FLUORESCENT KINETICS OF CHLOROPHYLL IN PHOTOSYSTEMS I AND II ENRICHED FRACTIONS OF SPINACH

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SUMMARY

The fluorescent emission kinetics of spinach subchloroplast Photosystems I and II particles have been studied on a picosecond time scale. Using picosecond laser pulses and an optical Kerr gate, the fluorescent decay times are measured to be 60±10 ps, and 200±20 ps for Photosystems I and II, respectively. The quantum yields are calculated to be 0.004 for Photosystem I and 0.013 for Photosystem II. Theory of exciton energy transfer and trapping is applied for the determination of intermolecular potential energy in the photosystems.

With the advent of picosecond laser pulses, direct observation of the time development of the primary energy conversion processes in photosynthesis on a picosecond time scale is now possible. An ultrafast optical Kerr gate [1] operated by modelocked Nd/glass laser pulses has permitted measurement of the fluorescent kinetics of whole chloroplasts prepared from spinach and esculent [2]. Absorption kinetics of the reaction centers of photosynthetic bacteria has also been studied with picosecond pulses [3]. This paper reports the first direct measurement of the fluorescent lifetime of chlorophyll a in Photosystems I and II enriched particles of spinach. The fluorescent lifetimes for Photosystems I and II are measured to be 60±10 ps, and 200±20 ps, respectively.

The experimental arrangement is schematically shown in Fig. 1. It consists of a mode-locked Nd/glass laser, a potassium dihydrogen phosphate crystal for generation of the second harmonic laser pulse, a sample cuvette, and a CS2 Kerr cell. Two laser beams of picosecond duration are used for the experiment: a 1.06 µm pulse with ≈ 8 ps duration, and its second harmonic 0.53 µm pulse with ≈ 5 ps duration. The 0.53 µm beam excites fluorescence emission in the Photosystem sample. The 1.06 µm beam times the Kerr gate by inducing a short-lived birefringence in the CS2 cell which is situated between crossed polarizers in the fluorescence path. The fluorescence can only pass through the crossed polaroids during the short time of the induced birefringence in the CS2 cell. At 10^6 W (the power of the 1.06 µm beam), about 1.5% of the incident fluorescent light in a 5 ps interval (half width at half maximum) transmits through the
gate. By adjusting the optical path length of the 1.06 μm beam with respect to the 0.53 μm beam, the fluorescence is sampled at different times. The zero time is defined as the coincidence in time and space of the 0.53 μm and 1.06 μm pulses at the Cs2 cell with water substituted for the sample in the cuvette.

Four signals, the fluorescent intensity at a particular time and wavelength, the total fluorescent intensity, and the intensity of the excitation (0.53 μm) and probe (1.06 μm) beams, are detected with fast photodiode or photomultiplier and displayed simultaneously on a Tektronix 556 oscilloscope. The Photosystem I enriched particles are prepared in the dark at 4 °C from fresh spinach by two methods: (1) the method of Anderson and Boardman [4], using digitonin, and (6) the method of Sene et al. [5], using a French pressure cell. The ratio of chlorophyll a to chlorophyll b is determined to be 5.8 for method i, and 6.2 for method ii [6]. The Photosystem I sample is placed in a 1 cm cuvette with a concentration of chlorophyll a at about 500 μg/ml. For Photosystem II particles, prepared with the French pressure cell method, the ratio of chlorophyll a to chlorophyll b is determined to be 2.2. In the experiment, the Photosystem II sample is placed in a 1 cm cuvette with the concentration of chlorophyll a at approx. 40 μg/ml. The sample in the cuvette is continuously circulated to and from an ice-cooled, black-dyed covered reservoir by means of a vibrostatic pump. The reservoir to cuvette volume ratio is 15 to 1. The time between laser flashes is about 1 min., which is about ten times the time required for emptying the cuvette by the pump. With a photon flux of 6 × 10^4 photons/cm²s, the total fluorescence from both photosystems is observed to be linearly proportional to the excitation photon flux, and the intercept at zero excitation photon flux is zero. All work of this experiment is performed in the dark.

