BRIEF COMMUNICATION

LOW-INTENSITY TWO-STEP ABSORPTION OF CHLOROPHYLL A IN VIVO

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ABSTRACT The intensity-dependent transmission of primary leaves of *Triticum aestivum* seedlings at $\lambda = 694$ nm was measured with single pulses of a Q-switch ruby laser. At photon flux densities above 2×10^{17} cm^{-2}s^{-1} a decrease of transmission was observed. The result is interpreted as a two-step absorption of cooperative units of 10^5–10^6 chlorophyll molecules.

The investigation of nonlinear optical absorption (intensity-dependent transmission) offers new access to the study of the primary processes of photosynthesis and of the structure of the antenna pigment system (1, 2). In what follows we shall report on transmission measurements of this type on chlorophyll A in vivo at $\lambda = 694.3$ nm with low intensities, i.e. in the intermediate region between linear and nonlinear absorption. The results suggest that in the chlorophyll of green plants a stepwise absorption of two photons occurs even at intensities comparable to that of the sunlight. Moreover, the results permit a preliminary estimation of the size of the absorbing unit in vivo.

Recently, Mauzerall (3) and Campillo et al. (4) were the first to observe nonlinear optical effects in the low energy region in chlorophyll in vivo. With nanosecond ($\lambda = 337$ nm) and picosecond pulses ($\lambda = 530$ nm), they found the quantum efficiency of fluorescence in *Chlorella pyrenoidosa* to decrease for energy densities above 10^{13} photons/cm^2, interpreting this decrease as a singlet-singlet annihilation (see also ref. 5). This effect is accompanied by a reduction of fluorescence lifetime (6).

Nonlinear absorption was measured on chlorophyll in vitro by several authors using ruby laser pulses (7–9). The beginning of nonlinearity, i.e. the deviation from the low-signal transmission, T_0, occurs at energy densities $\geq 10^{15}$ photons/cm^2 in this case, depending especially on the type of solvent. We have carried out measurements on chlorophyll in vivo (primary leaves of *Triticum aestivum* seedlings) in the range of

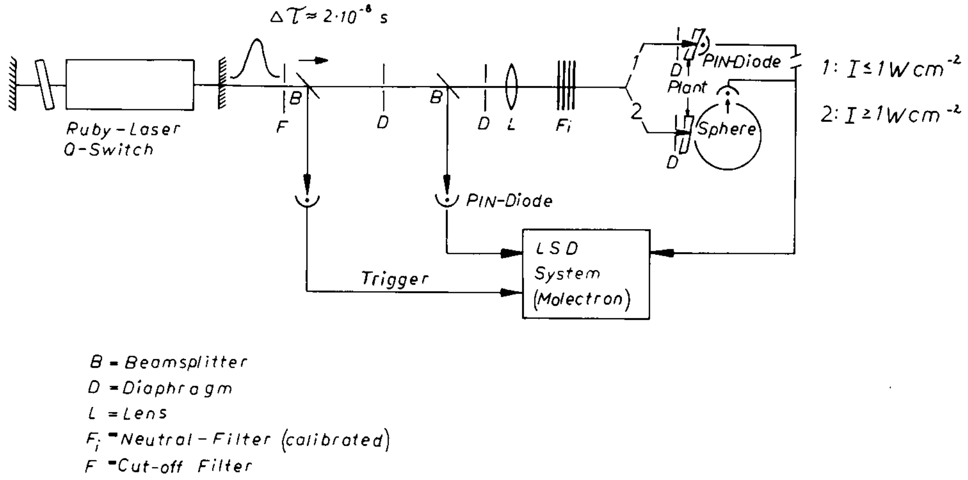

FIGURE 1 Experimental setup for nonlinear absorption measurement.

energy densities between 5 × 10^8 and 5 × 10^{18} photons/cm^2 (photon flux densities ranging from 2.5 × 10^{16} to 2.5 × 10^{26} cm^{-2}s^{-1}, or power densities ranging from 8 × 10^{-3} to 8 × 10^7 W/cm^2). We found that in this case the range of beginning nonlinearity lies at energy densities as low as 4 × 10^9 photons/cm^2 (photon flux density of 2 × 10^{17} cm^{-2}s^{-1}). The experimental setup is shown in by Fig. 1. The measurements were carried out with a 20-ns single-pulse of a Q-switch ruby laser, the intensity of

