ENERGY TRANSFER IN PHOTOSYNTHESIS: PIGMENT CONCENTRATION EFFECTS AND FLUORESCENT LIFETIMES*

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Received 16 April 1975

1. Introduction

The first steps in photosynthesis involve the transfer of energy from bulk pigment molecules to photosynthetic reaction centers. Because the energy transfer processes are of subnanosecond duration, it has been difficult to make accurate measurements. With the introduction of picosecond techniques such as the optical gate to the study of the fluorescent properties of photosynthetic materials [1,2], it has become possible to directly measure rapid energy transfer processes. In this letter we report results obtained on photosynthetic systems by a powerful streak camera technique [3]. These results indicate that the decay of the fluorescence from two photosynthetic species, *Anacystis nidulans* and *Chlorella pyrenoidosa*, is nonexponential with much shorter lifetimes than reported previously. Furthermore, studies of the dependence of the lifetime on concentration of several pigments including chlorophylls *a* and *b*, show that at the high pigment concentrations known to be present in living cells, the lifetime can be exceedingly short allowing us to conclude that the high dye concentrations are responsible for the short decay times observed experimentally in the living cells.

2. Materials and methods

The experimental arrangement consists of a mode-locked Nd:glass laser followed by a second harmonic generating crystal, the samples, and a lens that collects the fluorescence beyond 640 nm onto the slit of a streak camera. The mode-locked laser is nearly identical to that described in a prior publication [4], the sole difference being that the mode-locking dye cell is 1 cm long and is not in contact with the back mirror. The 530 nm pulses are 5 psec in duration.

The streak camera is a Los Alamos Scientific Laboratory device with an S-20 photocathode response and a 10 psec resolution time. A signal from a photodiode triggers the camera when a pulse is exciting the sample. The converted image provided by the streak camera is photographed on Eastman Kodak #2484 emulsion. The response of the film is calibrated with a step density wedge developed with the film. An accurate measure of the intensity response of the camera is determined by uniformly illuminating a step density wedge placed before the slit with 530 nm picosecond pulses. These calibrations and densitometer traces of the resultant negatives yield the temporal dependence of the fluorescent intensity.

The samples were prepared as follows: Viable samples of *Chlorella pyrenoidosa* (Sorokin's high-temperature strain ICC No. 1230) and *Anacystis nidulans* (Kratz and Allen strain ICC No. 625) were grown on agar slants in an illuminated environmental chamber maintained at 26°C. Slants were inoculated four to five days preceeding use so that the material would be in a rapid state of cell division. The cells were washed from the slants with sterile physiological saline and diluted to about 10^6 cells per cm^3.

Cell suspensions of lyophilized (non-living) *Chlorella* and *Anacystis* were prepared from cells grown in mass liquid culture [5]. They were removed from the growth medium, which had been rapidly

* Work performed under the auspices of the U.S. Energy Research and Development Administration.

North-Holland Publishing Company – Amsterdam

cooled to 4°C, by centrifugation. The cells were washed in physiological saline and then lyophilized. For these studies the lyophilized cell material was suspended in cold physiological saline (5°C) to a concentration of 30 mg of cells per cm^3. Just before use, the samples were warmed to room temperature.

Chlorophylls *a* and *b* were obtained in pure form from *Chlorella* by a large-scale modification of that previously described [6]. The dried chlorophylls were stored in the dark under vacuum at −20°C. Oxygen-free chloroform was used to prepare the homogeneous chlorophyll solutions in the desired concentrations; these were kept in the dark (N_2 atmosphere) during preparation and use to minimize the possibility of interference from degradative products. Sample containers were usually 1 mm long, except shorter cells were used at the highest chlorophyll concentrations.

3. Results

Experimental results showing the dependence of the lifetime of chlorophyll *b* on concentration in a chloroform solution are shown in fig.1. Each curve is a densitometer trace of a streak photograph of the fluorescence. The fluorescence rises abruptly ($\leqslant$ 10 psec); careful calibration indicates that the intensity decays non-exponentially – the lifetime ranges from tens of picoseconds at high concentrations, to about a nanosecond at low concentrations. Similar results were obtained for the dependence of the lifetime on concentration for chlorophyll *a* in chloroform. All results are summarized in table 1.

