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FREE-ENERGY CHANGE OF PHOTOSYSTEM II AS MEASURED BY DELAYED LUMINESCENCE

MICHAEL A. MARCHIARULLO and ROBERT T. ROSS

Department of Biochemistry, The Ohio State University, 484 W. 12th Avenue, Columbus, OH 43210 (U.S.A.)

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The intensity of light emission was used to determine the ground-to-excited state free-energy difference of Photosystem II in the alga *Scenedesmus quadricauda*. Prompt fluorescence measurements give the driving force of that photosystem under illumination while delayed fluorescence indicates the free energy stored in reaction products. Comparison of the light-on and light-off cases suggested that the immediate products were 0.13 eV lower in free energy than the excited state. In the physiological light range, Photosystem II can store 1.0 eV, or about 60% of that needed for photosynthesis, and still shows a ΔG of 0.7 eV after 3 h.

Light emission can be used to calculate the free-energy difference between the ground and excited states of any photochemical system [1,2]. This free energy, ΔG, is important in any process where light energy is stored.

Previously [2], the equation for ΔG has been written as:

$$\Delta G = kT\ln(\phi_{lum}R_{in}/R_{therm}) \quad (1)$$

where ϕ_{lum} is the quantum yield of luminescence, R_{in} is the rate of ground-to-excited state transitions induced by all mechanisms, and R_{therm} is the rate of thermally induced ground-to-excited state transitions. R_{therm} is:

$$R_{therm} = \int\sigma(\lambda)I_{bb}(\lambda)d\lambda \quad (2)$$

where $\sigma(\lambda)$ is the absorption cross-section at λ, and $I_{bb}(\lambda)$ is the intensity of radiation emitted by a black body at temperature T. The integrand of Eqn. 2 is the fluorescence spectrum, and R_{therm} is most easily evaluated by determining the emission spectrum, and the absorbance at one wavelength on the long-wavelength tail [3]. R_{in} is the rate of quanta absorption, given by:

$$R_{in} = \int\sigma(\lambda)I_{ext}(\lambda)d\lambda + R_{therm} \quad (3)$$

where I_{ext} is the intensity of radiation of an actinic light source at λ. Usually, R_{therm} is negligible in Eqn. 3.

Therefore, to calculate a ΔG value, apparently one needs to know the quantum yield of fluorescence, the absorption spectrum of the sample, the intensity and wavelength distribution of the actinic light, and the temperature. By assuming a 100% fluorescent sample ($\phi_{lum} = 1$), Ross and Calvin [2] found chloroplasts, under an illumination of 1.6 W/m^2 of 680 nm light, produced a maximum potential difference of 1.32 eV in PS I and 1.36 eV in PS II. A quantum yield other than 1 changes ΔG by $kT\ln\phi_{lum}$.

Abbreviation: PS, photosystem.

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The rate of fluorescence emission is:

$$R_{lum} = \phi_{lum} R_{in} \quad (4)$$

Substituting Eqn. 4 into Eqn. 1, we find that:

$$\Delta G = kT\ln(R_{lum}/R_{therm}) \quad (5)$$

Eqn. 5 is equivalent to Eqn. 8′ of Ref. 1, and makes it clear that ΔG can be evaluated in any luminescent system by measuring only the rate of emission, and R_{therm}. Absorption and action spectra, and quantum yields, are not needed. Actual ground-to-excited state free-energy differences can be determined in the presence of actinic light by measuring prompt fluorescence. The ΔG of a photosystem can be followed in the absence of actinic light by measuring the intensity of chemiluminescence.

In the case of photosynthesis, the ΔG generated under illumination by light of physiological intensity represents the driving force behind chemical synthesis. When the actinic light is off, the intensity of luminescence is a measure of the free energy stored in the primary reactions. Comparison of the light-on and light-off cases shows a loss of free energy needed to stabilize the products.

We report here the results of prompt and delayed fluorescence measurements on a suspension of algae. The measurements were used to determine ΔG under illumination, and up to 3 h after the external light was extinguished.

