,250\text{ cm}^{-1}$ at 77 K [30], $13,700\text{ cm}^{-1}$ in ethanol [31], and $13,300\text{ cm}^{-1}$ in acetonitrile and THF [23]. Previous theoretical studies of hypericin involve ab initio geometry optimizations and conformational studies of hypericin and deprotonated hypericin [10,32,33]. It has been shown that the most stable tautomers of hypericin are those with carbonyl groups in positions 7 and 14 (as depicted in Fig. 1) [33]. The most stable neutral and anionic conformers of this tautomer are bay H-bonded for the neutral case and bay region deprotonated for the anion, respectively [10,31,34].

In order to obtain a better knowledge of the photodynamic mechanism of hypericin, it is crucial to understand its physical, chemical and biological properties, including the interaction between the excited form of the sensitizer and O_2 [35]. In this sense, accurate computational calculations on well defined species can be a very valuable complementary tool to experimental studies, as they allow for determination of properties and discrimination between mechanisms at a molecular and electronic level. In this work we have applied hybrid density functional theory methods to the study of various ground and excited state properties of hypericin, with the aim to provide further insight into the possible processes that may govern the production of singlet oxygen and superoxide anion. The methodology has previously proven to yield results of high quality and reliance both for hypericin [10] and in the study of photodynamic properties [36].

2. Theoretical approach

All structures have been optimized without symmetry constraints using the Becke three-parameter hybrid functional

(B3LYP [37]) method, which is well known to provide accurate ground state geometries, in combination with the 3-21G(d) [38] and 6-31G(d) [39] basis sets. The large size of hypericin (54 atoms and 260 electrons) imposes constraints as to how large basis sets that are manageable in the quantum chemical computations and thus the frequency calculations were performed at the lower level of theory. Data used for estimation of standard Gibbs energies, ionization potentials, electron affinities, and excitation energies were calculated at the B3LYP/6-31 + G(d,p) level based on the B3LYP/6-31G(d) geometries. The use of diffuse functions is known to be essential for accurate determination of the energetics, particularly for anionic and excited states. Vertical excitation energies were computed in the time-dependent [40] density functional theory (TD-DFT) formalism [41,42], using the optimized ground state geometries. TD-DFT in combination with the B3LYP hybrid functional and the 6-31 + G(d,p) basis set has previously been shown to provide accurate energies to within $\sim 0.2\text{ eV}$ (5 kcal/mol) [36,43,44].

The effect of the solvent was included through B3LYP/6-31 + G(d,p)//B3LYP/6-31G(d) single-point calculations using the integral equation formalism of the polarised continuum model (IEF-PCM) of Tomasi and co-workers [45,46]. Two different values for the dielectric constant were used, $\epsilon = 78.39$ and $\epsilon = 4.34$ (corresponding to bulk water and a hydrophobic environment, respectively). The bulk effects have previously shown to be negligible for excitations [36], and were not included in the calculations of excitation energies. All calculations have been performed with the Gaussian03 program package [47].

3. Results and discussion

3.1. Structure and energetics

The two different neutral hypericin conformers differ by the presence (Hyp I) or absence (Hyp II) of an intramolecular bond in the bay region (cf. Fig. 2). The structures of both these conformers, and their respective radical anions and radical cations, and the bay deprotonated anion (Hyp III) were optimized.

Some key structural parameters are presented in Table 1. Overall a very good agreement is observed between the currently optimized structures, previous theoretical work, and experimental data. As seen in Fig. 2, the hypericin molecule has a slightly distorted structure defined by the two dihedral angles $D_1(\text{C3-C-C-C4})$ and $D_2(\text{C10-C-C-C11})$ (Fig. 1), caused by interactions between the $-\text{OH}$ and $-\text{CH}_3$ groups, respectively. The presence of the intramolecular hydrogen bond in Hyp I causes a slight reduction of D_1 compared with the Hyp II conformer. Deprotonating the bay region reduces the repulsive distortion further, $D_1 = 16.2^\circ$ instead of 25.8° . On the other hand, the distortion on the methyl side ($D_2 = 33.0^\circ$ and 33.7°) is essentially unaffected by these changes.

