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Intermolecular and Intramolecular Energy and Electron Transfer Reactions between Porphyrin and Fluorescein

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ABSTRACT

A porphyrin (TTP)–fluorescein (FL) heterodimer covalently linked with a flexible polyatomic chain has been synthesized and characterized. The rate constants and the efficiencies of the inter- and intramolecular energy and electron transfer processes were determined. Their UV-Vis absorption, steady-state and time-resolved fluorescence spectra were investigated. The UV–Vis absorption and ^1H *NMR spectroscopy suggest that there is some exciton coupling between the two chromophores in this system, whilst fluorescence spectroscopy shows that the FL unit transfers singlet-state excitation energy to the TTP. From time-resolved fluorescence studies,it is concluded that the heterodimer exists in solution in different, nonequilibrating, conformations. The effects of the solvent polarity on the intramolecular energy and electron transfer efficiencies are discussed. The results showed that on selective excitation of the FL chromophore, only a very efficient singlet state energy transfer process from FL to TTP was observed in different solvents, but on selective excitation of the TTP chromophore, only in a polar solvent (DMF) could the intramolecular electron transfer reaction occur.The difference of intramolecular interaction in solvents of various polarity may be explained in terms of conformational change due to the nature of solvent interaction. © 1997 Elsevier Science Ltd*

Keywords: Intermolecular, intramolecular, energy and transfer electron, porphyrin–fluorescein heterodimer.

INTRODUCTION

The most efficient primary electron donor in bacterial photosynthesis is a bacteriochlorophyll dimer, the 'special pair' [1]. Green-plant and bacterial

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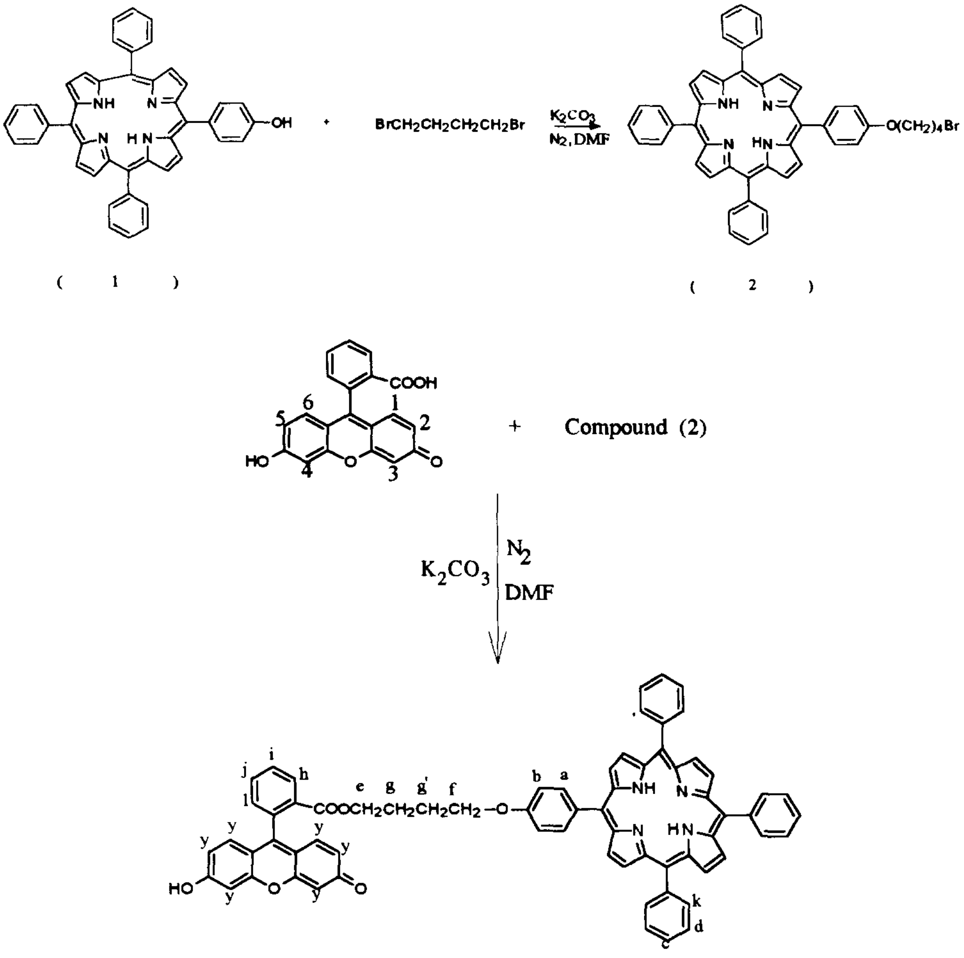
photosynthetic organisms employ closely associated porphyrin as carriers of photons and electrons. Spectroscopic evidence has shown that the porphyrins are held in carefully controlled arrangements that optimise the efficiency of photon or electron transfers. Characterisation of these natural systems has been aided by the synthesis of many different covalently linked porphyrin dimers and high-order oligomers [2–8], as they can serve as model systems for the reaction centre of photosynthesis. In most systems,the donor and the acceptor groups were attached either to rigid systems, so that their spatial relationship was known but could be varied only by using different spacers, or to a flexible chain so that their spatial relationship could not be determined. More recently, several mixed dimers containing zinc porphyrin linked



