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EXCITATION ENERGY TRANSFER BETWEEN PIGMENT SYSTEM II UNITS IN BLUE-GREEN ALGAE

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Summary

Efficiency in excitation energy transfer from closed to open reaction center II in blue-green and red algae was estimated by the method developed by Joliot and Joliot (C.R. Acad. Sci. (1964) 258, 4622—4625) after slight modification; the number of open reaction centers II was counted from the mean O_2 yield of repetitive short flashes.

The efficiency in energy transfer in *Chlorella pyrenoidosa* was the same in our measurement as that reported by Joliot and Joliot (0.55 ± 0.02). However, the values obtained with four blue-green algae and one red alga were very small, in a range of 0.00—0.07. The low efficiency was always obtained independently of the size of the apparent photosynthetic unit which was varied by growth conditions. Results indicated that pigment system II forms a unit in which only one reaction center II is operative.

Introduction

Photosynthetic pigment system of blue-green and red algae consists of chlorophyll *a*, phycobilins and carotenoids. Phycobilins act as a major component of pigment system II. However, their location in the photosynthetic organelles is different from that of chlorophyll *a* and carotenoids. Phycobilins are bound to hydrophilic proteins. Electron microscopic analyses have revealed that phycobiliproteins in a highly associated form are located on the surface of thylakoids; each unit of the protein associates takes a granular structure (phycobilisome) and is arranged at a regular distance (40—50 nm) on the thylakoid surface [1].

We found that the size of one phycobilisome is almost equal to the phyco-

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

bilin size per one reaction center II [2]. This suggests that together with chlorophyll a in thylakoids, each phycobilisome forms a unit of pigment system II. In blue-green and red algae, the amount of pigment system II chlorophyll a is very small (approx. 10% of total chlorophyll a in *Anabaena variabilis* [2] 16% of *Anacystis nidulans* [3] and approx. 5% of *Porphyridium cruentum* [4]). Our estimate indicated that in *A. variabilis*, only 20 molecules of chlorophyll a function in one pigment system II unit [2]. Pigment system II chlorophyll a is probably located only at the thylakoid locus where phycobilisome resides. These features suggest that in blue-green and red algae, pigment system II units are isolated in the excitation energy transfer between them. To examine this possibility, we tried to estimate the efficiency in excitation energy transfer between pigment system II units in blue-green and red algae. Results reported here indicate that pigment system II units in these algae show a typical separate package nature in the energy transfer between them.

Materials and Methods

Algal culture. Algal strains used in the present experiments were originally supplied from the Algal Collection at the Institute of Applied Microbiology, University of Tokyo [5]. Cells were grown autotrophically under continuous illumination of incandescent light at 26°C. Air containing 0.5% CO_2 was continuously supplied. Algal strains, growth conditions and specific growth rates are summarized in Table I. *Anabaena cylindrica* was grown under N_2-fixing conditions with modified Detmer's medium [5] free from KNO_3, and *P. cruentum* was grown in artificial seawater medium of Provasoli [6] enriched with 2-fold $NaNO_3$ and 5-fold K_2HPO_4. Cells at the late log-growth phase were used in all experiments.

Polarographic measurements. Cells washed with a fresh culture medium were suspended in the fresh medium at a concentration equivalent to 3 μg chlorophyll a/ml. In our experimental procedure described below, this algal concen-

TABLE I

CONDITIONS FOR AND SPECIFIC GROWTH RATES OF ALGAL GROWTH

For experimental details, see Materials and Methods.

Species	Medium	Light intensity (lux)	Specific growth rate (day^{-1})
Chlorella pyrenoidosa	Myers' 1 N medium	3000	0.66
Anabaena cylindrica	Modified Detmer's medium minus KNO_3	1500 6000	0.38 0.80
Anabaena variabilis	Modified Detmer's medium	1500 6000	0.43 0.76
Anacystis nidulans	Modified Detmer's medium	1500 6000	0.58 0.90
Plectonema boryanum	Modified Detmer's medium	1500	0.54
Porphyridium cruentum	Enriched artificial sea water medium of Provasoli *	1500	0.37

* cf. ref. 6.

tration gave 40—50% (in most cases, 42—45%) light absorption at the wavelength for phycobilin peak. Until measurements were taken cell suspensions were kept under the same light conditions as the algal culture.

