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A study of photoinduced electron transfer and redox properties of hypericin

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Abstract

Hypericin (HYP), is a derivative of polyhydroxy substituted perylenequinones. In acetonitrile, the fluorescence of HYP can be quenched by electron donor: *N,N*-diethylaniline (DEA) and acceptors: methyl viologen (MV), anthraquinone (AN) (less than 1.2×10^{-3} mol l^{-1}), and the quenching rate constants are K_q (DEA) $=3.52\times10^{10}$ l mol^{-1} s^{-1}, K_q (MV) $=7.16\times10^{11}$ l mol^{-1} s^{-1}, K_q (AN) $=1.05\times10^{11}$ l mol^{-1} s^{-1} respectively, indicating that hypericin can act either as an electron acceptor like quinones or as an electron donor like phenones. Hypericin in ground state maybe forms charge transfer complex with the higher concentrated anthraquinone which has a plane configuration. The stoichiometry is 1:2.6 (HYP:AN) determined by Job's photometric titration plots. The methyl viologen cation radical formed in the photoinduced interaction between hypericin and methyl viologen was also detected by the absorption spectrum.

Keywords: Hypericin; Photoinduced electron transfer; Charge transfer complex

1. Introduction

Hypericin (HYP), 7,14-dione-1,3,4,6,8,13-hexahydroxy-10,11-dimethyl-phenanthrol [1,10,9,8-opgra]perylene (Fig. 1), is a natural pigment isolated from Hypericum, a genus of plants widely distributed throughout the planet. Its closely related pigments have been also found in some Australian insects [1,2]. Its synthetic chemistry, photodynamic action and other physical activities have been investigated and reviewed [2]. Recently, substantial advances have been made on various aspects of hypericin. The most intriguing was its activities against cancers and the acquired immunodeficiency syndrome (AIDS) [3,4], although there are some disputes about the crucial role of light for in vivo and in vitro activities of hypericin. It is noteworthy that the activity of hypericin also occurs with exclusion of oxygen and in both the presence and absence of light, but is promoted by irradiation and an increase in concentration (without irradiation) [5].

Hypericin is polyhydroxy substituted quinone molecule with an extended π system which can act as a photosensitizer and as an efficient electron donor/acceptor, whereas its function as a sensitizer in the photochemical production of singlet oxygen and superoxide has been studied by several research groups [6–8]. Its electron-donating/accepting properties have received less attention [9,10]. It is well known that phenols are good electron donors while quinones act as an efficient electron acceptor, interestingly hypericin contains both phenolic and quinoid moieties. This structural characteristic places it in an unique class of amphi-electronic compounds. It can lead different electron transfer process with different substrates. In this paper we chose some typical electron acceptors/donors to investigate the photoinduced electron transfer interaction between them and hypericin.

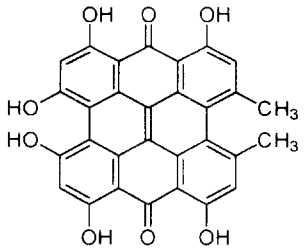

Fig. 1. Structure of hypericin.

2. Experimental details

2.1. Material

Hypericin was extracted from a Chinese herb, the structure was characterized by spectra of UV, IR, MS, ^{1}H NMR. ^{1}H

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Table 1
The redox potentials of hypericin and electron donors/acceptors and calculated data of the free energy changes of electron transfer reactions between hypericin and donors/acceptors and the rate constants of fluorescence quenching[a]

	$E_{(D^{+}/D)}$ (V)	$E_{(A/A^{-})}$ (V)	ΔG (eV)	K_q (l mol^{-1} s^{-1})
Sensitizer				
Hypericin	+0.93	−0.92		
Donors				
Diethylaniline	+0.56[c]		−0.66	3.52×10^{10}
Diphenylamine	+1.004[c]		−0.13	
Acceptors				
Methyl viologen		−0.44[c]	−0.77	7.16×10^{11}
Anthraquinone		−0.85[c]	−0.34	1.05×10^{11}
Tetrachlorobenzoquinone		+0.01[c]	−0.122	1.43×10^{10}[b]

[a] $E_{(D^{+}/D)}$ (V) and $E_{(A/A^{-})}$ (V) are the redox potentials (vs. SCE).
[b] Ref. [10].
[c] Ref. [14].