Compound (3):FL-TTP

Scheme 1

to zinc phthalocyanine via an oxygen atom and/or via a flexible chain of variable length have been reported. More generally, the approach likely to act as a model system would imply having a bichromophoric molecule consisting of a donor and an acceptor (D–A) held in proximity with specific properties: namely (i) D and A should exhibit a large difference in the ground-state absorption spectrum so that each moiety could be selectively excited and (ii) D and A should be attached in such a manner so that conformation and spatial relationship could be varied and controlled.

Until recently, relative few efforts have been devoted to the study mixed dimers containing porphyrin and xanthene. Our present interest is focused on a system consisting of a metal-free porphyrin (TTP) linked together with fluorescein (FL) via a flexible polyatomic chain (FL-O-$(CH_2)_4$-O-TTP). Morever, the porphyrin–fluorescein heterodimer is peculiar in having a ground-state absorption spectrum that covers the entire visible region, while allowing selective excitation of each individual chromophore. In the present paper, a study of the inter- and intramolecular energy and electron transfer processes in mixed monomers and heterodimer is reported.

EXPERIMENTAL

Materials

The structure of the heterodimer is shown in Scheme 1. Fluorescein was purchased from Beijing Chemical Plant (China). All organic solvents used were of analytical grade and were used as received. The required high-purity solvents were prepared by further purification of the commercial products, and no impurities were detected by absorption and/or fluorescence spectroscopy.

Synthesis

Model compound, ethyl ester of FL(FLEt)

This was synthesized with reference to the procedure of Acree and Slagle [9] from the disodium salt of FL. For FLEt, UV–Vis $\lambda_{\mathrm{max}}^{\mathrm{MeOH}}$ nm: 500. IR $\nu_{\mathrm{max}}^{\mathrm{KBr}}$ cm^{-1}: 1710(C=O). ^{1}H NMR($CDCl_3$): δ0.9 (*t*, 3H, CH_3); 4.1 (*q*, 2H, CH_2); 6.5–8.3 (*m*, 10H).

Compound 2 (Br-(CH_2)4-O-TTP)

A mixture of 0.291 g(0.46 mmol) of compound **1**, 5.45 ml of 1.4-dibromobutane and 0.5 g of anhydrous potassium carbonate was stirred magnetically in 20 ml *N*,*N*-dimethylformamide for 24 h, during which time a steady stream of nitrogen gas was passed into the solution. The reaction mixture was then poured into 170 ml of water and filtered. The porphyrin was dried to remove excess dibromobutane and was dissoved in chloroform and chromatographed on a silica gel column using chloroform as the eluant to give 0.185 g of pure **2**. (Compound **2** moves with the solvent front and unreacted **1** remains on the column as a slow moving purple band). ^{1}H NMR: δ8.8–8.9 (*m*, 8H, β-H); 8.2–8.4 (*m*, 6H, d); 8.0–8.3 (*d*, 2H, b); 7.2–7.8 (*m*, 11H); 3.95 (*t*, 2H,-CH_2O); 3.25 (*t*, 2H, -CH_2-Br); 2.89 (*m*, 2H, -HN-); 1.6-1.8 (*m*, 4H, -CH_2-).