O_2 evolution was measured by a Teflon-covered oxygen electrode (YSI 4004) in a closed cylindrical vessel (20 mm height and 12 mm diameter; total volume, 2 ml) with two light sources at 26°C. The one was xenon short flash. Flash source was a Sugawara Storoboscope MS-230. The flash duration was 4.4 μs at 1/2. An ultraviolet-cut filter (Toshiba UV-39) was inserted between the electrode vessel and the flash source. Heat irradiance was cut by a lucite block (10 mm thickness). The other was an I_2-W lamp light (Ushio 5V 150W E.H.). Heat irradiance was removed by an infrared cut filter and a lucite block (10 mm thickness). The light intensity was varied by a neutral density filter (Hoya Glass ND series). The flash source gave an intensity of at least $3.8 \cdot 10^3$ erg/cm^2 per flash at the surface of the electrode vessel; an actual intensity must be higher than the above value because we set a concave mirror on the opposite side of the flash source so as to give focusing of the reflecting light at the electrode vessel. This flash intensity gave an estimation that far more than 95% of targets (reaction centers I and II) can be hit by each flash, even when we count only the light absorbed by phycobilins which are present in vivo as a phycobilisome unit (30 nm in diameter, cf. ref. 1). The estimate was experimentally proved by the relationship between O_2 flash yield and flash intensity (Kawamura, M., Mimuro, M. and Fujita, Y., in preparation).

Maximum O_2 flash yield was obtained for *Chlorella pyrenoidosa* at a repetition rate as high as 20 Hz in our measuring system. However, we found that the maximum O_2 flash yield was obtained only at a repetition rate as low as 10 Hz in all blue-green and red algae, and thus, we applied repetitive flashes at 5 Hz to all measurements. Details of conditions for our repetitive flash experiments will be reported elsewhere (Kawamura, M., Mimuro, M. and Fujita, Y., in preparation).

Cells placed in the vessel were kept in the dark for 7 min for the dark standardization and the temperature equilibration. After that, repetitive flashes for 3 min and continuous light for 3 min were alternately given with a 5 min dark interval.

The efficiency (p) in excitation energy transfer from closed to open reaction center II can be obtained from Joliot's equation between a steady-state and light-limiting photosynthesis (V) and a fraction of open reaction center II (e), $V/V_{max} = e/1 - p(1 - e)$ where V_{max} is the rate of steady state photosynthesis under maximum number of open reaction centers II [7]. Joliot, Joliot and Kok [8] reported that the dark reaction between two reaction centers in spinach chloroplasts and *Chlorella* cells has a low apparent equilibrium constant (K). Thus, the O_2 flash yield does not necessarily count the total number of reaction centers II. In the experiments of Joliot and Joliot [7] and Joliot [9], the fraction of open reaction centers II was estimated from the O_2 yield of a single short flash superimposed on a weak continuous light, and thus, the number of open reaction centers II under continuous illumination can be equal to that under the flash illumination. Our method consists of alternate measurements of O_2 evolution under continuous and repetitive flash illumination. Thus, the fraction of open reaction centers II is not necessarily equal under the two,

steady-state and quasi-steady state, conditions. The white light used for the continuous illumination had the color temperature of 3400 K; the energy distribution in a visible wavelength region indicates that photons absorbed by phycobilins and transferred to pigment system II chlorophyll *a* are a little larger (1.1—1.4 times) than those absorbed by pigment system I chlorophyll *a*. The light quanta must be equally distributed to pigment systems I and II, so that the fraction of open reaction centers II under the continuous illumination can approximate that under the repetitive short flashes.

Chlorophyll content was determined with acetone extracts with use of the absorption coefficient of Mackinney [10].

Results and Discussion

Fig. 1 shows relative rates of steady-state photosynthesis in *C. pyrenoidosa* as a function of relative number of open reaction centers II. A varied proportion of open reaction centers II was set by addition of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) at various concentrations, and expressed as a value relative to that without DCMU addition. The pattern is identical to that reported by Joliot and Joliot [7] and Joliot [9]. The *p* calculated by the least-square method is 0.55 ± 0.02, in good agreement with the value reported by Joliot and Joliot [7]. Our method is valid for the experiment for green algal system.