Densitometer traces of fluorescent emission from live *Anacystis* and *Chlorella* are shown in fig.2. The fluorescence rises abruptly, and then decays to the 1/e point in 74 ± 5 and 41 ± 5 psec for the *Anacystis* and *Chlorella* samples, respectively. Calibration shows these curves are not nearly as non-exponential as the chlorophyll in chloroform solution data, but differ slightly from a simple exponential. Measurements for the nonliving *Anacystis* samples indicate fluorescence curves identical within experimental error (77 ± 15 psec) to living samples. Depolarization measurements for the *Anacystis* indicate that the fluorescence is depolarized within the first 10–15 psec after excitation.

Table 1
Comparison of fluorescent lifetime measurements with equation 1

Sample	Concentration (moles/liter)	Fluorescence lifetime (psec)	Calculated lifetime (psec)
Chlorophyll *a*	0.1	12 ± 5	13[a]
Chlorophyll *a*	0.016	275 ± 50	244[a]
Chlorophyll *a*	0.002	685 ± 145	678[a]
Chlorophyll *b*	0.1	15 ± 5	9[b]
Chlorophyll *b*	0.023	120 ± 20	119[b]
Chlorophyll *b*	0.0038	572 ± 56	567[b]
Anacystis, living		74 ± 5	86[c]
Chlorella		41 ± 5	86[c]
Anacystis, lyophilized		77 ± 15	86[c]

[a] Calculated from the Förster equation $\exp(-At - CBt^{1/2}) = \exp(-1)$, where C is molar concentration, t is in psec, and $A = 1{,}262 \times 10^{-3}$, $B = 2{,}767$.
[b] Calculated as in [a] but $A = 1.219 \times 10^{-3}$, $B = 3.412$.
[c] From Bay and Pearlstein [16].

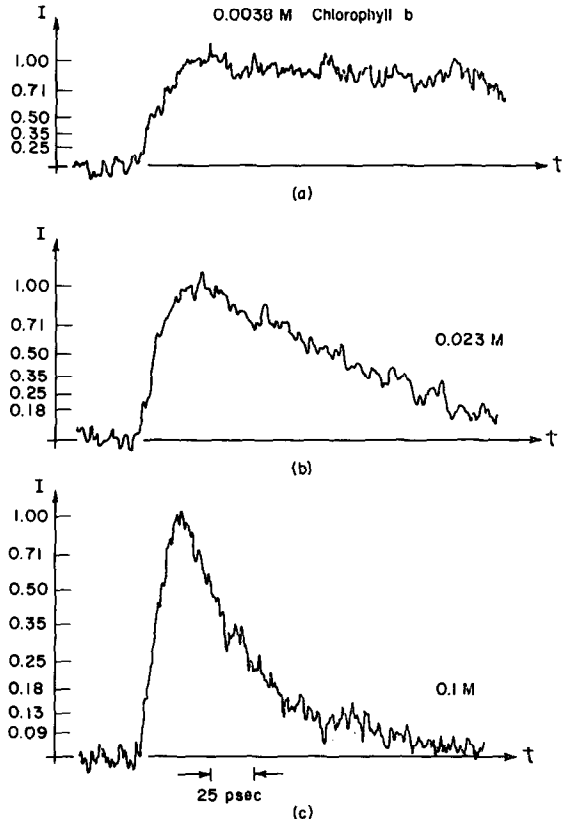

Fig.1. Densitometer traces of streak photographs of chlorophyll *b* in chloroform showing strong dependence of fluorescent lifetime with concentration. Figs.1a, 1b and 1c show, respectively, concentrations of 0.0038, 0.023 and 0.1 M. Samples were excited with 5 psec, 530 nm light pulses.

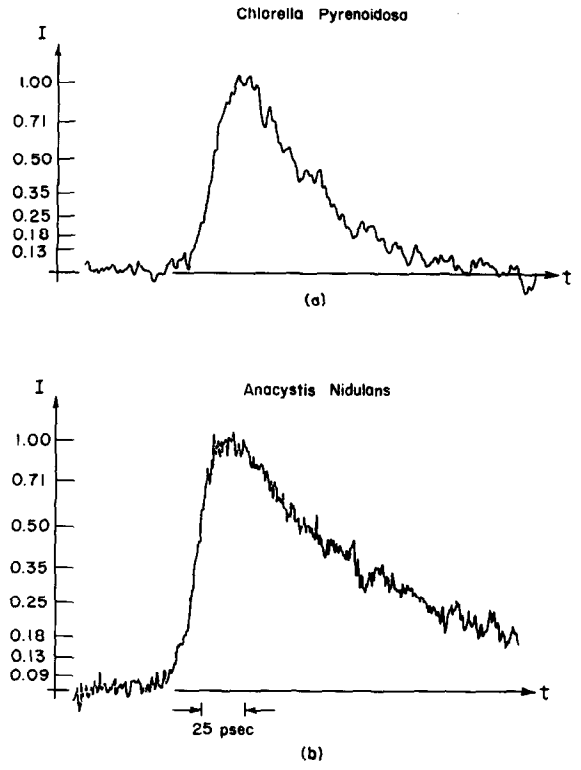

Fig.2. Densitometer traces of streak photographs showing fluorescent lifetimes of living *Chlorella Pyrenoidosa* (fig.2a) and *Anacystis Nidulans* (fig.2b).