NMR (deuterated acetone) δ: 7.37 ppm (s, 2H), 6.64 ppm (s, 2H), 2.73 ppm (s, 6H). FAB mass spectrum (FAB MS): $|M-1|=503$. UV–Visible (acetonitrile): $\lambda_{max}=594$ nm and 550 nm. IR (KBr): 3424 cm^{-1}, 2923 cm^{-1}, 1619 cm^{-1}. All the data coincided with that of hypericin purchased from Atomergic Chemetals (HPLC grade). The purity was tested by TLC [11] and HPLC (C_{18} reversed-phase column, CH_3OH as eluate).

Anthraquinone (AN) was supplied by Chinese Academy of Military Medicine. Methyl viologen (MV) and *N,N*-diethylaniline (DEA) were bought from Shanghai biochemical pharmaceutical factory. Diphenylamine was from Beijing chemical factory. Acetonitrile was dried as in Ref. [12]. Other solvents were of analytical grade and only redistilled before use.

2.2. Spectrometric measurements

Absorption spectra were recorded on an UV-160A UV–Visible spectrophotometer (HITACHI). Fluorescence spectra were obtained with Perkin-Elmer LS-5 spectrometer. Fluorescence lifetime was determined using a HORIBN NAES-1100 single-photon counting apparatus. The samples for spectrometric measurements were 10^{-6} mol l^{-1} of sensitizer and 10^{-4} mol l^{-1} of quenchers and were purged with argon for 20 min before measurements. The photolysis was carried out with irradiation of an unfiltered 450 W high pressure sodium lamp (maximum emission is around 550 nm) in a 1-cm cuvette.

3. Results and discussion

It has been reported that the oxidation and reduction potential of hypericin are +0.93 V [13] and −0.92 V [9], respectively, indicating that hypericin can be either oxidized or reduced easily. In other words, it can either act as an electron donor or an acceptor depending on the presence of substrates in the system. The typical electron donors, *N,N*-diethylaniline (DEA), diphenylamine and acceptors, methyl viologen and anthraquinone were employed here to study the redox properties of hypericin.

3.1. Energetics

The redox potentials of hypericin and electron donors/acceptors are listed in Table 1 and the free energy changes ΔG of electron transfer reaction calculated with Rehm–Weller equation are also shown in Table 1.

Rehm–Weller equation [15]:

$$\Delta G = E_{(D^{+}/D)} - E_{(A/A^{-})} - \Delta E_{0-0} - C$$

$E_{(D^{+}/D)}$ is the oxidation potential of donor. $E_{(A/A^{-})}$ is the reduction potential of acceptor. ΔE_{0-0} is the singlet state energy of sensitizer (ΔE_{0-0} of hypericin is 2.08 eV determined by the overlap of the normalized absorption and the emission spectra). C is the solubilizing energy of $D^{+}A^{-}$ and usually regarded as 0.06 v in polar solvent [16].

Table 1 shows us that from the thermodynamic point of view, hypericin may act either as an electron acceptor when reacts with strong electron donors like *N,N*-diethylaniline or as an electron donor when reacts with electron acceptors like methyl viologen, and anthraquinone. In addition, the singlet state energy of all acceptors/donors we used here is less than that of hypericin, so when hypericin was excited, there was no energy transfer between hypericin and acceptors/donors.