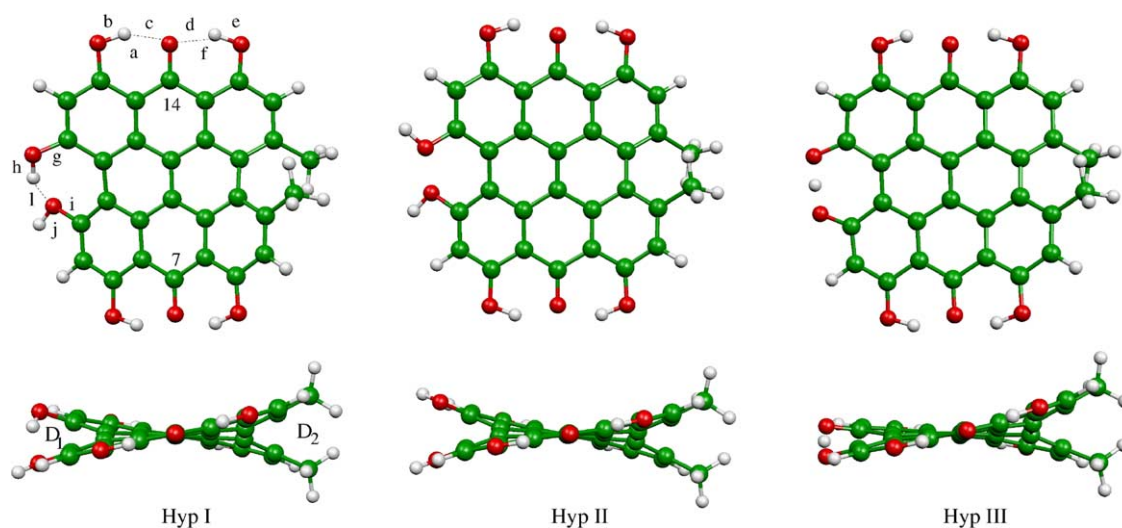


Fig. 2. B3LYP/6-31G(d) optimized structures of the hypericin molecule (I, II, and III).

Compared to experimental data, the B3LYP/3-21G(d) data (not shown) was found to slightly overestimate bond distances, with the exception of the O...O distances (*a* and *f*). B3LYP/6-31G(d) on the other hand gave results in close agreement with experimental data (2.553 Å), $d(\text{O}\cdots\text{O})=2.542$ and 2.553 (Hyp I) and 2.549 and 2.559 (Hyp II). At B3LYP/6-311G(d,p) level this distance is 2.581 Å [10]. The O...O distances *a* and *f* agree well with the requirements for adiabatic proton transfer (~ 2.5 Å). Our results for conformer Hyp I are in very good agreement with those previously obtained by Etzlstorfer and Falk [33], and by Uličný and Laaksonen [10]. The intramolecular hydrogen bonds *c* and *d*, 1.638 and 1.651 Å, are in the range (between 1.5 and 1.8 Å) predicted by Etzlstorfer and Falk [33]. Again a very good accord is obtained for the C–O, O–H and O...H distances when comparing with the ones reported by Etzlstorfer and Falk [33] ($h=0.96$, $i=1.377$, and $l=1.70$ Å),

and by Uličný and Laaksonen [10] ($g=1.350$, $h=0.972$, $i=1.377$, $j=0.977$, and $l=1.670$ Å). Hyp II has exactly the same structure as that predicted by Petrich and co-workers [32] some years ago. The effect of deprotonation at the bay region is a shift of the peri hydrogens towards the central carbonyls, and that the remaining bay hydrogen attains a strong bond to both the hydroxylic oxygens ($R_{\text{H}-\text{O}}=1.175$ Å). This markedly lowers the torsional deformation, D_1 from 25.8 to 16.2° (Fig. 2). A similar structure was suggested by Uličný and Laaksonen [10] and by Etzlstorfer and Falk [33].

Energetically the most stable neutral hypericin is the one with a hydrogen bond (Hyp I) in the bay region with the non-bonded conformer ~ 3.7 kcal/mol higher in energy.

Evaluation of the different redox processes involving solvated electrons and protons ($e^-_{(\text{aq})}$ and $\text{H}^+_{(\text{aq})}$) are listed in Fig. 3, based on the scheme developed by in our group [48].

The standard Gibbs energy balance of reduction (Eq. (1)), suggests, that it is easier to reduce Hyp I, -42.7 kcal/mol, than Hyp II, -40.5 kcal/mol.

