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Jeong-Woo Choi $^{\rm a}$, Yun-Suk Nam $^{\rm a}$, Dongho Kim $^{\rm b}$, Won Hong Lee $^{\rm a}$ & Masamichi Fujihira $^{\rm c}$

^a Dept. of Chem. Eng., Sogang Univ., C.P.O. BOX 1142, Seoul, 100-611, Korea

^b National Creative Research Initiatives Center for Ultrafast Optical Properties Characterization, Korea Research Institute of Standards and Science, P.O. BOX 102, Yusong, Taejeon, 305-600, Korea

^c Dept. of Biomol. Eng., Tokyo Institute of Technology, Yokohama, 227, Japan

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Transient Fluorescent Characteristics of GFP/Viologen/Cytochrome-c Hetero-LB Films

JEONG-WOO CHOI^a, YUN-SUK NAM^a, DONGHO KIM^b, WON HONG LEE^a and MASAMICHI FUJIHIRA^c

 ^aDept. of Chem. Eng., Sogang Univ., C.P.O. BOX 1142, Seoul 100-611, Korea,
 ^bNational Creative Research Initiatives Center for Ultrafast Optical Properties Characterization, Korea Research Institute of Standards and Science,
 P.O. BOX 102, Yusong, Taejeon 305-600, Korea and ^cDept. of Biomol. Eng.,
 Tokyo Institute of Technology, Yokohama 227, Japan

The charge transfer characteristics of molecular hetero-LB films composed of green fluorescence protein (GFP), viologen, and cytochrome-c were investigated by transient fluorescence measurement. In this work, transient fluorescence decay of GFP homo film and GFP/viologen/cytochrome-c hetero films were measured to investigate the charge separation rate and transport mechanism by using time-correlated single photon counting method (TCSPC).

<u>Keywords:</u> Green fluorescent protein (GFP); Cytochrome c; Time resolved fluorescent; Electron transfer; TCSPC

INTRODUCTION

In the biological photosynthesis, photoelectric conversion and long-range electron transfer occur not only efficiently but also unidirectionally through the functional groups of biomolecules. Electron sensitizer/electron acceptor structured hetero films have the similar structure with the photoinduced

electron transfer system of the bacterial photosynthetic reaction center^[1]. The green fluorescent protein (GFP) is the final light emitting protein in the bioluminescent jellyfish *Aequorea victoria*. GFP absorbs blue light and emits green light (510nm). Since GFP shows very highly efficient quantum yield, approximately 80%, it is very reasonable approach to use GFP as an electron sensitizer in elucidation of the electron transfer mechanism of natural biological photosynthesis.

In this work, the hetero-Langmuir-Blodgett (LB) films consisting of GFP/viologen/cytochrome-c was constructed. The transient fluorescence decay profile of hetero-LB films was measured to investigate the charge separation rate and transport mechanism.

EXPERIMENTAL DETAILS

Viologen (Electron relay; R) and cytochrome-c (Electron acceptor; A) films were deposited onto the quartz plate by LB method with a circular type Langmuir trough (Model 2022, Nima Tech.,UK). The viologen/cytochrome-c hetero-LB films were soaked into the GFP (Electron sensitizer; S) solution (1 μ M, pH8.0) for 1hr, and then the GFP molecules were electrostatically adsorbed onto the surface of hetero-LB films.

Transient fluorescence decay profiles were recorded by using TCSPC technique with a femtosecond Ti:Sapphire laser pulse excitation. The fluorescence lifetimes of S homo, S/R hetero, and S/R/A hetero films were determined from the response profiles at 510nm.

RESULTS AND DISCUSSION

Fig.1 shows the energy diagram of S/R/A structured hetero-films. By light illumination, the electrons of S molecules are excited from their ground

state to excite state (S*). The photo-excited electrons return to their ground state and then green fluorescence is emitted at 510nm.

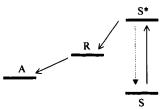


FIGURE 1. The energy diagram of S/R/A structured hetero-film

However, the electron acceptor is exposed to the excited sensitizer, some of photo-excited electrons of S^* can be separated $(S^+/R^-/A)$ and transferred $(S^+/R/A^-)$ to A molecule via their redox potential difference. Thus the photoinduced one-way electron transfer can be generated. By measuring the transient fluorescence, the charge separation rate of S molecule to A molecule could be calculated and the photoinduced one-way electron transfer could be verified.

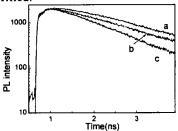


FIGURE 2. Transient fluorescence decay profiles: a, S homo-film; b, S/R hetero-film; c, S/R/A hetero-film

Transient fluorescence of each film was obtained at 510nm. The charge separation rate (k_a) of excited S to R/A can be written as $k_a=1/\tau_t$ - $k_s=1/\tau_t$ - $1/\tau_s$. Where k_a is the electron transport rate from excited S to R/A, τ_t and τ_s are the fluorescence lifetime of S in hetero and homo-films, respectively, and k_s is the

fluorescence decay rate of excited S in homo-films. Based on the above equation, the charge separation rate can be obtained from the fluorescence lifetime of S in homo and hetero-film. The transient fluorescence decay profiles were shown in Fig.2.

The fluorescence lifetime of S homo-films was c.a 1.54ns. The time constant and charge transfer rate of each film were shown in Table 1. The fluorescence decay profile of S homo-film was well described as single exponential component, whereas the decay profiles of S/R and S/R/A hetero-film were well described as double exponential components.

TABLE 1. Time constants and charge transfer rates of each film

Films structure	Component 1	Component 2
S homo	1.54ns	<u>-</u>
S/R hetero	0.17ns	1.38ns
k _a (s ⁻¹)	5.25 × 10 ⁹	
S/R/A hetero	0.105ns	1.274ns
k _a (s ⁻¹)	8.87×10^9	-

The charge separation rate of S/R/A hetero-films was faster than that of S/R hetero and S homo-films. In this result, the one-way electron transport from S to A via R was verified, and this result can be extended to develop bioelectronic devices, such as a photodiode and memory device.

Acknowledgements

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