In Fig. 2, the relative fluorescence of Photosystem I particles at 683 nm is plotted as a function of time for different sample preparations. Fig. 3 shows the time dependence of the relative fluorescence of Photosystem II particles at 695 nm. The fluorescence decay can be readily fitted to the equation, \( I = A + B \cdot \exp(-t/\tau) \), where \( I \) is the relative fluorescence, \( A \) and \( B \) are constants and \( t \) is the decay time. For Photosystem I, the constant background \( A \), which amounts to a maximum of 20% of the initial fluorescence, is probably due to the fluorescence of the solubilized chlorophyll.
molecules in the suspension. For Photosystem II particles, the value $A$ is approximately zero. Since the method of preparation of Photosystem I requires a much longer time in high speed centrifugation, it is entirely plausible that solubilized chlorophyll molecules show up substantially higher in the Photosystem I preparation than the Photosystem II preparation. Shown in the same figure on a semilogarithmic graph, the relative fluorescence intensity with background subtracted represents a single exponential with an average decay time $\tau = 60 \pm 10$ ps for Photosystem I, and $200 \pm 20$ ps for Photosystem II. The time at which the fluorescence is maximum, $t_{\text{max}}$, is observed to be about $13 \pm 5$ ps for both photosystem preparations. It should be noted that both methods of preparation of Photosystem I yield essentially the same decay time constant and rise time. For Photosystem II particles, a measurement of fluorescence at 683 nm shows essentially the same kinetics of that at 695 nm.

Prior to the development of the picosecond optical gate, phase fluorometry has been the traditional method to deduce sub-nanosecond fluorescent lifetimes. For Photosystem I, Borisov et al. estimated a lifetime of 30 ps [7]. Miller et al. have reported an average lifetime of fluorescence from whole chloroplast of 350 ps under low exciting light intensity [8]. Presumably, the observed fluorescence from whole chloroplasts
Fig. 3. Fluorescence intensity of Photosystem II particles vs time. As in Fig. 2, the error bar is one standard deviation of the mean. The emission wavelength is 695 nm. The solid curve is a theoretical curve fitting \( I = IB + A + B \cdot \exp[-(t - \tau_{0})/\tau] \), where \( IB \), the instrumental background, is 2.5, \( A = 0 \), \( B = 30 \), \( \tau = 200 \) ps, and \( \tau_{0} = 15 \) ps. The semilogarithmic plot of \((I - IB - A)\) has a decay time \( \tau = 200 \pm 30 \) ps.

is that of Photosystem II, because of the much higher fluorescent efficiency and the longer lifetime in Photosystem II particles. The results of this experiment have thus confirmed the previously measured lifetimes in Photosystems I and II. At this point it should be emphasized that the picosecond gate method provides direct measurement of the full kinetics of fluorescence. The fluorescent lifetime can also be deduced from quantum yield measurements from the relation \( \tau = \varphi \tau_{0} \). The intrinsic fluorescent lifetime of a chlorophyll a molecule, \( \tau_{0} \), has been measured to be 15 ns [9]. The quantum yield of fluorescence of Photosystems I and II, measured by Boardman et al. [10], are 0.0032 and 0.016, respectively. Therefore, the calculated fluorescence lifetimes are 51 ps and 255 ps, respectively, in good agreement with the results of this experiment. From the measured lifetimes, the quantum yields are calculated to be 0.004 for Photosystem I and 0.013 for Photosystem II.

Regarding the problem of photosynthetic energetics, the mechanism of energy transfer from the light harvesting pigments to the trapping center is believed to be that of the excitonic resonance transfer [11]. The excitation is believed to spread throughout a photosynthetic unit in a time \( t_{0} = Ah/V \), where \( V \) is the intermolecular interaction energy, and \( A \) is a multiplication factor to account for the extent of delocalization of the excitation from a single site to the whole unit. This time \( t_{0} \) in part contributes
to the risetime of the fluorescence. Other contributing factors are: (1) The energy transfer time from accessory pigments to chlorophyll a pigments; (2) the time of thermalization in the first excited singlet manifold of an individual molecule; and (3) the response time of the Kerr gate. Letting the total risetime of fluorescence be \( \tau_r \), the time dependence of the fluorescence intensity \( I \) is proportional to \( \left( e^{-t/\tau_r} - e^{-t/\tau_0} \right) \), where \( \tau_0 \) is the fluorescence decay time. The time at which the fluorescence is maximum is

