Fluorescence Resonance Energy Transfer between Cationic Porphyrins Accelerated with DNA as a Template

Francis D'Souza,*[1] Lisa M. Rogers,[1] D.-M. Shafiqul Islam,[2] Yasuyuki Araki,*[2] Osamu Ito,[2] and Takehiko Wada[2]
[1]*Department of Chemistry, Wichita State University, Wichita, KS 67260-0051, USA.*
[2]*Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Sendai 980-8577*

(Received January 21, 2008; CL-080065; E-mail: francis.dsouza@wichita.edu, araki@tagen.tohoku.ac.jp)

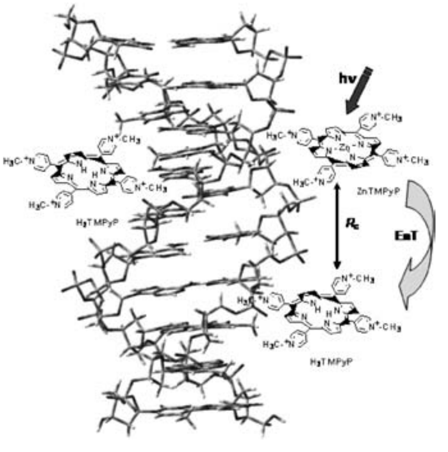
Resonance energy transfer between two cationic porphyrins, viz., (*meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin and its zinc derivative, along a model DNA, poly(dA-dT) as a template is demonstrated. The rate of energy transfer (k_{EnT}), quantum yield (Φ_{EnT}), and critical transfer distance are evaluated to be $1.78 \times 10^9\ \mathrm{s}^{-1}$, 0.74, and 23 Å, respectively, indicating efficient energy transfer process.

The interaction of cationic porphyrins with DNA can occur through three types of binding modes, namely, intercalation, outside binding in the groove, and outside binding with self-stacking along the DNA surface.[1,2] Circular dichroism (CD) studies have been vastly utilized to arrive at the different modes of binding.[3] The widely studied cationic porphyrins, *meso*-tetrakis(*N*-methylpyridinium-4-yl)porphyrin (H_2TMPyP) and its zinc derivative, ZnTMPyP, when associated with (dA-dT)-rich synthetic DNA [poly(dA-dT)],[3] as shown in Scheme 1, in which although perpendicular arrangements are predominantly depicted, various orientations including parallel ones are possible (see Supporting Information, Figure S1).[4] The intrinsic binding constant for M(TMPyP) (M = 2H or Zn)–poly(dA-dT) ranges $(2–8) \times 10^5\ \mathrm{M}^{-1}$ in aqueous pH 7.4 buffer[3c] suggesting strong ion-pair interactions.

The ground and excited state interactions between cationic porphyrins or drugs, positioned differently in the DNA helix, are important not only for their significance in photodynamic therapy, but also to understand the structure–reactivity aspects of these DNA bound molecules.[5] In the present study, for the first time, the possibility of fluorescence energy transfer between two cationic porphyrins bound to a model DNA, poly(dA-dT), which is confirmed by the emerged CD signals (See SI, Figure S2),[3c] is investigated using time-resolved fluorescence techniques. The two porphyrins, H_2TMPyP and ZnTMPyP, have different absorption and emission properties and are suitable candidates to examine the occurrence of fluorescence energy transfer[6] when bound to model DNA as a template.

Figure 1a shows the absorption spectra of ZnTMPyP, H_2TMPyP, and equimolar mixture of these species in the presence of poly(dA-dT) in pH 7.4 phosphate buffer.[7] Compared with the spectrum obtained in the absence of poly(dA-dT) (See SI, Figure S3),[4] the bands are sharp and blue shifted by 2–5 nm suggesting deaggregation of the self-associated cationic porphyrins due to electrostatic binding to the negatively charged poly(dA-dT). In the visible region, the ZnTMPyP revealed a band at 568 nm, where H_2TMPyP absorption is found to be minimal. That is, excitation at this wavelength is expected to predominantly excite ZnTMPyP.

