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Effect of adaptation to high light intensity on the kinetics of energy transfer from phycobilisomes to photosystem II in *Anabaena cylindrica*

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Abstract. Transfer efficiencies between phycobilisomes and photosystem II antenna chlorophylls were determined on membrane fragments isolated from low and high light adapted *Anabaena* cells. The observed increase in energy transfer in high light adapted cells is a consequence of shorter interchromophore distances and a decrease in the number of jumps of the exciting photons. Calculation of the rates of energy transfer and the coupling energies indicate that the weak interaction inferred for energy transfer between phycobilisome and photosystem II in low light adapted cells is replaced by an intermediate interaction in high light adapted cells.

Key words: Adaptation to light intensity, energy transfer, phycobilisome, coupling energy, migration of photons

Introduction

It is now generally accepted that excitation energy transfer from phycobiliproteins to the antenna chlorophyll in photosystem II (PS II) proceeds by inductive resonance (Dale and Teale 1970; Grabowski and Gantt 1978 a) in blue-green algae grown under physiological conditions. The rate of this kind of energy transfer is proportional to the inverse sixth power of the intermolecular distance and to the overlap of the absorption spectrum of the acceptor and fluorescence spectrum of the donor. Our earlier studies indicated that the light energy absorbed by phycobilisomes (PBS) is transferred to Chl-a with higher efficiency in high light adapted (HLA) compared to low light adapted (LLA) *Anabaena* cells. This increased energy transfer is possibly due to a more compact structural contact between PBS and PS II particles (Laczkó and Kaiseva 1987).

In this paper we report our analyses of the energy transfer mechanism between PBS and PS II Chl-a in low and high light adapted *Anabaena cylindrica* cells. Interaction energies on the PBS-Chl level were determined to decide what kind of mechanism applies to energy transfer from PBS to PS II Chl-a. The mean transfer time and the number of jumps of exciting photons in PBS before capture in the PS II antenna complex were also evaluated.

Materials and methods

Anabaena cylindrica (strain PCC 7122, Pasteur Institute, Paris) LLA and HLA cells were cultivated as described earlier (Laczkó and Barabás 1981).

Intact PBS and PBS-membrane complex were isolated as previously described (Laczkó and Kaiseva 1987). Fluorescence spectra were measured with a Perkin-Elmer spectrofluorimeter (MPF-3). The temperature of the samples was maintained at 25 °C. To avoid errors caused by reabsorption and secondary fluorescence the concentration of the samples were adjusted so that their absorption was 0.1 at the excitation wavelength of 590 nm.

The absolute quantum yield of PBS fluorescence was determined according to Grabowski and Gantt (1978 a) but instead of Rhodamine B, cresyl violet was used as a standard.

Results and discussion

The efficiency of energy transfer from PBS to PS II Chl-a in LLA and HLA *Anabaena cylindrica* was determined from the fluorescence spectra of the donor (PBS) in intact membrane fragments (when the acceptor Chl is present) and from that of the isolated PBS

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Abbreviations: LLA, low light adapted; HLA, high light adapted; PBS, phycobilisome; PS, photosystem

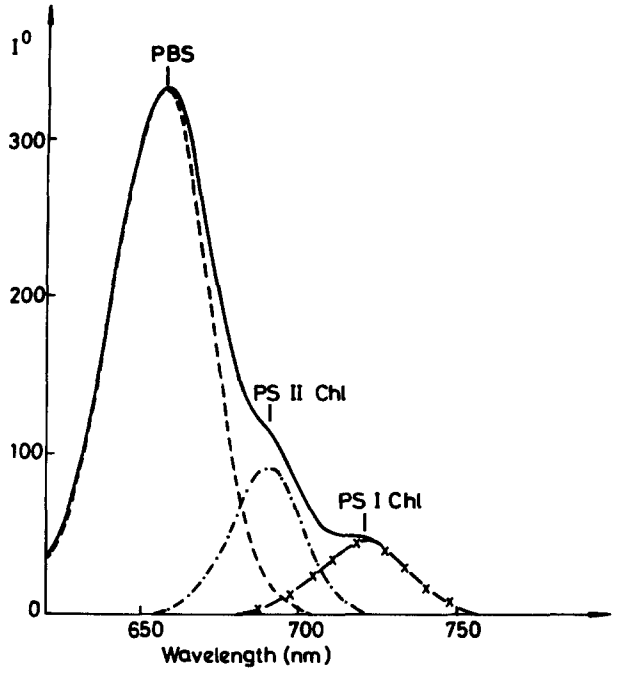

Fig. 1. Deconvoluted fluorescence spectrum of the intact (in 0.75 *M* phosphate) membrane fragments from LLA *Anabaena* cells. Excitation 590 nm

