Biochimica et Biophysica Acta, 459 (1977) 376–389

BBA 47260

ESTIMATION OF CHLOROPHYLL *a* DISTRIBUTION IN THE PHOTOSYNTHETIC PIGMENT SYSTEMS I AND II OF THE BLUE-GREEN ALGA *ANABAENA VARIABILIS*

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(Received August 23rd, 1976)

SUMMARY

Chlorophyll *a* distribution in pigment systems I and II was estimated with the blue-green alga *Anabaena variabilis* by two methods: first, with intact cells using delayed light emission as an index reaction; second, by measuring the 2,6-dichlorophenolindophenol-Hill reaction and the cytochrome *c* photooxidation in membrane fragments.

The first estimation indicated that 0.053 ± 0.014 of total chlorophyll *a* functions as a component of pigment system II, and the second method, 0.086 ± 0.012. Though the values were somewhat different in the two methods, both estimations indicated that pigment system II chlorophyll *a* occupies a very small fraction of total chlorophyll *a*.

INTRODUCTION

Photosynthetic pigment system of blue-green algae consists of phycobiliproteins, chlorophyll *a* and carotenoids. These pigments form two groups of pigment system as in the case of other green plants; pigment system I for supplying excitation energy to reaction center I and pigment system II for reaction center II [1]. It is well known that phycobiliprotein is a component of pigment system II [2, 3]. Intact cells emit the fluorescence characteristic of pigment system II chlorophyll *a* [4], and membrane fragments free from phycobiliproteins are still highly active in the reaction driven by reaction center II [5, 6]. Thus, some portion of chlorophyll *a* has to function as a component of pigment system II.

Ogawa et al. [7] succeeded in separation of "Photosystem I" particles from *Anabaena variabilis* and reported that 20 % of total chlorophyll *a* functions in pigment system II. Similar results were also obtained by Öquist [8]. These findings suggest that the amount of chlorophyll *a* functioning in pigment system II is markedly small

Abbreviations: DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

when compared with that of pigment system I chlorophyll *a*. However, the detergent treatment involved in their experiments may have caused an erroneous estimation. We tried to estimate chlorophyll *a* distribution in the two pigment systems of *A. variabilis* with use of velocities of the reactions driven by the action of a single reaction center. The index reactions adopted were (i) delayed light emission due to reaction center II [9–11] in intact cells, and (ii) 2,6-dichlorophenolindophenol (DCIP) photoreduction and cytochrome *c* photooxidation in membrane fragments.

MATERIALS AND METHODS

Algal culture. Cells of *Anabaena variabilis* (M-2 IAM Collection, University of Tokyo) were grown autotrophically in the modified Detmer's medium [12] under 2000 lux of incandescent light at 27 °C. The air containing 0.5 % CO_2 was supplied continuously. Cells grown for 4 to 5 days were used for the experiments.

Delayed light emission. Cells were washed once with the fresh culture medium and suspended in the same medium at the concentration giving $A_{678\ nm} = 0.1$ (2 mm optical path). The suspension was kept in the light (incandescent light, 1000 lux) before use.

The delayed light emission was measured by a modified Becquerel-type phosphoroscope set up by Dr. N. Murata [cf. 11]. Time span for one cycle was 2.5 ms (0.9 ms excitation and 1.6 ms darkness). Delayed light emission was measured (0.1 to 0.9 ms after the excitation). The suspension was excited with monochromatic light isolated from a 500 W xenon lamp light by an interference filter (Japan Vacuum Optics; 630 nm, half band-width 6 nm; 676 nm, half band-width 7 nm; 689 nm, half band-width 7 nm) and a Hoya HA-50 IR cutting filter or with colored light isolated by a color filter (Toshiba VR-60) and a Hoya HA-50. The excitation intensity was measured with a Kipp and Sons compensated thermopile. Before measurement, cell suspensions in the light were first kept in the dark for 5 min at room temperature, then transferred to the cuvette placed in a Dewar bottle of a constant temperature. After equilibration of the temperature (2.5 min), the measurement was started. The sample temperature was monitored with a gold-constantan platinum thermocouple.

DCIP photoreduction and cytochrome c photooxidation. Photosynthetic membrane fragments were prepared by the method previously reported [5] after a slight modification. Cells in a medium containing 0.6 M sucrose, 20 mM sodium phosphate (pH 6.5), 20 mM NaCl and 2 mM $MgCl_2$ were disrupted by intermittent sonication. Resulting homogenates were fractionated by differential centrifugation. Membrane fragments were obtained as precipitates of a 144 000 $\times g$ centrifugation (60 min). They were suspended in the preparation medium free of $MgCl_2$ and stored at −25 °C until use.

