Dyes and Pigments 112 (2015) 54–58

Contents lists available at ScienceDirect

Dyes and Pigments

journal homepage: www.elsevier.com/locate/dyepig

Photoinduced electron transfer between flavin and tryptophan in a poly(vinyl alcohol) film

Youmin Lee, Hwiin Lee, Minyung Lee*

Department of Chemistry and Nanoscience, Global Top 5 Program, Ewha Womans University, Seoul 120-750, Republic of Korea

ARTICLE INFO

Article history:
Received 22 February 2014
Received in revised form
12 June 2014
Accepted 19 June 2014
Available online 25 June 2014

Keywords:
Time-resolved fluorescence
Lumiflavin
Riboflavin
Flavin mononucleotide
Poly(vinyl alcohol)
Photoinduced electron transfer

ABSTRACT

The fluorescence of an electronically excited dye is influenced by surrounding quenchers through photoinduced electron transfer. Flavins participate in many biochemical reactions and undergo photoinduced electron transfer in the presence of tryptophan. Using three flavins (lumiflavin, riboflavin, and flavin mononucleotide), we investigated the photoinduced electron transfer of flavin-tryptophan which were embedded in poly(vinyl alcohol) films. The decrease in the fluorescence lifetimes of flavins becomes significant as the tryptophan concentration increases. Furthermore, we used the rate constants obtained from the measured fluorescence lifetimes to describe our observations based on the free energy change of the reaction, specific interactions, and excluded effects for the photoinduced electron transfer processes.

1. Introduction

Photoinduced electron transfer (PET) processes are widely occurring phenomena in chemistry and biology [1–7]. The excited chromophore could be an electron donor (D) or acceptor (A), depending on the nature of the two molecular moieties involved in the PET. In oxidative electron transfer, an electron is transferred from the excited chromophore to the ground-state acceptor. In reductive electron transfer, an electron is transferred from the ground state donor to the excited chromophore. The efficiency of the electron transfer is usually monitored by the fluorescence of the chromophore. Reductive electron transfer is common for many fluorophores such as fluorescein, rhodamine dyes, bodipy derivatives, oxazines, methyl red dyes, and flavins. Considerable attention has been focused on the PET between dyes and aromatic amino acids such as tyrosine (Tyr) and tryptophan (Trp), due to their biological importance. The quenching of a dye's fluorescence can be either static or dynamic, and both processes provide information on the intermolecular distance between the electron D-A pair [8–14]. In other words, when the ground-state complex between the donor and acceptor pair is neglected, greater fluorescence quenching indicates faster electron transfer.

As a naturally found chromophore, flavin exists in a variety of forms that are involved in many biochemical reactions [15,16]. The nature of flavin fluorescence is affected by nearby amino acids. Phenylalanine does not quench flavin fluorescence but Tyr and Trp strongly quench the fluorescence via PET. Sulfur-containing amino acids such as methionin and cysteine also quench flavin fluorescence, although the degree of quenching is much weaker than that induced by Tyr or Trp [17]. Studies of dynamic fluorescence quenching provide valuable information on the conformations of biomacromolecules. Fig. 1 shows the molecular structures of three flavins that we have chosen to investigate the PET process. Those flavins have an isoalloxazine (lumichrome; 7,8-dimethylalloxazine) as the chromophore in common with different substituents at the 10 position of the nitrogen atom. The molecular sizes increase in the order of lumiflavin (LF) < riboflavin (RF; vitamin B_2) < flavin mononucleotide (FMN).

There are numerous studies on the PET between flavin and Tyr or Trp pairs. Zhong and Zewail employed ultrafast frequency upconversion and transient absorption spectroscopy and investigated the electron transfer dynamics of flavoproteins at the femtosecond time scale [18]. Similar work was also performed by Tanaka et al. with flavodoxin protein from *Megasphaera elsdenii* [19]. The fluorescence quenching studies for flavins are not limited to natural flavoproteins. Butterfield et al. synthesized a Trp-containing peptide that mimics flavoprotein [20] and observed efficient quenching of the flavin in the model peptide. Recently,

* Corresponding author. Tel.: +82 2 3277 2383; fax: +82 2 3277 2384.
E-mail address: mylee@ewha.ac.kr (M. Lee).

http://dx.doi.org/10.1016/j.dyepig.2014.06.023
0143-7208/

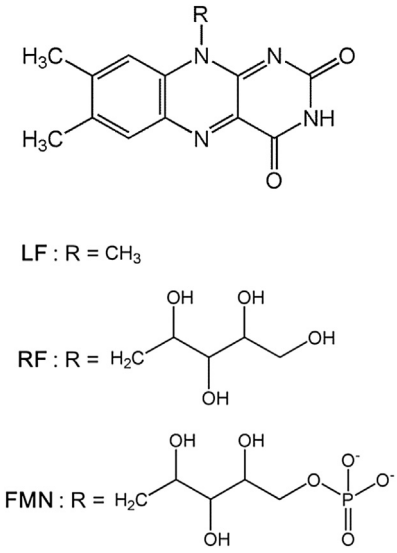

Fig. 1. The molecular structures of LF, RF and FMN.

