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ENERGY- AND ELECTRON-TRANSFER SYSTEMS IN ALGAL PHOTOSYNTHESIS

II. OXIDATION–REDUCTION REACTIONS OF TWO CYTOCHROMES AND INTERACTIONS OF TWO PHOTOCHEMICAL SYSTEMS IN RED ALGAE

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SUMMARY

The functioning of cytochromes *f* and *b* in the electron-transfer chain of red algae, Porphyridium and Porphyra, was studied. Effects of inhibitors, temperature, *etc.* on the oxidation–reduction reactions of these cytochromes are shown. The interaction of two photochemical systems through dark electron-transfer in the 10-msec range was demonstrated. The reaction time of electron-transfer reactions was measured with pulsed excitation. Two electron-transfer paths, a non-cyclic path connecting two photochemical systems and a cyclic electron-transfer path around photochemical system I are postulated and a scheme for photosynthetic electron transfer in Porphyridium and Porphyra is presented.

INTRODUCTION

The oxidation–reduction reactions of an *f*-type cytochrome, cytochrome-553, in living cells and isolated particles of Porphyridium and Porphyra, have been shown. Its function as an electron carrier between photochemical reaction systems I and II has been demonstrated[1–3]. Roles of the cytochrome of *b*-type in the light-induced and dark phases of electron transfer were studied and some of the data are presented here. The actions of several inhibitors and physical conditions on different steps of electron transfer were analyzed and a scheme for the electron-transfer system in red algae is presented. The time required for the interaction of two photochemical systems has been studied by WITT and collaborators[4,5]. The direct interaction time was measured here by the use of background illumination and pulsed excitation of photosynthesizing cells. The interaction of two photochemical systems in the time range of 10 msec was demonstrated.

Abbreviations: BIMU, 5-bromo-3-isopropyl-6-methyluracil; CMU, 3(4′-chlorophenyl)-1,1-dimethylurea; CCCP, carbonylcyanide *m*-chlorophenylhydrazone; FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; CCP, carbonylcyanide phenylhydrazone.

METHODS

Five species of red algae were used in this study. Unicellular alga, *Porphyridium cruentum*, was cultured in a medium described by JONES, SPEER AND KURY[6] under illumination with air introduced into a flask or in a slowly rotating flask. Four species of Porphyra were also used and their thalli, single-cell layered, were quite suitable for spectrophotometric measurements. *Porphyra umbilicalis* was collected from the natural environment near Atlantic City, N.J. *Porphyra yezoensis*, *Porphyra tenera* and *Porphyra suborbiculata*, used in the earlier phase of this work, were either cultured in the laboratory or collected from natural surroundings (the bay of Tokyo). The sample was suspended in the sea-water medium of JONES, SPEER AND KURY[6] under a gas phase of air. For spectroscopic studies, a double-beam spectrophotometer and a rapid single-beam spectrophotometer developed by DEVAULT AND CHANCE[7] were used. Continuous and pulsed light intensities were measured by a Reedeer thermopile and a TRG ballistic thermopile, respectively, both calibrated by an irradiance standard lamp (National Bureau of Standards). Chlorophyll *a* content was determined in 80 % acetone extract using specific extinction coefficients of MACKINNEY[8].

RESULTS

Oxidation–reduction reactions of cytochromes of f- and b-types

Fig. 1 shows the illuminated *minus* dark difference spectra of Porphyridium under continuous illumination. The three curves correspond to the total change (steady state), rapid "light-off" phase and slow "light-off" phase. The total change and the rapid change had peaks at 405 mμ, 422 mμ and 553 mμ, and the slow phase