Both reactions at room temperature (20 °C) were followed spectrophotometrically by a Hitachi 356 spectrophotometer in a two-wavelength mode (600 and 500 nm for DCIP photoreduction, and 550 and 540 nm for cytochrome *c* photooxidation). Samples were placed in a four-side-transparent cuvette (10 mm for measuring beam and 5 mm for actinic beam). Actinic lights were isolated by a Toshiba VR-65 and an interference filter (Japan Vacuum Optics; 676 nm, half band-width 7 nm; 689 nm half band-width 7 nm) from a 500 W xenon lamp light (UXL 500, Ushio Electric Co.). Heat irradiance was cut by a water filter (10 cm thick), a Hoya HA-20

and a lucite block (3 cm thick). The light energy was measured by a silicon blue cell (Sharp Electric Co.) calibrated by a standard thermopile (Kipp and Sons).

The reaction mixture for DCIP photoreduction (1.5 ml) contained membrane fragments equivalent to 5.4 μg chlorophyll *a*, 75 nmol diphenylcarbazide, 30 μmol sodium phosphate (pH 6.5), 30 μmol NaCl and 900 μmol sucrose. When necessary, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU, 150 nmol) was added. The DCMU-sensitive reaction was adopted as the one induced by reaction center II action. The extinction coefficient of DCIP at pH 6.5 (600 vs. 500 nm) was calculated from the value, $\varepsilon_{600} = 16.5\ mM^{-1}\ cm^{-1}$ [13].

The reaction mixture for cytochrome *c* photooxidation (1.6 ml) contained membrane fragments equivalent to 5.4 μg chlorophyll *a*, 150 nmol horse heart cytochrome *c* (Sigma Chemical Co., Type III), 150 nmol methylviologen, 15 μmol sodium ascorbate, 30 μmol Tris · HCl buffer (pH 7.6), 30 μmol NaCl and 900 μmol sucrose. The kinetics of the photooxidation were the first order type in the presence of an excess ascorbate. The reaction rate was calculated under the assumption that the kinetics consist of the zero order oxidation and the first order reduction. The extinction coefficient of cytochrome *c* (550 vs. 540 nm) was calculated with use of the value, $\varepsilon_{550\ (red-ox)} = 21.1\ mM^{-1}\ cm^{-1}$ [14].

Chlorophyll *a* content was estimated with the absorption coefficient of Mackinney [15].

Estimation of chlorophyll a distribution in the two pigment systems. When the actinic light is red (longer than 580 nm), the light quanta absorbed by carotenoids become negligible so that the reaction driven by reaction center I or II depends only on the excitation energy of phycobiliproteins and chlorophyll *a*. The energy transfer from phycobiliproteins or chlorophyll *a* to each reaction center can be formulated as shown in Fig. 1. Light quanta absorbed by phycobiliproteins are transferred to pigment systems II and I chlorophyll *a* at the efficiency α and γ, respectively. A part of the quanta (β) is also transferred from pigment system II to pigment system I chlorophyll *a*. Under light limiting conditions, the reactions driven by reaction centers I and, II, respectively, can be written

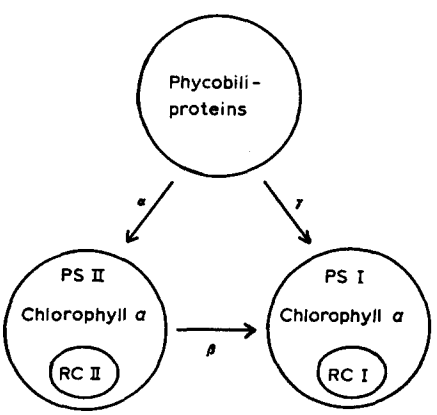

Fig. 1. A model for excitation energy transfer in phycobiliproteins-chlorophyll *a* pigment system. For explanations, see text.

$$V_{\mathrm{I}} = \Phi_{\mathrm{I}} I a_{\mathrm{I}} \tag{1}$$

and

$$V_{\mathrm{II}} = \Phi_{\mathrm{II}} I a_{\mathrm{II}} \tag{2}$$

where V_{I} and V_{II} are the velocities of the reactions driven by reaction centers I and II, respectively, and I is the rate of incident quanta, a_{I} and a_{II} the fractions of quanta transferred to reaction centers I and II, respectively, and Φ_{I} and Φ_{II} the reaction yields of quanta transferred to reaction centers I and II, respectively.

According to the model in Fig. 1, a_{I} and a_{II} are expressed by the following equations:

$$a_{\mathrm{I}} = a_{\mathrm{CI}} + \beta(a_{\mathrm{CII}} + \alpha a_{\mathrm{P}}) + \gamma a_{\mathrm{P}} \tag{3}$$

and

$$a_{\mathrm{II}} = (1-\beta)(a_{\mathrm{CII}} + \alpha a_{\mathrm{P}}) \tag{4}$$

where a_{CI}, a_{CII} and a_{P} are the fractions of quanta absorbed by pigment systems I and II chlorophyll *a* and phycobiliproteins, respectively.

