PHOTOSYNTHETIC STUDIES WITH A 10-PSEC RESOLUTION STREAK CAMERA*

V. H. Kollman, S. L. Shapiro, and A. J. Campillo

University of California
Los Alamos Scientific Laboratory
Los Alamos, NM 87544

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SUMMARY

The fluorescence decay times for the species *Anacystis nidulans* and *Chlorella pyrenoidosa* are measured by means of a streak camera to be 75 ± 10 and 41 ± 5 psec, respectively. Concentrated solutions of chlorophyll *a* are shown to have fluorescence decay times as short as 10 psec. Fluorescence decay curves similar to that of the living cells can be generated by mixing cellular component molecules at comparable concentrations to that present in living cells.

INTRODUCTION

Because pigment molecules present in the photosynthetic apparatus have fluorescence decay times in the nanosecond and subnanosecond range, it has been difficult, until recently, to obtain accurate measurements of their fluorescence lifetimes. Such measurements are valuable for understanding the energy transfer mechanisms in a cell. With the advent of the modelocked laser and picosecond time measurement techniques, such as the optical gate (1) and the streak camera (2,3), it is now possible to accurately measure photosynthetic lifetimes (4). Optical gate measurements (4-6) for escarole and spinach chloroplasts indicate a possible complex decay mechanism. In this paper we report lifetime measurements for the photosynthetic systems, *Anacystis nidulans* and *Chlorella pyrenoidosa* obtained with a streak camera. The fluorescence decay curves for these species are smooth monotonically decreasing functions with time. We have found that similar decay curves can be produced by mixing the component pigment species at about the same concentrations and in the same proportions as present in the living cell. Concentration

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quenching is responsible for the production of the short lifetimes observed.

MATERIALS AND METHODS

The experimental arrangement is as follows: A modelocked Nd:glass laser emits picosecond pulses at 1060 nm. These pulses are converted by a KDP crystal into second harmonic pulses at 530 nm. These 5-psec green pulses excite the sample, and the fluorescence at wavelengths greater than 640 nm is collected onto the slit of a streak camera. A streak camera (2,3) operates in the following way: Light from an event enters a slit and is focused onto a photocathode, where electrons are released. The electrons are accelerated through an anode and are then deflected by a voltage ramp which is actuated to trigger by a voltage pulse derived from a picosecond pulse incident on a photodiode. The increasing voltage in the ramp streaks the electrons across a phosphorescent screen so that the electrons released at different times strike the screen at different positions. A photograph of the resulting phosphorescent streak then gives a measure of the lifetime of the event. Our streak camera is a homemade Los Alamos Scientific Laboratory device with an S-20 photocathode and a resolution time of less than 10 psec. A portion of the beam is directed to a photodiode and the streak camera is timed by delay cables to sweep when a single pulse from the modelocked laser excites the material. Streak camera photographs of the fluorescence produced by the single pulse are taken on Eastman Kodak #2484 film. The streak camera and film exposure responses are carefully calibrated with step density wedges and a light source.

The samples were prepared in the following way: *Chlorella pyrenoidosa* (7) (Sorokin's high temperature strain ICC No. 1230) and *Anacystis nidulans* (8) (Kratz and Allen strain ICC No. 625) were grown in spinner flask culture (500 cm^3 volume) at 37°C and 39°C, respectively. Carbon dioxide gas was automatically added to the cultures to maintain the pH and to provide the carbon source. Lighting was by natural outdoor fluorescent and incandescent lamps, giving 10,000 lux at the flask surface. For these studies, the cells were harvested while in exponential growth phase, cooled to 4°C, and centrifuged from the growth medium. The cells were resuspended in sterile physiological saline to about 10^6 cells per cm^3.

Cell suspensions of nonliving *Chlorella pyrenoidosa* and *Anacystis nidulans* were prepared from cells harvested during exponential growth, washed with physiological saline (5°C), and then lyophilized. The dried lyophilized cells were suspended in saline and diluted to 30 mg (lyophilized weight) per cm^3.

Chlorophyll *a* was obtained in pure form from *Chlorella pyrenoidosa* by a large-scale modification of that previously described (9,10). The dried chlorophylls (11) 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.