As shown in Figure 1b, the emission bands of both porphyrins are well-defined as compared to the ones recorded in the absence of poly(dA-dT) (see SI, Figure S4).[4] ZnTMPyP showed two emission bands at 612 and 656 nm, while the bands for H_2TMPyP were located at 652 and 714 nm, respectively. Interestingly, in the equimolar mixture in the presence of poly-(dA-dT), the emission of ZnTMPyP at 612 nm is found to be quenched over 60% of its original intensity, accompanied by much intense emission bands of H_2TMPyP (see titration spectral changes, Figure S5), suggesting excited energy transfer from poly(dA-dT)-bound ZnTMPyP to H_2TMPyP as shown in Scheme 1. Increasing addition of poly(dA-dT) decreased the emission intensity of ZnTMPyP at 612 nm with concurrent increase of H_2TMPyP emission at 714 nm. These results convey the role of poly(dA-dT) acting as a template to hold the energy-donor–acceptor cationic porphyrins electrostatically, and to



Scheme 1.

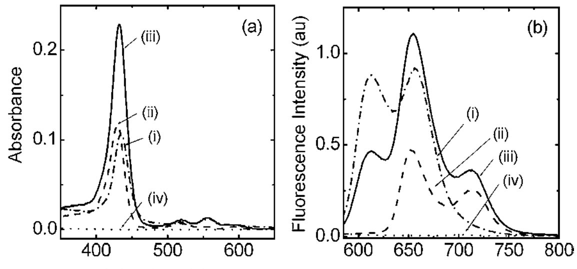


Figure 1. (a) Absorption spectra of (i) ZnTMPyP (5 µM), (ii) H_2TMPyP (5 µM), and (iii) equimolar mixture of H_2TMPyP and ZnTMPyP in the presence of poly(dA-dT) (45 µM) in pH 7.4 phosphate buffer. (b) Emission spectra of the samples defined in (a); $\lambda_{\mathrm{ex}} = 568$ nm. Plot (iv) shows absorption and emission of poly(dA-dT) alone.

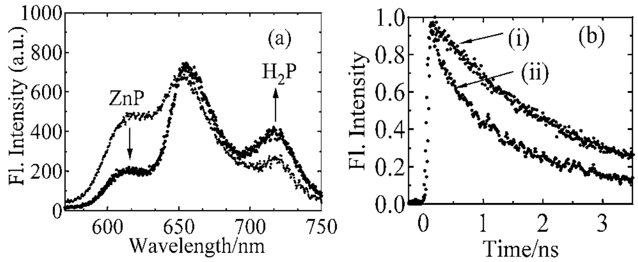


Figure 2. (a) Time-resolved fluorescence spectra of a mixture of ZnTMPyP (ZnP; 5 μM) and H_2TMPyP (H_2P; 5 μM) in presence of poly(dA-dT) (45 μM) in pH 7.4 phosphate buffer; $\lambda_{ex} = 400$ nm. Blue spectrum = 0–1 ns and green spectrum 1–3 ns (normalized at 650 nm). (b) Fluorescence decay monitored at 612 nm of ZnTMPyP (5 μM) in the presence of H_2TMPyP (5 μM); (i) without and (ii) with poly(dA-dT) (45 μM) in pH 7.4 phosphate buffer; $\lambda_{ex} = 400$ nm.

facilitate energy transfer from ^{1}ZnTMPyP* to H_2TMPyP along poly(dA-dT) (see excitation spectrum, see Figure S6).[4]

Time-resolved fluorescence studies provided a direct proof for the occurrence of resonance energy transfer from the poly(dA-dT) bound ^{1}ZnTMPyP* to H_2TMPyP. As shown in Figure 2a, the spectrum obtained at 0–1 ns is found to be dominated by ZnTMPyP bands at 612 and 656 nm, with relatively weak emission at 714 nm corresponding to H_2TMPyP, directly excited by the 400-nm laser light. At the 1–3 ns time interval, substantial quenching of ZnTMPyP at 612 nm accompanied by increased emission of H_2TMPyP at 714 nm was observed. That is, by about 3 ns the energy transfer process seems to be complete.

