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Photoinduced electron transfer in alkanoylpyrene aggregates in conjugated polypeptides

Valentine I. Vullev*,† and Guilford Jones, II*

Department of Chemistry and Photonics Center, Boston University, 590 Commonwealth Avenue, Boston, MA 02215, USA

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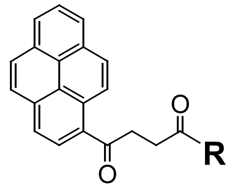
Abstract—An alkanoylpyrene moiety was used as a principal chromophore for labeling photoactive amphipathic polypeptides with a high propensity for self-assembly. Electrochemical, emission and transient absorption data showed that photoinduced electron transfer within pairs of pyrene pendants accounts for a lower fluorescence quantum yield observed for aggregated chromophores and for the formation of radical-ion, intermediates. © 2002 Elsevier Science Ltd. All rights reserved.

The role of chromophore aggregation in macromolecular and supramolecular photochemistry has been well established.[1] Because of the readily distinguishable emission of pyrene aggregates,[2] derivatives of this chromophore have been favored as photoprobes in macromolecular research[3] and in photoinduced electron-transfer studies of synthetic polypeptides.[4]

Another set of pyrene derivatives, alkanoylpyrenes (OPy), constitute alternative candidates to serve as photoprobes since their photophysical properties are sensitive to medium polarity and hydrogen-bonding characteristics of the microenvironment.[5] In contrast to alkylpyrene, however, the emission quantum yield of the aggregate of alkanoylpyrene is about an order of magnitude smaller than the emission quantum yield of its monomer.[6] Our investigation of photoactive polypeptides that utilize OPy as a principal chromophore led us to the conclusion that the significant quenching of the alkanoylpyrene fluorescence of the aggregated species is a result of photoinduced electron transfer between the OPy moieties in the chromophore assembly. This publication concentrates on photochemical studies relevant to a medium-dependent intra-aggregate electron transfer of an amphipathic polypeptide, TT2oX15N (Scheme 1), whose self-assembly in aqueous media provides a template for formation of alkanoylpyrene aggregates.[6] The alteration of the aqueous environment for this study was conducted by addition of surfactant, sodium dodecylsulfate (SDS), at concentrations for which peptide was expected to complex with the amphiphile in premicellar forms. Oxopyrene butanoic acid (OPBA) and its ethyl ester were used as model compounds for further photophysical and electrochemical measurements (Scheme 1). The preparation and characterization of the polypeptide as well as the model compounds are described elsewhere.[6]

The driving force for photoinduced charge transfer, ΔG_{et}, between an electron donor, D, and an acceptor, A, can be estimated from the electrode potentials and the zero-to-zero transition energy, $\mathscr{E}_{00}$, of the species that is photoexcited:[7]

$$\Delta G_{et} = F(E^0_{D^{+\bullet}/D} - E^0_{A/A^{-\bullet}}) - \mathscr{E}_{00} - \delta_C \qquad (1)$$



OPBA : –R = –OH

OPB-OEt : –R = $-OC_2H_5$

TT2oX15N : –R = –Orn·Orn·Gly·Gly·Lys·Ala·*Leu*·Phe·Glu·Gln·*Ile*·Ala·Glu·*Leu*·Asn·Lys·Glu·*Ile*·Glu·Glu·*Leu*·Lys·Lys·Glu·*Ile*·Glu·Glu·*Leu*·Lys·Lys·Lys·Ala-OH

Scheme 1.

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* Corresponding authors. Tel.: 1-617-495-9433; fax: 1-617-495-2500 (V.I.V.); Tel.: 1-617-353-8656; fax: 1-617-353-6466 (G.J.); e-mail: vullev@fas.harvard.edu; jones@chem.bu.edu

† Present address: Department of Chemistry and Chemical Biology, Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA.

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where F is the Faraday constant, $E^0_{D^{+\bullet}/D}$ and $E^0_{A/A^{-\bullet}}$ are the appropriate potentials of the donor and the acceptor, respectively, and δ_C represents the Coulombic electrostatic interaction between the charged species that are produced.

