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## BBA Report

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# Picosecond fluorescent kinetics of *in vivo* chlorophyll

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SUMMARY

Fluorescent emission kinetics at 685 nm of *in vivo* escarole chloroplast chlorophyll has been studied on a picosecond time scale using a frequency-doubled, mode-locked Nd: glass laser (4-ps wide pulses) and an optical Kerr gate. The fluorescent risetime is less than 10 ps and the decay time of the fluorescence envelope is 320 ± 50 ps. An apparent dip in the time dependence of the decay kinetics occurs at about 50 ps after the flash. This may be the result of energy transfer between carotenoids or chlorophyll *b* and chlorophyll *a* or an indication that two independent species are fluorescing.

During the past 15 years many groups have estimated the mean fluorescent lifetime of *in vivo* chlorophyll. Techniques have involved both direct flash (hydrogen flash lamps) and phase methods with authors reporting lifetimes of between 0.3 to 1.9 ns [1–10] in various non-bacterial photosynthetic systems. Besides possible species differences, it is known that fluorescent lifetimes are dependent on the intensity of the exciting flash[2,3,11] and on photosynthetic activity[12,13]. Detailed examination of the chlorophyll fluorescence kinetics on a sub-nanosecond time scale has not been possible even when direct flashes were used, since the flash pulse widths were in the order of a nanosecond (deconvolution techniques could not extract accurately the temporal behavior of the fluorescence in the sub-nanosecond time domain). This communication reports on the fluorescent kinetics of chlorophyll in escarole (*Cichorium endivia* L.) chloroplasts probed on a picosecond time scale.

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The experimental arrangement discussed elsewhere[14] consists of a mode-locked Nd: glass laser, a KDP crystal for generation of the second harmonic, a cuvette and a $CS_2$ cell optical Kerr gate[15,16]. Chloroplasts were prepared by the methods of Avron[17] and Cramer and Butler[18]. Two laser beams of picosecond duration are used in the experiment – a 0.53 - $\mu$m beam with a 4-ps pulse width and a 1.06 - $\mu$m beam 6 ps wide. The 0.53 - $\mu$m beam activates fluorescence emission in the chloroplast sample which is continuously cycled to and from a dark, ice-cold reservoir. The 1.06 - $\mu$m beam times the gate by inducing a short-lived birefringence in the $CS_2$ cell which is situated between crossed polarizers in the fluorescence path. The fluorescence (which is circularly polarized) can pass through the crossed polaroid configuration only during the short time of induced birefringence in the $CS_2$ cell. Chlorophyll fluorescence is thus sampled at different times simply by adjusting the pathlength of the 1.06 - $\mu$m beam with respect to the 0.53 - $\mu$m beam. The zero point is defined as the coincidence in time and space of the 0.53 - $\mu$m and 1.06 - $\mu$m beams at the $CS_2$ cell with water substituted for chloroplasts in the cuvette. The total fluorescent intensity, the fluorescent intensity at a particular time delay and wavelength and the intensities of the 0.53 - $\mu$m and 1.06 - $\mu$m beams were detected and displayed simultaneously on a Tektronics 556 oscilloscope. All work was done in the dark with an average actinic intensity (600 ergs/cm$^2$) low enough such that the total fluorescence was linearly proportional to the intensity of the 0.53 - $\mu$m flash. Under the experimental conditions, the actinic beam (0.5 cm in diameter at the cuvette) did not cause stimulated emission when Rhodamine 6G, a common laser dye, was substituted for the chloroplast sample. Therefore, stimulated emission cannot occur in the chloroplast chlorophyll since it has a much lower quantum efficiency than Rhodamine 6G.

The relative fluorescent intensity of escarole chloroplast chlorophyll at 685 nm is plotted as a function of time in Fig. 1. Although the results are preliminary, several observations can be made. The initial peak occurs very close to time zero which indicates that the risetime of fluorescence in this sample is less than 10 ps, the resolving time of the instrument. The solid curve represents the decay envelope of the fluorescent state and the dashed curve an apparent dip in the time dependence of emission. A decay time ($\tau$) of 320 ± 50 ps is obtained for the envelope (time at which the envelope has reached 0.37 of its maximum), a value which is close to that reported by Müller *et al.*[11] for sugar beet leaves (but less than for sugar beet chloroplasts) and for chlorella[11] at low actinic intensities. The dip at approximately 50 ps, although not expected, cannot be ignored since it is reproducible in escarole and is apparent in preliminary studies with spinach chloroplasts.