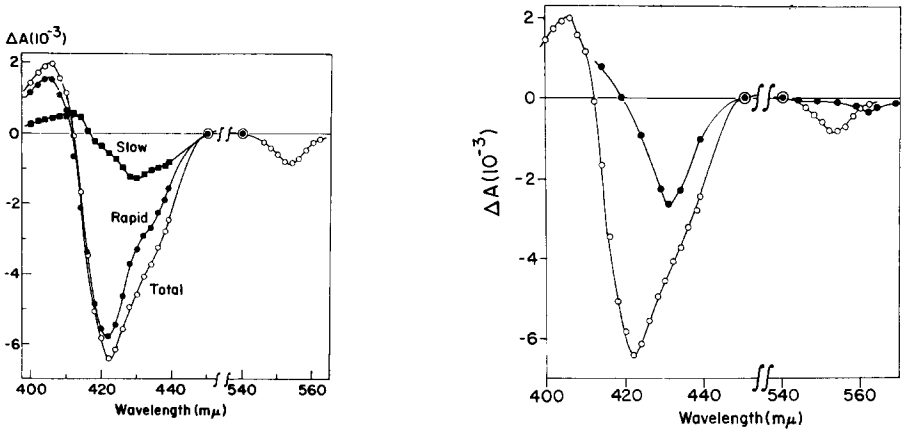

Fig. 1. Light-induced cytochrome oxidation in *Porphyridium cruentum*. Double-beam spectrophotometer recordings (reference wavelengths, 450 mμ and 540 mμ). Excitation light, 690 mμ, $1.81 \cdot 10^{15}$ quanta/cm² per sec, continuous illumination. Chlorophyll *a*, 4.37 μg/ml; path length, 2 mm; temp., 23°. ○, illuminated *minus* dark difference spectrum (steady state); ●, rapid "light-off" phase; ■, slow "light-off" phase.

Fig. 2. Effect of CCCP on light-induced cytochrome oxidation in *Porphyridium cruentum*. Double-beam spectrophotometric measurements (reference wavelengths, 450 mμ and 540 mμ). ○, no CCCP; ●, 12.5 μM CCCP. Steady-state difference spectra under continuous illumination. Other details same as in Fig. 1.

had maxima at 412 mμ and 429 mμ (see also Table I). These bands are interpreted to correspond to oxidation of cytochrome *f* (cytochrome-553) and cytochrome *b*, respectively.

The effect of carbonylcyanide *m*-chlorophenylhydrazone (CCCP) or carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) was somewhat unique in Porphyridium (Fig. 2). The light-induced absorbance decrease (steady-state under continuous illumination) had maxima at 430 mμ and 562 mμ in the presence of CCCP or FCCP, as contrasted to 422 mμ and 553 mμ in untreated cells. This result, obtained under continuous illumination with a rather long time constant (50 msec), suggests the faster reduction of cytochrome *f* in the presence of carbonylcyanide phenylhydrazone (CCP). This interpretation was verified by the rapid spectrophotometric analysis using the laser flash technique. In Fig. 3, Trace B indicates the rapid oxidation of cytochrome *f* and the dark reduction after flash. C is the time course of cytochrome reaction in the presence of CCCP. Here, indeed the reduction of cytochrome *f* is much faster than in the untreated cells. In Trace D, a recording in the presence of CCCP at the wavelength of 430 mμ, a rapid and a slow oxidation–reduction cycle were clearly observed. The rapid cycle had maxima at 421 mμ and 552 mμ and the slow cycle had maxima at 430 mμ and 562 mμ. The maximum oxidation was attained