The $\mathscr{E}_{00}$ of a chromophore P is proportional to the energy required to transfer an electron from its highest occupied (HOMO) to its lowest unoccupied (LUMO) molecular orbital, and concomitantly, its oxidation potential, $E^0_{P^{+\bullet}/P}$, is proportional to the energy needed to remove an electron from its HOMO, while the reduction potential, $E^0_{P/P^{-\bullet}}$, is proportional to the energy needed to place an electron on its LUMO. Thus, it is reasonable to express the relation between these three quantities in the following manner:[8]

$$F(E^0_{P^{+\bullet}/P} - E^0_{P/P^{-\bullet}}) = \mathscr{E}_{00}(\mathrm{P}) + \delta_S{}^{9} \tag{2}$$

where the correction term, δ_S, is determined by internal (i.e. exchange and Coulomb integrals as well as the configuration interaction energies)[10] and environmental factors (i.e. the difference in the solvation energies between the ion-radicals and the neutral species).[8]

From Eqs. (1) and (2) it follows that the driving force for electron transfer between photoexcited and ground state species of the same chromophore is determined from δ_C and δ_S:

$$\Delta G_{et} = \delta_S - \delta_C = \delta_S - \frac{e^2}{4\pi\varepsilon_0\varepsilon r} = \delta_S - \frac{\kappa}{\varepsilon r} \tag{3}$$

where e is the transferred charge along distance r, ε is the dielectric constant of the medium, and $\kappa = 14.4$ eV Å.

The reduction and oxidation potentials of OPy were estimated from cyclic voltammetry (CV) measurements of OPB-OEt and $\mathscr{E}_{00}$ was determined from spectroscopic data (see Figs. 3 and 4).[11] As a result, from Eq. (2), $\delta_S \approx 0.02$ eV, a small positive value that suggests that electron transfer within a pair of singly excited OPy species can be important for singlet-state quenching. The net driving force, ΔG_{et}, will however depend strongly also on the value of δ_C (usually, also small—e.g. <0.2 eV for aqueous media with $r = 1$ Å) (Eq. (3)).

Emission spectroscopy was used to monitor the aggregation of alkanoylpyrene in aqueous solutions of TT2oX15N where OPy exhibited two characteristic fluorescence bands: at 450 and 530 nm, ascribed to the monomer and aggregate, respectively (Fig. 1a).[6] Addition of small amounts of a negatively charged surfactant, SDS, to the polypeptide solution resulted in an increase in the intensity of the aggregate emission.[12] A phenomenon of induced aggregation of alkylpyrene derivatives in the presence of surfactants under their critical micelle concentration has been reported.[13] However, in this case, the intensity of the monomer OPy emission remains practically constant in the region of SDS concentration (C_{SDS}) where the intensity of aggregate emission increases almost three-fold (Fig. 1a and b), suggesting that the surfactant does not induce formation of more alkanoylpyrene aggregates, rather, it alters their environment (and/or geometry). Furthermore, the secondary structure of the polypeptide was essentially undisturbed upon addition of small amounts of SDS (Fig. 1c) implying that the polypeptide dimer is retained. This transition (at $C_{SDS}/C_{TT2oX15N} \approx 2.5$) is consistent with a previously reported formation of pre-