A crude fraction of lipids and pigments was obtained from lyophilized *Anacystis nidulans* by extraction in a nitrogen atmosphere. The cells were washed three times with oxygen-free absolute ethanol and twice each with oxygen-free absolute ethanol-chloroform (9:1 v/v) and benzene. The extracts were pooled, concentrated to dryness under reduced pressure, and taken up in a minimum amount of oxygen-free ether. The ether-insoluble material was removed by centrifugation and the extract concentrated to dryness and left under vacuum for 12 h to ensure complete solvent removal. The lipid-pigment fraction accounted for 13 percent of the dry weight of cells.

The crude lipid-pigment extract was dissolved in oxygen-free chloroform to make a final concentration of 1 M for chlorophyll *a* (12,13). The natural ratios of carotenes, xanthophylls, and lipids were present in solution.

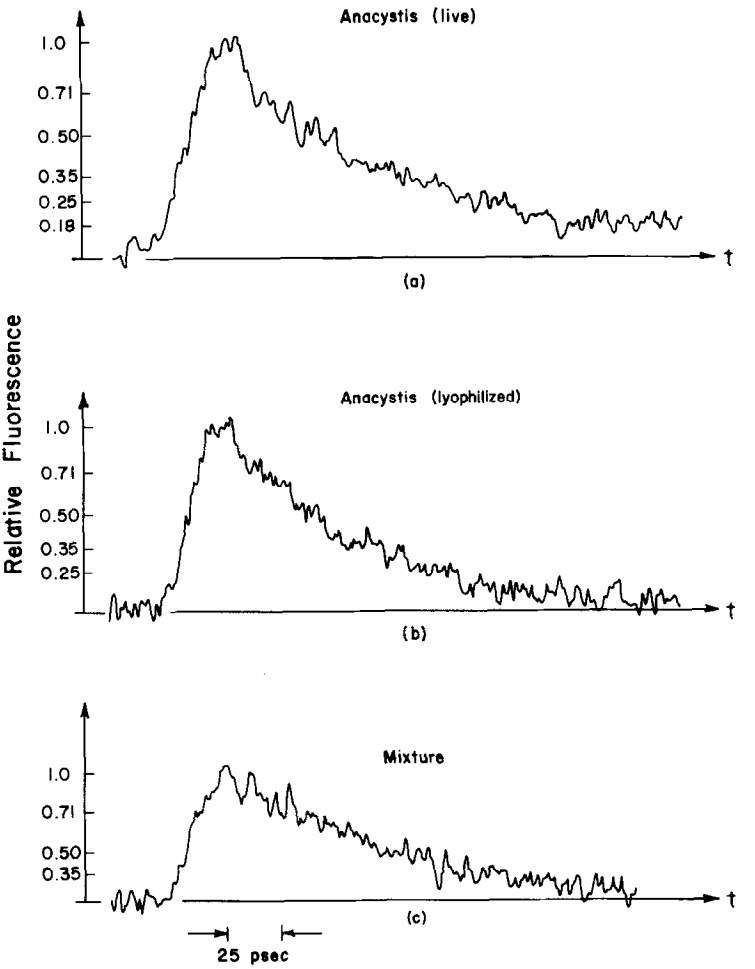

Figure 1: Densitometer traces of streak photographs showing fluorescent lifetimes of living (Fig. 1a) and lyophilized (Fig. 1b) samples of *Anacystis nidulans*. The samples were excited with 5-psec 530-nm light pulses. Fig. 1c shows the fluorescence of a mixture of carotenes, xanthophylls and lipids as described in the text.

RESULTS

Experimental results showing the fluorescence as a function of time from several samples are shown in Fig. 1. Each curve is a densitometer trace obtained from streak camera photographs. Careful calibration shows that the curves cannot be fit by a simple exponential decay, but are very nearly of the same form as predicted by Förster (14,15). As determined from calibration, the fluorescence from *Anacystis nidulans* shown in Fig. 1a rises in less than

10 psec and then decays (i.e., time from peak to e^{-1} point) in 75 ± 10 psec. Nearly identical results are obtained from the lyophilized *Anacystis* as shown in Fig. 1b. In Fig. 1c a decay curve is shown for the pigment mixture described in the experimental section. The similarity to the *Anacystis* curves is evident. Decay curves for living *Chlorella pyrenoidosa* samples yielded a lifetime of 41 ± 5 psec. Preliminary results, based on only two photos, indicate that lyophilized *Chlorella pyrenoidosa* samples had a comparable lifetime (80 ± 30 psec). The measurements were also made with a cross polaroid placed after the *Anacystis* sample with the electric field of the incident exciting radiation perpendicular to the polaroid. Under these conditions the decay curve had the same shape and the fluorescence was depolarized immediately (≤ 10 psec).