1. *Calculation from delayed light emission*

The delayed light emission depends on the excitation of reaction center II. Thus, the reaction can be expressed by Eqns. (2) and (4) as

$$V_{\mathrm{II}} = \Phi_{\mathrm{II}} I (1-\beta)(a_{\mathrm{CII}} + \alpha a_{\mathrm{P}}) \tag{5}.$$

The yield of the quanta absorbed by total chlorophyll *a* (Φ'_{II}) is expressed as

$$\Phi'_{\mathrm{II}} = \Phi_{\mathrm{II}} \frac{a_{\mathrm{CII}}}{a_{\mathrm{C}}} (1-\beta) \tag{6}$$

where a_{C} is the fraction of quanta absorbed by total chlorophyll *a*. Then,

$$V_{\mathrm{II}} = \Phi_{\mathrm{II}} I \alpha (1-\beta) a_{\mathrm{P}} + \Phi'_{\mathrm{II}} I a_{\mathrm{C}} \tag{7}.$$

The absorption spectrum of pigment system II chlorophyll *a* is not exactly identical with that shown by total chlorophyll *a* in vivo. The absorption maximum of the former (around 673 nm, cf. p. 387) is located at a somewhat shorter wavelength than the latter (680 nm). Thus, Φ'_{II} is not unity with actinic light of a different wavelength. For estimation of this deviation under the three monochromatic lights used in this experiment, we used an approximation by considering the pigment system II chlorophyll *a* to show an absorption spectrum identical in its pattern with that of total chlorophyll *a* in vivo but shifted by 7 nm to shorter wavelength. The rates of change in the light absorption due to the 7 nm-shift were calculated as 0.98, 0.98 and 0.69 under 630, 676 and 689 nm lights, respectively.

Thus, Φ'_{II} of 630 nm light is equal to that of 676 nm light but larger than that of 689 nm light by a factor of 1.42.

Measurements of delayed light emission under two monochromatic lights give the following relationship:

$$\frac{V_{\mathrm{II}\lambda_1}}{V_{\mathrm{II}\lambda_2}}(=R_V)=\frac{I_{\lambda_1}\alpha a_{\mathrm{P}\lambda_1}(1-\beta_{\lambda_1})+\dfrac{\Phi'_{\mathrm{II}\lambda_1}}{\Phi_{\mathrm{II}}}I_{\lambda_1}a_{\mathrm{C}\lambda_1}}{I_{\lambda_2}\alpha a_{\mathrm{P}\lambda_2}(1-\beta_{\lambda_2})+\dfrac{\Phi'_{\mathrm{II}\lambda_2}}{\Phi_{\mathrm{II}}}I_{\lambda_2}a_{\mathrm{C}\lambda_2}}, \tag{8}$$

so that

$$\frac{\Phi'_{\mathrm{II}\lambda_1}}{\Phi_{\mathrm{II}}}=\alpha\frac{I_{\lambda_1}a_{\mathrm{P}\lambda_1}(1-\beta_{\lambda_1})-R_VI_{\lambda_2}a_{\mathrm{P}\lambda_2}(1-\beta_{\lambda_2})}{\dfrac{\Phi'_{\mathrm{II}\lambda_2}}{\Phi'_{\mathrm{II}\lambda_1}}R_VI_{\lambda_2}a_{\mathrm{C}\lambda_2}-I_{\lambda_1}a_{\mathrm{C}\lambda_1}} \tag{9}$$

and

$$\frac{\Phi'_{\mathrm{II}\lambda_2}}{\Phi_{\mathrm{II}}}=\alpha\frac{I_{\lambda_1}a_{\mathrm{P}\lambda_1}(1-\beta_{\lambda_1})-R_VI_{\lambda_2}a_{\mathrm{P}\lambda_2}(1-\beta_{\lambda_2})}{R_VI_{\lambda_2}a_{\mathrm{C}\lambda_2}-\dfrac{\Phi'_{\mathrm{II}\lambda_1}}{\Phi'_{\mathrm{II}\lambda_2}}I_{\lambda_1}a_{\mathrm{C}\lambda_1}} \tag{10}.$$