The relationship between V/V_{max} and *e* in *A. nidulans* was very different. As shown in Fig. 2, V/V_{max} values are almost equal to *e* values in a wide range of DCMU concentration. The transfer efficiencies calculated from Fig. 2 are 0.07 ± 0.03 in weak light cells and 0.02 ± 0.03 in strong light cells. Kinetics of fluorescence induction in *A. nidulans* appears monomolecular (cf. ref. 11). According to Murata (Murata, N., personal communication), the *p* estimated from the kinetics is less than 0.1, which agrees with our results. Similar low efficiency in energy transfer was also obtained with three other blue-green algae (Table II). The values are less than 0.10 in all cases. Though the values
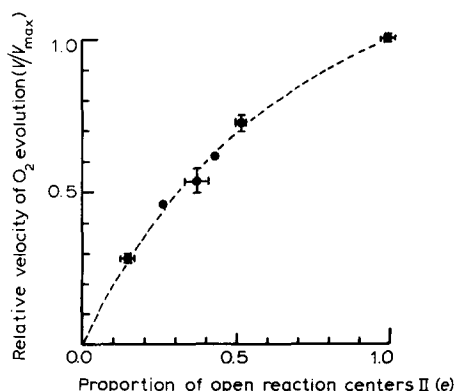

Fig. 1. Relative velocity of steady state O_2 evolution (V/V_{max}) under light-limiting conditions as a function of relative number of open reaction centers II (*e*) in *Chlorella pyrenoidosa* grown at 3000 lux. For experimental details, see text. Broken line shows a theoretical curve for *p* = 0.55.
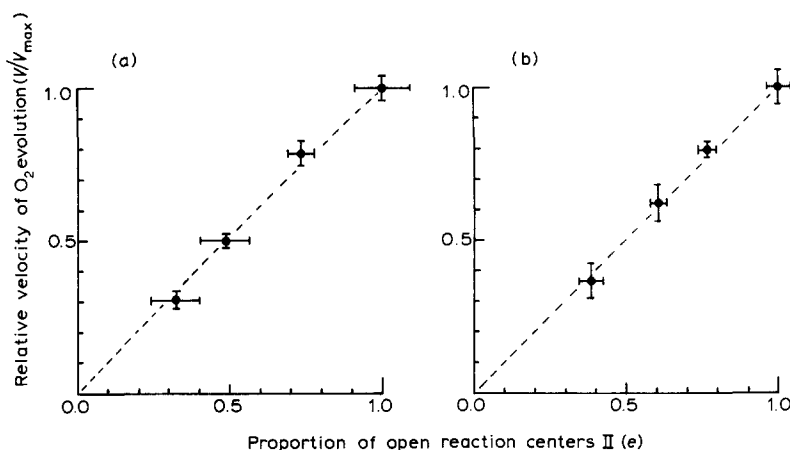
Fig. 2. Relative velocity of steady state O_2 evolution (V/V_{max}) under light-limiting conditions as a function of relative number of open reaction centers II (e) in *Anacystis nidulans* grown at 1500 (a) and 6000 lux (b). For experimental details, see text. Broken lines showed theoretical curves for $p = 0.07$ (a) and 0.02 (b), the most probable p values estimated by the least-square method.

were somewhat variable, they clearly indicate that energy transfer from closed to open reaction centers II hardly occurs in blue-green algae. A low efficiency was again obtained with the red alga *P. cruentum* (Table II) suggesting that this feature is not limited to the system in blue-green algae but a general character of pigment system II in blue-green and red algae.

Apparent photosynthetic unit (phycobilins plus chlorophyll a/O_2) in blue-green algae is markedly variable depending on the light conditions for algal growth (Kawamura, M., Mimuro, M. and Fujita, Y., in preparation); it is smaller in cells grown under strong light (cf. Table II) similarly to the case of *C. pyrenoidosa* reported by Myers and Graham [12]. This variation may be

TABLE II

TRANSFER EFFICIENCY (p) OF EXCITATION ENERGY BETWEEN PIGMENT SYSTEM II UNITS

Values are calculated from the equation of $V/V_{max} = e/1-p(1-e)$. In a, are values for grown under weak light (1500 lux) except for *C. pyrenoidosa* (3000 lux). In b, are values for cells grown under strong light (6000 lux). Values were obtained from 6 measurements. For experimental details, see text.

