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ENERGY TRANSFER IN THE PHOTOCHEMICAL APPARATUS OF FLASHED BEAN LEAVES

RETO J. STRASSER and WARREN L. BUTLER

Department of Biology, University of California, San Diego, La Jolla, Calif. 92093 (U.S.A.)

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SUMMARY

Fluorescence and energy transfer properties of bean leaves greened by brief, repetitive xenon flashes were studied at −196 °C. The bleaching of *P*-700 has no influence on the yield of fluorescence at any wavelength of emission. The light-induced fluorescence yield changes which are observed in both the 690 and 730 nm emission bands in the low temperature fluorescence spectra are due to changes in the state of the Photosystem II reaction centers. The fluorescence yield changes in the 730 nm band are attributed to energy transfer from Photosystem II to Photosystem I. Such energy transfer was also confirmed by measurements of the rate of photooxidation of *P*-700 at −196 °C in leaves in which the Photosystem II reaction centers were either all open or all closed. It is concluded that energy transfer from Photosystem II to Photosystem I occurs in the flashed bean leaves which lack the light-harvesting chlorophyll *a*/*b* protein.

INTRODUCTION

The leaves of young dark-grown bean plants which have been partially greened by being subjected to a repetitive series of brief xenon flashes (a 1ms flash every 12 min) for several days are not capable of photosynthetic O_2 evolution when first illuminated [1]. These leaves accumulate chlorophyll *a* but little if any chlorophyll *b* [2] and they are characterized morphologically at the submicroscopic level by parallel arrays of unfused primary thylakoids [3]. The flashed leaves appear to have a functional primary photochemical apparatus in that *c*-550, as demonstrated by light-induced absorbance changes at −196 °C, is present [4] and plastid preparations from these leaves are capable of performing Photoystem I and Photosystem II mediated photoreactions if suitable electron donor and electron acceptor systems are supplied [5–9]. The inability for O_2 evolution appears to be due to a lesion in the photosynthetic electron transport system between water and Photosystem II which can be repaired by irradiation with continuous light for a few minutes [1]. A study of changes

Abbreviation: DCMU: 3(3,4-dichlorophenyl)-1,1-dimethylurea.

which occur during this initial induction period, correlating absorbance changes and structural changes with the onset of O_2 evolution, was reported recently [10].

Our model for the photochemical apparatus of photosynthesis assumes that chlorophyll is organized in Photosystem I and Photosystem II units as well as a light-harvesting chlorophyll complex, which can transfer excitation energy to either of the two types of photosystem units [11, 12]. The light-harvesting chlorophyll complex is the chlorophyll *a/b* protein characterized by Thornber and co-workers [13, 14]. The photosynthetic apparatus of the flashed leaves contains active Photosystem I and Photosystem II units but not the chlorophyll *a/b* protein [15]. The purpose of the present work was to explore the fluorescence and energy transfer properties of these leaves which lack light-harvesting chlorophyll *a/b* complexes.

MATERIALS AND METHODS

Bean seeds (*Phaseolus vulgaris* var. red kidney) were soaked overnight and planted in vermiculite. After four days in darkness the plants were irradiated with a repetitive series of 1 ms flashes from a xenon lamp once every 12 min for 6 days.

All measurements were carried out on leaf samples in the form of a 1 cm disc frozen to liquid nitrogen temperature in our vertical cuvette and Dewar system [16]. Light-minus-dark difference absorption spectra were measured with our computer-linked single-beam spectrophotometer described previously [16]. Fluorescence measurements were carried out using two triple-arm fiber-optic light-pipe assemblages described previously [17] (each triple-arm combination comes together at a 1 × 10 cm glass rod which serves as a mixing chamber). One light-pipe combination rests on top of the frozen sample in the cuvette; the other views the bottom of the sample through the bottom of the Dewar (see Fig. 1). Three of the light-pipe arms were used for excitation of fluorescence and three were used for the measurement of fluorescence. The 633 nm light from the Ne-He laser (used in combination with a 633 nm interference filter) gave a uniform irradiation at the surface of the leaf of 2 mW/cm^2. This intensity was used for actinic purposes only. For kinetic measurements, where the measuring, beam was used to change the fluorescence yield as well as to excite the fluorescence, the intensity of this beam was reduced to 100 μW/cm^2 with a 5 % neutral density filter. For measurements of fluorescence, where it was required that the yield of fluorescence at the F_0 level at −196 °C should not be altered by the measurement, the intensity of the laser beam was reduced further to 5 μW/cm^2 with two 5 % neutral density filters. Actinic light at 730 nm (30 nm halfwidth) was obtained from a xenon lamp with two Calflex heat reflecting filters, two Corning 5850 filters, two Corning 2030 filters and a Corning 2600 filter. This source gave an intensity of 4 mW/cm^2 at the surface of the leaf. A tungsten lamp with a Bausch and Lomb monochromator set at 705 nm was used in conjunction with a 710 nm interference filter to provide a weak (10 μW/cm^2) fluorescence excitation beam at 705 nm. The 710 nm interference filter was used to give a steep cut-off of the shorter wavelengths in the 705 nm pass band from the monochromator. This band was intended to excite only Photosystem I and some fluorescence from Photosystem II could be detected if the 710 nm interference filter was omitted.