Heterodimer FL-TTP

A mixture of 0.098 g(0.125 mmol) of compound **2** and 0.4 g(1.25 mmol) of FL was stirred and refluxed in 25 ml dried *N*,*N*-dimethylformamide in the presence of anhydrous potassium carbonate and a small amount of potassium iodide under a nitrogen atmosphere for 48 h. Chromatographic examination of an aliquot of the reaction mixture,using silica gel plates and toluene, showed that the reaction had gone to about 90% completion. The reaction mixture was poured into water, filtered, and the residue washed with water, dried and chromatographed on a silica gel column using chloroform as eluant giving three bands. The highest R_f band (which moved with the solvent front) contained the by-product TTP-FL-TTP in low yield; the mid R_f band contained unreacted **2** and the FL-TTP; and the lowest R_f band contained FL. The band containing the FL-TTP was collected and rechromatographed on a silica gel column with elution using benzene; the frontal band was assigned as the heterodimer **3** (monitored by UV-Vis spectroscopy) and was collected; the resultant product showed only one spot TLC, yield 0.020 g (15.6%)

UV-Vis $\lambda_{max}^{CHCl_3}$ nm: 420, 460, 515, 592, 646; IR ν_{max}^{KBr} cm^{-1}: 3417, 2918, 1716 and 1501; ^{1}H NMR($CDCl_3$): δ8.8 (*m*, 8H, β-H), 8.0–8.2 (*d*, 7H, d,l), 7.8–8.0 (*d*, 2H, b), 7.6 (*m*, 11H, a,c,k), 7.2–7.5 (*m*, 2H, i,j), 7.0(*d*, 1H, h), 6.5–6.8 (*m*, 6H, y), 3.9 (*t*, 2H, e), 3.7–3.8 (*t*, 2H, f), 1.4 (*t*, 2H, g); 0.8 (*t*, 2H, g′). MS(FD): 1019 [M−1].

Instruments and methods

The absoption spectra were measured on a Hitachi-557 spectrometer. IR spectra were performed with a Perkin-Elmer 557 grating spectrophotometer. ^{1}H NMR spectra were run on a Varian XL-400 spectrometer, unless otherwise specified. The solvent was deuteriochloroform with TMS as an internal standard; the chemical shifts are given in ppm. Mass spectra were performed on a Hitachi 80(FD).The fluorescence spectra were recorded using a Perkin-Elmer LS-5 and/or Hitachi 850 spectrofluorometer with excitation and emission slits of 5 nm The fluorescence quantum yields were determined by a comparative method using TTP as standard. Fluorescence lifetime measurements were determined using a Horiba NAES-1100 time-correlated singel-photon counting instrument.

RESULTS AND DISCUSSION

Intermolecular interaction between FL and TTP

In the presence of TTP, on selective excitation of FLEt at 460 nm, the fluorescence intensity and lifetime of FLEt decrease concomitantly with an increase in the concentration of the TTP; the fluorescence of TTP at about 656.5 nm was clearly observed. There was no change in the shape of the FLEt fluorescence spectra, even with the higher concentration of the TTP used, and thus it is concluded that no exciplex is involved in the quenching mechanism. Steady and dynamic fluorescence quenching plots of FLEt are shown in Fig. 1. Within the low concentration limit of the quencher, the steady, and dynamic fluorescence quenching followed the Stern–Volmer equations (1) and (2), respectively.

$$I_0/I = 1 + K_{sv}[Q] = 1 + k_q^s\tau_0[Q] \tag{1}$$

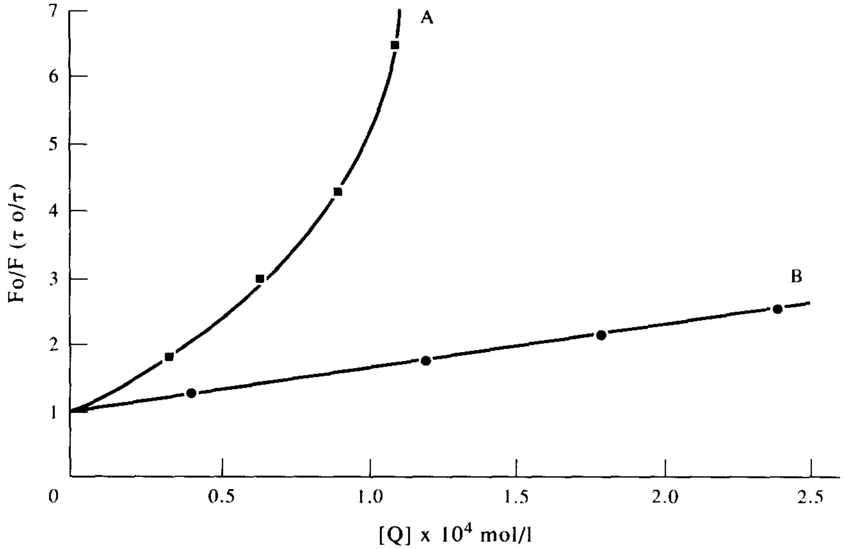


Fig. 1. Steady (A) and dynamic (B) fluorescence quenching plots of FLEt by TTP at room temperature.

