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Communication

Elucidation of photo-induced electron transfer in a loop-forming peptide: Dye-Ala-Gly-Gln-Tyr

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ABSTRACT: We investigated photo-induced electron transfer (PET) in a dye-labeled peptide, fluorophore-Ala-Gly-Gln-Tyr, employing time-resolved fluorescence. As an effort to develop new functional dyes, we studied an acriflavine derivative for the electron-acceptor in the excited state from tyrosine, an electron-donor in the ground-state. The pH dependence of the fluorescence lifetime of the model peptide indicates that electron transfer between the excited dye and tyrosine occurs when the tyrosine is deprotonated. The proton-coupled electron transfer appears to be sequential rather than concerted. We also report direct time measurements on the end-to-end loop formation processes of the peptide in water.

Photo-induced electron transfer (PET) is often investigated by monitoring fluorescence of UV-visible dyes in the presence of quenching species. It has been reported that fluorescence of electronically excited dyes is quenched by some amino acids such as tryptophan and tyrosine via PET [1-7]. Dyes used for such purpose include fluorescein, rhodamine dyes, bodipy derivatives, oxazines, methyl red dyes, and flavins. PET has been widely used to gain information on the conformations of biomacromolecules that bear an excited chromophore and a quencher. There have been numerous studies on PET involving tryptophan and its rate processes are relatively well understood. On the other hand, PET involving tyrosine has been subjected to much debate. Some reports have suggested that the tyrosine participates directly in the PET [3,4]. Other reports insist that the fluorescence quenching of the excited dye by tyrosine is not simply PET, but must be proton-coupled electron transfer (PCET) [1,6,8]. It appears that this issue needs to be resolved with a well-defined molecular system.

Tyrosine is used sparsely for intermolecular quenching because of its low solubility in water (2.5 mM at 25 ℃). Thus, it is more desirable to investigate the intramolecular PCET process that involves Tyr as the fluorescence quencher. In this work, we prepared a dye-labelled tetrapeptide, fluorophore-Ala-Gly-Gln-Tyr in which an electron transfer is expected to occur between the photoexcited dye and Tyr. An acriflavine derivative was used as the fluorophore. The fluorescence lifetimes of the model compound were measured as a function of pH and the PET rate constants were obtained from the measured fluorescence lifetimes at various pH values. On the basis of our observations, we address some unresolved issues of the PCET such as proton-coupling kinetics, dependence of the electron transfer rate on pH, and time scale of the quenching rate constant.

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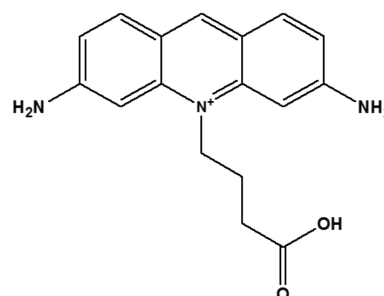
Protein conformations are often in dynamic equilibrium between unfolded and folded structures. Simple peptides have been designed to idealize such a two-state model that comprises open (unfolded) and closed (folded) forms. A pentapeptide, Cys-Ala-Gly-Gln-Trp (CAGQW), is probably the shortest amino acid sequence that satisfies such a model. The end-to-end contact formation process of the pentapeptide has been previously investigated by experiments and MD simulations [9-13]. Based on the pentapeptide, we replaced the N-terminal Cys with Atto 465 (A465) and the C-terminal Trp with Tyr to study PET from Tyr to excited A465. As an acriflavine derivative, A465 is capable of N-termialconjugation. Acriflavine is a cationic and basic dye, originally developed as an antiseptic agent. Later, it was used for an efficient laser dye and as a probe for DNA intercalation. The spectroscopic properties of acriflavine (or A465) such as absorption/emission spectra, fluorescence quantum yield, and fluorescence lifetime in solution are well documented [14-17]. Figure 1 shows the molecular structure of Atto 465 used for the preparation of A465-Ala-Gly-Gln (AF-AGQ) and A465-Ala-Gly-Gln-Tyr (AF-AGQY). Quencher-deficient AF-AGQ is a reference peptide for AF-AGQY in which the PET occurs.



ATTO 465 (A465)

AF-AGQ : A465-Ala-Gly-Gln
AF-AGQY : A465-Ala-Gly-Gln-Tyr

Figure 1. The molecular structure of acriflavine used for the synthesis of dye-labeled peptides; AF-AGQ and AF-AGQY.

Figure 2 shows the fluorescence decay curves of AF-AGQ and AF-AGQY at five different pH values.The fluorescence lifetimes of AF-AGQ are 4.26, 4.24, 4.55, 4.42, and 3.96 ns at pH 3.5, 5.5, 7.5, 9.5, and 11.5. The fluorescence lifetime shows little variance on pH with the average value of approximately 4.3 ns. However, the fluorescence decay curves of AF-AGQY showed distinct multiple exponential forms in various pH solution. Thus, they were fitted to sum-of-exponentials and the obtained amplitudes and fluorescence

lifetimes were shown in Table 1. The amplitudes and lifetimes of each component exhibit an interesting feature.The two lifetime components of AF-AGQY are independent of pH, but the amplitudes exhibit dramatic change. It seems that the short and long lifetime components (τ_1 and τ_2) represent quenched (contact) and unquenched (non-contact) conformations and the amplitudes (α_1 and α_2) are related to the population fractions of each conformation.