Table 1
Structural parameters of Hyp (I, II, and III)

Distances	B3LYP/6-31G(d)			Previous work		
	(I)	(II)	(III)	(I) [33]	(I) [10]	(III) [10]
O...O (<i>a</i>)	2.542	2.549	2.520		2.590	
O–H (<i>b</i>)	0.999	1.001	1.002	0.99		
O...O (<i>c</i>)	1.638	1.642	1.599	1.5–1.8		
H–O (<i>d</i>)	1.651	1.659	1.608	1.5–1.8		
O...O (<i>e</i>)	0.999	0.998	1.004	0.99		
O...O (<i>f</i>)	2.553	2.559	2.529		2.590	
C–O (<i>g</i>)	1.350	1.353	1.297		1.350	1.297
O–H (<i>h</i>)	0.971	0.971	–	0.96	0.972	–
C–O (<i>i</i>)	1.377	1.353	1.297	1.377	1.377	1.297
O–H (<i>j</i>)	0.977	0.970	1.176	0.977	0.977	1.175
O...O (<i>l</i>)	1.660	–	1.175	1.70	1.670	1.175
Dihedrals						
D_1	25.8	29.8	16.2			
D_2	33.0	32.8	33.7			

Distances in angstroms and dihedrals in degrees. Experimental value of O–O (*a* and *f*) is 2.553 Å.

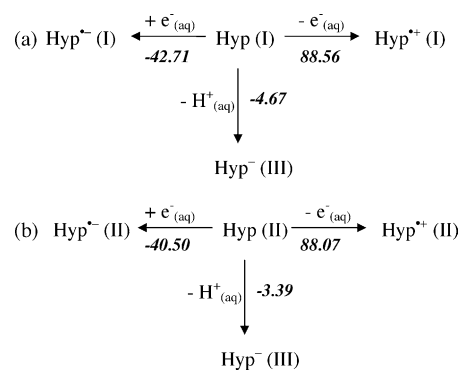
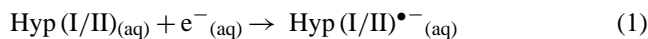


Fig. 3. IEF-PCM/B3LYP/6-31+G(d,p)//B3LYP/6-31G(d) standard Gibbs energies diagram (ΔG), in kcal/mol, of reduction, oxidation and deprotonation of Hyp I and II ((a) and (b), respectively), in aqueous solution.

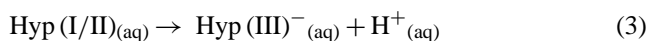
Table 2
Hyp I calculated vibrational frequencies in cm^{-1}

	HF/3-21G [32]	B3LYP/3-21G(d)	Exp. [30]
O–H str. (bay)	3500–3930	3554	~3600
C–H str.	3200–3400	3064–3265	
O–H str. (peri)		2602–2735	~2608
Backbone	80–1750	53–1720	143–1931
O–O vib.	400–500	410–510	

On the other hand, the standard Gibbs energies of oxidation (Eq. (2)), show that in aqueous solution, Hyp II is slightly more easy to oxidise ($\Delta G = 88.1$ kcal/mol) compared to Hyp I (88.6 kcal/mol).



The standard Gibbs energy change for the deprotonation reaction of Hyp I and Hyp II in aqueous solution were calculated by Eq. (3).



The deprotonation Gibbs free energy was -4.7 kcal/mol for Hyp I and -3.4 kcal/mol for Hyp II. As expected it is easier to extract the non H-bonded proton from Hyp I ($\text{O–H} = 0.977$ Å) than any of the hydroxyl protons in Hyp II ($\text{O–H} = 0.970$ Å).

3.2. Vibrational spectrum

Vibrational spectroscopy can provide valuable information to clarify structural details, which are otherwise inaccessible. The 156 normal vibrations (54 atoms) expected for hypericin are thus far mostly unassigned, with the exception of the characteristic hydroxyl and carbonyl vibrations [1]. In Table 2 we report the calculated unscaled B3LYP/3-21G(d) vibrational frequencies for Hyp I.

