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The charge transfer characteristics of molecular homo-films composed of green fluorescence protein (GFP) were investigated by time-resolved fluorescence measurement. In this work, time-resolved fluorescence decay of GFP homo film was measured to investigate the solid-state fluorescence lifetime and to observe the charge transfer by using time-correlated single photon counting method (TCSPC).

Keywords: Green fluorescent protein (GFP); Lifetime: Time resolved fluorescent; TCSPC; Charge transfer

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.

In this work, the homo-films consisting of GFP was constructed. The time-resolved fluorescence decay profile of GFP homo-film at various wavelengths was measured to investigate the fluorescence lifetime and charge separation rate.

EXPERIMENTAL DETAILS

Recombinant Green Fluorescent Protein (rEGFP, CLONTECH, USA) was used as fluorescent molecules. The GFP homo-film was deposited onto the quartz substrate by casting method. Time-resolved fluorescence measurement was performed with vacuum condition.

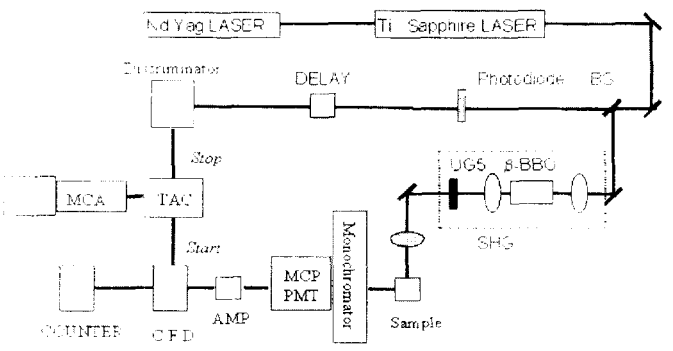

FIGURE 1. Experimental setup for time resolved fluorescence measurement.

Fig. 1 shows the experimental setup for the time-resolved fluorescence. Time-resolved fluorescence decays were measured with GFP homo films.

Fluorescence decay profiles were recorded by using a time-correlated single photon counting (TCSPC) technique with a femtosecond Ti:Sapphire laser pulse excitation. The laser pulse width is 150fs and the average power was 500mW at 82MHz operation. To excite the GFP molecules of films, the laser pulses were frequency doubled by using a β-BBO(β-barium borated) crystal. All the standard electronics used for the TCSPC system were purchased from EG&G Ortec(TN, USA). The observed instrumental response function (IRF) for the excitation pulse was ca.52ps(FWHM) at 350nm. This method allowed us to measure the fluorescence decay with a time resolution of ca.10ps after deconvolution.

RESULTS AND DISCUSSION

Fig.2 shows the time-resolved fluorescence decay profiles of GFP homo-films at various wavelengths.

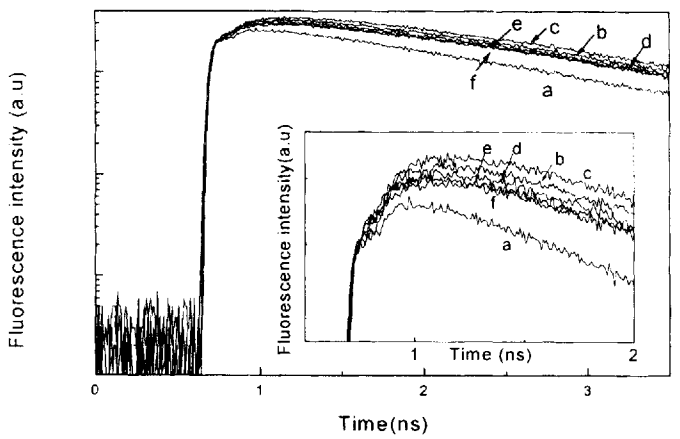

FIGURE 1. The time-resolved fluorescence decay profiles; a, 500nm; b, 510nm; c, 520nm; d, 530nm; e, 540nm; f, 550nm.

The fluorescence lifetimes were shown in Table 1. The fitting equation of fluorescence decay curves can be represented as :

$f(t)=a_1\exp(-t/\tau) + a_2\exp(-t/\tau)$

where a is the amplitude(intensity) and τ is the time constant of each component. The fluorescence decay profiles of GFP homo-films were well described by the fitting function and the single exponential components. The fluorescence lifetimes of GFP homo-films were c.a 1.56ns, 2.33ns, 2.50ns, 2.18ns, 2.01ns, and 2.09ns in 500nm, 510nm, 520nm, 530nm, 540nm, and 550nm, respectively. In the aqueous solution, GFP has 508nm maximum fluorescence intensity. However, in the solid state, GFP fluorescence of 520nm was more intensively detected than those of other wavelength.

TABLE 1. Fluorescence lifetimes at various wavelengths

Wavelength(nm)	500	510	520	530	540	550
Lifetime(ns)	1.56	2.33	2.50	2.18	2.01	2.09

In this result, we can conclude that the fluorescence of solid-state GFP molecules at vacuum conditions has 520nm maximum fluorescence intensity, and the lifetime of 500nm is faster than those of other wavelengths.

Acknowledgements

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