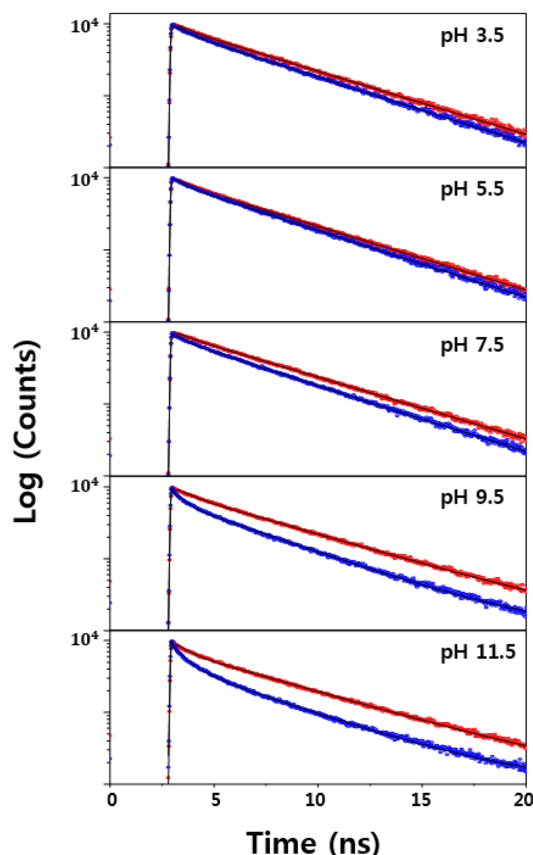


Figure 2. Fluorescence decay curves of AF-AGQ (slow decay)and AF-AGQY (fast decay) in water at different pH values.

Table 1. AF-AGQY in water at different pH values.

pH	α_1	τ_1 (ns)	α_2	τ_2 (ns)	<τ> (ns)
3.5	0.20	0.56	0.80	4.63	3.79
5.5	0.20	0.59	0.80	4.62	3.82
7.5	0.19	0.50	0.81	4.58	3.79
9.5	0.37	0.46	0.63	4.24	2.85
11.5	0.51	0.67	0.49	4.25	2.41

Table 1 shows both the short and long lifetimes are invariant to pH, but the amplitude ratio of the two components exhibit dramatic pH dependence. The ratio does not change up to pH 7. However, above pH 7, the amplitude of the short component increases rapidly at the expense of the amplitude decrease of the long component. This strongly suggests that the deprotonation of tyrosine is a determining factor for the photo-induced electron transfer. The proton-coupled electron transfer (PCET) process is sequential rather than concerted because the electron transfer occurs from the already deprotonated tyrosine to the acceptor dye. Our results may provide some useful information on the PCET process [18-27].

According to Marcus ET theory, the electron transfer process becomes spontaneous when $\Delta G^0 < 0$ [28]. The free energy change of the reaction can be obtained through the Rehm-Weller equation:

$$\Delta G^0 = e[E_{ox} - E_{red}] - E_{0,0} + C \quad (1)$$

Where E_{ox} and E_{red} are the oxidation and reduction potentials of the donor and acceptor, respectively [29]. $E_{0,0}$ is the zero-zero transition energy for the electron acceptor (fluorophore). C is the Coulombic energy term, which is negligible in polar environments. On the basis of the molecular structure shown in Figure 1, the reduction potential of A465 is likely similar to that of acriflavine (-1.10 V) [15]. The one-electron oxidation potentials (E_{ox}) of TyrOH and $TyrO^-$ are 1.46 V and 0.72 V, respectively [30]. When the $E_{0,0}$ value of A465 used is 2.61 eV [15], the ΔG^0 value is -0.05eV for A465-TyrOH and -0.79 eV for A465-$TyrO^-$. This indicates that PET does not occur for A465-TyrOH because ΔG^0 is close to zero. On the other hand, the PET for A465-$TyrO^-$ is thermodynamically favorable because of the exergonic ΔG^0. In this calculation, the E0,0 value is assumed to be pH-independent.

The quenching rate constant, k_q, can be experimentally obtained from the time-resolved data:

$$k_q = \frac{1}{\tau_F} - \frac{1}{\tau_0} \quad (2)$$

Where τ_F and τ_0 represent the fluorescence lifetime of the A465 in the presence and absence of Tyr, respectively. When the decay is not single exponential, one must use the average lifetime $<\tau>$ to calculate the quenching rate constant in Equation (2):

$$<\tau> = \sum_i \alpha_i \tau_i / \sum_i \alpha_i \quad (3)$$

where α_i and τ_i indicate the amplitude and lifetime of the *i*th-component of the exponential decays, respectively. The $<\tau>$ of AF-AGQY at different pH values were shown in Table 1.