The time course measurements of the fluorescence yield changes at 693 and 735 nm excited by the 633 nm beam at 100 μW/cm^2 were measured by the photo-

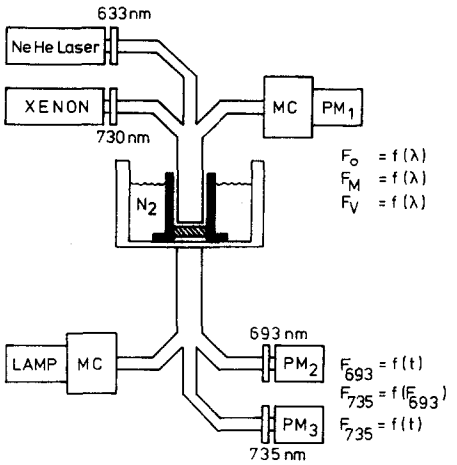


Fig. 1. Schematic diagram of instrumentation showing the sample (hatched area) in the cuvette frozen to liquid nitrogen temperature. Two triple-arm, fiber-optic light pipes are used, one at the top of the sample, the other at the bottom of the Dewar.

tubes PM2 and PM3, respectively, in Fig. 1. These were Hamamatsu, extended-red-sensitive S-20 phototubes with appropriate blocking and interference filters. The output from these phototubes was measured either as a function of time with a dual-pen strip-chart recorder or against one another with a Hewlett-Packard 7047A X-Y recorder (0.1 s time response). Emission spectra excited by the weak (5 μW/cm^2) 633 nm beam were measured with a computer-linked Bausch and Lomb High Intensity Monochromator plus a Kodak Wratten 70 filter and a Hamamatsu galium arsenide phototube (R666S). This monochromator and phototube combination has a flat response within 5 % from 675 to 745 nm. Emission spectra from the frozen leaf discs at both the F_0 and F_M levels were measured on line with the computer.

The rate of photooxidation of *P*-700 in the frozen leaf discs was measured by the absorbance change at 703 nm (both monochromators shown in Fig. 1 were set at 703 nm). The intensity of the measuring beam was sufficiently weak that it did not transform *P*-700; the *P*-700 was transformed by a repetitive series of 3 μs xenon flashes from a General Radio Stroboslave, type 1534, (at the position of the xenon lamp in Fig. 1) at a rate of 1/s similar to the assay system described previously [18]. The leaf discs were infiltrated with 10 μM DCMU and 10 mM hydroxylamine 30 min before freezing. The dark adapted samples were kept in complete darkness; the preilluminated samples were given a 1 min irradiation followed by a 30 s dark period just prior to freezing. In both cases, *P*-700 was fully reduced but the Photosystem II reaction centers were either all open in the dark adapted samples or all closed in the preilluminated samples.

The total chlorophyll content of the leaves was measured by the method of Arnon [19]; the ratio of chlorophyll *a* to chlorophyll *b* was determined in ethanolic extracts by the sensitive fluorometric method of Boardman and Thorne [20].