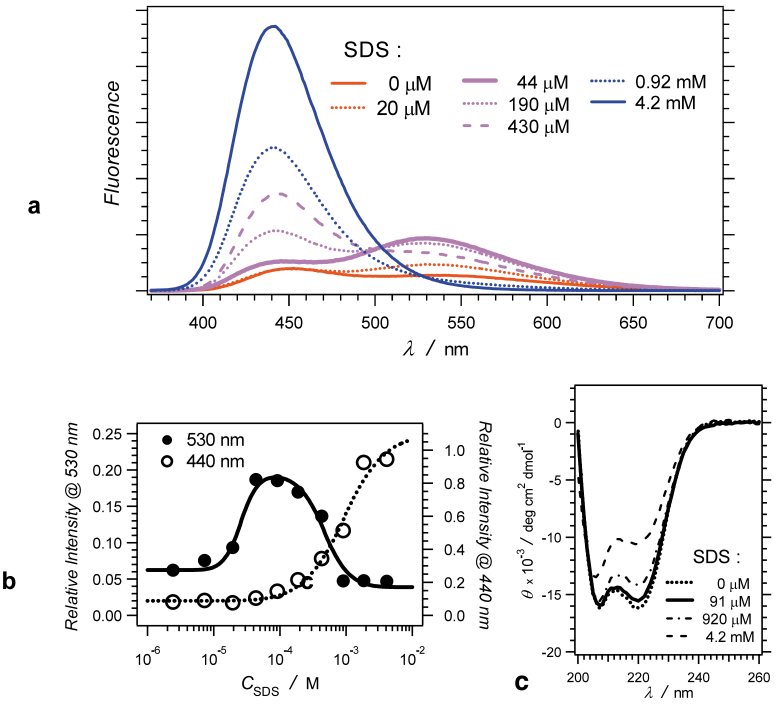

Figure 1. Emission and circular dichroism (CD) properties of TT2oX15N, 10 µM, in aqueous media (100 mM phosphate buffer, pH 7) in the presence of various amounts of SDS: (a) fluorescence spectra, $\lambda_{ex} = 355$ nm; (b) the change of the intensities of the monomer and aggregate emission bands at 450 and 530 nm, respectively, with addition of SDS; and (c) CD spectra.

Table 1. Photophysical properties of alkanoylpyrene aggregate observed in aqueous solutions of TT2oX15N (10 μM) in the presence and absence of SDS

C_{SDS}/μM	Emission data					Transient decay times at:	
	Φ_{fl}[a] (total)	Φ_{fl}[b] (aggregate)	$\tau_{(540nm)}$/ns	k_{fl}[c] $\times 10^{-6}$/s^{-1}	k_{nr}[d] $\times 10^{-6}$/s^{-1}	450 nm[e] τ_i/μs (α_i)	500 nm[e] τ_i/μs (α_i)
0	0.050	0.030	13.8±0.2	2.2	70.	76 (0.23) 2200 (0.77)	120 (0.26) 2100 (0.74)
44	0.12	0.085	19.2±0.3	4.4	48.	41 (0.23) 1600 (0.77)	20 (0.53) 1300 (0.47)

[a] Coumarin 102 and 153 solutions in ethanol were used for standards.[6]

[b] $\Phi_{fl}^{(aggregate)} = \varphi_a \Phi_{fl}^{(total)}$, where $\varphi_a = (S_{aggregate}/S_{total})\ (A_{total}/A_{aggrergate})$, A is the absorption at λ_{ex} and S is the integrated emission intensity.[6]

[c] Emission rate constant, $k_{fl} = \Phi_{fl}/\tau$.

[d] Non-radiative rate constant, $k_{nr} = (1-\Phi_{fl})/\tau$.

[e] The transient decays were fit to a biexponential function, $\Delta OD(t) = \Delta OD_0 \Sigma_{i=1}^{2} \alpha_i e^{-t/\tau_i}$ and $\Sigma_i \alpha_i = 1$.

