Effect of Aging on the Fluorescence Lifetime of Chloroplasts

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Z. Naturforsch. **37 c**, 881–883 (1982); received January 4/May 24, 1982

Photosynthesis, Fluorescence Lifetimes, Aging, Sonicated Chloroplasts

The lifetime of fluorescence from pea chloroplasts at room temperature increases with age. The ratio of the fluorescence intensities of the slow to fast decay components, decreases with age of the chloroplasts. This observation can account for the wide range of lifetimes reported in the literature. The variability of biological material (cultured and prepared with similar procedures) introduces considerable variation in the fluorescence lifetimes measured with pea chloroplasts.

Sonication of chloroplasts results in an increase in both the fast and slow fluorescence lifetimes.

Introduction

The fluorescence decay from chlorophyll in chloroplasts is biphasic [1]. The fast fluorescence lifetime is interpreted as arising from exciton quenching by 'open' traps in photosynthetic units. The fluorescence is attributed to chlorophyll *a* antenna of photosystem I. The slow fluorescence life time is interpreted as arising from excitation in photosynthetic units, when the photochemical reaction centers are 'closed'. The slow fluorescence is attributed to light harvesting chlorophyll of photosystem II [2].

In the present studies, changes in fluorescence lifetimes and intensities are used to monitor alterations of the fluorescence centers associated with aging and sonication of chloroplast.

Materials and Methods

Chloroplasts were prepared from pea leaves as described by Nakatani and Barber [3], and resuspended in low salt buffer (LSB); 0.33 M sorbitol, 50 mM Hepes (N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid) adjusted to pH 7.6 with KOH. Chloroplasts were stored in a light tight ice bucket (0 °C) until used in an experiment.

The instrument used to resolve picosecond fluorescence was described in detail by Tredwell *et al.* [4] and Porter *et al.* [5]. A single 6 ps pulse, wavelength 530 nm, was used to excite fluorescence. Fluorescence from the sample passed through a sharp red cut-off filter into an Imacon streak camera (Model 600, John Hadland P.I. Ltd.). The streak rate was 20 ps/channel. Excitation intensity was less than 2×10^{14} photons cm^{-2} to avoid exciton annihilation. Unless indicated otherwise, nine to twelve fluorescence decays are summed together to improve the signal to noise ratio. The lifetimes are calculated from the summed data. The fluorescence decay can not be described as a single exponential. The fluorescence data were deconvoluted and analyzed by finding the best fit to the expression $y(t) = A_1 \exp -t/\tau_1 + A_2 \exp -t/\tau_2$, where A and τ are the fluorescence intensity and lifetime, respectively. The subscripts 1 and 2 refer to the slow and fast fluorescence decays, respectively.

Results and Discussion

1. Variability of biological material

Pea chloroplasts were prepared under carefully controlled conditions on different days, over a period of several months. Fluorescence lifetime measurements were carried out on the native chloroplasts, without the addition of any inhibitors, electron donors or acceptors. The experimentally determined A's and τ's, for the different chloroplast preparations exhibited a variation that far exceeded the accuracy of the measurement. In Table I is summarized some of the data obtained over several months with chloroplasts prepared following identical procedures. It can be seen that there is more than a 30% variation in the slow fluorescence lifetime and almost a 100% variation in the fast lifetime.

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Table I. Fluorescence lifetimes of freshly prepared chloroplasts.

Date	τ_1, ps	τ_2, ps	A_1/A_2
May 11	770	192	0.150
June 22	782	120	0.129
July 14	576	80	0.149
August 14	740	88	0.053
August 29	694	70	0.111
Average	$\tau_1 = 712 \pm 74$ ps		
Average	$\tau_2 = 110 \pm 44$ ps		
Average $A_1/A_2 = 0.118 \pm 0.036$			

Fluorescence lifetimes were determined from the sum of 9 to 12 fluorescence decays. A_1 and A_2 refer to the intensities of the slow and fast fluorescence decays, respectively.

Table II. Fluorescence decay as a function of number of laser flashs.