RESULTS AND DISCUSSION

The chlorophyll content of the flashed leaves was 10 to 15 % of that found in

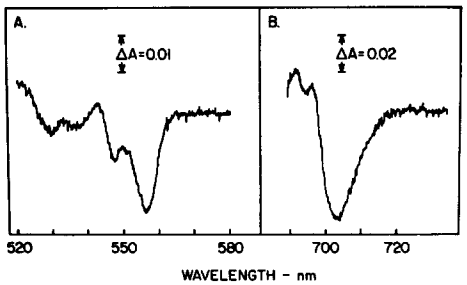


Fig. 2. Light-minus-dark difference spectra of a flashed leaf at −196 °C due to a saturating irradiation with 633 nm light. A. The difference spectrum in the range from 520 to 580 nm shows the photoreduction of *c*-550 and the photooxidation of cytochrome *b*-559. B. The difference spectrum in the range from 690 to 730 nm shows the photooxidation of *P*-700.

mature green bean leaves, on an equal area basis, and the ratio of chlorophyll *a* to chlorophyll *b* was always above 50 as opposed to a ratio of about 3 for the green leaves. We take these latter measurements to confirm the absence of any significant amounts of the light-harvesting chlorophyll *a*/*b* protein in the flashed leaves. These leaves, however, appear to have normal, photochemically active Photosystem I and Photosystem II reaction centers. Irradiation of a flashed bean leaf at −196 °C produces the same light-induced absorbance changes that are observed with mature green leaves. The light-minus-dark difference spectrum in Fig. 2A shows the photoreduction of *C*-550 and the photooxidation of cytochrome *b*-559 and the spectrum in Fig. 2B shows the photooxidation of *P*-700.

At room temperature the fluorescence properties of a flashed leaf are significantly different from a normal green leaf in that they show very little fluorescence of variable yield at the onset of irradiation with strong light, presumably because the block in the electron transfer chain between water and Photosystem II limits the supply of electrons for the photochemical reduction of the primary electron acceptor of Photosystem II. At a low temperature, however, which limits the electron transfer reactions to only those occurring in the reaction center complexes, a block between water and Photosystem II should have no influence so that if the flashed leaves have

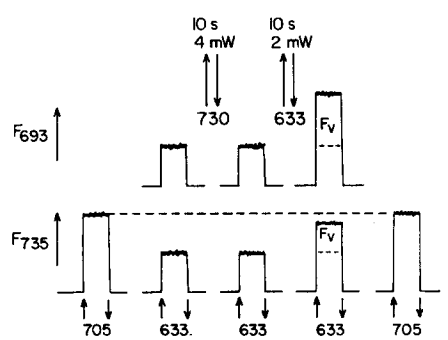
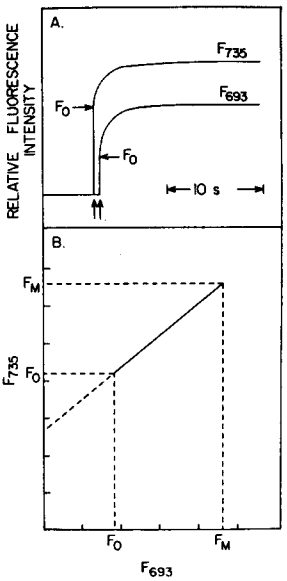


Fig. 3. Intensity of fluorescence from a flashed leaf at −196 °C at 693 and 735 nm excited by 705 nm light (10 μW/cm^2) and 633 nm (5 μW/cm^2). 10 s irradiations were given with 4 mW/cm^2 of 730 nm light and 2 mW/cm^2 of 633 nm light as indicated to transform *P*-700 and *C*-550, respectively.

