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# The influence of reducing the chlorophyll concentration by photobleaching on energy transfer to artificial traps within Photosystem II antenna systems

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**The influence of reducing the chlorophyll concentration by photobleaching on the relative excitation-energy-transfer rates to the artificial quinone trap dibromothymoquinone was examined in both a Photosystem II–grana preparation and the isolated light harvesting chlorophyll *a/b* protein complex. Energy transfer as a function of decreasing chlorophyll was biphasic. Photobleaching of up to 30% of the total chlorophyll produced no detectable decrease in the energy-transfer rate. For photobleaching in excess of 30% the transfer rate decreased as a linear function which extrapolates to zero at 100% photobleaching, in agreement with a diffusive Förster-type transfer model in a bidimensional pigment matrix (Rubin, L.B., Braginskaya, O.V., Isakova, M.L. and Efremov, N.A. (1985) Photochem. Photobiol. 42, 77–87). The initial photobleaching-insensitive phase may therefore suggest that in the non-bleached antenna systems energy transfer could have a significant non-Förster component. It is also concluded that the physiological effects of photobleaching on photosynthetic activity via a reduced energy transfer efficiency are not likely to be significant.**

## Introduction

The photosynthetic antenna systems of green plants consist essentially of a large number of chlorophyll molecules the function of which is to absorb light and efficiently transfer the excitation energy to a small number of reaction centres. In normally grown sun plants there are, on an average, about 200–250 antenna chlorophylls per reaction centre [2], all of which seem to be bound to specific polypeptides to form a number of chlorophyll-protein complexes [3,4]. It is generally accepted that the number of antenna chlorophylls which transfer energy to Photosystem II is somewhat greater than for Photosystem I [4] due mainly to the massive presence of the light harvesting chlorophyll *a/b* protein complex II (LHCPII) which is preferentially associated with Photosystem II.

The array of antenna chlorophylls is made up of two chemically distinct chlorophyll species, chlorophyll *a* and chlorophyll *b*, with chlorophyll *a* being present in at least five different spectroscopic forms [5,6]. These chlorophyll species, including chlorophyll *b*, can be arranged, with respect to wavelength, in a series in which the absorption maxima differ by 7–11 nm. It is generally thought that excitation is transfered within the antenna matrix according to the dipole–dipole $R^{-6}$ theory of Förster [7], where $R$ is the donor–acceptor distance. While fast (ps) fluorescence studies provide strong experimental evidence favouring such a mechanism in the phycobilin-chlorophyll antenna system of red algae [8] similar techniques have not to date been successful in elucidating transfer mechanisms for the chlorophyll matrices of green plant antennae. Sauer [9] has suggested that energy transfer within the chlorophyll protein complexes may be via a delocalised exciton coupling-type mechanism due to the small chlorophyll–chlorophyll distances (less than 20 Å) with the Förster model being applicable over the greater distances (in excess of 20 Å) between the chlorophyll-protein complexes.

Exposure of leaves or chloroplasts to high light intensity is known to lead to inactivation of the reaction centres [10,11] and to the photobleaching of the antenna

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Abbreviations: DBMIB, dibromothymoquinone; PS II (I), Photosystem II (I); BBY grana, grana called after the authors of Ref. 17; LHCPII, light-harvesting chlorophyll *a/b* protein complex II.

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chlorophylls [12–15]. This second process is thought to be brought about by some form of 'activated oxygen', possibly the singlet form. All the various spectroscopic chlorophyll forms are susceptable to photobleaching [15]. Apart from the obvious result of reducing the antenna absorption cross section, photobleaching may also be expected to modify the energy-transfer rate and efficiency within the chlorophyll matrices. With this in mind we have examined the influence of photobleaching on the relative energy-transfer rate within two preparations of Photosystem II antenna. The motivation was twofold: (1) to examine the effect of reducing the chlorophyll concentration on energy transfer in these antenna systems; and (2) to study just how sensitive energy transfer is to photobleaching, a naturally occurring phenomenon for plants growing in high light intensity environments. To this end we have determined the relative energy-transfer rate to the artificial energy trap dibromothymoquinone (DBMIB) by examining the steady-state fluorescence quenching induced by this compound. It is demonstrated that photobleaching of up to 30% of the chlorophyll in these antenna systems does not lead to a detectable decrease in the transfer rate to the artificial traps. For photobleaching in excess of this value the transfer rate decreases in a linear fashion with decreasing chlorophyll concentration.