$$\tau_0/\tau = 1 + k_q^d[Q] \tag{2}$$

where I_0 and I are the relative fluorescence intensities in the absence and presence of the quencher, τ and τ_0 are the lifetimes of the FLEt singlet excited states in the presence and absence of the quencher, k_q^s and k_q^d are bimolecular steady and dynamic quenching rate constants.

The value of k_q^s (3.6×10^{12} S^{-1} mol^{-1} litre) is not agreement with k_q^d (1.3×10^{12} S^{-1} mol^{-1} litre) obtained from the dynamic state measurements, indicating that the ground-state interaction between two chromophores existed. Furthermore, the ^{1}H NMR spectra of FLEt in $CDCl_3$ was compared with that of an equimolar mixture of TTP and FLEt, and the ^{1}H NMR data of FLEt in monomer and mixture are listed in Table 1. The results indicate that the chemical shifts of the FLEt hydrogen atoms on the heteroanthracene ring in the mixture are at higher field than in the monomer. The fluorescence and ^{1}H NMR studies thus provide evidence for the ground-state interaction between TTP and FLEt. Since there is considerable overlap between the fluorescence of FLEt and the ground-state absorption spectra of TTP, and the electron transfer from FLEt to TTP is not possible according to Weller's equation, so in these systems, the singlet–singlet energy transfer from FLEt* to TTP contributes to the fluorescence quenching of FLEt by TTP.

Intramolecular interaction in the heterodimer

Ground state absorption spectra of the heterodimer

The electronic absorption of TTP (418, 515, 551, 592, 646 nm), and fluorescein (456, 482 nm) overlap significantly throughout the visible region. The ground state absorption spectra of the heterodimer (FL-O-$(CH_2)_4$-O-TTP) in dilute benzene solution is compared with that of an equimolar mixture of TTP and the model compound, FLEt, in Fig. 2. This shows that in dilute solution FL-TTP exhibits an absorption spectra similar to that of the 1:1 molar mixture of FLEt and TTP. However, close examination of the spectra

TABLE 1
^{1}H NMR Data of FLET/TTP (1:1) Mixture and FLET Monomer

	Chemical shift (ppm)	
H	*FLEt/TTP (1:1)*	*FLEt*
1	6.67	6.78
2	6.67	6.76
3	6.70	6.84
4	6.69	6.80
5	6.99	7.05
6	6.97	7.07

shows that the molar extinction coefficients of the porphyrin in the dimers are only *ca* 75–80% of the corresponding bands in the mixture of monomers. In addition,the B-bands of the dimers have a slight bathochromic shift (2 nm) in dilute benzene solution. Thus absorption spectroscopy provides evidence for electronic interaction in the ground state between the two chromophores in the heterodimer. There are many similar examples in the literature, e.g. Little [10] has found that for the $n=3$ porphyrin dimer the B-band intensity is only *ca* 70% of the expected value. So in our system, these absorption spectral changes can be ascribed to weak exciton coupling