The highest calculated frequencies correspond to O–H stretches in the bay region (the *h* and *j* bonds of Fig. 2), ~ 3554 cm^{-1} , while the stretches of the C–H bonds in the rings correspond to 3265, 3215 cm^{-1} , and from the methyl groups 3064–3172 cm^{-1} , respectively. The values are in good agreement with previous results obtained with HF/3-21G

by Petrich and co-workers [32] (3500–3930 cm^{-1} for O–H stretches and 3200–3400 cm^{-1} for C–H stretches), and experimental data obtained by Arabei and co-workers [30] (~ 3600 cm^{-1}). The normal modes that correspond to peri-region O–H stretches (*b* and *e* in Fig. 2) were found in the 2602–2735 cm^{-1} range, similar to the average frequency interval found by Arabei and co-workers [30] (~ 2608 cm^{-1}) and to the calculated value 2600 cm^{-1} obtained by Uličný and Laaksonen [10]. The typical stretching vibration of a hydroxyl group is about 3600 cm^{-1} . In hypericin this band is however shifted to smaller wavenumbers due to the intramolecular hydrogen bonding. The vibrational modes corresponding to the internal breathing, bending, inversion, twisting and stretching of the aromatic backbone are in the 53–1720 cm^{-1} range (in good agreement with other studies [34]). In several cases these vibrations are coupled to other normal modes like C=O stretching motions, distortions of the methyl groups, O–H bending motions, and O–O vibrations [32]. We also found very clear O–O vibrations in the range of 410–510 cm^{-1} . The differences in vibrational frequencies between the two neutral forms of hypericin are less than ~ 20 cm^{-1} .

3.3. Formation of excited states

The photosensitizer reactions are initiated by excitation to the first excited singlet state (S_1) and intersystem crossing to the first excited triplet state (T_1) of hypericin. In Table 3 we report the excitation energies and oscillator strengths for the six lowest singlet and triplet excited states of Hyp I and deprotonated Hyp III. Since the $S_0 \rightarrow T_n$ transitions are spin-forbidden, the probabilities for these transitions are all zero.

The calculated first ($S_0 \rightarrow S_1$) and second ($S_0 \rightarrow S_2$) lowest singlet excitations are in very good agreement with the experimental data reported by Wynn and Cotton [8], $\lambda_{\text{max}} = 500$ –600 and 425–485 nm, and Arabei and co-workers [30], $\lambda_{\text{max}} = 590$ and 480 nm, respectively. The $S_0 \rightarrow S_1$ excitation has been assigned to the first $\pi \rightarrow \pi^*$ transition that is polarised along the molecular C_2 axis (the short axis of

Table 3
Vertical excited state energies (singlets and triplets) in eV and nm computed at the B3LYP/6-31 + G(d,p) level

Excited states	E_{0-0}	λ	f	λ_{exp}
S_1	2.23 (2.19)	556 (566)	0.244 (0.237)	500–600 [8], 590 [30]
S_2	2.76 (2.56)	450 (485)	0.166 (0.075)	425–485 [8], 480 [30]
S_3	2.86 (2.75)	433 (451)	0.048 (0.013)	
S_4	3.08 (3.01)	402 (412)	0.015 (0.025)	
S_5	3.19 (3.12)	388 (397)	0.022 (0.003)	
S_6	3.35 (3.24)	370 (383)	0.011 (0.152)	
T_1	1.62 (1.49)	765 (833)		730 [31], 752 [23], 754.8 [30]
T_2	2.03 (2.08)	611 (596)		
T_3	2.26 (2.31)	548 (538)		
T_4	2.53 (2.33)	491 (531)		
T_5	2.76 (2.68)	449 (462)		
T_6	2.97 (2.70)	418 (459)		

Data is given for Hyp I and in parentheses for Hyp III.

the hypericin molecular skeleton), and the second transition, perpendicular to the first, is the one polarised along the axis containing the carbonyl groups (the long axis of the hypericin molecule). The $S_0 \rightarrow S_1$ and $S_0 \rightarrow S_2$ transitions are the most probable ones ($f=0.244$ and 0.166), with the remaining showing much lower probabilities. The result for the lowest triplet excitation of Hyp I, 765 nm, is also in very good agreement to that observed experimentally by Arabei and co-workers [30] (754.8 nm), Jardon and co-workers [31] (730 nm), and Darmanyan and co-workers [23] (752 nm). The gap between $S_1 \rightarrow T_1$ is approximately 210 nm (0.62 eV). The differences in excitation energies between hypericin conformers Hyp I and Hyp II are in most cases less than 0.1 eV. Effect of deprotonation is to reduce $E_{0-0} \sim 0.1-0.2$ eV.