4. Discussion

The results for chlorophyll *a* and *b* solution are readily explainable by concentration quenching, a term applied to the phenomenon whereby an increase in concentration leads to a decrease in quantum efficiency. In chlorophyll this quenching mainly occurs because an excited molecule in solution can decay by transferring its energy to another solute molecule by a resonant nonradiative transfer process, as treated quantum mechanically by Förster [7]. This type of transfer results from dipole–dipole coupling between two molecules, and therefore the rate of energy transfer is proportional to $1/r^6$, where r is the spacing between the two molecules, and hence is a sensitive function of concentration. Förster's equation [7] describing the decay of excited state molecules with time for frozen molecular orientations* [8] is given by

$$n(t) = n_0 \exp\left[-t/\tau - 0.846 \sqrt{\pi} N \frac{R_0^3}{R_g^3} (t/\tau)^{1/2} \right] \quad (1)$$

where τ is the lifetime, N is the number of molecules within a sphere of radius R_g and R_0 is an interaction distance calculable from the absorption and emission spectra. This equation predicts a nonexponential decay, as has been verified in a dye mixture by Rehm and Eisenthal [9] and for the case of F-center interactions

* The molecular orientation is considered frozen in the time scale of interest (< 100 psec) due to the large size of the chlorophyll molecule (MW ~ 900) and in live cells, structural considerations.

by Fröhlich and Mahr [10]. At high concentrations, the second term due to the dipole-dipole interaction dominates, while at low concentrations, the normal decay dominates.

For chlorophyll *a* the time to decay to the 1/e point as originally calculated by Förster [7] is much shorter than is experimentally observed. For example, at 0.1 M the Förster expression predicts 0.6 psec (0.9 psec with the 0.846 correction factor in eqn. (1)). This is perhaps not too surprising since it is now fairly well established that chlorophyll aggregation occurs in chlorophyll solutions [11]. This mechanism would tend to reduce the effective concentration of the monomeric species. A dimer, for example, would play the role of the acceptor in a Förster transfer [12]. At a nominal concentration of 0.1 M, we estimate the concentration of dimers is, from [11], 0.03 M and this results in a decay time from eqn. (1) of 10 psec, in reasonable agreement with that experimentally observed. Also, all our data for chlorophyll *a* can be fit with eqn. (1) if we assume $R_0 = 27.4$ Å and $\tau^{-1} = 1.262 \times 10^9$/sec. Each curve in fig.1 can be fit as a function of time with a non-exponential of the form $\exp(-At - CBt^{1/2})$ and parameters found in Table I. Thus the evidence for a chlorophyll-oligomer Förster-type mechanism in the solution systems is strong.

To explain results for *Anacystis* and *Chlorella*, we note that chlorophylls *a* and *b* are present in live cells in high concentrations [13]. Therefore in light of the short decay curves for chlorophylls *a* and *b* at 0.1 M concentration, it is not surprising that high concentrations are responsible for the short observed lifetimes of the living cells. The fact that the observed emission becomes depolarized immediately leads one to conclude that numerous transfers between molecules have occurred. In the cell, the dimeric reaction center [14,15] takes on the role of an acceptor, and the energy is funneled between different pigments going from molecule to molecule before reaching the reaction center. Our results for *Anacystis* and *Chlorella* are close to a value of 86 psec calculated for the Förster transfer of energy to a reaction center by Bay and Pearlstein [16] based on an energy migration model. In their model, a large number of intermolecular transfer steps results in fluorescence which has a form that is nearly exponential [16], as we have observed in living cells. The finite number of migration steps (40–80) would yield curves closer to exponential than the form predicted by eqn. (1). A recent exciton diffusion calculation based on a Förster mechanism by Knox [17] predicts a decay time of 35 psec for parallel chlorophyll dipole orientations, also in good agreement with our data.