Figure 3 shows the obtained k_q at different pH values using Equation (2) and (3). The k_q value does not change up to pH 7, but increases rapidly above pH 7, virtually reflecting the change of the amplitude ratio (Figure 3B). The $k_q$calculated from the average lifetime can be regarded as the apparent rate constant that could be obtained through steady-state measurements. Our results show that, while the electron transfer rate is constant,the relative population of contact and noncontact conformations varies with respect to pH environments. At pH 10, which is the pK_a value of tyrosine, there is no abrupt change in the apparent rate constant. The pH dependence of the data arises from the concentration change of the deprotonated tyrosine. The contact formation time is the inverse of the electron transfer rate constant. The contact formation time between Trp and Cys in a pentapeptide was observed to be approximately 40 ns by Eaton and coworkers [9]. However, previous MD simulations and experiments have presented much faster contact formation times [10-12]. A recent MD simulation showed that the contact formation time

could occur on a subnanosecond time scale. [13]. The measured value of the contact formation time (500 ps) in basic condition is in excellent agreement with the MD simulations. Our important findings may provide new insights on PCET involving tyrosine. Previously, the pH dependence of PCET has been described on the basis of a Pourbaix diagram. Our time-resolved studies using the loop-forming peptide, AF-Ala-Gly-Gln-Tyr, do not indicate significant electron-proton coupling. That is, the PET rate constant does not depend on pH and the electron transfer only occurs after the tyrosine is deprotonated. Detailed information on the population distribution of the conformations of the peptide in water can be accessed by separate work in the future.

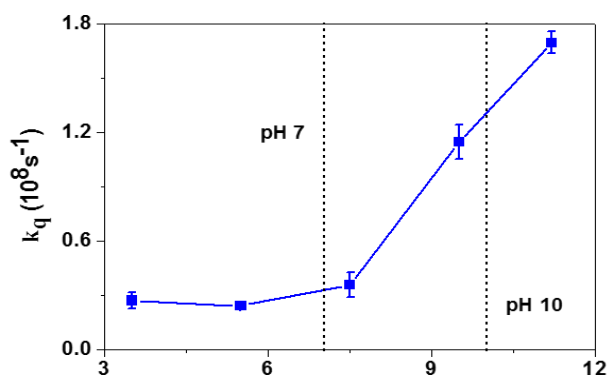


Figure 3. The quenching rate constants of AF-AGQY in water at different pH values.

In summary, we used a dye-labelledtetrapeptide, fluorophore-Ala-Gly-Gln-Tyr, to investigate photo-induced electron transfer involving acriflavine and tyrosine. The fluorescence decay profiles obtained by TCSPC consist of two lifetime components (0.5 and 4.5 ns) which represent quenched (contact) and unquenched (non-contact) conformations, respectively. Both the short and long lifetimes are invariant to pH, but the amplitudes of the two components exhibit pH dependence. Both amplitudes do not change in acidic and neutral conditions. However, above pH 7, the amplitude of the short component increases rapidly at the expense of the amplitude decrease of the long component. Dependence of the fluorescence lifetime of our model peptide on pH showed that the PET only occurs between the dye and the deprotonated tyrosine (YO^-), strongly indicating that the process is sequential proton coupled electron transfer. We also report the contact formation time of the model peptide, which is in excellent agreement with MD simulations.

KEYWORDS: acriflavine, tyrosine, fluorescence quenching, peptide conformation, proton-coupled electron transfer

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SUPPORTING INFORMATION

The synthesis of peptides was performed using conventional Fmoc chemistry and a peptide coupling method on the solid phase. The synthesized peptides were confirmed by MALDI-TOF analysis which was performed on an Axima Performance mass spectrometer (Shimadzu). The purified peptides which were freeze-dried in a lyophilizer were incubated with Atto 465 NHS ester (0.3 eq.) and DIPEA (50 eq.) in acetonitrile in a dark environment at room temperature for 1 h. After the solvent was removed by blowing N_2 gas, the crude conjugates were similarly purified through a C18 reverse-phase column on a Shimadzu binary HPLC system equipped with a UV-visible detector to give the desired fluorescent dye-peptide conjugates which were freeze-dried in a lyophilizer and solubilized in dimethyl sulfoxide (DMSO). Samples were prepared at concentration of 60 μM by dilution in distilled water at pH 3.5, 5.5, 7.5, 9.5, and 11.5.

The fluorescence lifetimes of samples were measured using time-correlated single photon counting (TCSPC) system. The light source was a picosecond diode laser at wavelength 442 nm (Picoquant LDH-P-C-440M & PDL800-B) and repetition rate 20 MHz. An inverted microscope (Nikon, TE2000-S) was used as a platform with a water immersion objective lens (NA 1.4, x60) for excitation of samples and fluorescence detection. The total fluorescence signal from samples was detected by a microchannel plate photomultiplier tube (Hamamatsu R3809U) and processed by a fast board (Becker-Hickl, SPC-830). The instrument response function (IRF) of our TCSPC system was approximately 90 ps. The fluorescence lifetimes were extracted from the measured decay curves through a nonlinear least square fit with deconvoluting IRF by using the FluoFit software (Picoquant).

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