Scenedesmus quadricauda, from the culture collection at the University of Texas, were grown in Kessler's medium as modified by Bishop and Senger [4]. Cultures were grown at 23°C and continuously shaken. Continuous illumination was provided by fluorescent lighting (General Electric, 'cool white') with an intensity of 1 W/m^2. New cultures were started every 3 days.

For experiments, algae were spun down and resuspended in growth media to an apparent absorbance of 1.0 at 680 nm in a conventional 1 cm square cuvette. The temperature was 21–23°C. Prior to illumination, the sample was kept in the dark for 10 min. For measuring delayed light emission 10 or more s after illumination, the sample was continuously preilluminated for 5 min. For measuring delayed light emission at shorter times, the sample was illuminated with alternating light-on and light-off cycles of equal length, and measurements from many such cycles were averaged. At light intensities of 1 W/m^2 or less, it was not necessary to shutter the detector during illumination, allowing both prompt and delayed fluorescence to be observed throughout the cycle.

Samples were illuminated with either a 100 W quartz-halogen lamp, or a green-light-emitting diode (Hewlett-Packard HLMP-2500). Corning 4-96, 3-67 and 4-97 colored-glass filters, and a Ditric 580 nm short-pass interference filter were placed between the light source and sample. A 4 cm water filter was also used with the quartz-halogen lamp. The central wavelength for the actinic light was 560 nm with a half-bandwidth of approx. 20 nm.

Light emitted by the sample was collected at 90° to the excitation beam with $f/0.7$ optics and filtered through 2 cm of 25 mM tris(1,10-phenanthroline) ferrous sulfate [5]. This long-pass filter has a transmittance of over 40% at all wavelengths longer than 695 nm, and an absorbance of over 2 for all wavelengths shorter than 640 nm. The advantage of this filter over other filters is a lack of filter fluorescence *.

The emission was measured with a photon-counting GaAs photomultiplier tube (RCA C31034-02) cooled to approx. –30°C, and having a dark counting rate of 6–10 s^{-1}. The spectral response of the total detection system was calibrated using a standard lamp (Optronic Laboratories) and its absolute efficiency was determined using cresyl violet perchlorate (Kodak, catalogue No. 11884) as a fluorescence standard of known quantum yield [6]. Counting rates were measured with a Tracor NS-570A digital signal analyzer. The data were then transferred to a computer, which made the necessary corrections to convert counting rates to a free-energy difference.

The fluorescence emission spectrum was measured on a Fluorolog 1902 fluorimeter (Spex Industries). The emission spectrum was corrected for the photomultiplier sensitivity.

Sample absorbance at 680 nm was measured in the scattered transmission accessory of a Cary 118 spectrophotometer; apparent absorbance at 800 nm was subtracted from the apparent absorbance at 680 nm to correct for residual scattering loss. It was assumed that 50% of the absorbance at 680 nm is due to chlorophyll associated with PS II [7], and that all prompt and delayed fluorescence is emitted from PS II. Errors in these assumptions do not introduce a significant error in the calculated free energy, since

* We thank J.N. Demas for suggesting this filter.

TABLE I

PS II GROUND-TO-EXCITED STATE FREE ENERGIES

ΔG calculated from fluorescence emission rates as described in text. Measurements made during and 10 ms after illumination. Actinic light 560 nm.

Illumination (W/m^2)	ΔG (eV)	
	Light on	Light off (10 ms)
10^2	1.32	1.06
10^0	1.20	1.04
10^{-1}	1.13	1.00
10^{-2}	1.06	0.92
10^{-3}	1.01	0.88
10^{-4}	0.96	0.83

most light emission is from PS II [8–12], and a change of intensity by a factor of 2 changes the ΔG computed by only 0.02 eV.