Most previous lifetime measurements in the literature have been made with nanosecond excitation pulses and nanosecond electronic detectors, making it exceedingly difficult to extract correct short lifetime information. In contrast our technique has a 10 ps resolution making it straightforward to extract the lifetimes reported here. Our lifetimes for *Anacystis* and *Chlorella* are far shorter than had been previously reported [18]. Picosecond technology has enabled us to make the first measurements for chlorophyll *a* and *b* at high concentrations, and our measurements indicate lifetimes for the algae which are not longer than those indicated by recent quantum efficiency measurements of 0.7% reported by Boardman et al. [19]. Our results are not, however, consistent with the much higher quantum efficiencies reported by Rabinowitch et al. [20].

It is interesting that in the case of the simple algae *Anacystis*, lyophilization did not increase the lifetime measurement. In chloroplast containing systems which have been similarly disrupted, we observe [21] longer lifetimes than for the living counterparts. This confirms that lyophilization induces a physical change in the energy transfer process as expected in chloroplasts.

Acknowledgments

We thank K. B. Eisenthal, R. A. Fisher, and J. McLeod for helpful discussions, and J. Hanners, R. C. Hyer, and H. Sutphin for experimental assistance.

References

[1] Seibert, M., Alfano, R. R. and Shapiro, S. L. (1973) Biochim. Biophys. Acta 292, 493–495; Seibert, M. and Alfano, R. R. (1974) Biophys. J. 14, 269–281.

[2] Yu, W., Ho, P., Alfano, R. R. and Seibert, M. (1975) Biochim. Biophys. Acta, in press.

[3] Bradley, D. J., Liddy, B. and Sleat, W. E. (1971) Opt. Commun. 2, 391–395; Schelev, M. Ya., Richardson, M. C. and Alcock, A. J. (1971) Appl. Phys. Lett. 18, 354–357.

[4] Shapiro, S. L., Hyer, R. C. and Campillo, A. J. (1974) Phys. Rev. Lett. 33, 513–515.
[5] Kollman, V. H. and Buchholz, J. R. (1972) Biotechnol. Bioeng. 14, 819–829.
[6] Strouse, C. E., Kollman, V. H. and Matwiyoff, N. A. (1972) Biochim. Biophys. Res. Comm. 46, 328–331.
[7] Förster, T. (1948) Ann. Physik 2, 55–75; (1949) Z. Naturforshg. 4a, 321–327.
[8] Steinberg, I. Z. (1968) J. Chem. Phys. 48, 2411–2413.
[9] Rehm, D. and Eisenthal, K. B. (1971) Chem. Phys. Lett. 9, 387–389; Eisenthal, K. B. (1970) Chem. Phys. Lett. 6, 155–157.
[10] Fröhlich, D. and Mahr, H. (1966) Phys. Rev. 141, 692–695.
[11] Ballschmiter, K., Truesdell, K. and Katz, J. J. (1969) Biochim. Biophys. Acta 184, 604–613.
[12] See also Kelly, A. R. and Porter, G. (1970) Proc. Roy. Soc. Lond A315, 149–161; Kelly, A. R. and Patterson, L. K. (1971) Proc. Roy. Soc. Lond A324, 117–126.
[13] Boardman, N. K. (1968) Advances in Enzymology Vol. 30, p. 1–79, Intersci. Publ. New York.
[14] Fong, F. K. (1974) Proc. Natl. Acad. Sci. USA 71, 3692–3695.
[15] Katz, J. J. and Norris, J. R. (1973) in: Current Topics in Bioenergetics, (Sanadi, D. R. and Packer, L. eds.) Vol 5, pp. 41–75, Academic Press, New York.
[16] Bay, Z. and Pearlstein, R. M. (1963) Proc. Natl. Acad. Sci. USA 50, 1071–1078.
[17] Knox, R. S. (1973) Primary Molecular Events in Photobiology, pp. 45–77 Elsevier Sci. Pub. Co., New York.
[18] See e.g. Nicholson, W. J. and Fortoul, J. I. (1967) Biochim. Biophys. Acta 143, 577–582; Singhal, G. S. and Rabinowitch, E. (1969) Biophys. J. 9, 586–591; Mar, T., Govindjee, Singhal, G. S. and Merkelo, H. (1972) Biophys. J. 12, 797–808.
[19] Boardman, N. K., Thorne, S. W. and Anderson, J. M. (1966) Proc. Natl. Acad. Sci. USA 56, 586–593.
[20] Latimer, P., Bannister, T. T. and Rabinowitch, E. I. (1957) in: Research in Photosynthesis, (Gaffron, H. ed.) pp. 107–111, Interscience Publishers, New York.
[21] Campillo, A. J., Kollman, V. H. and Shapiro, S. L., Biochem. Biophys. Res. Comm., to be published.