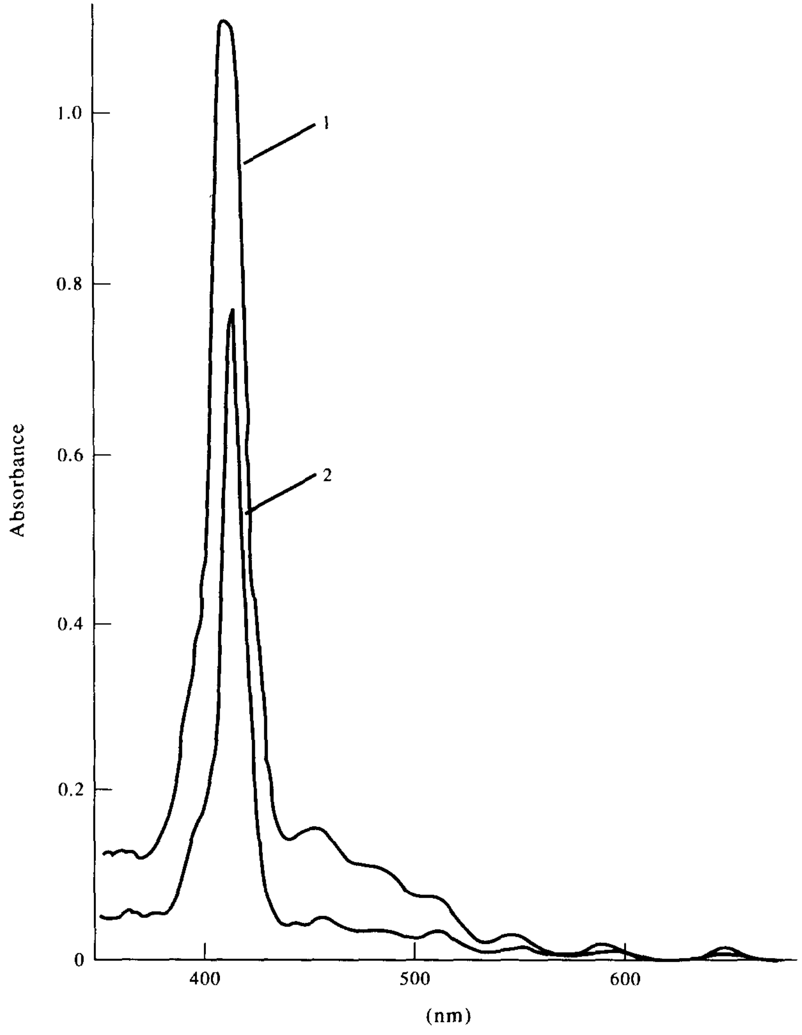


Fig. 2. Absorption spectra of FL-TTP mixture (1:1) [1] and FL-C4-TTP [2] in benzene solution.

interaction between the porphyrin ring and the fluorescein ring in the heterodimer.

Steady-state fluorescence spectroscopy

On excitation of FL-TTP at 460 nm,a strong decrease in the FL fluorescence is observed accompanied by an increase in the TTP emission with a peak at 656.5 nm (Fig. 3).Since there is considerable overlap between the fluorescence of FLEt and the ground-state absorption spectra of TTP(Fig. 4) indicating that singlet–singlet excitation energy transfer from FL to TTP should be extremely efficient. From the room temperature spectra, and assuming a random orientation of reactants in dilute benzene solution, the Forster equation (3) can be used to determine the average donor–acceptor distance R_o at which the rate constant for energy transfer(k_{ENT}) equals the rate constant for non-radiative decay of the donor.

$$R_o^6 = \frac{9 \ln 10 K^2 \Phi_D}{128\pi^5 N n^4} J \tag{3}$$

where Φ_D is the fluorescence quantum yield of FLEt in benzene in the absence of acceptor, n is the refractive index of the solvent ($n = 1.424'$ for benzene), N is Avogadro's constant and K^2 is the orientation factor that equals 2/3 for random geometries. The numerical value of overlap J can be calculated from eqn (4):

$$J = \frac{\int_0^\infty F_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda}{\int_0^\infty F_D(\lambda) d\lambda} \tag{4}$$

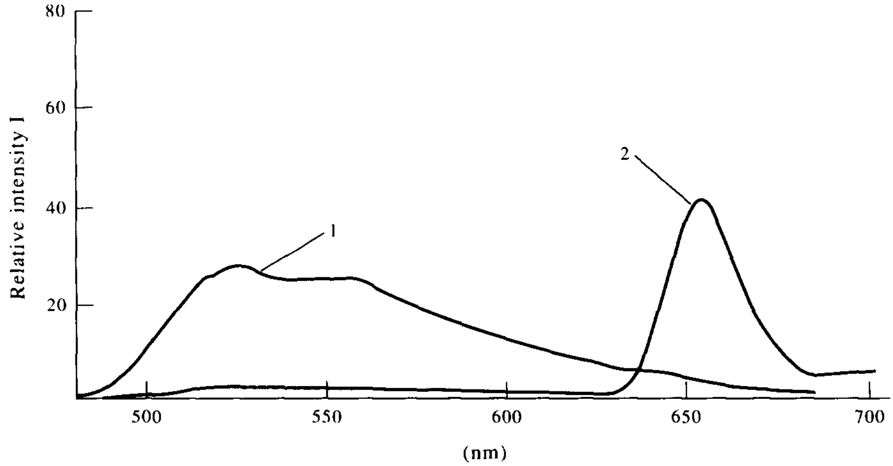


Fig. 3. Fluorescence spectra of FLEt [1] and FL-C4-TTP [2] in benzene solution ($\lambda_{ex} = 460$ nm).