Sengupta et al. investigated binding of flavins to human serum albumin, employing time-resolved fluorescence and molecular dynamic simulation [21].

PET is basically a pairwise interaction but there are many situations in which a fluorophore is surrounded by many electron donors. Fig. 2 shows such a case where each arrow represents the probability of transferring an electron. The distance distributions for each electron donor-acceptor pair determine the overall electron transfer rate. In solids, the rate constant only depends on the donor-acceptor distance distribution because the diffusion process can be neglected. In this work, the fluorescence lifetimes of flavins embedded in poly(vinyl alcohol) (PVA) films were measured in the presence of Trp by using a time-correlated single photon counting (TCSPC) system and the PET rate constants were obtained from the measured fluorescence lifetimes as a function of quencher concentration. We describe our observations with respect to the free energy change of the reaction, specific interactions and excluded volume effects for the PET processes.

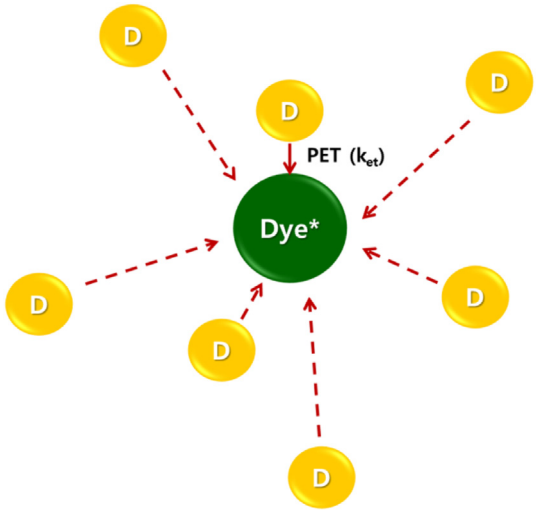

Fig. 2. A schematic diagram of the PET of a single acceptor surrounded by many donors.

2. Experimental

2.1. Materials and sample preparation

Lumiflavin, (-)-riboflavin, flavin mononucleotide, L-tryptophan, and poly(vinyl alcohol) (Mw 146,000–186,000) were purchased from Sigma–Aldrich. All chemicals were used without further purification. Each stock solution of flavins, Trp, and PVA were prepared in distilled water under sonication. PVA polymer films containing dye and Trp were prepared on a cover glass by spin coating. The as-prepared polymer films were dried at 70 °C for 3 h. The thickness of the dye-doped PVA films was approximately 600 nm and each of the polymer films contained approximately 10 μM fluorophores. This concentration is sufficiently low compared to the electron donors, which are 0–20 mM in solution.

2.2. Fluorescence lifetime measurements

The fluorescence decay curves were obtained using a TCSPC system consisting of three parts. First, a picosecond diode laser operating at wavelength of 442 nm (Picoquant LDH-P-C-440M & PDL800-B) was used as the light source. Second, an inverted confocal microscope (Nikon, TE2000-S) with an oil immersion objective lens (NA 1.4, 60×) was used as a platform for sample excitation and fluorescence detection. The sample on top of the microscope was continuously scanned by an atomic force microscopy (AFM) controller (XE-100, Park Systems) over an area of $60 \times 60\ \mu m^2$ at 1 Hz to avoid possible photochemical bleaching and glass softening effects. A long pass cut-off filter (Semrock BLP01-473R) was used to block the excitation beam. A microchannel plate photomultiplier tube (Hamamatsu R3809U-51) was used to detect the total fluorescence signal parallel to the excitation polarization. Third, a fast TCSPC board (Becker-Hickl, SPC-830) was used to obtain the fluorescence signal. The instrument response function (IRF) of the TCSPC system was approximately 90 ps. The measured decay curves were analyzed using the FluoFit software (Picoquant). The fluorescence lifetimes were extracted from the measured decay curves by a nonlinear least square fit after deconvoluting the IRF.

3. Results and discussion

Both of Trp and Tyr have been widely used as flavin quenchers. Here, Trp was chosen instead of Tyr because Trp has much higher solubility in water than Tyr (>twentyfold). In addition, Trp is less prone to undergo proton-coupled electron transfer (PCET) in which Tyr is heavily involved. Fig. 3 shows the fluorescence decays of the three flavin fluorophores (LF, RF, and FMN) in the absence and presence of Trp in PVA. All of the decay curves of flavins did not fit well to a single exponential decay function, and thus they were fitted using a triple-exponential form. Then, the amplitude-averaged fluorescence lifetime was calculated using

$$<\tau> = \sum_i \alpha_i \tau_i \Big/ \sum_i \alpha_i \qquad (1)$$

where α_i and τ_i indicate the amplitude and lifetime of the ith-component of the exponential decays, respectively.

From the measured decay curves of flavins, the average lifetimes were extracted and plotted in Fig. 4. The radiative and nonradiative decay processes of the flavins reportedly depend on the physical characteristics of solid films [22]. Our data showed that those of LF, RF, and FMN significantly differ when embedded into the polymer. The fluorescence lifetimes of the flavins without Trp were 3.28 ns (LF), 2.87 ns (RF), and 1.27 ns (FMN) in PVA. RF is a LF derivative