## Materials and Methods

LHCPII, the principal PS II antenna complex, was prepared from spinach leaves according to Ref. 16. The preparation was finally suspended in a medium containing 0.05 M sucrose, 5 mM Tricine (pH 7.5) and 10 mM MgCl.

The BBY grana were prepared from freshly harvested spinach leaves according to the method of Berthold et al. [17] but omitting the last Triton-X treatment. This preparation contains both LHCPII and PS II core protein complexes and is substantially free of PS I activity. It is capable of oxygen evolution and has an $F_m/F_0$ ratio only slightly less than that of freshly prepared thylakoids.

Photobleaching was performed by illuminating the sample (4 $\mu$g chlorophyll per ml), maintained at 0 °C, with white light from a xenon lamp filtered across a Calflex X heat filter (Balzers) and a 2 cm pathlength of water. The incident intensity was about 3500 $\mathrm{W \cdot m^{-2}}$. The extent of chlorophyll photobleaching was determined by measurement of the area under the absorption spectra in the wavelength interval 640–720 nm.

Absorption spectra were measured with a Jasco UVIDEC-510 spectrophotometer at a slit width of 2 nm. Opal glass diffusors were utilised to minimise light scattering. The small residual signal at 750 nm was subtracted from the spectra.

The decomposition of the absorption spectra in gaussian components was performed using a non-linear least-square analysis program for the estimation of the gaussian parameters. A spectrum is fitted by a linear combination of asymmetric gaussian functions, each obtained as the sum of the right and left sides of two gaussian functions of equal height and mean value but with different FWHM. Each function is characterized by four free parameters: the wavelength at which the function peaks, the height of the band and the two independent half-widths. The program runs on a Vax 8650 computer (Digital) and enables us to use a maximum combination of 10 bands. The analysis gives the total area of the spectrum, the area and the left and right half-width of each of the bands, the $\chi^2$ of the fit and the graph of the residuals. The last two parameters are used to judge the goodness of the fit [18].

Titration of chlorophyll fluorescence of LHCPII and the BBY grana preparation was carried out with the quinone DBMIB as previously described [19]. For LHCPII the successive quencher additions were 0.9 $\mu$M while for BBY grana they were 0.7 $\mu$M. In the case of the BBY grana DCMU (25 $\mu$M) was also added. Fluorescence was excited with a narrow band (half band width, 2 nm) interference filter (Oriel) with peak transmittance at 650 nm in combination with a Balzers B-40 filter (650 nm: half-band width, 8 nm). The fluorescence emission was monitored at 745 nm (Balzers B-40, half-band width 8 nm combined with a Corning 5-56 filter). The choice of this wavelength was determined by the necessity of measuring fluorescence outside the chlorophyll absorption band and thus obviating problems associated with different levels of fluorescence reabsorption after photobleaching. All fluorescence values were normalised to the absorptance of the sample at 650 nm. In a series of preliminary measurements it was observed that the quencher titration characteristics at the 745 nm emission wavelength were similar to those measured at an emission wavelength of 682 nm (the emission peak at room temperature) both before and after pronounced chlorophyll photobleaching. Thus we conclude that the population of fluorescing molecules measured is the same at both wavelengths.

## Results

It is well accepted that to a good first approximation the fluorescence yield ($F$) of an excitation energy-transferring chlorophyll antenna matrix may be represented by

$$F = \frac{k_f}{\Sigma k + C k_q} \tag{1}$$

where $k_f$ is the fluorescence rate constant. $\Sigma k$ is the sum of all intramolecular decay processes, $k_q$ is the trapping constant of the artificial traps averaged over the matrix and is a function of the energy-transfer process, $C$ represents the concentration of the artificial traps in the pigment matrix [20–25]. It is thus clear that when the fluorescence is titrated with an artificial trap the slope of the reciprocal of the fluorescence versus the concentration of the added quencher is a function of $k_q/k_f$. We have used this approach to examine the influence of decreasing the chlorophyll concentration by photobleaching on energy transfer in both spinach LHCPII and the BBY grana preparations. Representative examples of such titrations are presented in Fig. 1 for BBY grana in which photobleaching had reduced the chlorophyll concentration by about 30% and 70%, respectively. After 30% photobleaching the straight line quencher plot has a similar slope to the non-photobleached sample. On the other hand a considerable decrease in the slope was encountered after 70% photobleaching. The different $1/F$ axis intercepts are due to a substantial fluorescence quenching induced by the high light intensity treatment.