Substituting the value of J into eqn (3) results in an R_0 value of 66.8 Å for FLTTP in benzene, showing that singlet energy transfer can occur over quite large distances. The values of R_0 and J for FL-TTP in different solvents are listed in Table 2.

As shown in Fig. 3,with selective excitation at 460 nm of the FL-TTP, a drastic decrease of the FL fluorescence at 526.5 nm is observed concomitant with an obvious increase of the TTP fluorescence (at about 656.5 nm),indicating that a very efficient intramolecular singlet–singlet energy transfer process from FL* to TTP occurs. At the same time, due to some overlap of absorption spectra of TTP and FL at 426 nm, controlled experiments were carried out as follows: on excitation of TTP solution at 460 nm, very weak intensities of fluorescence at 656.5 nm were recorded and submitted to correct the data obtained from the heterodimer. Comparing fluorescence from

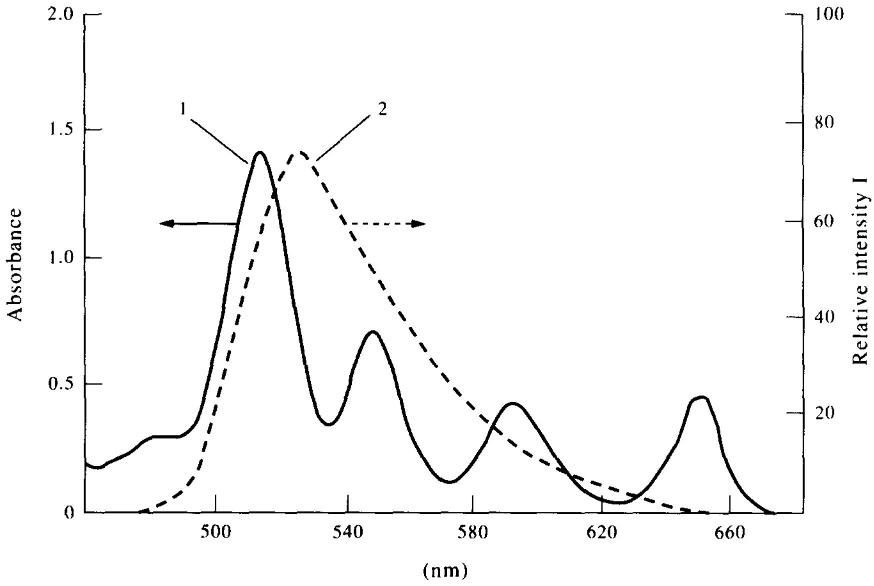


Fig. 4. Absorption spectra of TTP [1] and fluorescence spectra of FLEt [2] ($\lambda_{ex} = 460$ nm) in benzene.

TABLE 2
Parameters Derived From Steady-state Fluorescence Spectra

Compound	*Solvent*	ϕ_{ENT} (%)	k_{ENT} (s^{-1})	ϕ_q (%)	k_q (s^{-1})	J ($mol^{-1}cm^6$)	R_0 (Å)	R (Å)
FLTIP	C_6H_6	98.4	1.25×10^{10}	97.8	1.21×10^{10}	1.26×10^{-9}	66.8	34.9
FLTFP	C_6H_6/CH_3CN_3(1:1)	98.2	1.23×10^{10}	97.6	1.19×10^{10}	1.23×10^{-9}	65.2	33.2
FLTTP	CH_3CN	98.2	1.23×10^{10}	97.4	1.17×10^{10}	1.23×10^{-9}	65.2	33.2

the monomer with fluorescence spectra recorded for the FL-TTP dimers in dilute benzene solution (Fig. 3) shows that, in all cases, the dimers show a drastic decrease in fluorescence associated with the FLEt ($\lambda_{em} = 526.5$ nm) and a corresponding increase in fluorescence associated with the TTP ($\lambda_{em} = 656.5$ nm). This observation is consistent with singlet energy transfer from the FL to the covalently linked TTP. One can determine ϕ_{ENT} by eqn (5) derived from the kinetic scheme of Fig. 3