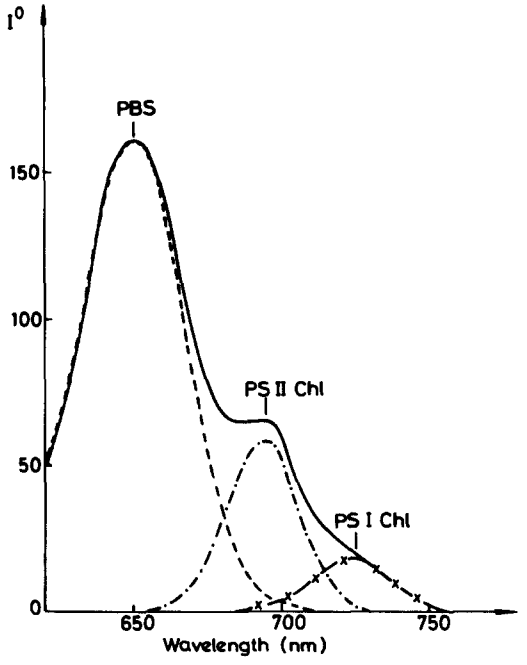

Fig. 2. Deconvoluted fluorescence spectrum of the intact (in 0.75 *M* phosphate) membrane fragments from HLA *Anabaena* cells. Excitation 590 nm

(without acceptor). The fluorescence spectra of the intact (in 0.75 *M* phosphate) membrane fragments were deconvoluted into the individual Gaussian curves of PBS, PSII and PSI Chl as Figs. 1 and 2 show. The characteristic parameters of these curves are given in

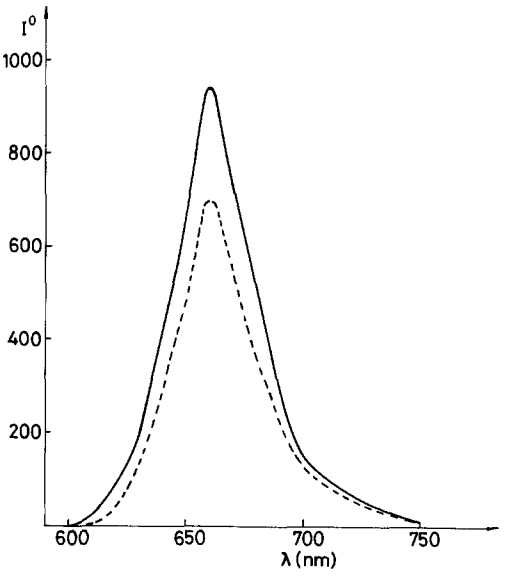

Fig. 3. Fluorescence spectra of intact PBS (in 0.75 *M* phosphate) isolated from LLA (——) and HLA (– – – –) *Anabaena* cells. Excitation 590 nm

Table 1. Characteristic parameters (maximum relative fluorescence intensity I_0 measured at λ_0; half band width, FWHM) of the deconvoluted fluorescence spectra of intact *Anabaena* membrane fragments in 0.75 *M* K-phosphate buffer (pH 6.8). Excitation at 590 nm

Pigments	LLA			HLA		
	λ_0 (nm)	I_0	FWHM (nm)	λ_0 (nm)	I_0	FWHM (nm)
PBS	657	84.2	36	651	40.8	42
PS II Chl	691	23.5	26	695	14.7	28
PS I Chl	721	11.6	32	724	4.4	29

Table 1. Fluorescence spectra of intact PBS (in 0.75 *M* phosphate) isolated from LLA and HLA cells are shown in Fig. 3. The transfer efficiencies were calculated by the equation:

$$E = 1 - \frac{F_D^A}{F_D}, \tag{1}$$

where F_D^A and F_D are the fluorescence of the donor (PBS) in the presence and absence of the acceptor (Chl in PS II), respectively. The amount of phycobiliprotein was the same in all preparations.