Flashs	τ_1, ps.	τ_2, ps.	A_1/A_2
1	1575	380	
2	820	160	0.32
3	675	45	0.25
4	540	35	0.04
5	550	30	0.03
7	600	30	0.025

The variation observed in the lifetimes of fresh chloroplasts (Table I) atests to the lack of uniformity or homogeneity of the biological preparations. The fluorescence lifetimes of chloroplasts are a function of a wide variety of variables including how the chloroplasts are prepared and handled, and the amount of time the biological material is maintained at room temperature in order to carry out an experiment. The rather wide range of lifetimes reported in the literature [2, 6–8] for chloroplasts (at laser intensities less than 2×10^{14} photons cm^{-2}) could well be related to the particular state or age of the biological preparation. Attempts to assign exact fluorescence lifetimes to chloroplast preparations with the data available at this time, is not too meaningful.

2. Effect of laser flashs on fluorescence decays

When the fluorescence decays are analysed individually, without summing, it is seen that at 40 °C there is a rapid decrease in the fluorescence lifetimes with time or the number of laser flashs (Table II). The ratio of the slow to fast fluorescence intensities, A_1/A_2 also decreases with each laser flash. The time between each laser flash is 5 to 12 min.

At the start of the experiment the chloroplasts are in the dark adapted (F_0) state, where the traps are open. The laser flash, like preillumination, tends to close the traps so after several flashs the chloroplasts could be at the F_m level. When chloroplasts are changed from the F_0 to the F_m level the fluorescence lifetime is reported to increase [2]. Since only decreases in lifetimes are observed, in Table II, it would appear that chloroplast aging at 40 °C is the controlling factor, rather than the closing of the traps by laser flashs. Since one is measuring a dynamic aging system, not a static stable chemical compound, it is apparent that the fluorescence decay will be different at the beginning and the end of a relatively long experiment.

3. Sonicated preparations

Sonication of chloroplasts results in an increase in the lifetimes of both the fast and slow fluorescence decay (Table III). Measurements were made on sonicated, but still turbid, chloroplast suspensions and on suspensions sonicated until they were clear. All chloroplasts were sonicated in a stream of nitrogen, in the dark and at 0 °C. The relative fluorescence intensities of the slow to fast decays, A_1/A_2 is only slightly modified by sonication. The longer the sonication the greater the effect on τ.

The object of the experiment with sonicated chloroplasts is to help illucidate the origin of the biphasic fluorescence decay. Sonication would not 'open' or 'close' traps. If fluorescence originates solely from the antenna chlorophyll immediately associated with reaction centers, then breaking the

Table III. Fluorescence lifetimes of sonicated chloroplasts.

Material	τ_1, ps.	τ_2, ps.	A_1/A_2
Fresh	654 ± 12	70 ± 5	0.111
Turbid	790 ± 16	84 ± 6	0.111
Clear	958 ± 20	144 ± 10	0.140

Fluorescence lifetimes were determined from the sum of 9 to 12 fluorescence decays. Fresh denotes un-sonicated chloroplasts. Turbid denotes chloroplasts that were sonicated, but the suspension was still turbid. Clear denotes chloroplasts that were sonicated until the suspension became clear.

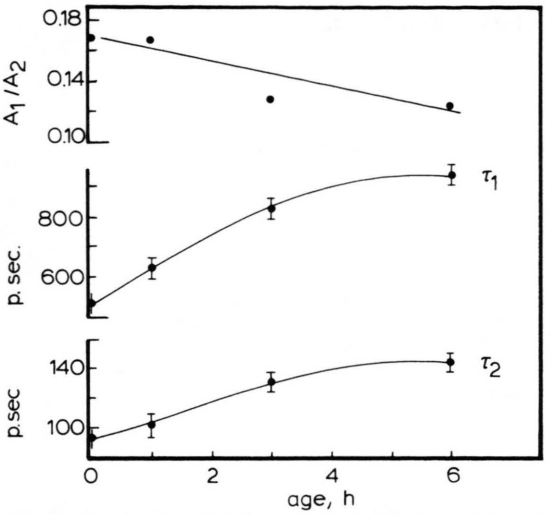

Fig. 1. Fluorescence lifetimes and relative intensities of the slow (τ_1) to fast (τ_2) fluorescence decays A_1/A_2 of pea chloroplasts as a function of time, at room temperature (26 °C). Each set of lifetimes was determined from the sum of nine to twelve fluorescence decays.