normal Photosystem II reaction centers, they should show normal light-induced fluorescence yield changes at low temperature. Indeed they do as data below confirm. In the work reported here we examined how the selective transformation of both Photosystem I and Photosystem II reaction centers affects the fluorescence yield of flashed leaves at −196 °C. At low temperature Photosystem I reaction centers could be transformed without affecting Photosystem II by irradiation with 730 nm light; Photosystem II reaction centers could then be transformed subsequently by an irradiation with red light. The fluorescence at 693 nm was assumed to be due primarily to emission from the antenna chlorophyll in Photosystem II while that at 735 nm was due to emission from the antenna chlorophyll in Photosystem I; excitation at 633 nm excited both Photosystem I and Photosystem II while excitation at 705 nm excited only Photosystem I. The results of a typical experiment are shown in Fig. 3. Excitation of a flashed leaf with a weak 705 nm beam at −196 °C produced a certain level of fluorescence at 735 nm shown as the step function increase in the presence of the excitation beam: the emission at 693 nm could not be measured with this excitation wavelength. Excitation with a weak 633 nm beam produced the levels of fluorescence at 693 and 735 indicated in the figure. (Throughout the experiment the intensities of the excitation beams were sufficiently weak that the photochemical state of the leaf was not altered by the measurements of fluorescence at −196 °C.) The leaf was then irradiated with 10 s of 4 mW/cm^2 of 730 nm light. (A separate experiment showed that this irradiation was saturating for the photooxidation of *P*-700 but had no influence on *C*-550.) The measurements of fluorescence at 693 and 735 nm excited by the weak 633 nm beam indicated that no fluorescence yield changes resulted from the actinic irradiation with 730 nm light. Thus, the phototransformation of Photosystem I reaction centers does not affect the fluorescence yield, even at wavelengths of emission from Photosystem I. The leaf was then irradiated with 2 mW/cm^2 of 633 nm light for 10 s to photoreduce the *C*-550. After this red irradiation the intensities of fluorescence at 693 and 735 nm excited by the weak 633 nm light were both greater, the increases being termed F_V at the two wavelengths. The increase of Photosystem I fluorescence at 735 nm following the phototransformation of Photosystem II reaction centers can be attributed to an increase of energy transfer from Photosystem II to Photosystem I [11, 12, 21]. A comparison of the intensities of fluorescence at 735 nm excited by 705 nm light at the beginning and at the end of the experiment shows no changes resulting from either the red or the far-red actinic irradiations.

This set of experiments with a flashed leaf is in agreement with results obtained with mature spinach chloroplasts showing that Photosystem I activity is not linked to any fluorescence yield changes at low temperature [21]. All fluorescence yield changes are controlled by Photosystem II activity. Furthermore, the fluorescence at 735 nm at −196 °C due to the direct excitation of Photosystem I by 705 nm light is not affected by the states of either the Photosystem I or Photosystem II reaction centers.

The kinetics of the light-induced fluorescence yield changes at 693 and 735 nm from the flashed leaf at −196 °C are shown in the upper part of Fig. 4. These measurements were made simultaneously from the same leaf by using two light-pipe arms (see Fig. 1) for the two wavelengths of emission. When these two measurements were plotted against one another on the two axes of an X-Y recorder, a straight line was obtained for the phototransformation from F_o to F_M (lower part of Fig. 4). This



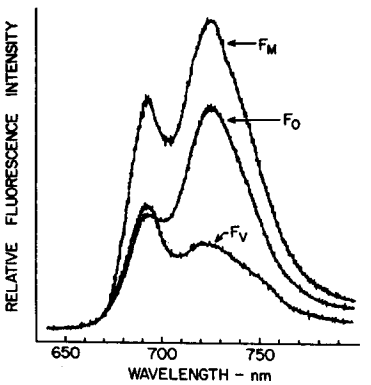


Fig. 4. A. Kinetics of fluorescence yield changes from a flashed leaf at −196 °C at 735 and 693 nm due to irradiation with 100 μW/cm^2 of 633 nm light. B. The same type of experiment except that the intensities of fluorescence at 735 and 693 nm were plotted on the two axes of an X-Y recorder.

Fig. 5. Fluorescence emission spectra from a flashed leaf at −196 °C due to excitation with 5 μW/cm^2 of 633 nm light. Spectra were measured before (F_o) and after (F_M) actinic irradiation of the frozen sample. F_V is the difference spectrum, $F_M - F_o$, plotted out directly from the computer.

linear X-Y plot shows that the kinetics of the light-induced fluorescence yield changes at 693 and 735 nm are identical as they should be if both were controlled by the rate of closure of the Photosystem II reaction centers. A similar straight line X-Y plot was obtained previously with spinach chloroplasts at −196 °C [21]. Emission spectra from the flashed leaf measured at the minimal, F_o, and maximal, F_M, levels at −196 °C with weak excitation at 633 nm are shown in Fig. 5. The emission spectrum of the F_V component was obtained from the difference spectrum, $F_M - F_o$.