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\tau_{\text{max}} = \tau \left( 1 - (1/\tau_0) (\tau/\tau_0 - 1) \right).
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From experimental results, \( \tau_r = 60 \) ps, \( \tau_0 = 200 \) ps for Photosystems I and II, respectively, and \( \tau_{\text{max}} \approx 13 \) ps, the fluorescence risetimes \( \tau_r \) are on the order of 5 ps, which is approximately equal to the half width of the gate response time. Therefore, the time required for a single excitation to spread out in the Photosystem particle is at most a few picoseconds.

Due to the very low concentration of trapping center in a photosynthetic unit, the probability of the excitation wave visiting a trapping site is low. The average time required for a center to trap the excitation energy is longer. The decay time of fluorescence indeed measures this average trapping time. Assuming a random walk model of the excitation, the average number of jumps for an excitation to reach a single trapping site in a two-dimensional array of, say, 300 chlorophyll a molecules has been calculated to be \( n \approx 600 \) [11]. Without accounting for the possibility of spectral heterogeneity among the pigment molecules, and assuming a 100% trapping efficiency, the pair jump time is \( \tau_p = t/n \approx 0.1 \) ps in Photosystem I, and 0.3 ps in Photosystem II, where \( \tau_0 \) the fluorescence decay times are taken to be the measured values, 60 ps and 200 ps for Photosystems I and II, respectively. The interaction potential between chlorophyll a molecules, therefore, can be estimated at \( V = \hbar c \sigma = 0.006 \) eV in Photosystem I and 0.002 eV in Photosystem II*. Applying this interaction energy to the previous ideas on the time \( \tau_0 \) for the excitation wave packet to spread out in a photosynthetic unit and assuming, \( \hbar \approx 25 \) [8], the times \( \tau_0 \) are approx. 2.5 ps and 7.5 ps for Photosystems I and II, respectively, which are consistent with the rise time data.

Previously, from the observation of a dip and rise in the fluorescence kinetics of whole chloroplast, Sibert and Alfano [2] have advanced a theory that the total fluorescence is composed of light emission from two independent components, probably Photosystems I and II. The 200 ps lifetime of Photosystem II is in excellent agreement with the long overall decay component observed in the previous work. But, present measurement of 60 ps as the fluorescent lifetime of Photosystem I particles is not readily identifiable with the suggested 10 ps component. Further, the observed fast fluorescent rise times in both systems cannot explain the rise and the dip in the fluorescence of the whole chloroplast. The theory suggested in ref. 2 requires the risetime of one component to be relatively long. To account for the discrepancies, it should be noted that the 10 ps time was estimated from two data points**. Furthermore, subchloroplast photosystem particles obtained in this experiment may not be the same photosystems in the environment of an intact whole chloroplast. Photosystem II contamination of Photosystem I particles is possible. Detergent and highpressure treatments may also alter the physical organization of pigments in the particle. It seems to suggest that the environment of a whole chloroplast is not a simple algebraic sum of Photosystems I and II, at least for the nature of fluorescence. This also implies that the procedure for isolating the photosystem.

* and **, See "Note added in proof". 
fractions alters the energy transfer processes found in intact chloroplasts. We believe further experiments on the fluorescent kinetics of the whole chloroplast, especially in the spectral domain, should be carried out for clarifying the relationship in fluorescent kinetics between the whole chloroplast and the Photosystem particles.

In conclusion, this study represents a first direct observation of the kinetics of Photosystems I and II fluorescence. The data are in good agreement with previous experiments [7, 10] on lifetime and quantum yield.

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* These calculated values of interaction energy correspond to a coupling of intermediate strength. The justification for employing the random-walk model (localized or weak coupling) has been presented in ref. 11.

** Recent measurements (Yu, W. and Alfano, R. R. unpublished) have indicated that the fluorescent decay profile from whole chloroplast is non-exponential, and only sometimes a dip and a rise are observed.

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REFERENCES