3.2. Photoinduced interaction between hypericin and electron donors

There was no change in the shape of either the absorption or the fluorescence spectra of hypericin in the presence of *N,N*-diethylaniline in acetonitrile. This observation provides a good evidence for the absence of ground-state interaction between hypericin and *N,N*-diethylaniline. However, the fluorescence of hypericin in acetonitrile was effectively quenched by *N,N*-diethylaniline and followed the Stern–Vol-

mer (SV) relationship (the quenching curves are shown in Fig. 2):

$$\Phi_0/\Phi = 1 + K_{SV}[Q] = 1 + K_q\tau_0[Q]$$

Where Φ_0 and Φ are the relative fluorescence quantum yields of hypericin in the absence and presence of quencher [Q], respectively. τ_0 is the fluorescence lifetime of hypericin in the absence of quencher [Q] and K_q is the rate constant of fluorescence quenching. We can get the K_q (DEA) = 3.52×10^{10} l mol^{-1} s^{-1} from Fig. 2, indicating the electron transfer was mainly a dynamic process. Unfortunately, the fluorescence quenching of hypericin by the weaker electron donor, diphenylamine could not be detected in our experiment.

3.3. Photoinduced interaction between hypericin and electron acceptors

It has been reported that fluorescence of hypericin can be quenched by 2,3,5,6-tetrachloro-1,4-benzoquinone [10]. To study further the abilities of hypericin as an electron donor, we employed another typical electron acceptor, methyl viologen, as a quencher to react with hypericin. It was found that methyl viologen could effectively quench the fluorescence of hypericin, but there was no change in the shape of fluorescence spectrum. The fluorescence quenching followed the Stern–Volmer relationship (the quenching curve are shown in Fig. 2). The fluorescence lifetime quenching followed the relationship too (Fig. 2). The rate constant of fluorescence quenching K_q (MV) = 7.16×10^{11} l mol^{-1} s^{-1}.

The absorption spectrum of mixtures of hypericin–methyl viologen were the simple addition of the component's spectra, implying there was no ground state interaction between hypericin and methyl viologen. All of these indicated that the fluorescence quenching of hypericin by methyl viologen was mainly dynamic quenching process and the excited hypericin can donate an electron to stronger electron acceptor, methyl viologen.

3.4. Interactions of hypericin with anthraquinone of different concentrations

The fluorescence quenching of hypericin (3.0×10^{-6} mol l^{-1}) by anthraquinone, was different from that by *N,N*-diethylaniline and methyl viologen. When anthraquinone was in low concentration (less than 1.2×10^{-3} mol l^{-1}) in acetonitrile, the shape of the quenched fluorescence of hypericin was not changed and the fluorescence quenching followed the Stern–Volmer relationship. The fluorescence lifetime quenching was fit for the quenching curve, too. The rate constant of fluorescence quenching was $K_q = 1.05\times10^{11}$ l mol^{-1} s^{-1}. While the concentration of Anthraquinone became higher, the shape of the fluorescence spectra was changed (see Fig. 3). The peaks were blue-shift, the intensities were slightly lower. When the concentration of anthraquinone was up to 2.4×10^{-3} mol l^{-1}, the main peak was

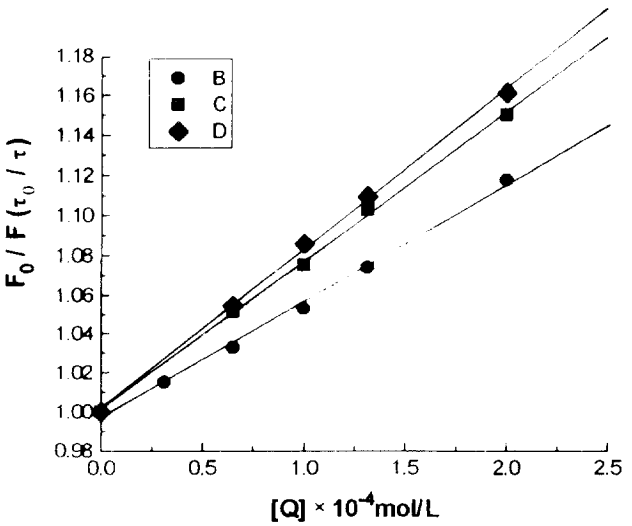


Fig. 2. Fluorescence quenching of HYP (in acetonitrile) by (B) DEA, (C) methyl viologen. (D) Fluorescence lifetime quenching of HYP by methyl viologen ([HYP] = 1.35×10^{-6} M, λ_{ex} = 450 nm, τ_0 = 5.5 ns).