It is apparent from the results in Figs. 4 and 5 that the fluorescence at 735 nm from the flashed leaves at −196 °C has an appreciable variable yield component which, according to our model for the photochemical apparatus [12, 21], can be assumed to be a manifestation of energy transfer from Photosystem II to Photosystem I. However, to confirm that such energy transfer does occur in the flashed leaves, we sought more direct evidence from an assay procedure used recently to demonstrate energy transfer from Photosystem II to Photosystem I in spinach chloroplasts at low temperature [18].

The flashed leaves infiltrated with DCMU and hydroxylamine behave in the same manner as chloroplasts in the presence of those inhibitors in that the high yield of fluorescence established by irradiation at room temperature persists in the dark after the irradiation and can be frozen in. Thus, flashed leaves infiltrated with 10 mM hydroxylamine and 10 μM DCMU were frozen to −196 °C in the dark adapted state or after a 1 min irradiation followed by a 30 s dark period at room temperature.

The *P*-700 was fully reduced in both samples but the Photosystem II reaction centers were all open in the dark-adapted sample and all closed in the preilluminated sample. The rates of photooxidation of *P*-700 (caused by repetitive flashes) for the two types of samples are shown in Fig. 6. As was found with spinach chloroplasts in a similar measurement [18], the rate of transformation of *P*-700 plots as a second order reaction. The second order plots of the data in the lower part of Fig. 6 show that the rate of photooxidation of *P*-700 was 33 % faster in the preilluminated sample, presumably because of the greater transfer of excitation energy from Photosystem II to Photosystem I when the Photosystem II reaction centers were closed. These data confirm the conclusion based on measurements of F_V at 735 nm at −196 °C that energy transfer from Photosystem II to I does occur in the unfused primary thylakoids of flashed leaves which lack the light-harvesting chlorophyll *a*/*b* protein.

The original model of the photosynthetic apparatus of mature spinach chloroplasts by Butler and Kitajima [11] depicted units of Photosystem I and II separated by the light-harvesting chlorophyll *a*/*b* protein and the question was asked whether energy transfer from Photosystem II to Photosystem I required the presence of the light-harvesting chlorophyll *a*/*b* complex to serve as a bridge between the two types of units. The present work answers that question: the light-harvesting chlorophyll *a*/*b* complex is not required for energy transfer from Photosystem II to I. In fact, our assays for energy transfer at −196 °C, i.e., the relative extent of F_V at 735 nm corrected for the relative extent of F_V at 693 nm or the stimulation of the photooxidation

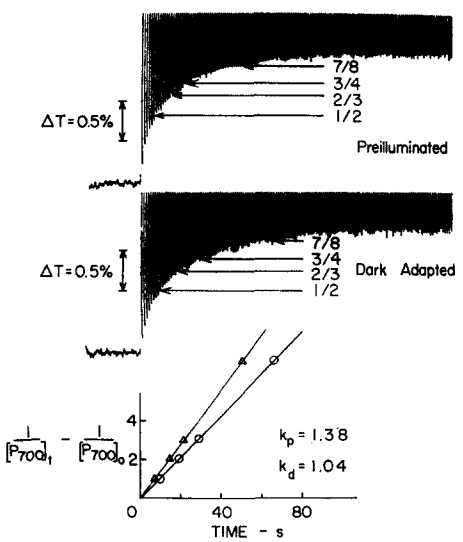


Fig. 6. Kinetics of the photooxidation of *P*-700 in a flashed leaf at −196 °C. The transmission of the sample is monitored at 703 nm with simultaneous irradiation from repetitive xenon flashes (1/s). The trace goes off scale during the 3 μs flash but recovers rapidly to give a valid reading between flashes. The leaf discs were infiltrated with 10 μM DCMU and 10 mM hydroxylamine 30 min before freezing to −196 °C. The dark adapted sample was kept in darkness before freezing; the preilluminated sample received a 1 min irradiation 30 s before freezing. Numbers shown on the rate curves indicate the fraction of transformation of *P*-700 at various times. Those data are used to obtain the second order rate plots at the bottom of the figure.

of *P*-700 by the closure of Photosystem II reaction centers, indicate that the yield of energy transfer is at least as great (possibly even slightly greater) in the flashed leaves as it is in mature spinach chloroplasts. These results are not in disagreement with the theory of Butler and Kitajima; rather they support an alternative model [22] in which the units of Photosystem I and II were placed adjacent to one another with the light-harvesting chlorophyll *a*/*b* complex surrounding the Photosystem II units but in partial contact with the Photosystem I units.

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