Kinetics of energy transfer was monitored by fluorescence decay experiments (Figure 2b). The decay of ZnTMPyP, in a solution containing equimolar H_2TMPyP as acceptor without poly(dA-dT), monitored at 612 nm followed monoexponential decay with a lifetime of 2179 ps, close to the lifetime of pristine ZnTMPyP. Addition of poly(dA-dT) to the solution facilitated decay of ZnTMPyP, and under these conditions, the decay could be curve-fitted to a biexponential function with lifetimes of 449 (51%) and 2048 ps (49%), respectively. The fast decaying component is attributed to energy transfer from ^{1}ZnTMPyP* to H_2TMPyP bound to poly(dA-dT). The rate of energy transfer (k_{EnT}) and quantum yield (Φ_{EnT}), calculated from these lifetime differences were found to be $1.78 \times 10^9\ s^{-1}$ and 0.75, respectively, indicating efficient resonance energy transfer process along poly(dA-dT).

The mechanism of energy transfer between porphyrins can be ascribed to a Förster-type mechanism involving dipole–dipole interaction.[1c] For this mechanism, it is possible to calculate the rate, k_{EnT}, with some accuracy as a function of the donor–acceptor distance (d_{DA}), knowing the emission quantum yield ($\Phi = 0.08$), lifetime ($\tau = 1720$ ps) of the donor ZnTMPyP, the spectral overlap integral between the donor emission and the acceptor absorption (J^F), and the refractive index of the medium (n) and the orientation factor (k^2) according to eq 1.[1c]

$$k_{EnT} = [(8.8 \times 10^{-25} k^2 \Phi)/(n^4 \tau d_{DA}{}^6)] J^F \qquad (1)$$

In these calculations, the J^F is calculated from the experimental emission and absorption spectra to be 2.6×10^{-14} $M^{-1}\,cm^{-1}$ (see Figure S7),[4,8] and k^2 as 2/3 assuming random orientation of the donor and acceptor dipoles. On the basis of these parameters, a critical transfer distance, R_c, (distance at which the energy transfer rate equals the intrinsic deactivation rate of the donor), was estimated to be about 23 Å, which is 7 base pairs meandering a pitch of double helix (33 Å). Interestingly, at least two arrangements are possible for the donor and acceptor entities, one in which the donor and acceptor are on the opposite sides of poly(dA-dT) and the second where both fluorophores are on the same side (Scheme 1). Since the estimated R_c is slightly longer than the radius of the double helix (20 Å), energy transfer across the (dA-dT) pair is also possible.[9] For the case of both fluorophores positioned on the same side, R_c is shorter than the pitch of double helix, suggesting occurrence of energy transfer between angularly oriented ^{1}ZnTMPyP* and H_2TMPyP within a pitch of double helix. Additionally, considering that the dipole orientation factor increases with approaching a pitch of double helix, long range energy transfer is also conceivable for almost complete ^{1}ZnTMPyP* with H_2TMPyP in the perpendicular orientation at a pitch (a pair of the right side of Scheme 1). Further studies along this line are in progress.

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4 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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7 Both porphyrins (Frontier Scientific Inc. (USA)) were purified on a Sephadex LH20 column using methanol as eluent prior to performing the spectral studies.

8 The spectral overlap integral, J^F was estimated from the absorption spectrum of the acceptor, H_2TMPyP ($\varepsilon(\lambda)$) and the normalized emission spectrum of donor, ZnTMPyP ($F(\lambda)$) using equation (see Figure S5).[4]

$$J = \int \varepsilon(\lambda) F(\lambda) \lambda^4 d\lambda$$

9 Parallel–parallel pair decreases the R_c favorable for this type energy transfer.

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