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Photosynthetic Units of Sun and Shade Plants[1]

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ABSTRACT

A computer analysis of fluorescence induction curves of leaves treated with 3-(3,4-dichlorophenyl)-1,1 dimethylurea was done for several species. These measurements gave the ratios of the total chlorophyll to photosystem II reaction centers. This communication is a preliminary survey of sun and shade plants and demonstrates a significant variation in this ratio. In the sun plants, the photosynthetic unit sizes (chlorophyll reaction centers) varied between 220 to 480. The shade plants gave numbers mostly in the range between 630 to 940. The computer analysis of the fluorescence data also gave the connectivity parameter of energy transfer between photosynthetic units of photosystem II which varied between 0.2 and 0.5 but did not show any obvious correlation to the photosynthetic unit size.

Plants are adapted to live in extremely different light environments, from the deep shade, such as is found on the floor of the redwood forest, to the extreme direct sunlight of open fields. For some plants this adaptation is absolute in the sense that a plant from one group will not survive or will grow much more slowly, showing detrimental effects, upon transfer to the opposite environment of the other group. The basis of this type of differentiation probably originates at the level of the photosynthetic machinery (1). One of the possible factors which was considered to contribute to the difference between the two extreme groups was the ratio of antennae Chl to the reaction center (PSU[3] size) and that sun plants would have the smallest PSU sizes (1). According to Boardman and Björkman *et al.* (1–4), the difference between the sun and shade plants was not in the ratio mentioned above but, rather, that the sun plants developed more photosynthetic capacity (in terms of higher level of electron transport enzymes and the enzymic activity associated with CO_2 fixation per Chl). They claimed that the ratio of reaction centers per Chl does not change significantly and, particularly, that this ratio for PS II is approximately the same. The same conclusion was achieved for a sun plant (*Atriplex patula*) adapted to grow at different light intensities (4).

Although we consider that the over-all photosynthetic rates do depend on the change of the activities of several factors and their adaptation to different light regimes, we would like to re-evaluate the conclusions regarding the PSU size. A previous communication (10) showed that a quantitative relation existed between photosynthetic rates and PSU sizes of PS II as measured by an improved and elaborated fluorescence induction method (*cf.* below). These measurements were done in four species of desert plants, in which the PSU size varied over a considerable range.

The fluorescence induction method (10) as used by us involves the following: (*a*) infiltration of a leaf with DCMU; (*b*) measurements with relatively high light intensity so that the fluorescence rise is completed in less than about 300 ms [The induction times (8) are typically in the range of 30–100 ms.]; (*c*) the actinic wavelength chosen so that it penetrates deeply through the leaf (*i.e.* a monochromatic light of wavelength about 550 nm); (*d*) the detected emission wavelength chosen at a wavelength of maximal absorption about 680 nm, with a highly monochromatic detection filter). (*e*) the photodetector placed above the irradiated surface at an angle to the actinic beam as large as practically possible. Conditions c through e guarantee that the fluorescence observed comes only from the places where the actinic light is homogeneous in intensity (provided, of course, that the intensity is uniform over its cross-section). In any other arrangement artifacts arise due to optical reasons.

To the strength of the method, we now add also the capability of computer analysis of the data (11) which, together with the usual graphical display, allowed us to obtain quickly and precisely values for the initial slope, the normalized area above the induction curve (induction time) and tables showing the relation between partial areas as a function of the fluorescence. With this technique, we examined whether any difference exist between sun and shade plants with regard to their PSU sizes.

MATERIALS AND METHODS

Leaves or short branches were picked from plants growing either in the deep shade of a redwood forest near Woodside, CA, or from plants growing in an open field of the Carnegie Institution. Individual leaves were cut to form a round shape having a diameter about 1.5 to 3 cm and then infiltrated with a DCMU solution (20 μM). The infiltration was accomplished by alternate evacuation for about 1 min and restoration of normal pressure repeating the process at least twice. This allowed the DCMU solution to infiltrate the leaf fully (10).