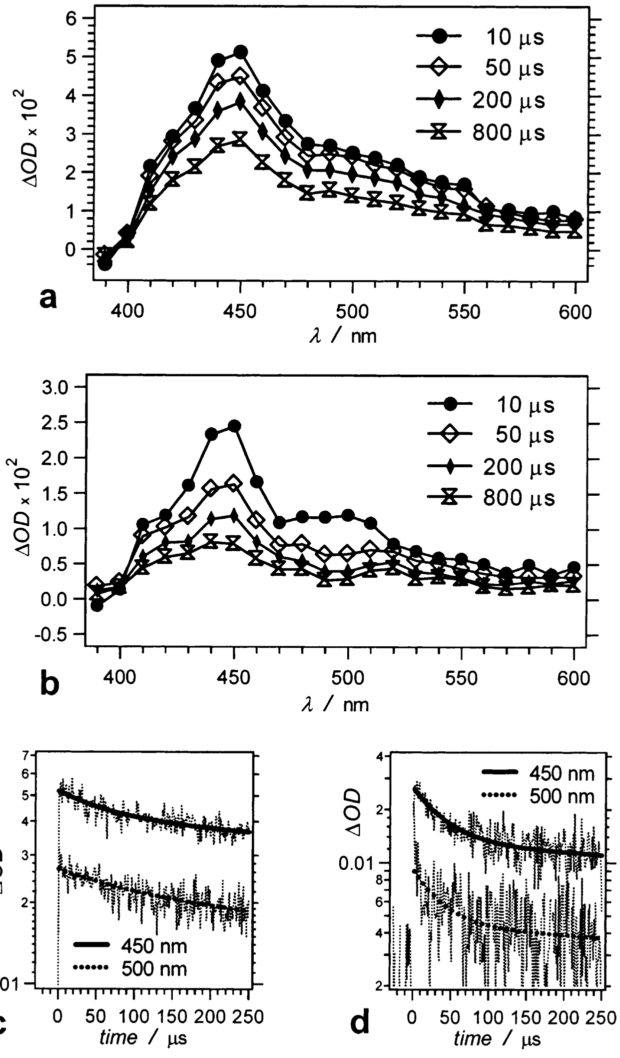

Figure 2. Transient absorption data for TT2oX15N in argon purged aqueous medium. Transient spectra of: (a) TT2oX15N, 10 μM; and (b) TT2oX15N, 10 μM in the presence of 44 μM SDS. The corresponding decays were fit to biexponential functions (Table 1): (c) for TT2oX15N, and (d) for TT2oX15N+SDS. (100 mM phosphate buffer, pH 7; λ_{ex} = 355 nm, 8 ns pulse, 10 mJ/pulse).

micellar aggregates involving polymers and pyrene derivatives.[14] When further amounts of surfactant were added, the aggregate emission disappeared and the monomer fluorescence band became significantly more intense (Fig. 1a and b). The breaking of the alkanoylpyrene aggregates (at $C_{SDS}/C_{TT2oX15N} \approx 50$) was accompanied by denaturation of the helical structure (Fig. 1c) suggesting a model in which fully developed micelles[14] absorb individual polypeptide molecules and disrupt the dimer template.

The change in the nature of the OPy aggregate and its environment, in the presence and absence of surfactant, is reflected in the alteration of its photophysical properties (Table 1). Addition of SDS should lead to a decrease in the polarity of the microenvironment around the alkanoylpyrene moieties, and hence, has several potential effects on various components of the non-radiative decay rate constant, k_{nr}: (1) a decrease in the dielectric constant, ε, will lead to more favorable electrostatic interaction between the radical-ion species (δ_C in Eq. (3)) leading to an increase in the electron transfer rate constant, k_{et};[15,16] (2) a less-polar environment will result in less-favorable solvation of the radical ions and thus, make δ_S more-positive (Eq. (3)) resulting in a decrease in the electron transfer rate; and (3)

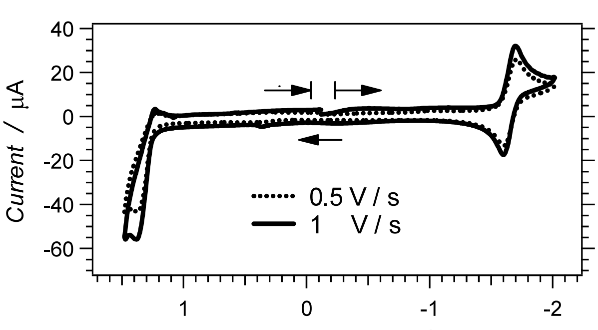

Figure 3. Cyclic voltammograms for alkanoylpyren: 1 mg ml^{-1} OPB-OEt in anhydrous acetonitrile in the presence of 100 mM $(C_4H_9)_4NPF_6$. Because of the exhibited reversibility, the half-way potential were approximated to the redox potentials: i.e. $E^0_{OPy+^\bullet/OPy} \approx E^{(1/2)}_{OPy+^\bullet/OPy} = 1.33 \pm 0.03$ V versus SCE, and $E^0_{OPy/OPy-^\bullet} \approx E^{(1/2)}_{OPy/OPy-^\bullet} = 1.65 \pm 0.004$ V versus SCE.

introduction of SDS hydrophobic chains in the vicinity of the carbonyls of the OPy chromophores will suppress hydrogen bond formation with the oxygen nonbonding orbitals that will lead to an increase in the rate of the intersystem crossing.[5] Apparently, the second factor is the overriding effect because of the decrease in k_{nr} observed on addition of SDS (Table 1).