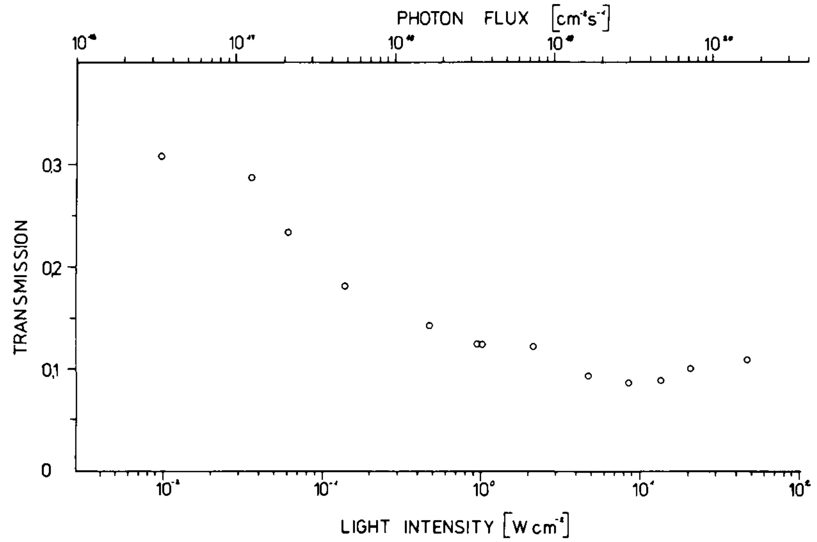

FIGURE 2 Intensity-dependent transmission of chlorophyll A in vivo (primary leaves of *Triticum aestivum* seedlings) at $\lambda = 694.3$ nm. Each circle represents an average of five measurements.

which was varied by means of calibrated neutral filters. Any influence of background ruby radiation (fluorescence or spiking regime) was carefully excluded. For intensity measurements before and after the measuring sample, photodiodes were used in combination with the laser spectroscopy detector system (Molectron Corp., Sunnyvale, Calif.). Either the measuring sample was placed immediately in front of the photodiode (in the range of 2.5×10^{16} to $3 \times 10^{18}\,cm^{-2}s^{-1}$), or an integrating sphere was inserted between the sample and the photodiode. Immediately in front of the measuring sample, the coherence of radiation was destroyed by a diffusing screen. The primary leaves of *Triticum aestivum* seedlings were grown for 12 days under 1,800 lux. For the measurement we took an area of about 7 mm^2 from a point 25 mm below the tip of the leaf. The low-signal transmission of all samples at $\lambda = 694$ nm lies in the interval of $T_0 = 30 \pm 1\%$; it was determined by an integrating sphere with a spectrophotometer type DK-2a (Beckman Instruments, Inc., Fullerton, Calif.).

The results of the measurements of intensity-dependent transmission in the range between 3.5×10^{16} and 2×10^{20} photons $cm^{-2}s^{-1}$ are shown in Fig. 2. At the lowest intensities the transmission is constant, corresponding to the low-signal level T_0. For intensities above 2×10^{17} (corresponding to an energy density of 4×10^9 photons/cm^2) a decrease of transmission is observed.[1] This course of the curve shows that a second absorption after the ground state absorption becomes effective, the cross-section of which, σ_2, is greater than that of the first transition (σ_1). Remarkably, this stepwise two-photon absorption occurs even in a intensity range comparable with that of sunlight (maximum of 10^{17} vis. quanta $cm^{-2}s^{-1}$).

It should be noted in addition that in the higher intensity range between 2×10^{20} and 2.5×10^{26}, several local extrema of transmission occur (1); however, $T(I) < T_0$ is satisfied up to the highest measured intensities.