The DCMU-infiltrated leaf was placed in a horizontal position so that its upper surface received the fluorescence exciting light which came from above. The leaf was covered by a black mask (checked to be nonfluorescent) which had a 7-mm diameter opening. The light source was a 650-w quartz-iodine lamp (type DWY). The light passed through an optical system containing an electronically controlled shutter, a water filter, and interference and glass filters [550-nm interference filter (Infra Red Industries) and 4-96 Corning glass filter]. A small portion of the light was diverted into a photocell which monitored the intensity and which served as a reference for the computer. Although the beam formed a circular shape, having a diameter of about 25 mm at the level of the sample, and would have produced a large fluorescence signal, we preferred to limit its size by the mask and thus ensure the uniformity of the exciting light over the exposed area. Measurements of the intensity were made with a small (3 × 3 mm) silicon photoelectric cell. Moving the silicon cell over the larger beam area (without cover) produced intensity variations of about ±15% from the average. It is probable that, over the limited exposed area

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[3] Abbreviation: PSU, photosynthetic unit.

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we used, this variation was even less. Uniformity is of utmost importance, as any deviation from it affects the kinetics of the fluorescence rise.

The photodetector was a red-sensitive photomultiplier (EMI 9558B) placed above the sample at an angle of about 60° from the vertical. The fluorescence produced was passed through Schott RG 5 filter and a narrow-band (5 nm, half-band), 675-nm interference filter (Infra Red Industries).

The filters of both exciting and detecting systems were checked for "false signal" due to scattered actinic light (that would produce an incorrect F_0 level), by adding extra filters (Corning 4-96 in the exciting system and RG 5 in the detecting system) and observing that the ratio of F_0/F_{max} remained the same.

The fluorescence signal was divided and sent simultaneously into an electronic processing system, which produced a graphic display of the fluorescence as a function of the time, and into a computer (11). In the last case the information was digitized into 1,024 points with intervals of time chosen at will. The signal of the last points was averaged and called F_{max}. To find F_0, a more complicated procedure was applied whereby the computer used the exciting light as a reference, sensed the point where the shutter was half opened, and fixed this point as time zero. The extrapolation of the fluorescence time curve, for the first points when the shutter was fully opened, to time zero gave the required F_0 and also served as a "corrected" fluorescence curve for the uncertainty period of the shutter opening time (11). This is a very small correction compared to the induction times of 30 to 1,000 ms inasmuch as the shutter opening time was of the order of 3 ms. A by-product of this procedure was that the initial slope of the fluorescence induction curve was also obtained and was expressed as an inverse of time (always normalizing the full extent of the variable fluorescence $F_{max} - F_0$ to unity).

In the computer analysis, care was taken to limit the time to a reasonable range where only the main increase phase of the fluorescence was completed. This need arose since in a few cases additional very slow increase or decrease phases of small amplitudes were observed following the main fluorescence rise. Although of small amplitudes, these could add or subtract significant area and change the induction time artificially. To eliminate rigorously the effect of these slow phases, the fluorescence curves were run with different termination times and the area was plotted as a function of the termination time. A back-extrapolation from the values obtained at large termination times gave the true value of the induction time, as proved by a simple mathematical analysis. Criteria were developed for the full-time scale to be used in each experiment, optimal to obtain the correct result.

The reason for the additional phases is not clear. They may reflect slow changes in the physiological ambient conditions of the membranes which influence the fluorescence parameters (*e.g.* radiationless transition probabilities). It is possible that DCMU infiltration was not absolutely complete. Alternatively the slow phase may be due to a different type of reaction centers (*e.g.* β centers of Melis and Duysens [12]) of minor abundance and, therefore, not counted in this analysis.

THEORETICAL OUTLINE

The fluorescence curve in the presence of DCMU gives information on the concentration of reaction centers and the interaction between groups of Chl antennae molecules around each reaction center. The concentration of reaction centers per Chl was obtained by using the following formula (see ref. 10 for more details):

$$n/\text{Chl} = [1 - (F_0/F_{max})] \times 0.5 \times 2300 \times \epsilon_i \times I \times \bar{\tau} \qquad (1)$$

where $1 - F_0/F_{max}$ is the maximal quantum yield of excitation trapping when all the reaction centers are opened. The value 0.5 is a tentative factor of light distribution into PSII (*cf.* ref. 10). The factor 2,300 comes from the conversion of decadic to natural logarithms and the conversion of volume units from cc to liters. ϵ_i is the extinction coefficient (at the wavelength of the actinic light) for Chl in its *in vivo* environment. For this, we take an average value of 8,000 $\text{M}^{-1}\,\text{cm}^{-1}$ at 550 nm (*cf.* ref. 10). To be sure, a precise value of this extinction coefficient is not available at the moment because of the spectroscopic artifacts that tend to distort it. Previously, we estimated it by measuring the absorption spectrum of chloroplast suspensions that were placed in an integrating sphere (10). This minimized errors but did not completely eliminate effects such as the lengthening of the optical path by internal scattering in the chloroplast particles, which tended to increase the apparent extinction coefficient, especially at the low extinction wavelengths. I is the incident light intensity in $\text{E/cm}^2\cdot$unit time and τ is the average induction time, given by the area above the induction curve when $F_{max} - F_0$ normalized to unity.