Transient absorption spectroscopy was used to test if, indeed, charge-transfer species are produced upon photoexcitation of alkanoylpyrene aggregates. When TT2oX15N was flash-photolyzed, the OPy triplet was the predominant species observed in the microsecond time-domain (Fig. 2a). However, at early time intervals, a sharp feature was observed at ~450 nm, resembling the radical-cation overlapping the triplet (see Fig. 5 for assignment of the transient spectra).[6] In addition, the transient curves collected at 450 and 500 nm manifested double-exponential decay properties (Fig. 2c), suggesting that there is more structural heterogeneity in the distribution of the chromophore aggregate, and/or that several transients contribute to the absorption decays at observation wavelength.

When TT2oX15N was flash-photolyzed under identical conditions in the presence of a surfactant, the appearance of the radical-ion transients at early times was more conspicuous (Fig. 2b). In addition to the OPy oxidized species peaking at 450 nm, a fast decaying broad absorption band at ~500 nm was observed and ascribed to the radical-anion. Again the transient curves exhibited double-exponential decays (Fig. 2d). The transient kinetics is summarized in the last two columns of Table 1. The longer decay times at 450 and 500 nm are similar for TT2oX15N in water, as well as for the polypeptide in aqueous SDS, and therefore, can be ascribed to the triplet transient. Apparently, the addition of SDS results in shortening of the lifetime of the triplet, a result consistent with an expected increase of the rate of the intersystem crossing to the ground state, due to suppression of hydrogen bonding with the OPy carbonyl moiety.[5]

The shorter lifetimes at 450 and 500 nm are thus attributed to the radical cation and anion, respectively. The difference in the lifetimes of the oxidized and the reduced OPy species is an indication that the radical transients observed in the microsecond time domain are species that escape the ion-pair cages, i.e. their decays do not necessarily involve charge recombination with an oppositely charged transient from the same aggregate.

The emission quantum yield of the aggregate of alkanoylpyrene is about an order of magnitude smaller than the emission quantum yield of its monomer.[6] Concomitantly, the relative yield of triplet formed upon photoexcitation of OPy monomer (i.e. aqueous solution of OPBA) appears to be higher than the triplet yield from the photoexcitation of the OPy aggregates (see Fig. 6). Therefore, the low fluorescence efficiency of the alkanoylpyrene aggregates is not due to increased triplet formation, rather, it can be ascribed to photoinduced electron transfer between the OPy species of the chromophore assembly.[17]

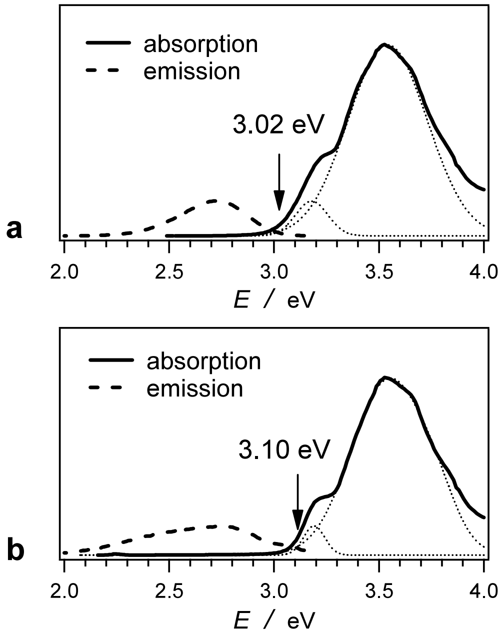

Figure 4. Emission and absorption spectra of OPBA in: (**a**) water in the presence of 100 mM phosphate buffer, pH 7; and (**b**) acetonitrile. The values for $\mathscr{E}_{00}$ were extracted from the energy of the crossing points of the normalized emission spectra and the $S_0 \leftrightarrow S_1$ transition obtained from deconvolution of the absorption spectra.

