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Time-resolved ultrafast blue-shifted fluorescence from pea chloroplasts

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The antenna chlorophylls in higher plants can produce fluorescence that is blue-shifted (anti-Stokes-shifted) relative to the exciting light. Global analysis of the time-resolved fluorescence spectra resolves an ultrafast fluorescence emission component with a lifetime of about 15 ps which is attributed to exciton equilibration in the antenna. These results support the hypothesis that the overall exciton decay kinetics in PS I and PS II is trap-limited as suggested by us earlier.

Time-resolved fluorescence; Anti-Stokes-shifted fluorescence; Exciton; Chlorophyll antenna

1. INTRODUCTION

Normally, fluorescence is red-shifted (Stokes-shifted) relative to the excitation light. Under some conditions, blue-shifted (anti-Stokes-shifted) fluorescence can be observed [1–3]. In dye molecules the blue-shifted fluorescence is often spectrally distorted as compared to the normal fluorescence [4]. In higher plants the absorption and fluorescence emission spectra overlap strongly. The absorption spectrum extends past 700 nm while the fluorescence emission peak is near 680 nm. After being absorbed, a photon creates an exciton which will have some finite equilibration time in the chlorophyll antenna (Chl). This equilibration time reflects both thermal equilibration with the environment (protein matrix) and spatial equilibration over the whole ensemble of antenna Chls. If the equilibration time is rapid relative to the exciton lifetime, then the fluorescence emission spectrum should be always the same, regardless of the excitation wavelength. This should be true even if the antenna is excited near the band-edge at 700 nm. In contrast, if the equilibration time is comparable to the overall exciton lifetime, limited by charge-separation processes, then there should be significant distortions in the spectral shape of the fluorescence emission when the antenna is excited at far-red wavelengths.

In this work we show that blue-shifted fluorescence with an undistorted emission spectrum can be observed from higher plants. We demonstrate directly that the previously observed lifetime components of 600, 250 and 80 ps [5–7] are constant, regardless of excitation wavelength and that a new ultrafast (15 ± 4 ps) component due to exciton equilibration within the antenna can be observed. We further demonstrate that an exciton created by far-red light (λ = 692 nm) is capable of closing photosystem (PS) II reaction centers and migrating over the entire PS II antenna.

2. MATERIALS AND METHODS

The fluorescence induction kinetics were measured on a laboratory-constructed spectrophotometer. The excitation

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Abbreviations: Chl, chlorophyll; PS, photosystem; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea

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light, provided by a tungsten lamp, was passed through a monochromator set at 692 nm with a bandpass of 4 nm. The photomultiplier was covered by a 685 nm interference filter. The use of a flow cell allowed the fluorescence signals from successive samples to be averaged together.

The steady-state fluorescence spectra were measured on the same set-up as the time-resolved spectra. The dye laser had a spectral width of less than 0.5 nm. The monochromator used to resolve the fluorescence emission had a bandpass of 4 nm.

The time-resolved fluorescence signals were measured using a single-photon timing apparatus and analyzed using the method of global analysis in [8].

Chloroplasts were prepared from laboratory grown peas as in [9]. All measurements were performed at a Chl concentration of 8 μg/ml, as determined according to Arnon [10].

3. RESULTS

Fluorescence emission is commonly measured at longer wavelengths than the exciting light. Such a situation is illustrated for pea chloroplasts in fig.1A, where excitation of the sample was at 630 nm and a fluorescence emission peak was observed at 680 nm. There is an emission shoulder, due to PS I [7], extending out to 750 nm. As mentioned previously, the antenna chlorophyll absorbs past 700 nm. If the exciton created by the absorbed light equilibrates rapidly within the antenna, then the fluorescence emission spectrum should always be the same, even when excited with far-red wavelengths. In fig.1B, we show the fluorescence emission spectrum from pea chloroplasts upon excitation at 692 nm. Note the similarity of this spectrum to that of fig.1A. In particular, the emission peak is still near 680 nm even for excitation at 692 nm. The only change in emission relative to fig.1A is an increase in relative fluorescence yield in the far-red shoulder. This increase is due to the relatively greater absorption by PS I at this wavelength. Time-resolved fluorescence lifetime data, using laser excitation at 692 nm and the single-photon timing technique, are shown in fig.2. The spectral shapes and lifetimes are similar to those previously reported for the four longer-lived components [5–7] upon excitation with 630 nm light. The expected increase in amplitude of the fast (82 ps) emission from PS I at this excitation wavelength is clearly seen. In addition, however, an ultrafast signal is resolved with both positive and negative amplitudes. The ultrafast lifetime component varied slightly from sample to sample, with an average lifetime of 15 ± 4 ps. Negative amplitude components indicate a rise time in the

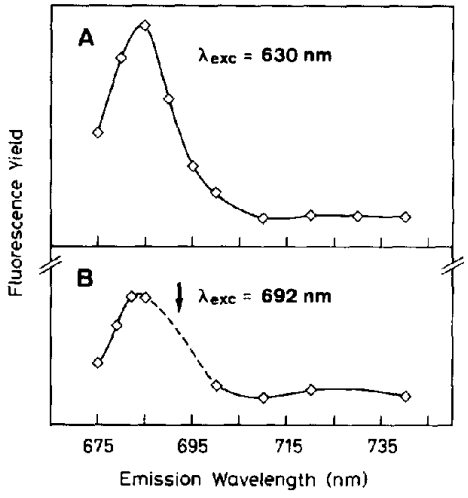

Fig.1. Fluorescence emission spectra from pea chloroplasts in the F_0 state (open PS II reaction centers). Excitation wavelength: 630 nm (A), 692 nm (B). The dashed part in (B) corresponds to the wavelength region of scattered excitation light where measurement is not possible.

signal, suggesting energy transfer from an absorbing pigment pool to the emitting pigment(s) [11].