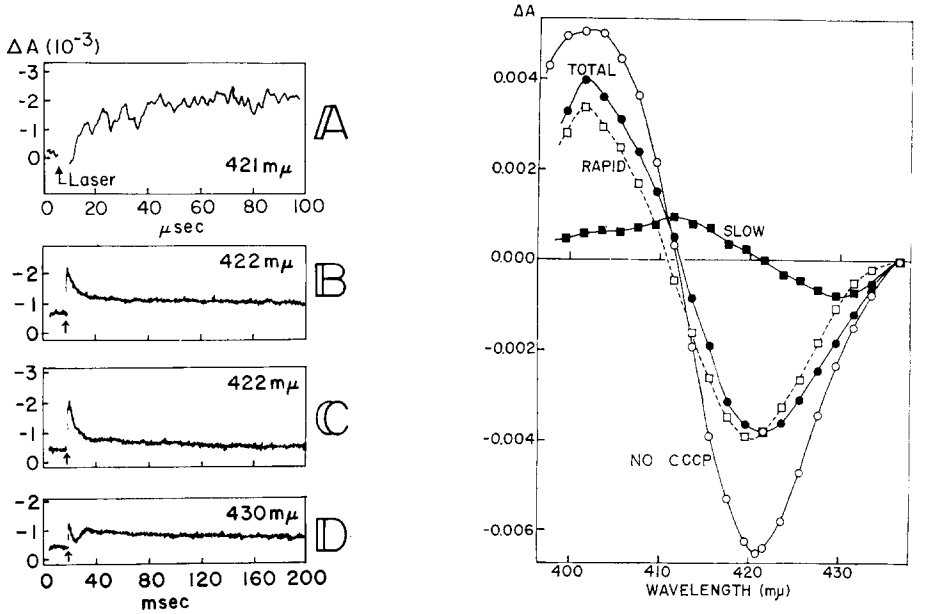

Fig. 3. Time course of cytochrome oxidation by laser flash in *Porphyridium cruentum*. Rapid single-beam spectrophotometric traces. Actinic wavelength, 694 mμ, $8.68 \cdot 10^{15}$ quanta/cm^2; duration, approx. 20 nsec. A: rapid sweep, measuring wavelength, 421 mμ; B: slow sweep, measuring wavelength, 422 mμ; C: slow sweep, 20 μM CCCP, measuring wavelength, 422 mμ; D: slow sweep, 20 μM CCCP, measuring wavelength, 430 mμ. Chlorophyll *a* concentrations: A, 7.88 μg/ml; B, C, D, 8.88 μg/ml. Path length, 1.6 mm. Temp., 23°.

Fig. 4. Light-induced absorbance change in *Porphyra yezoensis* in the presence and absence of CCCP. Double-beam spectrophotometric measurements (reference wavelength, 436 mμ). Illuminated *minus* dark difference spectra. Excitation light, 690 mμ, $1.67 \cdot 10^{15}$ quanta/cm^2 per sec; thalli, two layers; temp., 20°. ○, no CCCP (steady state); other curves with 6.25 μM CCCP; ●, total change (steady state); □, rapid "light-off" phase; ■, slow "light-off" phase.

TABLE I

LIGHT-INDUCED CYTOCHROME OXIDATION–REDUCTION REACTIONS IN PORPHYRIDIUM AND PORPHYRA

Laser excitation experiments measured by single-beam spectrophotometer, excitation wavelength, 694 mμ; continuous illumination experiments measured by double-beam spectrophotometer; excitation light, > 680 mμ. Room temperatures.

Illumination	*Sample*	*Phase*	*Position and direction of max. abs. change (mμ)*				*Half-rise time (μsec)*	*Half-decay time (msec)*	*Time for max. abs. change after flash (msec)*
Laser flash	*Porphyridium cruentum*	Rapid	402(+)	421(−)		552(−)	14–15	10.5	0.1
	Porphyridium cruentum	Rapid		421(−)		552(−)	18	9.7	0.1
	+20 μM CCCP	Slow		430(−)		562(−)	7000	164	20
	Porphyra umbilicalis	Rapid	404(+)	421(−)		552(−)	16	86	0.1
Continuous illumination	*Porphyridium cruentum*	Steady state	405(+)	422(−)	523(−)	553(−)			
		Rapid "off"	405(+)	422(−)	523(−)	553(−)			
		Slow "off"	412(+)	429(−)					
	Porphyridium cruentum +12.5 μM CCCP	Steady state		430(−)		562(−)			
	Porphyra yezoensis	Steady state	403(+)	421(−)	523(−)	552(−)			
	Porphyra yezoensis	Steady state	403(+)	422(−)	523(−)	552(−)			
	+6.25 μM CCCP	Rapid "off"	402(+)	420(−)					
		Slow "off"	412(+)	430(−)					
	Porphyra tenera	Steady state	403(+)	421(−)	523(−)	552(−)			
	Porphyra suborbiculata	Steady state	403(+)	421(−)					

in less than 100 μsec with a half-rise time of 14 μsec for the rapid phase (Trace A). The slow cycle reached its maximum oxidation at 20 msec after flash. This is interpreted as the very rapid oxidation of cytochrome *f* and its slower reduction and the simultaneous oxidation of *b*-type cytochrome. The reduction of cytochrome *f* and the oxidation of *b*-type cytochrome had a half-change time of 9.7 msec and 7.0 msec, respectively. This indicates that the transfer of electrons between these two cytochrome species is in the msec time range (see Table I).