Except for α, β_{λ_1} and β_{λ_2}, we can obtain experimentally all the values on the right side of these equations. For α, we can use the efficiency of excitation energy transfer from phycobiliproteins to chlorophyll *a* estimated with the fluorescence yield by Duysens (0.80, ref. 16) or by Tomita and Rabinowitch (0.86, ref. 17); in the present work we used the latter value, 0.86. According to Murata [18] and Bonaventura and Myers [19], β value depends on the state of pigment system. Under excitation of pigment system II pigments, β is largest (State II) and, under pigment system I excitation, it becomes smallest (State I). In our experiments for delayed light emission, however, the excitation time is too short (within 3 s) for the shift from the dark-adapted state to state I or II [cf. 18]. Thus, the difference in the values of β_{λ_1} and β_{λ_2} is expected to be so small that $(1-\beta_{\lambda_1})$ and $(1-\beta_{\lambda_2})$ are considered equal. Thus, Eqns. (9) and (10) can be written as

$$\frac{\Phi'_{\mathrm{II}\lambda_1}}{\Phi_{\mathrm{II}}}=\alpha(1-\beta)\frac{I_{\lambda_1}a_{\mathrm{P}\lambda_1}-R_VI_{\lambda_2}a_{\mathrm{P}\lambda_2}}{\dfrac{\Phi'_{\mathrm{II}\lambda_2}}{\Phi'_{\mathrm{II}\lambda_1}}R_VI_{\lambda_2}a_{\mathrm{C}\lambda_2}-I_{\lambda_1}a_{\mathrm{C}\lambda_1}} \tag{11}$$

and

$$\frac{\Phi'_{\mathrm{II}\lambda_2}}{\Phi_{\mathrm{II}}}=\alpha(1-\beta)\frac{I_{\lambda_1}a_{\mathrm{P}\lambda_1}-R_VI_{\lambda_2}a_{\mathrm{P}\lambda_2}}{R_VI_{\lambda_2}a_{\mathrm{C}\lambda_2}-\dfrac{\Phi'_{\mathrm{II}\lambda_1}}{\Phi'_{\mathrm{II}\lambda_2}}I_{\lambda_1}a_{\mathrm{C}\lambda_1}} \tag{12}.$$

From Eqn. (6):

$$\frac{a_{\mathrm{CII}\lambda_1}}{a_{\mathrm{C}\lambda_1}}=\alpha\frac{I_{\lambda_1}a_{\mathrm{P}\lambda_1}-R_VI_{\lambda_2}a_{\mathrm{P}\lambda_2}}{\dfrac{\Phi'_{\mathrm{II}\lambda_2}}{\Phi'_{\mathrm{II}\lambda_1}}R_VI_{\lambda_2}a_{\mathrm{C}\lambda_2}-I_{\lambda_1}a_{\mathrm{C}\lambda_1}} \tag{13}$$

and

$$\frac{a_{\mathrm{CII}\lambda_2}}{a_{\mathrm{C}\lambda_2}} = \alpha \frac{I_{\lambda_1} a_{\mathrm{P}\lambda_1} - R_V I_{\lambda_2} a_{\mathrm{P}\lambda_2}}{R_V I_{\lambda_2} a_{\mathrm{C}\lambda_2} - \frac{\Phi'_{\mathrm{II}\lambda_1}}{\Phi'_{\mathrm{II}\lambda_2}} I_{\lambda_1} a_{\mathrm{C}\lambda_1}} \tag{14}$$

which allow the calculation of $a_{\mathrm{CII}\lambda}$. For calculation of the chlorophyll *a* ratio, $a_{\mathrm{CII}\lambda}$ was corrected for normalization of the absorption spectrum of pigment system II chlorophyll *a* to that of total chlorophyll *a* ($a_{\mathrm{CII}\lambda\mathrm{cor}}$); correction factor for $a_{\mathrm{CII}\ 630}$ and $a_{\mathrm{CII}\ 676}$ is 1/0.98, and for $a_{\mathrm{CII}\ 689}$, 1/0.69. The molar ratio (R) of pigment system II chlorophyll *a* to total chlorophyll *a* is obtained by the following equation:

$$R = \frac{\log(1 - a_{\mathrm{CII}\lambda\mathrm{cor}})}{\log(1 - a_{\mathrm{C}\lambda})} \tag{15}.$$

2. *Calculation from oxidation-reduction reaction*

Our membrane fragments had almost completely lost phycobiliproteins during the preparation treatment, and the measurements for each reaction were done with actinic lights preferentially exciting chlorophyll *a* (actinic maxima: longer than 676 nm). Thus, a_{P} in Eqns. (3) and (4) can be approximated to zero, so that

$$V_{\mathrm{I}} = \Phi_{\mathrm{I}} I(a_{\mathrm{CI}} + \beta a_{\mathrm{CII}}) = \Phi_{\mathrm{I}} I\{a_{\mathrm{C}} - (1-\beta) a_{\mathrm{CII}}\} \tag{16}$$

and

$$V_{\mathrm{II}} = \Phi_{\mathrm{II}} I(1-\beta) a_{\mathrm{CII}} \tag{17}$$

According to the generally accepted assumption that one photon drives one electron in the primary photoact, Φ_{I} can be equal to Φ_{II}. Under the same actinic conditions,

$$\frac{V_{\mathrm{II}}}{V_{\mathrm{I}}} = \frac{(1-\beta) a_{\mathrm{CII}}}{a_{\mathrm{C}} - (1-\beta) a_{\mathrm{CII}}} \tag{18}.$$