The observation that the quencher titration slope decreases after pronounced photobleaching could in principle be explained in terms of an increased fluorescence rate constant ($k_f$) or a decreased trapping constant ($k_q$). We have therefore analysed by Gaussian decomposition the room temperature absorption spectrum of the BBY grana after pronounced photobleaching. As demonstrated previously all the commonly observed Gaussian components observed in spectra measured at low temperatures are also present in the room temperature spectra (Fig. 2). It can also be seen that photobleaching of about 50% of the total chlorophyll did not significantly change either the peak position or the half-band width of the major bands. It would therefore seem unlikely that either the overall fluorescence rate constant or the quinone chlorophyll interaction was significantly altered. Small changes in the relative contribution of the major gaussian components after photobleaching are observable in the BBY grana preparation (Fig. 2 and Ref. 15). Such changes are almost absent however with LHCPII [15]. On the basis of these observations we interpret the decrease in the quencher titration slope after pronounced photobleaching as being due to a decreased energy-transfer rate to the artificial traps within these antenna systems. The relative decrease in the transfer rate is given by the slope of the photobleached sample normalised to the slope of the non-photobleached sample.

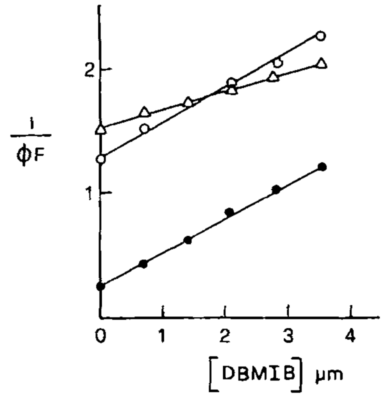

Fig. 1. The effect of two different extents of chlorophyll photobleaching on the titration of the reciprocal of the chlorophyll fluorescence with dibromothymoquinone. Measurements were performed with BBY-grana. ●, dark control; ○, sample photobleached by 32%; △, sample photobleached by 71%.

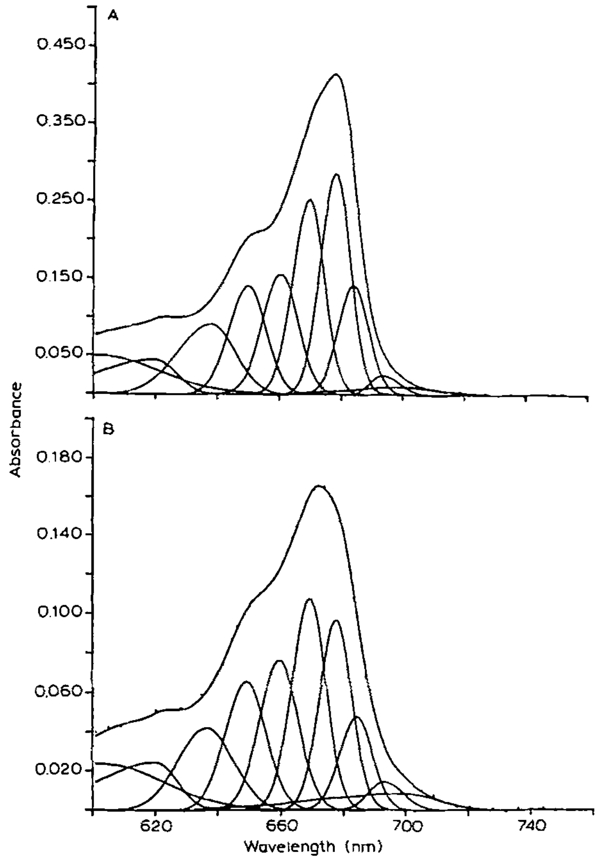

Fig. 2. The influence of pronounced chlorophyll photodestruction on the gaussian decomposition of the absorption spectrum of a BBY-grana preparation. (A) Dark control ($\chi^2 = 0.95$); (B) sample photobleached by 53% ($\chi^2 = 1.1$).

In Fig. 3 data are presented for a large number of such quencher titration experiments performed with both BBY grana and LHCPII for different levels of photobleaching. With both antenna systems the titration slope remains approximately constant for photobleaching of up to about 30%. For higher levels of photobleaching the slope decreases in an apparently linear fashion, extrapolating to a slope value of zero at 100% photobleaching.