The transfer efficiencies are given in Table 2. It can be seen that energy transfer from PBS to PS II Chl-a is more effective in HLA cells than in LLA cells. It is known that the migration of excitation energy from PBS to Chl-a proceeds by inductive resonance in cells grown under physiological conditions (Dale and Teale 1970; Grabowski and Gantt 1978a). We wanted to

Table 2. Experimental parameters for PBS→PSII Chl energy transfer in intact LLA and HLA *Anabaena* membrane fragments. E, efficiency of energy transfer; $|\mu_{PBS}|$ and $|\mu_{Chl}|$ electric transition dipole moments for PBS and Chl, respectively; $J(\lambda)$, spectral overlap between PBS fluorescence and Chl absorption spectra; ϕ_D, the absolute quantum yield of the PBS fluorescence

Membrane fragments	E	$\lvert\mu_{PBS}\rvert$ (D)	$\lvert\mu_{Chl}\rvert$ (D)	$J(\lambda)$	ϕ_D
LLA	0.71	7.54	4.88	9.72×10^{-13}	0.65
HLA	0.98	9.49	4.88	9.44×10^{-13}	0.64

Table 3. Calculated parameters for PBS→PSII Chl energy transfer in intact LLA and HLA *Anabaena* membrane fragments. R_0, the Förster critical distance; R, the distance between two chromophores; U, coupling energy; k, rate of energy transfer; t_T, mean time of energy transfer; τ_j, jump time; n, number of jumps

Membrane fragments	R_0 (Å)	R (Å)	U (cm^{-1})	k (s^{-1})	t_T (s)	τ_j (s)	n
LLA	58.5	50.4	0.48	2.5×10^{8}	4.17×10^{-9}	1.12×10^{-11}	372
HLA	58.2	27.9	3.53	2.7×10^{12}	1.3×10^{10}	1.5×10^{-12}	87

know whether the more efficient energy transfer in cells adapted to high light intensity is associated with different kinetics. To decide what kind of mechanism applies to energy migration in HLA cells we first evaluated the PBS-Chl interaction energy. In the case of weak interaction the value of this energy is as low as $1\,cm^{-1}$ or less, the rates of energy transfer are in the range $10^6-10^{11}\,s^{-1}$ and are proportional to the inverse sixth power of the intermolecular distance ($k\sim R^{-6}$) (Borisov and Godik 1973). In the case of intermediate and strong interaction the coupling energy is higher than $1\,cm^{-1}$, the rate constants are in the range $10^{11}-10^{13}\,s^{-1}$ and $\geqq10^{15}\,s^{-1}$, respectively and are proportional to the inverse third power of the intermolecular distance ($k\sim R^{-3}$). The interaction energy in the electric dipole-dipole approximation was calculated from the equation (Van Grondelle 1985):

$$U=\frac{\varkappa\,|\mu_{PBS}|\,|\mu_{Chl}|}{n^2\,R^3}, \tag{2}$$

where $\varkappa$ is a factor which depends on the mutual orientation of donor and acceptor molecules. Assuming a random chromophore orientation the value of $\sqrt{2/3}$ for $\varkappa$ was used. The refractive index for PBS is $n=1.576$ (Grabowski and Gantt 1978b). The electric transition dipole moments $|\mu_{PBS}|$ and $|\mu_{Chl}|$ for the longer wavelength electronic transition were calculated from the absorption spectra of PBS and chlorophyll and expressed in Debye units (D) (Moscowitz 1960). The donor-acceptor separation, R was calculated from the equation:

$$R=R_0\left(\frac{1}{E}-1\right)^{1/6}. \tag{3}$$

The Förster critical distance, R_0, is given by the formula:

$$R_0^6=8.785\times10^{-25}\,\varkappa^2\,\phi_D\,n^{-4}\,J(\lambda), \tag{4}$$

where ϕ_D is the absolute quantum yield of the donor (PBS) fluorescence in the absence of the acceptor. We found that ϕ_D was not significantly different in LLA and HLA membrane fragments and was in the range 0.63–0.66. The value of 2/3 for $\varkappa^2$ (random orientation of chromophores) was used both in LLA and HLA fragments. Although a more ordered orientation is assumed in HLA cells the error caused in R is less than 15%. $J(\lambda)$ (given in Table 2) is the spectral overlap between PBS fluorescence and Chl absorption spectra in intact membrane fragments. The values obtained for R and U are given in Table 3. It can be seen that the interaction energy is less than $1\,cm^{-1}$ in LLA fragments, and the energy transfer from PBS to Chl-a proceeds by weak interaction in accordance with earlier findings (Grabowski and Gantt 1978b). However, in the case of HLA cells this value is higher than $1\,cm^{-1}$, indicating that energy transfer occurs either by intermediate or strong coupling. It is possible to distinguish between the two mechanisms by calculating the rate constants. Substituting the interaction energy obtained for HLA fragments into the equation valid for strong coupling (Borisov and Godik 1973) we get:

$$k_{si}=\frac{4U}{h/2\pi}\sim10^{12}\,s^{-1}. \tag{5}$$

Hence the energy transfer cannot occur by strong interaction, and intermediate coupling energy transfer can be assumed. The value of $\sim10^{12}\,s^{-1}$ obtained gives the upper limit of the transfer rate in HLA fragments. The true value is probably lower, because the transfer rate with intermediate interaction is proportional to an additional factor, $S_{VV'}$ (the Franck-Condon integral of the vibrational functions of excited and unexcited molecules), whose value falls in the range 0–1. In intermediate intermolecular coupling, excitation cannot be considered as localized at the individual molecules but is partly delocalized over the molecular aggregates and energy is transferred by vibrational excitons.

The mean transfer time (t_T) for PBS to PSII Chl energy transfer was calculated according to Grabowski and Gantt (1978b). Actually this is the time which is needed for the exciton to be transferred from the

external layer of PBS to Chl-a upon excitation by 590 nm light. The mean number of jumps of the exciton in PBS during this time is the ratio of the mean transfer time and the jump time, τ_j. According to the uncertainty principle, τ_j are related to intermolecular coupling energies in the following manner (Borisov and Godik 1973):

$$\tau_j = \frac{h/2\pi}{U}. \quad (6)$$

The mean transfer time, the jump time and the number of jumps are given in Table 3. It can be seen that the mean transfer time is much shorter in the HLA than in LLA fragments owing to the shorter jump time and the decreased number of jumps. The shorter jump time can be explained by the shorter distance (R in Table 3) between the bilin chromophores and chlorophyll-a. The data of Table 3 show that the interchromophore distance of 50.4 Å calculated for the case of LLA cells falls to 27.9 Å in the case of HLA cells. A possible reason for the decreased number of jumps is that approximately half of the phycobiliprotein and chlorophyll are decomposed in HLA *Anabaena* cells (Laczkó and Barabás 1981).

The decreased number of jumps and the decreased interchromophore distances result in a shorter energy transfer path in HLA cells compared to LLA cells. Similar results were reported by Mimuro et al. (1985) who found that the change in size of the phycocyanin compartment or the path lengths of energy transfer can be the reason for the differences in energy transfer efficiencies. The position, orientation, distance and environment of the chromophores and hence the efficiency of energy transfer are determined by the secondary and tertiary structure and aggregation state of the polypeptides (Mimuro et al. 1986; Zuber 1986). We previously found that the phycobiliproteins form higher aggregates in PBS, and the structural contact between PBS and PS II particles is tighter in HLA cells than in LLA cells (Laczkó and Kaiseva 1987). It is possible that these structural changes of chromophore polypeptides are the consequence of carotenoid (especially xanthophyll) incorporation into the light harvesting assembly of PS II in HLA cells (Laczkó 1985). The structural changes of the polypeptide moiety of the chromophores result in a less meandering and more efficient energy transfer from PBS chromophores to PS II antenna chlorophyll in HLA *Anabaena* cells. The weak interaction inferred for energy transfer between PBS and PS II in LLA cells is replaced by the intermediate coupling in the HLA cells, giving the observed higher efficiency of energy transfer.

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