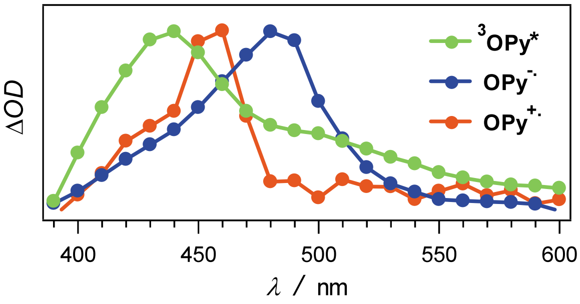

Figure 5. Normalized transient absorption spectra (at 5 μs) obtained on laser flash photolysis of alkanoylpyrene in aqueous media (λ_{ex} = 355 nm, 8 ns pulse, 10 mJ/pulse): **triplet** (**^{3}OPy***): 25 μM OPBA in 50 mM phosphate buffer, pH 8; **radical-anion (OPy$^{-•}$)**: 20 μM OPBA in the presence of 3 mM 8-hydroxyjulolidine, 50 mM phosphate buffer, pH 12; and **radical-cation (OPy$^{+•}$)**: 20 μM OPBA in the presence of 6 mM $CuSO_4$ and 5% NH_3. (All samples were purged with argon prior to data collection.) Despite the overlap of the absorption spectra of the triplet and the radical-ion transients of OPy, their spectral shapes were quite distinguishable.

In summary, the application of alkanoylpyrene as a polypeptide aggregation photoprobe in aqueous media has been demonstrated, as well as the use of a surfactant for modulation of the microenvironment of the

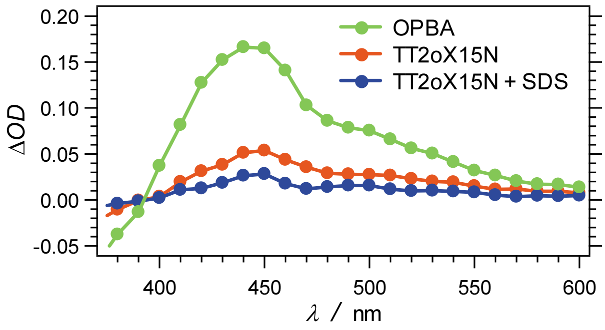

Figure 6. Comparison of transient absorption spectra (at 5 μs) of monomeric (OPBA) and aggregated (TT2oX15N) alkanoylpyrene in argon-purged aqueous media. The optical density at λ_{ex} was kept ~0.1 for all samples.

principal chromophore without disturbing the secondary conformation of the polypeptide template. Photoinduced transients observed by laser flash photolysis were associated with charge separation within self-assembled pairs of pyrene moieties, and held responsible for the significant fluorescence quenching observed upon chromophore aggregation.

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12. Since the N-terminal of TT2oX15N has a higher density of positively charged residues,[6] it is expected that the negatively charged surfactant will preferably bind in the vicinity of the alkanoylpyrene chromophore (Scheme 1).
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15. Since ΔG_{et} is small, intra-aggregate electron transfers are expected to fall in the normal region of the Marcus model,[16] i.e. an increase in driving force will result in an increase in the electron transfer rate constant.
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17. Charge transfer pathways initiated from the triplet of the chromophore were disregarded as a possibility. Triplet excited state of OPy lies about 1 eV below its singlet excited state, making the following process thermodynamically unfeasible: $^{3}\mathrm{OPy}^{*} + \mathrm{OPy} \rightarrow \mathrm{OPy}^{-\bullet} + \mathrm{OPy}^{+\bullet}$. Furthermore, the transient kinetics did not exhibit any dependence on the excitation power (10–50 mJ/pulse), implying that triplet–triplet annihilation (i.e. $^{3}\mathrm{OPy}^{*} + {}^{3}\mathrm{OPy}^{*} \rightarrow \mathrm{OPy}^{-\bullet} + \mathrm{OPy}^{+\bullet}$) is not the source of the observed charge-transfer species.