$$\phi_{ENT} = \frac{F_A/\phi_A}{F_A/\phi_A + F_D/\phi_D} \tag{5}$$

where F_D and F_A are the fluorescence intensity of FL and TTP in dimer on excitation of FL, respectively; φ_D and φ_A are the fluorescence quantum yield of FLEt and TTP in monomers, respectively, these are measured from the normalized and corrected fluorescence spectra by using the known value ($\varphi_f = 0.92$) of the standard compound (FL in benzene).The energy transfer efficiency ϕ_{ENT} can be used to calculate the average rate constant for intramolecular energy transfer k_{ENT}

$$\phi_{ENT} = \frac{k_{ENT}}{k_{ENT} + \tau_D^{-1}} \tag{6}$$

where τ_D is the fluorescence lifetime of the FLEt monomer. At the same time, one can determine k_q and φ_q by eqn (7):

$$k_q = \tau_D^{-1}\left(\frac{\phi_D}{\phi_{DA}} - 1\right) \phi_q = \frac{k_q}{k_q + \tau_D^{-1}} \tag{7}$$

where ϕ_D and ϕ_{DA} are the fluorescence quantum yields of FL chromophore in the monomer and heterodimer, respectively, k_q and ϕ_q are the quenching rate constant and quenching efficiency with which the FL fluorescence is quenched by TTP in heterodimers, respectively.

The results are listed in Table 2. The steady-state fluorescence data show that intramolecular singlet–singlet energy transfer from FL to TTP occurs efficiently in various solvent. The value of ϕ_{ENT} is nearly equal to the value of ϕ_q indicating the singlet–singlet energy transfer process is only one pathway of FL fluorescence quenching. That efficient singlet energy transfer does occur in these systems is confirmed by the corrected excitation spectra shown in Fig. 5. Here the corrected fluorescence excitation spectra of the heterodimer is compared with their absorption spectra. Monitoring the emission at 656.5 nm(TTP fluorescence) and scanning through 440–660 nm, it can be seen that, after normalizing at 592 nm,the agreement between the two spectra is good for the heterodimer in benzene, indicating that energy transfer is very efficient; the spectra almost concide, showing that energy transfer fron FL to TTP is nearly quantitative. These excitation spectra give good support to the values of ϕ_{ENT} collected in Table 1. The values obtained from steady-state fluorescence spectroscopy can be used to estimate the average separation distance (R) between the TTP and FL units:

$$k_{ENT} = \tau_D^{-1}(R_0/R)^6 \tag{8}$$

The steady-state fluorescence data given in Table 2 show that excitation energy transfer from FL to a covalently linked TTP can occur with high efficiency. The actual efficiency (ϕ_{ENT}) depends upon the orientation and separation distance between the two rings. Our derived R values suggest that the two chromophores of FL-TTP reside about 3.4 nm apart. For the heterodimer a space filling molecular model shows, that the extended separation distance is 3.6 nm [11], the value of R in FL-TTP is 3.4 nm, which is nearly equal to the calculated value; this may imply that the conformation of FL-TTP is extended in solvents.

Fluorescence quenching and intramolecular electron transfer

As mentioned above, FL fluorescence is quenched by porphyrin through an intramolecular energy transfer process, whereas, on selective excitation of the heterodimer or a 1:1 molar mixture of FL and TTP at 592 nm, only the