chloroplast into small fragments, by sonication, would have little effect on the fluorescence lifetimes. The fluorescence yield, however, should decrease. On the other hand, if fluorescence can originate from antenna chlorophyll in general, then removing some of the exciton trapping centers, by sonicating the chloroplast into small fragments, could lead to an increase in both the fast fluorescence lifetime and fluorescence yield. A similar interpretation was invoked to explain changes in the fluorescence spectrum induced by sonication [9]; it was shown that sonication produced both, a decrease of fluorescence intensity at 724 nm, and an increase in fluorescence intensity at 690 nm.

4. Effect of aging on 'summed' fluorescence decays

When 9 to 12 fluorescence decays are summed together, it is observed that with chloroplasts at room temperature (26 °C), both the fast and slow fluorescence lifetimes increase with time (age). In addition, the intensity of the slow fluorescence component (A_1) decreases relative to the fast component (A_2), as a function of chloroplast age (Fig. 1).

The proportion of 'closed' traps of photosystem II to 'open' traps of photosystem I, is given by the ratio of the fluorescence intensity of the slow decay to the intensity of the fast decay *i.e.* A_1/A_2 [1]. The increase in both the fast and slow fluorescence lifetimes (Fig. 1) during aging at room temperature, indicates that there is a decrease in exciton quenching by both the 'open' and 'closed' traps.

An increase in fluorescence lifetimes may result from a decrease in the absorption coefficient of the pigment molecule. The absorption coefficient (and wavelength of the absorption maximum) is dependent on the index of refraction (and dielectric constant) of the ambient medium. A change in the lipoprotein environment of chlorophyll which modifies the index of refraction, might change the fluorescence lifetime. Modifications of lipids have been identified with the aging process [10–12].

Acknowledgement

I would like to express my appreciation to Sir George Porter for making this work possible by providing encouragement, space, facilities and gracious hospitality at The Royal Institution in London. I am also obliged to Dr. C. Treadwell for his invaluable help by maintaining the picosecond apparatus.

[1] G. S. Beddard, G. R. Fleming, G. Porter, G. F. W. Searle, and J. A. Synowiec, Biochim. Biophys. Acta **545,** 165–174 (1979).
[2] G. F. W. Searle, C. J. Tredwell, J. Barber, and G. Porter, Biochim. Biophys. Acta **545,** 496–507 (1979).
[3] H. Y. Nakatani and J. Barber, Biochim. Biophys. Acta **461,** 510–512 (1977).
[4] C. Treadwell, J. Synowiec, G. Seale, G. Porter, and J. Barber, Photochem. Photobiol. **28,** 1013–1020 (1978).
[5] G. Porter, J. A. Synowiec, and C. Treadwell, Biochim. Biophys. Acta **459,** 329–336 (1977).
[6] I. Moya, Govindjee, C. Vernotte, and J. M. Briantias, FEBS Letters **75,** 13–18 (1977).
[7] W. Yu, F. Pellegrino, and R. R. Alfano, Biochim. Biophys. Acta **460,** 171–181 (1977).
[8] A. J. Campillo, V. M. Kollman, and S. L. Shapiro, Sci. **193,** 227–229 (1976).
[9] S. S. Brody, M. Brody, and J. Levine, Biochim. Biophys. Acta **94,** 310–312 (1965).
[10] P. J. Quinn and W. P. Williams, Prog. Biophys. Molec. Biol. **34,** 109–173 (1978).
[11] S. Hoshina, T. Kaji, and K. Nishida, Plant Cell Physiol. **16,** 465–474 (1975).
[12] R. E. McCarty and A. T. Jagendorf, Plant Physiol. **40,** 725–735 (1965).