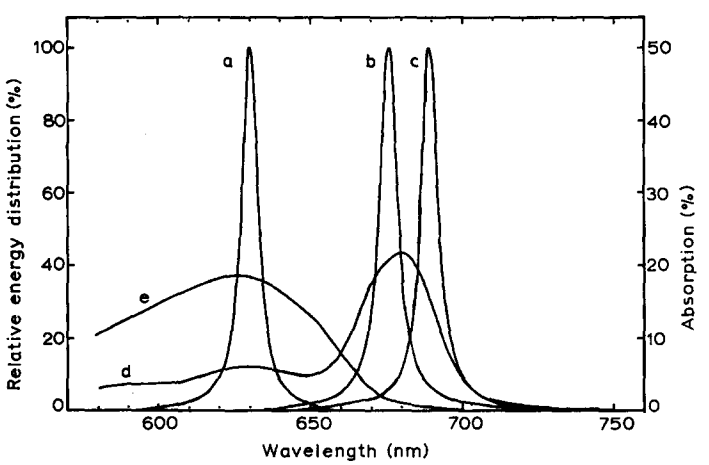

Fig. 2. Energy distribution of actinic lights and percent absorption spectra of washed membrane fragments and phycobilisomes obtained from *Anabaena variabilis*. Curves a-c, energy distribution patterns of 630, 676 and 689 nm lights; d, absorption spectrum of membrane fragments; e, phycobilisomes.

Under chlorophyll *a* excitation (State I), β becomes small enough to be approximated to zero. Thus,

$$\frac{V_{II}}{V_I} = \frac{a_{CII}}{a_C - a_{CII}} \tag{19}$$

which allows calculation of a_{CII}. For calculation of chlorophyll *a* ratio, $a_{CII\lambda cor}$ was used (cf. p. 381).

Estimation of a_C and a_P. The fractions of quanta absorbed by chlorophyll *a* (a_C) and phycobiliproteins (a_P) were estimated from the energy distribution spectrum of the actinic light (Fig. 2, a-c) and absorption spectrum of each component. For a_C, the absorption spectrum of membrane fragments washed four times with buffer were used (Fig. 2d). As the actinic lights used were red, longer than 580 nm, the light absorption by membrane fragments can be assumed to be due to chlorophyll *a* alone. The absorption spectrum of phycobiliproteins was measured with the phycobilin aggregates (phycobilisome) prepared by a density-gradient centrifugation [cf. 20] (Fig.2e).

RESULTS

Experiments with intact cells (delayed light emission)

Typical induction kinetics of delayed light emission are shown in Fig. 3. Upon start of excitation, the emission rapidly increased to an initial rise level (designated as I). After forming a distinct trough, it again increased rapidly to form a peak (P). Then a slow decrease occurred to its final steady state level. This induction occurred only after the dark incubation of the cells at least for 4 min. Within 1 min dark incubation, the initial rise became higher and the rise time longer. The occurrence of a distinct trough between the I and the P and of a rapid initial rise, is somewhat different from the induction kinetics reported with spinach chloroplasts [cf. 21].

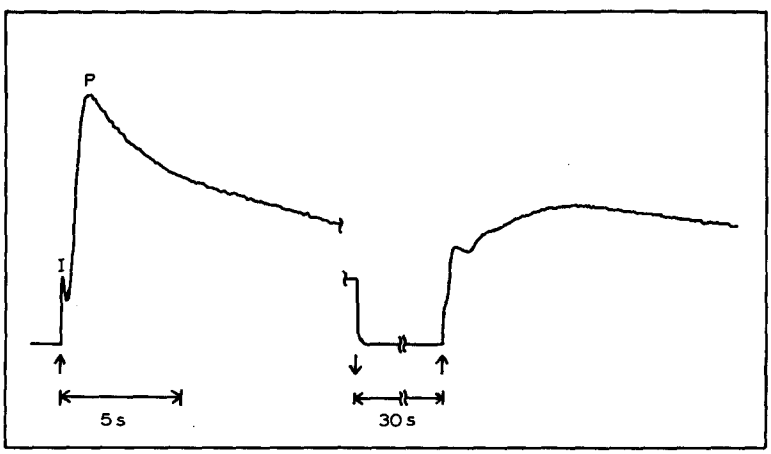

Fig. 3. Induction kinetics of delayed light emission (25 °C) of intact cells of *Anabaena variabilis*. Excitation, longer than 590 nm (5600 erg/cm^2 per s). Upwards arrows, light on; downward arrows, light off. Experimental details, see text.

TABLE I

EFFECTS OF DCMU, GRAMICIDIN J AND REACTION TEMPERATURE ON MAGNITUDES OF I AND P IN INDUCTION KINETICS OF DELAYED LIGHT EMISSION

For experimental procedures, see text.