Actually, $\bar{\tau}$ was corrected for light attenuation effects in the leaf (10) by division by the factor $(1 + [\epsilon_i/\epsilon_f] \cos \theta)$ where ϵ_f is the extinction coefficient for the fluorescent light (*i.e.* 685 nm) and θ is the average angle between the direction of the fluorescent light in the leaf, as viewed by the photomultiplier, and the normal to the leaf surface. This correction factor for our case was rather small, in the range of 7%.

The excitonic interaction between different PSU values is measured here by obtaining the parameter p, first introduced by Joliot and Joliot (5). One way to define p (as in ref. 5) is the average energy transfer probability for an exciton in a "closed" unit to migrate out and go to a different neighboring PSII unit. This concept leads to the following formula (6):

$$f = \frac{F_{max} - F(t)}{F_{max} - F_0} = \frac{a}{1 - p(1 - a)} \qquad (2)$$

where f is the normalized fluorescence function (as defined in 8). It is the complementary to the normalized variable fluorescence, *i.e.* $f = (1 - f_v)$. The term a is the complementary to the normalized partial area at a time t, formed by the fluorescence curve, the maximal fluorescence, time zero, and time t axes, *i.e.* it is the fraction of units in the "open" form at any time. The f *versus* a relation is linear when $p = 0$ but deviates more strongly from a linear relation as p increases. The extreme points (0:0) and (1:1) remain the same. The value of p may be estimated then by comparing the experimental f *versus* a curves to theoretical ones.

Another way to estimate p, which is perhaps more convenient, is by comparing the reciprocal of the initial slope of the normalized variable fluorescence to the induction time. One can prove from equation 2 that the ratio of the induction time to the reciprocal of the initial slope is $1 - p$ and can calculate p from it:

$$p = 1 - \frac{\bar{\tau}}{T} \qquad (3)$$

where $\bar{\tau}$ is the induction time and T is the inverse of the initial slope, *i.e.* the time when the tangent to the induction curve at time zero crosses the F_{max} line. This is particularly convenient when DCMU infiltration is not 100% complete. In this case, the slow secondary phases, although very small in extent, may introduce a perturbation in the analysis of f *versus* a relation if one is not careful enough. The initial slope in this case is not so sensitive to the presence of the slow phases.

RESULTS AND DISCUSSION

A sample of two fluorescence induction traces is shown in Figure 1. The most distinctive variation among the various species is the fluorescence induction time. The factor $1 - F_0/F_{max}$, representing the photochemical efficiency, varied in a minor way between 0.53 to 0.69 with most of the values close to 0.6 (data not shown).

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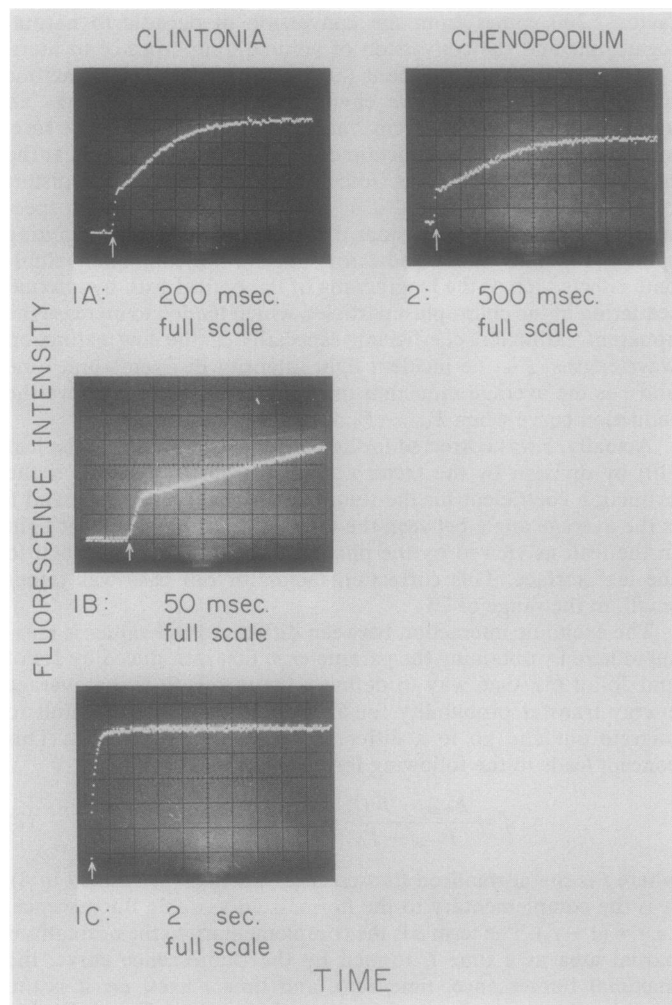