Experimental results showing the dependence of the lifetime on concentration is shown for chlorophyll *a* in a chloroform solution in Fig. 2. The

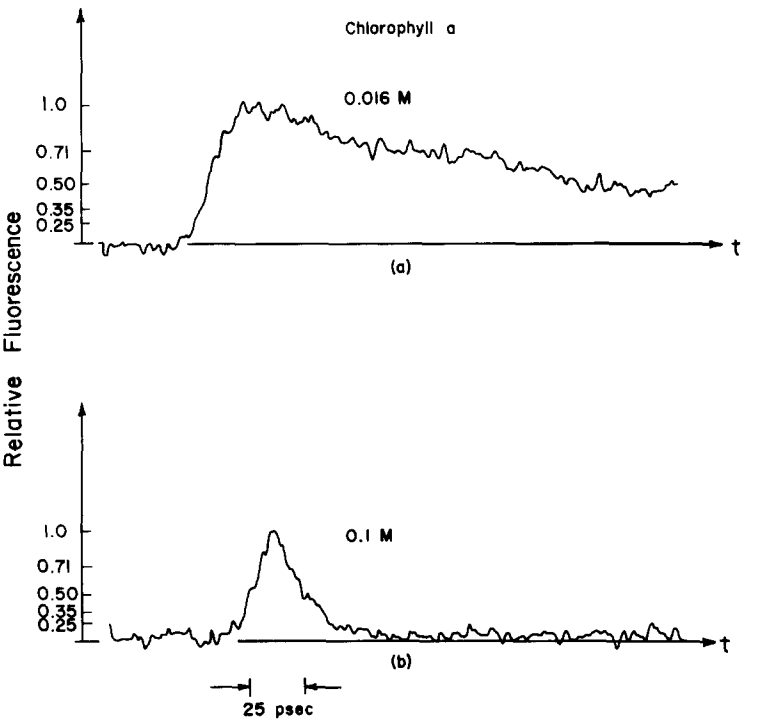

Figure 2: Densitometer traces of streak photographs of chlorophyll *a* in chloroform showing strong dependence of fluorescent lifetime with concentration. Figs. 2a and 2b show, respectively, concentrations of 0.016 and 0.1 Molar.

fluorescence lifetime dramatically changes from 275 ± 50 psec at 0.016 M concentration to 12 ± 5 psec at 0.1 M. The lifetime is 685 ± 145 psec at 0.002 M.

DISCUSSION

To explain our results, we note that the high concentration solutions of chlorophyll *a* resulted in short lifetimes. A quantum mechanical basis for this type of concentration quenching was formulated by Förster who treated nonradiative energy transfer in chlorophyll (14,15). Such quenching, as measured by the decrease in quantum efficiency, has been measured (16,17) for chlorophyll *a* and chlorophyll *b*. Concentration quenching is the result of resonant transfer of energy between molecules which takes place through a dipole-dipole interaction. A strong dependence with concentration results because the dipole-dipole transfer rate is proportional to $1/r^6$ where r is the spacing between the molecules. For chlorophyll *a* it can be shown that the nonradiative transfer rate dominates over the radiative rate when the spacing between molecules is less than about 60 Å. At such high concentrations the spacing between molecules goes inversely as the cube root of the concentration, so a change of concentration of a factor of 6 leads to a decrease in lifetime by a factor of 36. This accounts, within experimental accuracy, for the drop of the lifetime from 275 psec to ~ 10 psec in chlorophyll *a* as the concentration was increased by about 6.

To give a simple explanation of the order of magnitude of the decay time for the fluorescence from *Anacystis nidulans* and *Chlorella pyrenoidosa*, we need only note that chlorophyll *a* is present in cells in about 0.1 M concentration (18). Therefore because of the rapid nonradiative transfer, the lifetime would be expected to be short in both the living and lyophilized cells. Further support for this view is found from depolarization measurements made with the streak camera for *Anacystis nidulans*. These measurements indicate that the fluorescence is depolarized from the earliest (≤ 10 psec)

moments of emission showing that numerous transfers between molecules have taken place.

Previous measurements (19-26) on photosynthetic systems, with the exception of the optical gate measurements (4-6), have indicated longer lifetimes than we report in this paper. We believe that the improvement in technique is responsible for the high resolution we obtain. Our results, which are consistent with the high concentrations known to be present in cells, support a Förster energy migration model for photosynthesis (27).

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