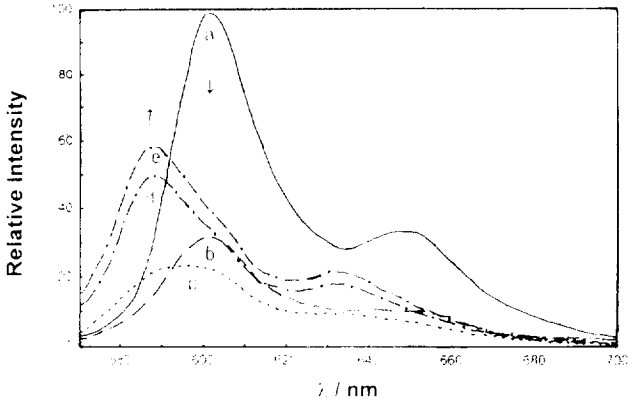


Fig. 3. Fluorescence spectra of HYP quenched by anthraquinone (in acetonitrile). Concentration of anthraquinone: (a) 0; (b) 6.0×10^{-4} mol l^{-1}; (c) 1.2×10^{-3} mol l^{-1}; (d) 2.4×10^{-3} mol l^{-1}; (e) 3.0×10^{-3} mol l^{-1}.

shift to 587 nm from 600.5 nm, and the intensity was increased. Following the concentration of anthraquinone went on higher, the shape of the fluorescence spectra was no longer changed, but the intensity kept going higher.

In acetonitrile, the absorption spectrum of hypericin has two main bands of λ_{max} = 594 nm and 551 nm respectively (see Fig. 4). When the concentration of anthraquinone was 6.0×10^{-4} mol l^{-1}, the two bands became lower, but the location was not changed. While the concentration of anthraquinone became higher, the absorbance of two bands at 594 nm and 551 nm kept going lower and meanwhile three new bands at shorter wavelength area, λ_{max} = 451 nm, 551 nm and 579 nm, emerged. The absorbance of three new bands went on higher with the further increasing of the concentration of anthraquinone.

Based on the observation of the changes of absorption and fluorescence spectra, we assumed that hypericin itself formed molecular aggregation because of the influence of anthraquinone when the concentration of it was 6.0×10^{-4} mol l^{-1}.

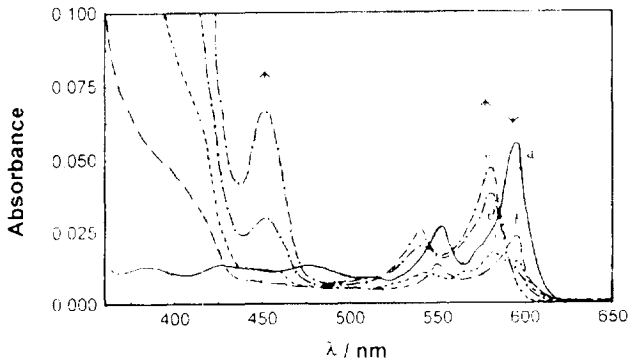


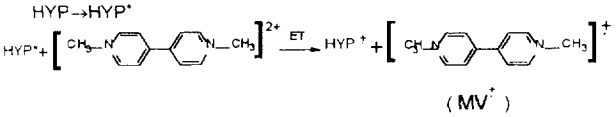
Fig. 4. Absorption spectra of HYP and anthraquinone in acetonitrile. Concentration of anthraquinone: (a) 0; (b) 6.0×10^{-4} mol l^{-1}; (c) 1.2×10^{-3} mol l^{-1}; (d) 2.4×10^{-3} mol l^{-1}; (e) 3.0×10^{-3} mol l^{-1}.