FIG. 1. Fluorescence induction curves for two species.

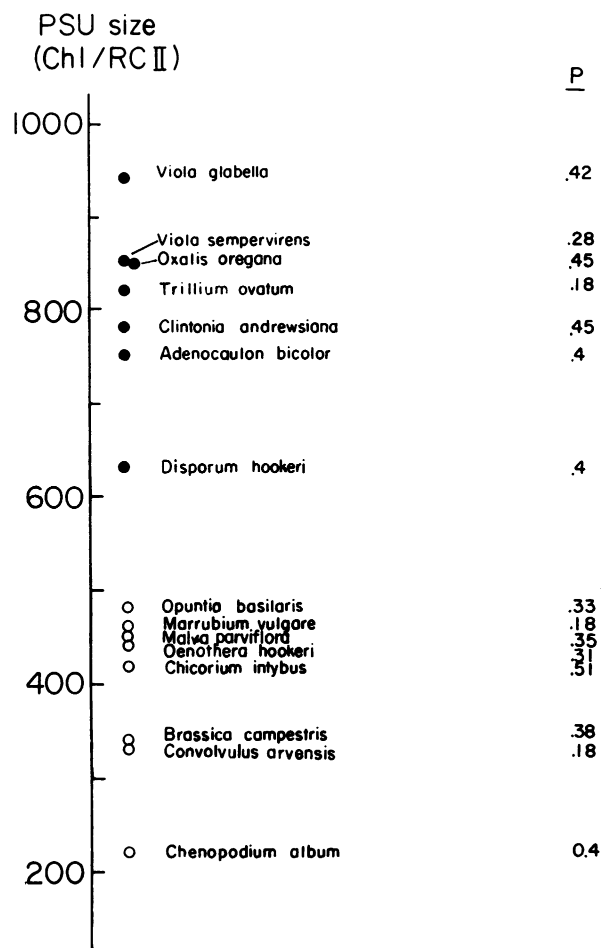

FIG. 2. PSU sizes (of PSII) for a variety of sun plants (○) and shade plants (●) and p values. RC, reaction center.

The results of photosynthetic unit determinations made on eight sun plants and six shade plants are shown in the diagram of Figure 2. This diagram shows that, for sun plants, the range of sizes is about 220 to 540 (Chl/reaction centers). The shade plants had a range of sizes usually between about 630 to 940. Thus, the range of all possible PSU sizes is divided into two more or less separate regions: the low values for sun plants and the high values for shade plants. The variations of the induction times and, hence, the PSU sizes within a given species was within 10%.

An interesting question is whether the PSU is a genotype characteristic in which only slight changes can occur when the plant is acclimated in a different environment than its natural one. As already mentioned, the results of Björkman *et al.* (5) on *Atriplex patula* grown under different light intensities showed relatively little variation of the PSU sizes, whereas other components of electron transport did change. The photosynthetic unit size may be a parameter which indicates a more basic characteristic of the plant and its evolutionary adaptation to the changes in light level. It is therefore possible that the early adaptation in evolution was done by mutations involving changes in photosynthetic unit size. Also, one can look for the possibility of defining the degree of "shade" or "sun" characteristics according to the photosynthetic unit concept.

Another characteristic of the pigment system of PSII is the number p, whether defined as an "energy transfer probability" between individual units or in any other manner. It is seen that there is no correlation whatsoever between the p values (ranging between about 0.2 to 0.5) and the PSU size. This is in contrast to an idea that as the number of pigments per reaction center increases the distances between units decrease and interactions among them become more probable (7). That p is variable between different species was already seen in fluorescence life-time studies (9). Whether p is also a characteristic property of a plant species remain to be seen in a more extensive work.

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