As reported above, the nonlinearity of the absorption of chlorophyll in vivo begins at an intensity lower by several orders of magnitude than that found for chlorophyll in vitro. This is considered due to the fact that the absorbing unit in vivo consists of a great number of molecules, whereas in solution it consists of single molecules, dimers, or relatively smaller oligomers. This number N of molecules per absorption unit—as well as the absorption cross section, σ_2—can be determined from the course of the function in Fig. 2. To this end, starting from the numerical solution of the coupled system of differential equations for the population densities of the absorption levels and the photon transport through the sample, a minimization procedure in the parameters σ_i and k_{ij} (rate constants) is added in order to reproduce the transmission behavior of the curve in Fig. 2. Such calculations are in preparation. For a preliminary estimation of the order of magnitude, we used the approximation of an optically thin

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[1] We made sure that the observed transmission decrease of green leaves really belonged to a nonlinear absorption of chlorophyll (*a*) by observation of an asymmetric change of the shape of the transmitted pulse in the intensity region around 1 W · cm^{-2}; and (*b*) by comparison with the transmission behavior of etiolated leaves under the same conditions. (The etiolated leaves were extracted by a mixture of methanol, acetone, ether, and petroleum ether in equal volume proportions, and rewashed in the same mixture).

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sample, supposing that with $I = 10^{18}$ photons/cm²s a proportion of 10 to 100% of all absorption units is involved in the second absorption. This yields $10^5 \leq N \leq 10^6$ and $6.5\,\sigma_1 \geq \sigma_2 \geq 1.5\,\sigma_1$.

We defer any statements on the plant-physiological and ecological importance of this two-step absorption in order to introduce into the discussion analogous intensity-dependent transmission curves at different wavelengths within the longest-wave absorption band of chlorophyll in vivo. It appears obvious that there is a relationship to the energy dissipation of the excited chlorophyll under intensive sunlight irradiation (valve reaction [10]). This relationship can be expected in the case of competition of the photon absorption rate $\sigma_2 I$ with the rate constants of the subsequent photosynthetic partial reactions (electron transport chain, CO_2 fixing), and might take place via energetically corresponding energy levels of deactivating molecules (carotinoids).

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REFERENCES

1. LEUPOLD, D., S. MORY, and P. HOFFMANN. 1976. Nonlinear absorption and stimulated emission of Chlorophyll-a. Conference on Luminescence, Szeged (Hungary). Aug. 24-27. *Acta Phys. Chem.* **23**:33.
2. HIEKE, B., P. HOFFMANN, D. LEUPOLD, S. MORY, and J. SCHOTTE. 1977. Absorption behaviour of Chlorophyll in vitro and in vivo at ruby laser excitation. *Photosynthetica* (Prague). In press.
3. MAUZERALL, D. 1976. Multiple excitations in photosynthetic systems. *Biophys. J.* **16**:87.
4. CAMPILLO, A. J., S. L. SHAPIRO, V. H. KOLLMAN, K. R. WINN, and R. C. HYER. 1976. Picosecond excitation annihilation in photosynthetic systems. *Biophys. J.* **16**:93.
5. SWENBERG, C. E., N. E. GEACINTOV, and M. POPE. 1976. Bimolecular quenching of excitons and fluorescence in the photosynthetic unit. *Biophys. J.* **16**:1447.
6. CAMPILLO, A. J., V. H. KOLLMANN, and S. L. SHAPIRO. 1976. Intensity dependence of the fluorescence lifetime of in vivo Chlorophyll excited by a picosecond light pulse. *Science (Wash. D.C.).* **193**:227.
7. LEBEDEV, O. L., YU. M. GRYAZNOV, and A. A. CHASTOV. 1968. Nonlinear transmission of Chlorophyll solutions to ruby laser light. *Opt. Spectrosk.* **24**:328.
8. ARSENAULT, R., and M. M. DENARIEZ-ROBERGE. 1976. Non-linear absorption and fluorescence of Chlorophyll a excited by a ruby laser. *Chem. Phys. Lett.* **40**:84.
9. LEUPOLD, D., S. MORY, P. HOFFMANN, B. HIEKE, and R. KÖNIG. 1977. Laser action and excited state absorption of Chlorophyll-a. *Chem. Phys. Lett.* **45**:567.
10. WITT, H. T. 1971. Coupling of quanta, electrons, fields, ions, and phosphorylation in the functional membrane of photosynthesis. Results by pulse spectroscopic methods. *Q. Rev. Biophys.* **4**:365.