## Discussion

In the present paper we examine the influence of decreasing the chlorophyll concentration in PS II an-

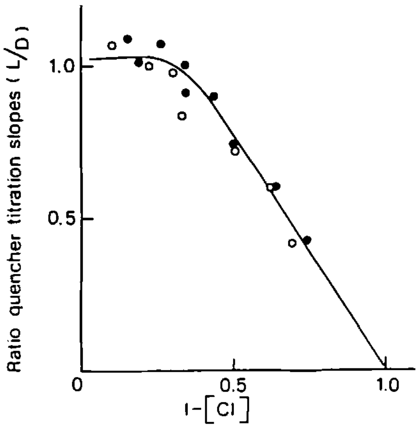

Fig. 3. The influence of different levels of chlorophyll photobleaching on the titration slope of the reciprocal of the chlorophyll fluorescence with the artificial energy trap dibromothymoquinone. 1 – [Cl] represents the chlorophyll photobleaching. Data are presented as the ratio of the titration slopes of photobleached samples ($L$) with respect to the dark controls ($D$). ●, LHCPII; ○, BBY grana.

tenna systems by photobleaching on the relative energy-transfer rate to the artificial trap DBMIB. From Fig. 3 the energy-transfer rate clearly displays a biphasic dependence on chlorophyll concentration. In the first phase photobleaching of up to 30% of the total chlorophyll in both BBY grana and LHCPII has little or no effect on energy transfer while in the second phase this parameter declines in a linear fashion with decreasing chlorophyll concentration.

Rubin et al. [1] have analysed energy transfer of a diffusive nature in a bidimensional pigment matrix. Starting from the usual differential equation for the excited state density decay kinetics one may write the limiting steady-state case as follows:

$$\alpha I = \frac{n}{\tau_0} + CnP_\infty \tag{2}$$

where $P_\infty$ is the rate of diffusive exciton capture by the traps; $\alpha I$ is an absorption parameter; $\tau_0$ is the intrinsic excited state lifetime; $C$ is the artificial trap concentration; $n$ is the concentration of donor-excited states. In this treatment we ignore the direct donor–trap transfer term [1] as the DBMIB/chlorophyll ratio was estimated to be well below 0.03, in the present study, for each quencher addition (unpublished observation).

The fluorescence yield ($F$) may therefore be written as

$$F = \frac{nk_f}{\alpha I} = \frac{k_f}{\Sigma k + CP_\infty} \tag{3}$$

Eqn. 3 is equivalent to Eqn. 1 if $k_q = P_\infty$.

As pointed out by Rubin et al. [1] $P_\infty$ may be expressed in terms of the Förster excitation transfer microparameters ($R_0$, $\tau_0$) and the matrix donor concentration ($C_D$) by

$$P_\infty \propto \frac{R_0^6}{\tau_0}\left(\frac{4\pi}{3}C_D\right) \tag{4}$$

where $P_\infty$ is seen to be a linear function of the matrix donor concentration. In the present paper we observe that for photodestruction levels above 30% (the second phase) the transfer rate decreases linearly with decreasing chlorophyll concentration. This is in good agreement with the prediction of the Förster $R^{-6}$ dipole–dipole theory, as described by Rubin et al. [1], at these chlorophyll concentrations. On the other hand the first phase, in which excitation transfer to the artificial traps seems not to depend on the antenna matrix chlorophyll concentration, is not readily understandable in these terms. This observation may therefore suggest that energy transfer in the non photobleached antenna systems has a significant non-Forster-type component.

Clearly the present data do not permit a detailed interpretation. However, as pointed out above, it has recently been suggested that the high chlorophyll concentrations within the pigment-protein complexes and the consequent low chlorophyll–chlorophyll distances (possibly considerably less than 20 Å) could be consistent with a delocalised transfer mechanism at least within the individual complexes. In this context it is interesting to note that for a delocalised transfer mechanism, in which the nearest neighbour interaction has an $R^{-3}$ dependence, the excitation transfer rate is concentration independent [26]. Possibly the loss of up to 30% of the chlorophyll within these energy-transfer domains does not significantly change this delocalised exciton-transfer property.

Apart from the mechanistic considerations it is clear that energy transfer in these PS II antenna systems is extremely resistant to photobleaching. A 50% reduction in the average excitation transfer rate to the artificial traps requires the photobleaching of about 70% of the total chlorophyll. As energy transfer is much faster than the intramolecular decay processes with which it competes to depopulate the excitated states the transfer efficiency will be even less sensitive to photobleaching than the transfer rate. We therefore suggest that photobleaching in nature is unlikely to influence photosynthetic activity significantly at the energy-transfer level.

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