Karamos and Turro reported [15] that, in some solvents, hypericin intends to form aggregate easily, the absorption spectrum of the aggregate was the same as the monomer except the lower intensity of absorption, and there was no fluorescence emission from the aggregate. So in our experiment, absorption and fluorescence spectra were not changed in shape but were lower in intensity due to the formation of the aggregate.

When the concentration of anthraquinone was upper to 2.4×10^{-3} mol l^{-1}, the possibility leading an interaction between hypericin and anthraquinone became larger. Since hypericin is a derivative of naphthodianthrone with a plane configuration and anthraquinone also has a plane configuration, in high concentration solution, they may form charge transfer complex in ground state, which changed the shape of absorption spectra. This charge transfer complex emit different fluorescence from HYPs and changed the shape of the fluorescence of hypericin in quenching experiment. In fact, the stoichiometry of the complex is 1:2.6 (HYP:AN) determined by Job's photometric titration plots [17]. In general, it means that the complex of HYP:AN is a mixture of two complex HYP:2AN and HYP:3AN.

3.5. Detection of methyl viologen cation radical (MV^{+}) generated in the photoinduced electron transfer interaction between hypericin and methyl viologen by absorption spectra

From above-mentioned, we have proved that in acetonitrile methyl viologen can get an electron from excited hypericin, which can be presented by:



It is well know that cation radicals are usually not stable, while the methyl viologen cation radical (MV^{+}) generated from one electron reduction of methyl viologen is stable enough to be examined by absorption spectrum [18]. In the irradiation of the mixed solution of hypericin and methyl viologen with high pressure sodium lamp, only hypericin can be excited. With the proceeding of the photolysis, the absorption spectrum of the reaction solution changed. The differential absorption spectra between before and after the irradiation was measured (shown in Fig. 5, irradiated 15 s). The spectrum with bands of $\lambda_{max}=397$ nm and $\lambda_{max}=605$ nm is fit the well known spectrum of methyl viologen cation radical [19] very well. So it is also an evidence for that the excited hypericin gave an electron to the ground state methyl viologen, which produced the methyl viologen cation radical and the hypericin cation radical. The lifetime of the hypericin cation radical was too short to be examined, only the absorption spectrum of the methyl viologen cation radical was recorded in our experiment.

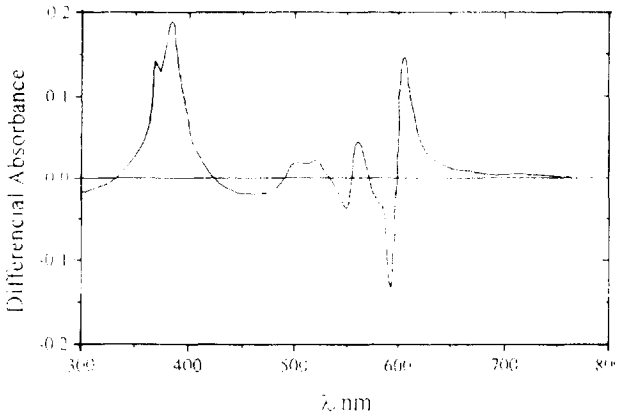


Fig. 5. Differential absorption spectra of photolysis of HYP and methyl viologen in acetonitrile (irradiated 15 s).

4. Conclusion

Strong electron donor and electron acceptor could quench the fluorescence of hypericin indicating that hypericin is an unique class of amphi-electronic compounds, it can either accept or donate an electron, depending on the presence of substrates in the system. Hypericin also could form charge transfer complex with plane configuration molecular which change the interaction mechanism.

Acknowledgements

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