Species	Transfer efficiency (p)	Apparent photosynthetic unit (phycobilins plus chlorophyll a/O_2 per flash)
(a)		
Chlorella pyrenoidosa	0.55 ± 0.02	—
Anacystis nidulans	0.07 ± 0.03	2240 ± 300
Anabaena cylindrica	0.07 ± 0.02	2200 ± 210
Anabaena variabilis	0.00 ± 0.01	1910 ± 190
Plectonema boryanum	0.00 ± 0.03	2420 ± 160
Porphyridium cruentum	0.01 ± 0.04	1930 ± 230
(b)		
Anacystis nidulans	0.02 ± 0.03	1180 ± 120
Anabaena cylindrica	0.00 ± 0.03	1220 ± 80
Anabaena variabilis	0.02 ± 0.01	1100 ± 120

accompanied with a modification in thylakoid architecture including pigment system II array in the thylakoids. As shown in Fig. 2b and Table II, however, no significant difference in p values was observed between cells grown under weak and strong light. Modification of pigment system II array in the thylakoids, if any, may be not so extensive as to cause a variation in energy transfer efficiency between reaction centers II.

The p values less than 0.1 probably mean that (1) each pigment system II unit associates with one reaction center II and (2) the energy transfer between pigment system II units does not occur with a weak coupling mechanism but it depends only on the reabsorption of fluorescence emitted by pigment system II chlorophyll a. An occurrence of uphill energy transfer from pigment system II chlorophyll a to phycobilins [13,14] suggests that phycobilin emission also somewhat contributes to the energy transfer. This separate package nature of pigment system II unit strongly supports our previous view that each reaction center II has a pigment system which consists of one phycobilisome and a small amount of chlorophyll a [2].

Our electron microscopic observation revealed that in *A. cylindrica*, each phycobilisome (mean diameter, 33 nm) was arranged at a mean distance of 53 nm on the thylakoid surface (unpublished data). Together with our findings described above, this geometry gives a tentative model for pigment system II unit array in the thylakoids (Fig. 3). Each pigment system II unit is located at a distance of 50 nm. Pigment system II chlorophyll a (about 20 molecules per one pigment system II unit [2]) is present only at the thylakoid surface for phycobilisome locus. Remaining area of thylakoid may be occupied by pigment system I. Thus, phycobilisome, which was originally defined as a morphological unit, now seems to be a functional unit.

An action spectrum for photosynthesis having a clear chlorophyll a peak was reported with *Phormidium ectocarpi* [15]. In this alga, the amount of pigment system II chlorophyll a must be markedly larger than that in the algae used in our experiments. If so, the excitation energy transfer between reaction centers
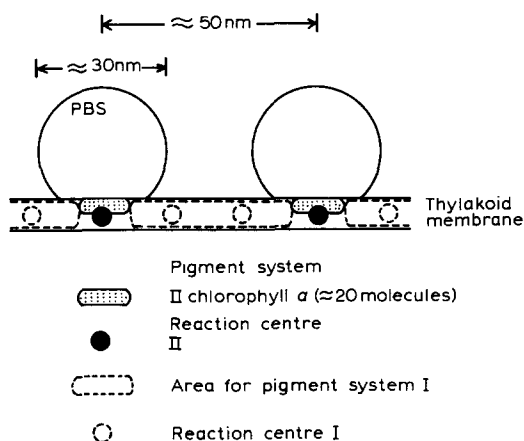

Fig. 3. A tentative model of the pigment system in blue-green algae. For explanation, see text. PBS, phycobilisome.

II may occur with a weak coupling at chlorophyll *a* level. We note that our model for pigment system II in blue-green and red algae is applicable to the system in which only a small fraction of chlorophyll *a* functions in pigment system II.

Acknowledgements

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