3.4. Oxygen-dependent type I reactions

In oxygen-dependent type I reactions the molecule is excited from the ground state (S_0) to the first excited singlet state (S_1), from which it can be interconverted to the first excited triplet state by intersystem crossing. The photosensitizer triplet state may be reduced by an electron donor, followed by electron transfer from the reduced photosensitizer to molecular oxygen, resulting in the formation of reactive superoxide anion radicals and regeneration of the photosensitizer in the S_0 ground state [36]. In Table 4 we list the computed ground state vertical and adiabatic electron affinities (VEA and AEA) of hypericin in different solvents, as well as the energy gain when reducing the lowest excited triplet state (VEA(T_1)).

The vertical electron affinities for Hyp I are in the range between 2.2 eV in vacuum to 3.4 eV in aqueous solution, whereas the adiabatic electron affinities are somewhat higher (2.3–3.5 eV). The difference between vertical and adiabatic electron affinities is a measure of the extent of radical anion relaxation, indicating how much the structure is modified by the addition of the negative charge (see discussion above). If Hyp I is first excited into the lowest triplet state (T_1), the vertical electron affinities increase to between 3.8 and 5.0 eV. The vertical electron affinity of the triplet, VEA(T_1), is a measure of the ability to extract an electron from the surroundings (the larger the energy gain for reduction of the T_1 state, the easier it is to extract an electron). As noted in several earlier studies, the EA increases significantly due to stabilization of the anionic species by the medium. After the reduction of hypericin, electron transfer to molecular oxygen will lead to the formation of the re-

active superoxide radical anion ($O_2^{\bullet-}$) and regeneration of hypericin (S_0).

The superoxide radical anion is in aqueous solution a precursor to hydrogen peroxide (H_2O_2), and consequently the hydroxyl radical (OH^\bullet). This reactive radical ($O_2^{\bullet-}$) can also act as a reducing agent, producing O_2 . When comparing the adiabatic electron affinity of Hyp I in aqueous solution (3.52 eV) and in vacuum (2.31 eV) with the calculated adiabatic electron affinity of oxygen in solution (3.91 eV) and in vacuum (0.59 eV) [36], we conclude that in aqueous solution, once the reduced drug is formed, electron transfer from the drug to molecular oxygen is associated with an energy gain ($\Delta_r E_{0k} = AEA(\text{Hyp}) - AEA(^3O_2)$), of about 0.4 eV. This is in agreement with experimental findings by Burel et al. [49]. The data for the triplet state indicates that this in turn will be able to quench superoxide anions (VEA(T_1) = 4.7–5.0 eV; VEA(O_2) = 3.91 eV). In vacuum, on the other hand, the oxidising power of O_2 is not sufficient to extract an electron from reduced hypericin.

3.5. Oxygen-dependent type II reactions

An excited state that is sufficiently long-lived to allow for intermolecular interactions is likely to be quenched by $O_2(^3\Sigma_g^-)$, and singlet oxygen photosensitizers are observed in virtually any chemical system where light is absorbed in the presence of molecular oxygen [50]. In type II reactions, the excitation energy is transferred from the triplet state of the sensitizer to molecular oxygen, forming singlet oxygen and regenerating the sensitizer in the ground state. Comparing the de-excitation energy of the Hyp I triplet state, 1.62 eV (see Table 3), with the energy needed to convert molecular oxygen, $O_2(^3\Sigma_g^-)$, to the reactive singlet state, $O_2(^1\Delta_g)$ (1.06 eV [36]), we conclude that probably the triplet state (T_1) excitation energy can be transferred from hypericin to molecular oxygen to generate singlet molecular oxygen. However given the strong EA of the T_1 state, this process will be in competition with formation of $\text{Hyp}^{\bullet-}$.

3.6. Direct electron transfer to $O_2(^3\Sigma_g^-)$

The direct electron transfer mechanism involves the ionization of the hypericin upon radiation and electron transfer from the drug to molecular oxygen. The extent of this ionization can be measured by comparing the vertical and adiabatic ionization potentials (VIP and AIP) of hypericin to the electron affinities of $O_2(^3\Sigma_g^-)$. In Table 5, computed data for vertical and adiabatic ionization potentials of the ground and triplet state of hypericin in different media are presented.