Two possible explanations for this observation are being explored. The dip may be a manifestation of energy transfer between a non-fluorescent and a fluorescent species (both absorbing) with chlorophyll *b* and carotenoids being candidates for the former and chlorophyll *a* for the latter. An alternate tack might involve two independent fluorescent species.

We thank Dr David Mauzerall for discussion of our results and acknowledge the technical assistance of Mr Peter Holliday and Mr Syd Hussian.

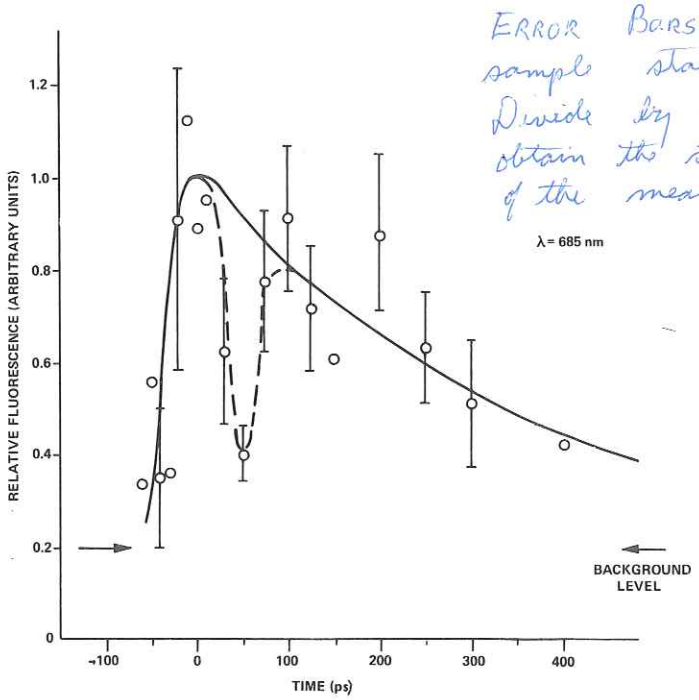


Fig. 1. The relative fluorescence intensity of *in vivo* escarole chloroplast chlorphyll is plotted as a function of time after excitation with a 4-ps wide pulse of 0.53-$\mu$m light. The solid curve represents the fluorescent decay envelope and the dashed line an apparent dip in the emission kinetics. Wavelength, 685 nm; bandwidth, 3.3 nm.

## REFERENCES


1 Brody, S.S. and Rabinowitch E. (1957) *Science* 125, 555
2 Dmitrievsky, O., Ermolaev, V. and Terenin, A. (1956) *Dokl. Akad. S.S.S.R.* 124, 585
3 Dmitrievsky, O., Ermolaev, V. and Terenin, A. (1957) *Dokl. Akad. Nauk. S.S.S.R.* (Biol. Sci. transl.) 114, 468
4 Tomita, G. and Rabinowitch, E. (1962) *Biophys. J.* 2, 483–499
5 Rubin, A.B. and Osnitskaya, L.K. (1963) *Mikrobiologiya* 32, 200–203
6 Butler, W.L. and Norris, K.H. (1963) *Biochim. Biophys. Acta* 66, 72–77
7 Murty, N.R. and Rabinowitch, E. (1965) *Biophys. J.* 5, 655–661
8 Nicholson, W.J. and Fortoul, J.I. (1967) *Biochim. Biophys. Acta* 143, 577–582
9 Singhal, G.S. and Rabinowitch, E. (1969) *Biophys. J.* 9, 586–591
10 Merkelo, H., Hartman, S.R., Mar, T. and Govindjee (1969) *Science* 164, 301–302
11 Müller, A., Lumry, R. and Walker, M.S. (1969) *Photochem. Photobiol.* 9, 113–126
12 Tumerman, L.A., Borisov, O.F. and Rubin, A.B. (1961) *Biofizika* 6, 645–649
13 Tumerman, L.A., Borisov, O.F. and Rubin, A.B. (1961) *Biophysics* (transl.) 6, 723–728
14 Alfano, R.R. and Shapiro, S.L. (1972) *Optics Commun.*, 6, ~~500–504~~ 98-100
15 Duguay, M.A. and Hansen, J.W. (1969) *Appl. Phys. Lett.* 15, 192–194
16 Duguay, M.A. and Hansen, J.W. (1969) *Optics Commun.* 1, 254–256
17 Avron, M. (1961) *Anal. Biochem.* 2, 535–543
18 Cramer, W.A. and Butler, W.L. (1967) *Biochim. Biophys. Acta* 143, 332–339

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