Conditions	Magnitude (arbitrary units)	
	I	P
Expt. 1		
25 °C	1.00	3.48
25 °C, DCMU, 10^{-7} M	0.17	0.71
25 °C, DCMU, 10^{-6} M	0.04	0.10
Expt. 2		
25 °C	1.00	4.92
25 °C, Gramicidin J, $3 \cdot 10^{-5}$ M	0.89	3.67
25 °C, Gramicidin J, $7 \cdot 10^{-5}$ M	0.75	2.00
Expt. 3		
35 °C	0.73	2.12
25 °C	1.00	2.32
10 °C	2.50	1.19

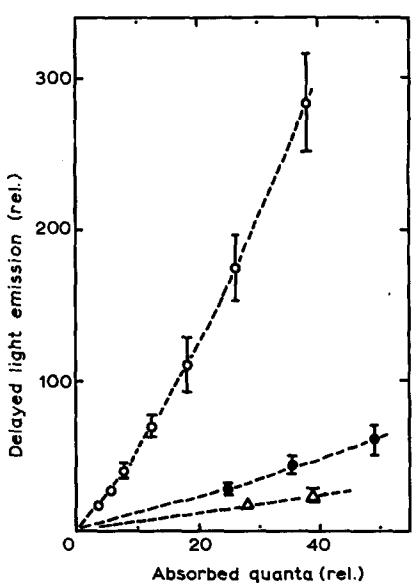

Fig. 4. Magnitude of I component as a function of absorbed quanta under three monochromatic lights. Reaction temperature, 10 °C. (○—○, 630 nm excitation; ●—●, 676 nm; △—△, 689 nm. For experimental details, see text.

TABLE II

RELATIVE QUANTUM YIELDS OF DELAYED LIGHT EMISSION (I COMPONENT) UNDER THREE MONOCHROMATIC EXCITATIONS

Figures were estimated from the data presented in Fig. 2, and expressed as relative to the value for 630 nm excitation. For experimental procedures, see text.

I level (rel.)	Relative quantum yield under monochromatic excitation	
	676 nm	689 nm
1.00	0.22	0.13
1.41	0.23	0.13
1.65	0.22	–
2.35	0.23	–
2.59	0.24	–
3.59	0.23	–
Mean	0.23±0.008	0.13

Effects of added DCMU and gramicidin J, and the reaction temperature on the magnitudes of the I and the P are presented in Table I. The I was sensitive to DCMU, but less to gramicidin J than the P. Lowering the reaction temperature caused an enhancement of the I. On the contrary, the P is temperature-dependent. The induction kinetics and the characters of the I described above were not altered under monochromatic excitation for either phycobiliproteins or chlorophyll *a*. These characteristics were essentially identical with those in spinach chloroplasts [11, 21, 22]. The character of the I indicates that it comes from a transition of the redox state of electron transfer components acting near the reaction center II. According to Itoh and Murata [22], this is formed by a transition of X^-Y^+ to XY (X: primary acceptor of reaction center II, and Y: primary donor). The P is, however, sensitive to gramicidin J and temperature-dependent, indicating that this reflects a high energy state of thylakoid membrane. Thus, the I at low temperature (10 °C) was used as the index for the reaction driven by reaction center II in the following experiments.

Fig. 4 shows the I size as a function of absorbed quanta. The I size did not increase linearly with increase in the absorbed quanta; the emission yield was lower

TABLE III

LIGHT ABSORPTION BY PHYCOBILIPROTEINS AND CHLOROPHYLL *a* UNDER THREE MONOCHROMATIC EXCITATIONS USED FOR EXPERIMENTS OF DELAYED LIGHT EMISSION

Figures are presented as proportions of absorbed quanta to incident quanta. Experimental procedures, see text.

Actinic light (nm)	a_P	a_C
630	0.396	0.162
676	0.067	0.467
689	0.022	0.418

TABLE IV

CALCULATION OF RATIO OF PIGMENT SYSTEM II (PS II) CHLOROPHYLL *a* TO TOTAL CHLOROPHYLL *a* FROM EXPERIMENTAL DATA ON DELAYED LIGHT EMISSION

Figures for λ_1, data obtained from 630 nm excitation; those for λ_2, obtained from 676 or 689 nm excitation. For experimental details, see text