Regardless of the wavelength of the absorbed light, once equilibration occurs the excitation energy is available for photochemistry. This is illustrated in fig.3, which shows a fluorescence induction curve from chloroplasts excited at 692 nm

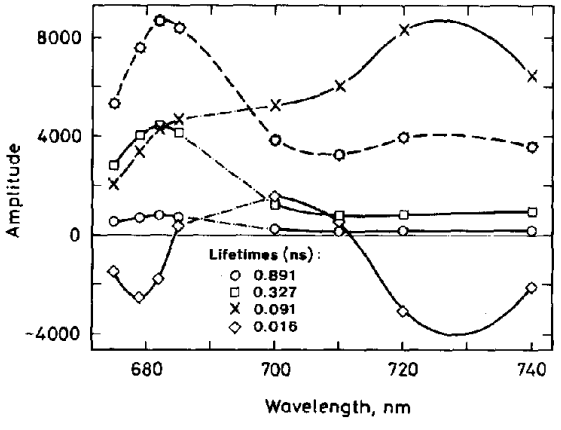

Fig.2. Time-resolved fluorescence decay-associated spectra [8] obtained from pea chloroplasts upon excitation at 692 nm under F_0 conditions. The global χ^2 is 1.1. The data points are given by symbols. The lines connecting the points are calculated from best-fit second-order polynomials. The dashed line is the steady-state emission spectrum calculated from the time-resolved spectra.

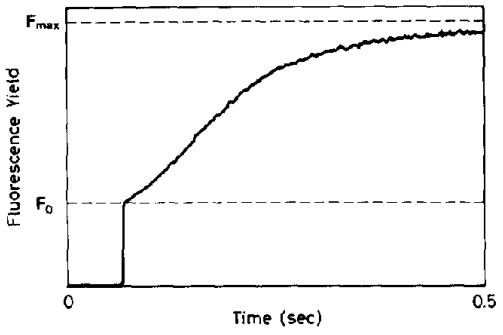

Fig.3. Biphasic blue-shifted fluorescence induction from pea chloroplasts in the presence of DCMU. Excitation wavelength, 692 nm; fluorescence detection wavelength, 685 nm. The connectivity parameter of the sigmoidal phase is 0.55.

in the presence of DCMU. The fluorescence emission was measured at shorter wavelengths, i.e. at 680 nm. The pronounced variable fluorescence indicates that charge separation is occurring at P-680, the reaction center of PS II. Note also the sigmoidicity of the fluorescence rise. This sigmoidicity demonstrates that an exciton created with a low-energy photon is not confined to the neighborhood of one reaction center, but rather can migrate through the antenna to sample several different PS II reaction centers as is well-known for excitation at shorter wavelengths [12].

4. DISCUSSION

In both higher plants and green algae, fluorescence decay components at F_0 (open PS II centers) with lifetimes of about 600, 250, and 80 ps, as well as a very small amplitude component of about 2 ns due to either closed centers or uncoupled chlorophyll, have been measured [5–7]. These components are due to the decay of the exciton created in the antenna by the absorption of light. Models of exciton migration in the photosynthetic antenna fall into two general classes, diffusion-limited and trap-limited [13]. In diffusion-limited models, the time for an exciton to migrate from the antenna to an irreversible trap at the reaction center (first passage time) determines the fluorescence lifetime. Fluorescence is emitted as the exciton passes through the antenna Chls on its way to the reaction center. Thus, an exciton created at the reaction center should have a shorter lifetime and a lower fluorescence yield [15].

In contrast, the first passage time is very short as compared to the overall exciton trapping time in trap-limited models. The exciton then equilibrates very rapidly within the entire antenna. The trap is assumed to be so shallow, however, that the exciton can encounter the reaction center many times before trapping by charge separation occurs. In a trap-limited model, an exciton will produce nearly the same fluorescence, regardless of whether it is created at the reaction center or in the outer antenna.

The lifetimes that we observe here for the three major longer-lived components (890, 320, 91 ps) are identical within the error limits to those observed upon short-wavelength illumination. This suggests that the equilibration time (first passage time) in the antenna is much faster than any of these lifetimes. In addition, we observe an ultrafast component of about 15 ps, which has also recently been observed in green algae [14]. This component has both positive and negative amplitude components, suggesting that it represents energy transfer from the pigments initially excited at 692 nm to the neighboring pigments. This ultrafast signal provides direct evidence for the fast antenna equilibration and thus supports the trap-limited model. We note here that the global analysis [16] method is most effective at resolving multiple components of the decay when their spectral shapes differ. By exciting at 692 nm we create conditions which maximize the difference in spectral shape of the very fast component from the previously observed, longer-lived components. This ultrafast component is probably a composite of emissions from both PS II and PS I reflecting the so far unresolved exciton equilibration process in the respective antenna systems.

This work demonstrates that exciton equilibration in the photosynthetic antenna of higher plants is a very rapid process, much faster than the 80 ps time characteristic of the PSI fluorescence decay. The 15 $\pm$ 4 ps signal is emitted during the exciton equilibration process. The extra energy that the blue-shifted fluorescence photons possess is obtained from thermal energy in the environment.

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