Table I shows the ΔG generated in the presence of different actinic light intensities, and 10 ms after illumination ended. Fig. 1 shows the decay of emission and ΔG with time. The data at 100 W/m^2 are partly our results and partly those of Haug et al. [8]. All other data were measured in our laboratory. At 100 W/m^2, we could only measure delayed fluorescence 1 s or longer after illumination. Haug

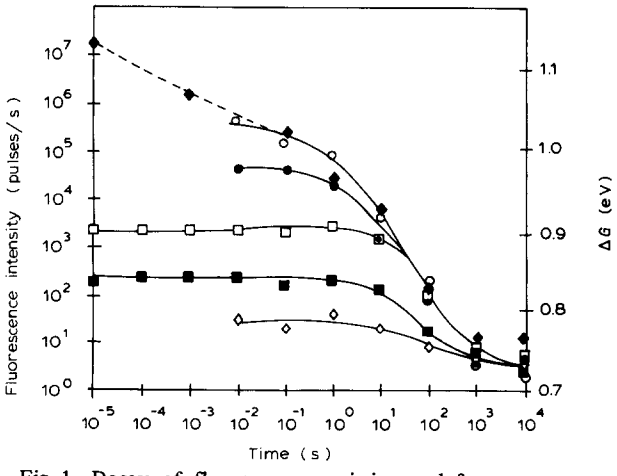

Fig. 1. Decay of fluorescence emission and free energy vs. time after illumination. Light intensities (W/m^2 at 560 nm): ♦, 100; ○, 1; ●, 10^{-1}; □, 10^{-2}; ■, 10^{-3}; ◇, 10^{-4}. Dashed line indicates data used from Haug et al. [8].

and co-workers [8] published data on the decay of emission for *Scenedesmus obliquus* from 4 ns up to 10 s after illumination, and their decay curve was matched to our measurement at 1 s for determination of emission intensity and ΔG at shorter times.

The ΔG computed by measurement of prompt fluorescence, and corrected to a 100% fluorescence yield, agrees with the calculations of Ross and Calvin [2] on spinach PS II to within 0.01 eV. The actual ΔG between ground and excited state chlorophyll is less than this maximum by $kT\ln\phi_{lum}$ (see Eqn. 1), or 0.10 eV for our measured prompt fluorescence quantum yield of 0.02.

Within nanoseconds after the actinic light is turned off, the population of the chlorophyll excited state decreases to a value determind by an approximate equilibrium with more stable high-energy chemicals. As the highest-energy pools are depleted, the population of the excited state and the emission intensity decrease.

At actinic intensities of 10^{-4} and 10^{-3} W/m^2, we found that the luminescence intensity falls to about 1/150 of the prompt fluorescence intensity in less than 10 μs. Similarly, Haug et al. [8] found that the delayed fluorescence at short delay times (20 ns to 1 μs) was 1/168 as intense as the prompt fluorescence.

The constancy of this delayed/prompt fluorescence ratio suggests that the first intermediates following singlet-excited chlorophyll are $kT\ln 150 =$ 0.13 eV lower in free energy. This may be due to a loss pathway [13].

The higher light intensities show a faster decay at short times, while the lower intensity curves show almost no decay until about 1 s after excitation is stopped. All show a large drop at 1 s, and have decayed to a common level at 10^3 s (17 min). The lifetimes of the upper S-states are about 3 s [14], and may be responsible for the intensity drop at 1 s. Delayed light is still emitted after 10^4 s (3 h), with an intensity indicating a ΔG of 0.7 eV. No attempt was made to characterize the source of this emission. Lavorel [15] has measured this very long-lived luminescence in *Chlorella*, and experiments in that laboratory indicate the source of positive charges at long times is the reduction of oxygen via the oxygen-evolving protein. The source of electrons is less certain.

What fraction of the energy needed for photosynthesis is provided by PS II? The physiological range of light intensities is that which produces 0.3–100 excitations/reaction center per s [14]. This is equivalent to an illumination at 560 nm of 0.2–50 W/m^2. In that range, Fig. 1 indicates that about 1.0 eV is stored by PS II. Avron [16] has estimated that ΔG for an electron going from H_2O to NADPH is 1.65 eV. If this is true, and PS II supplies 1.0 eV of this amount, then PS I needs to supply 0.65 eV. For every drop of 0.06 eV in free energy, the measured fluorescence will decrease by a factor of 10. If PS I stores 0.35 eV less than PS II, its luminescence may be only 10^{-6} as intense. This might explain the rarity of reports of delayed light emission from PS I.

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