Actinic light (nm)	I size (V) (rel.)	Absorbed quanta (rel.)	$a_{CII\lambda}/a_{C\lambda}$	$a_{C\lambda}$	$a_{CII\lambda}$	$a_{CII\lambda cor}$	Ratio of PS II chlorophyll *a* to total chlorophyll *a*
							$\log(1-a_{CII\lambda cor})/\log(1-a_C)$
λ_1 630	40± 5	7.6	0.052	0.162	0.0084±0.0034	0.0086±0.0035	0.049±0.020
λ_2 676	61±11	49.0	0.052	0.467	0.024 ±0.011	0.024 ±0.011	0.039±0.018
λ_1 630	27± 4	5.6	0.048	0.162	0.0078±0.0012	0.0080±0.0012	0.045±0.007
λ_2 689	24± 4	38.8	0.067	0.418	0.028 ±0.004	0.041 ±0.006	0.077±0.011
Mean							0.053±0.014

under weaker excitation. The same relationship was also observed, when excited by a colored light (a Toshiba VR-66 filter; longer than 650 nm), indicating that this is not due to a monochromatic excitation. To obtain a relative yield, we adopted the reciprocal of quantum number required for the same I size. At least in the tested range of the I size, this treatment gave consistent values for relative yield (Table II). Thus, the I size in this range can hold the relationship with the quanta absorbed by each pigment as expressed by Eqn. (5). Yields for the I were higher with the quanta absorbed by phycobiliproteins than those absorbed by chlorophyll *a*, indicating that a large part of the quanta absorbed by chlorophyll *a* is not effective for reaction center II. Values of a_P and a_C obtained from the experiments with three monochromatic lights are summarized in Table III. When values for 630 and 676 nm excitation were used, a_{CII}/a_C took 0.052 (Table IV). However, it became a little larger in the calculation from data of 630 and 689 nm excitation (0.048 for 630 nm and 0.067 for 689 nm). In the latter case, the energy maximum of 689 nm light was not located at the absorption maximun of the sample, so that the calculation for absorbed quanta may contain a larger error. In Table IV, ratios of pigment system II chlorophyll *a* to total chlorophyll *a* calculated from a_C and a_{CII} in each experiment are summarized. Though the values are somewhat different in each calculation, they indicate that pigment system II contains only a small fraction of the chlorophyll *a*.

Experiments with membrane fragments (oxidation-reduction reactions)

Membrane fragments showed the activity higher than 250 μmol DCIP reduced/mg chlorophyll *a* per h for the DCIP-Hill reaction. The Hill activity of our membrane fragments appeared high enough for the present purpose. However, we measured DCIP-photoreduction in the presence of diphenylcarbazide whic compensates for the inactive water oxidation system as the electron donor.

Under our experimental conditions, the velocities of both DCI hotoreduction and cytochrome *c* photooxidation did not form a linear relationsh. ith the absorbed quanta. The curve of the light-dependency fitted well for a saturation curve against the absorbed quanta (Fig. 5). Thus, we obtain quantum yields of the two

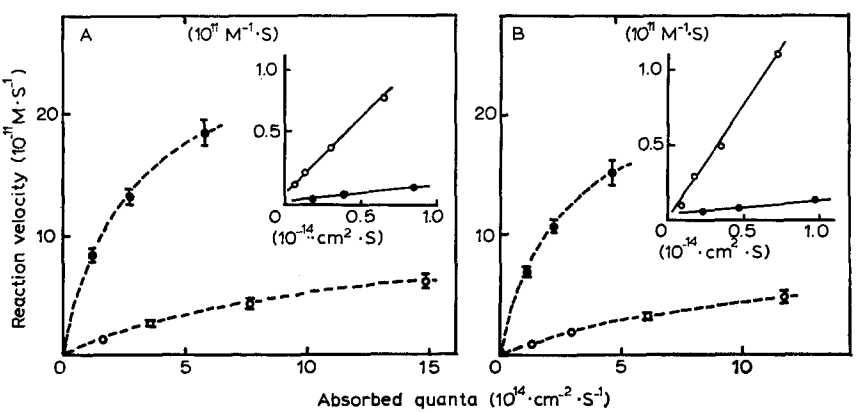

Fig. 5. DCIP photoreduction (○—○) and cytochrome *c* photooxidation (●—●) as a function of absorbed quanta. Chart A, under 676 nm illumination, and chart B, 689 nm illumination. Inserts, double-reciprocal plots. For experimental details, see text.

TABLE V

CALCULATION OF RATIO OF PIGMENT SYSTEM II (PS II) TO TOTAL CHLOROPHYLL *a* FROM EXPERIMENTAL DATA ON OXIDATION-REDUCTION REACTIONS

For experimental details, see text.

Actinic light (nm)	V_{II}/V_I	a_C	a_{CII}	$a_{CII\lambda cor}$	Ratio of PS II to total chlorophyll *a*
					$\log(1-a_{CII\lambda cor})/\log(1-a_C)$
676	0.091±0.008	0.305	0.025±0.003	0.026±0.003	0.070±0.009
689	0.078±0.012	0.265	0.019±0.003	0.028±0.004	0.092±0.014
Mean					0.086±0.012

reactions from a double-reciprocal plot against the absorbed quanta. Though data are not shown, action peaks of DCIP photoreduction and cytochrome *c* photooxidation appeared at 673 and 680 nm, respectively. These fit well for those reactions which are driven by a single action of reaction centers II and I in *Nostoc* membrane fragments [23].