The difference between VIP and AIP express the relaxation of the cation (the larger this difference is, the more the structure is affected by the ionization). We observe that the difference between VIP and AIP (Table 5) is approximately 0.1 eV (2.3 kcal/mol), suggesting that the structural relaxation induced by the ionization of hypericin is very small. The reason for this is the rigidity of the aromatic ring structure,

Table 4

Vertical and adiabatic electron affinities, VEA and AEA, in eV, of the ground state of Hyp I (and Hyp II in parentheses) and of the lowest excited triplet state in different media at the B3LYP/6-31 + G(d,p) level

	VEA(T_1)	VEA	AEA
$\epsilon = 0$	3.78 (3.64)	2.16 (2.02)	2.31 (2.17)
$\epsilon = 4.34$	4.71 (4.60)	3.09 (2.98)	3.24 (3.12)
$\epsilon = 78.39$	4.99 (4.91)	3.37 (3.28)	3.52 (3.42)

$$\text{VEA}(T_1) = \text{VEA}(S_0) + {}^3E_{0-0}$$

Table 5

Vertical and adiabatic ionization potentials, VIP and AIP, in eV, of the ground state of Hyp I (and Hyp II in parentheses) and of the lowest excited triplet state in different media at the B3LYP/6-31 + G(d,p) level

	VIP(T ₁)	VIP	AIP
$\epsilon = 0$	5.45 (5.30)	7.07 (6.93)	6.95 (6.81)
$\epsilon = 4.34$	4.34 (4.22)	5.96 (5.84)	5.86 (5.73)
$\epsilon = 78.39$	3.98 (3.87)	5.60 (5.50)	5.50 (5.40)

$$\text{VIP}(T_1) = \text{VIP}(S_0) - {}^3E_{0-0}.$$

and thus possible structural reorganisations are very small. From Table 5 we can see that hypericin is easier to ionize the larger the dielectric constant of the medium; the inclusion of the water solvent reduces the VIP and AIP by about 1.5 eV (~ 56 kcal/mol).

Our data suggests that O₂ is basically not able to oxidize Hyp I, albeit the energy difference of the triplet state in aqueous solution is very small. For Hyp II, the AEA of O₂ is 0.04 eV higher than the VIP from T₁ state, indicating that Hyp II (T₁) can in principle produce superoxide directly.

3.7. Hypericin auto-ionization

When hypericin is excited to the first triplet state, this may be reduced by another hypericin molecule in the ground or the Hyp I triplet state. Reducing the Hyp I first triplet state there is an energy gain of 3.78 eV in vacuum, 4.71 eV in hydrophobic environment, and 4.99 eV in aqueous solution (VEA(T₁), Table 4). On the other hand, Hyp I VIP's are 7.07 eV in vacuum, 5.96 eV in hydrophobic environment and 5.60 eV in aqueous solution. Based on these results we conclude that it is unlikely that a triplet Hyp I can be reduced by ground state Hyp I. For the Hyp I triplet reduction by another Hyp I triplet, the energy gain for reduction (VEA(T₁), Table 4) and the VIP(T₁)'s (5.45 eV in vacuum, 4.34 eV in hydrophobic environment, and 3.98 in aqueous solutions, Table 5), suggest that in solution ($\epsilon = 78.39$ and 4.34) the electron transfer between two excited Hyp I molecules will be possible, leading to auto-ionization reaction. This, however, will require high local concentration of the photosensitizer.

4. Conclusions

We have in the present letter explored the energetics, geometries, vibrational spectra, and excitation spectra of hypericin molecules employing density functional theory, with inclusion of the IEF-PCM to mimic the effects of a solvent, and the time-dependent formalism for the study of singlet and triplet excited states. Our structural and vibrational data are in very good agreement with previous calculated and experimental results. Our study suggests that the hypericin triplet state is easily reduced and that there is an energy gain in electron transfer from the ionized drug to molecular oxygen in aqueous solution. In addition, other probable reactions involve triplet excitation energy transfer from hypericin to

molecular oxygen providing a source of singlet molecular oxygen, and direct oxidation of hypericin (T₁) by oxygen thereby generating superoxide anions. We also explored the effect of triplet excitations on hypericin auto-ionization reactions. It was concluded that hypericin is easier ionized when in aqueous solution than in hydrophobic environment or in vacuum, and that it is improbable that a triplet hypericin can be reduced by another molecule in the ground state. The data suggest that in solution ($\epsilon = 78.39$ and 4.34) electron transfer between two triplet excited hypericin molecules, leading to auto-ionization, is possible.

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