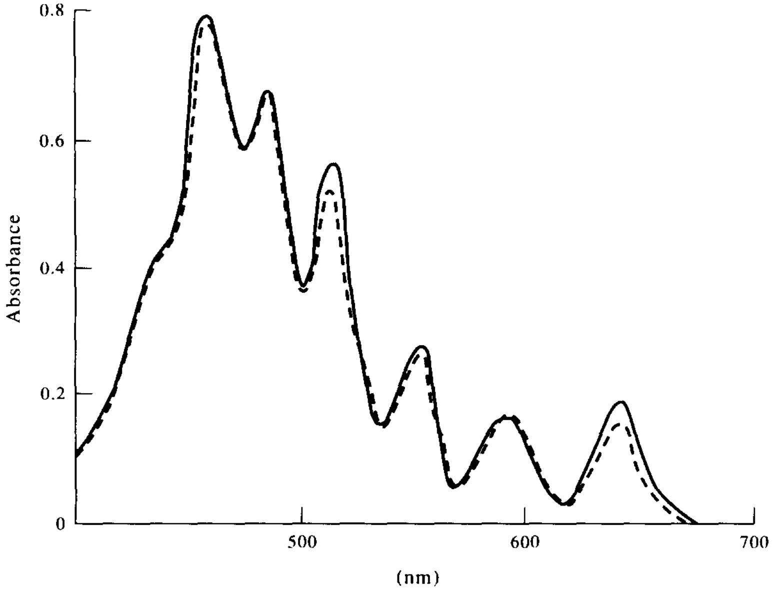


Fig. 5. Comparison of the excitation (λ_{em} = 656.5 nm) (- - -) and the absorption (———) spectra of FL-C_4-TTP in dilute benzene solution. The spectra was normalized at 592 nm.

porphyrin chromophore was excited in the dimer or monomer. Comparison of the fluorescence from the 1:1 molar mixture with fluorescence spectra recorded for the FL-TTP dimer in dilute DMF solution shows that the fluorescence intensity of TTP at 656.5 nm in the dimer was lower than in the monomer. Electronic energy transfer, exciplex formation and electron transfer processes can be perceived as possible pathways of the TTP fluorescence quenching in these systems. As the singlet-state energy level of porphyrin lies below the FL's level, there is no spectral overlap between the emission of the porphyrin and the absorption of the FL. Hence intramolecular singlet–singlet energy transfer from TTP to FL could not occur, and the fluorescence spectra show no evidence for the formation of exciplex, so it is suggested that the porphyrin fluorescence is quenched by FL via intramolecular electron transfer. The standard free energy change (ΔG_{ET}) value on one electron transfer from a singlet excited donor molecule D* to an electron acceptor molecule A can be calculated from the Weller's equation:

$$G_{\mathrm{ET}} = E(\mathrm{D/D^+}) - E(\mathrm{A^-/A}) - E_s - C \tag{9}$$

where E_s indicates the excitation energy and C denotes the small additional coulombic stabilization of the $\mathrm{D^+A^-}$ pair(0.06 eV in DMF)

The ΔG values show that on excitation of TTP, electron transfer from FL to TTP is possible in polar solvent, whereas electron transfer from TTP to FL is thermodynamically unfavourable.

Table 3 shows that on selective excitation of TTP at 592 nm of the heterodimer in benzene, the fluorescence lifetime of the porphyrin remains unchanged, demonstrating that electron transfer could not occur in benzene. However, in dilute DMF solution, the fluorescence decay measurements exhibit two components, one short-lived component (9.2 ns) and one long-lived component (11.7 ns). The electron transfer rate constant can be calculated from eqn (10):

$$k_{\mathrm{ET}} = 1/\tau_{\mathrm{f}} - 1/\tau_{\mathrm{f}}^{0} \tag{10}$$

where τ_{f}^{0} and τ_{f} denote the fluorescence lifetimes of the TTP monomer and TTP in the heterodimer, respectively, and the results are listed in Table 3.

TABLE 3

Parameters Derived From Fluorescence Decay Measurements

Compound	*Solvent*	λ_{ex}*(nm)*	λ_{em}*(nm)*	τ_{f} *(ns)*	k_{ET} (s^{-1})	ϕ_{ET} *(%)*
TTP	C_6H_6	592	658	11.8	—	—
TTP	CH_3CN	592	658	11.7	—	—
FLTTP	C_6H_6	592	658	11.8	—	—
FLTTP	CH_3CN	592	658	9.2(11.6)	2.21×10^{7}	20.4

From Table 3,it can be seen that the fluorescence lifetime of the porphyrin moiety in the heterodimer is shortened in a polar solvent. This behaviour may result from the existence of different conformations. The intramolecular D–A compound studied in this work is linked by a flexible chain, which gives rise to flexibility of the distance and orientation of the FL with respect to the porphyrin plane, and permits the possibility of several conformations. The distance between the two chromophores and their mutual orientation during the lifetime of the excited states determine the electron transfer efficiency. In a polar solvent such as DMF, the two chromophores of the dimer are held in a closely packed conformation on excitation. which accounts for the intramolecular electron transfer reaction. But in a non-polar solvent (such as benzene), the extended conformation of the heterodimers shows very little electron transfer efficiency because of the unfavourable orientation factor. The lifetime of the long-lived component in the heterodimer (about 11.7 ns) coincided with the lifetime of TTP monomer, which may arise from an unfavourable conformation to electron transfer of the two moieties adopted in the heterodimer.

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