V_{II}/V_I, a_C and a_{CII} under 676 and 689 nm illuminations are summarized in Table V. The a_{CII} values were calculated from a_C and V_{II}/V_I under the assumption that β is approximated to zero. Molar ratio of pigment system II chlorophyll *a* to total chlorophyll *a* thus obtained is larger than that obtained from the experiments for delayed light emission (Table IV vs. V). However, it again indicates that pigment system II chlorophyll *a* is only a small portion of total chlorophyll *a*.

DISCUSSION

The discrepancy in the results obtained from the two types of experiments may be partly due to the approximation of the β value. However, this cannot be the main cause, because only a small variation in β is expected. Another cause may come from the assumption that the yields of the quanta transferred to reaction centers (Φ_I and Φ_{II}) are unity in the two oxidation-reduction reactions in membrane fragments. The added cytochrome *c* may form backward electron flow against the action of *P*-700; cytochrome *c* may be reduced by O_2^- formed at the reducing side of reaction center I [24, 25]. This may not be zero in the presence of methylviologen, so that the apparent quantum yield of reaction center I probably has to be lowered. Under our experimental conditions, therefore, Φ_I may be somewhat smaller than Φ_{II} and may give a higher ratio of pigment system II to pigment system I chlorophyll *a* in the experiments for membrane fragments.

A. variabilis grown under our culture conditions contains *P*-700 at 1 per 140 chlorophyll *a*, and the reaction center II has been assumed to be present at a half number of *P*-700 [26]. The latter relationship was confirmed by the experiments for flash yield of oxygen evolution (M. Kawamura and Y. Fujita, unpublished data). These data allow us to formulate a quantitative constitution of the two pigment systems in *A. variabilis*: 280 molecules of chlorophyll *a* function in one unit; each *P*-700 has 130 light-capturing chlorophyll *a* (pigment system I), and 20 chlorophyll *a*

for one reaction center II (pigment system II). Phycobiliproteins have been generally recognized as components of the pigment system II [2, 3]. Cells grown under our culture conditions contained phycocyanin and allophycocyanin at 0.64 and 0.11 chromophore per chlorophyll *a*. Thus, we can expect that a unit of pigment system II consists of 180 phycocyanin chromophores, 30 allophycocyanin chromophores and 20 chlorophyll *a* molecules. One phycobilisome has been assumed to consist of 7 [27] to 14 [28] hexamers of phycobiliproteins. The chromophore number in a unit of pigment system II in *A. variabilis* corresponds to about 12 hexamers (10 for phycocyanin and 2 for allophycocyanin) indicating that one unit of pigment system II is formed by one phycobilisome and 20 chlorophyll *a* molecules. When 20 chlorophyll *a* are arranged two-dimensionally on the surface of thylakoid membranes, their space occupation is at most 100×100 Å. This is smaller than the thylakoid space occupied by one phycobilisome [cf. 28]. Pigment system II chlorophyll *a* may be located only at the thylakoid locus where phycobilisome resides. This geometrical size also suggests a possibility that some of pigment system I chlorophyll *a* is located at the same locus of thylakoid membrane so that a direct transfer of excitation energy from phycobiliproteins to pigment system I chlorophyll *a* is possible.

The number of chlorophyll *a* molecules in pigment system II obtained from our experiments is smaller than those estimated by Ogawa et al. [7] and Öquist [8]. Their estimations, however, were done with use of chlorophyll *a* content in Photosystem I particles isolated by the method involving detergent treatment. Detergent treatment causes more or less a release of chlorophyll *a* from thylakoid membranes. We consider that their Photosystem I particles had lost some part of chlorophyll *a* so that the fraction of pigment system II chlorophyll *a* became larger.

The in vivo fluorescence at -196 °C of blue-green algae is generally characteristic in that chlorophyll *a* excitation produced far lower emission at 685 and 695 nm due to pigment system II chlorophyll *a* than the emission at 730 nm due to pigment system I chlorophyll *a*. This suggests that a small distribution of chlorophyll *a* in pigment system II is a general character of blue-green algae. Similar characteristics of the in vivo fluorescence are often observed with red algae too [4]. The model proposed for the photosynthetic pigment system in *A. variabilis* may be widely applied to the system consisting of phycobiliproteins and chlorophyll *a*.

ACKNOWLEDGEMENTS

We wish to express our thanks to Dr. N. Murata, Department of Biophysics and Biochemistry, Faculty of Science, University of Tokyo for his help in the measurement of delayed light emission and the stimulation of discussion. This work was partly supported by grants for scientific research from the Ministry of Education, Japan, to Y. F.

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