In the case of Porphyra, CCCP or FCCP introduced a different pattern. By the addition of CCP (6–17 μM), the light-induced steady-state change at 421 mμ was decreased to about 60–80 % of its original value. At the same time, a change in the time course of the cytochrome reaction was observed. In the absence of CCCP or FCCP, the time course of the "light-off" reaction was more or less monophasic, whereas in the presence of CCP, the kinetics showed a biphasic change and the rapid and the slow phases were clearly separated. The rapid and the slow phases, plotted separately, show distinctly different spectral shapes (Fig. 4). The rapid phase had a negative peak at 420 mμ and a positive peak at 402 mμ. The slow phase had a negative maximum at 430 mμ and a positive one at 412 mμ. The total change had negative maxima at 421–422 mμ, 523 mμ and 552 mμ and a positive maximum at 403 mμ in both the presence and absence of CCCP (or FCCP). These data indicate a role of the *b*-type cytochrome as well as cytochrome-553 in the light-induced electron transfer in Porphyra. The response of the *b*-type cytochrome is smaller than the cytochrome-553 change. CCCP and FCCP did not significantly alter the action spectrum of cytochrome-553 oxidation or reduction.

In both Porphyridium and Porphyra, CCP induced the increased reduction rate of cytochrome *f* and the decreased reduction rate of cytochrome *b* after cessation of illumination. But the degrees of action of CCP were different in these organisms. Therefore, we obtained different types of steady-state spectral changes under continuous illumination, even though the actions of these inhibitors of these two organisms are qualitatively the same.

Besides the data shown here, similar analyses were carried out for other species and the summarized data are presented in Table I. No significant difference was observed in the cytochrome reactions of four species of Porphyra.

Reaction time of cytochrome f oxidation by laser flash

Following a short pulsed flash of 694 mμ (about 20 nsec duration), a rapid cytochrome *f* oxidation was observed in Porphyridium and Porphyra (Fig. 5; Table I). A typical recording of the rapid oxidation is shown also in Fig. 3, Trace A. The time required for completion of oxidation was less than 100 μsec. The half-oxidation time was 14–16 μsec in both species, and the half-reduction time was 10 msec for Porphyridium and 86 msec for Porphyra. No inhibitors were found to affect the rapid cytochrome oxidation.

Effect of temperature on steady-state change, "light-on" and "light-off" rates

The effect of temperature on the light-induced cytochrome reaction was quite remarkable. In Fig. 6, the time course at four different temperatures is presented. At higher temperatures, higher rates of the dark reduction of cytochrome and smaller steady-state changes were observed, though the initial rate of the cytochrome

oxidation remained unchanged in the temperature range of 1–34°. The "on" and "off" rates and the steady-state changes are plotted against the reciprocal of absolute temperature in Fig. 7. From the straight line approximating the "off" rate–reciprocal temperature relationship, the apparent activation energy of the dark reduction of cytochrome-553 was calculated to be 5.39 kcal/mole. The reduced steady-state absorbance change at higher temperatures can be explained by the constant rate of

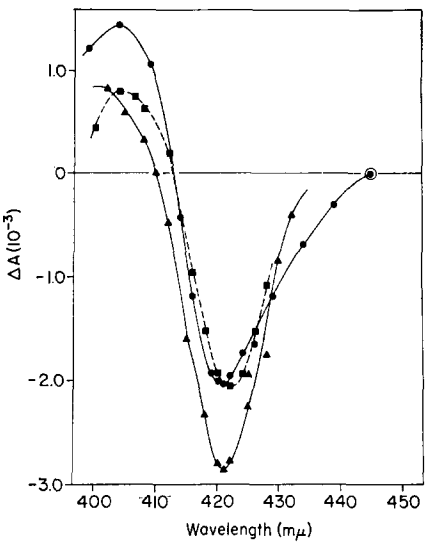

Fig. 5. Cytochrome oxidation by continuous illumination and laser flash. ●, *Porphyridium cruentum*, double-beam spectrophotometric measurements (reference wavelength, 445 mμ), excitation light, 690 mμ; continuous illumination, $1.81 \cdot 10^{15}$ quanta/cm² per sec; chlorophyll *a*, 2.60 μg/ml; path length, 10 mm. ▲, *Porphyridium cruentum*, single-beam spectrophotometric measurements; laser activation, 694 mμ; $8.68 \cdot 10^{15}$ quanta/cm²; duration, approx. 20 nsec; chlorophyll *a*, 19.87 μg/ml; path length, 1.6 mm. ■, *Porphyra umbilicalis*, single-beam spectrophotometric measurements; laser activation, 694 mμ, $8.68 \cdot 10^{15}$ quanta/cm²; duration, approx. 20 nsec; thallus, one layer; temp., 23°.

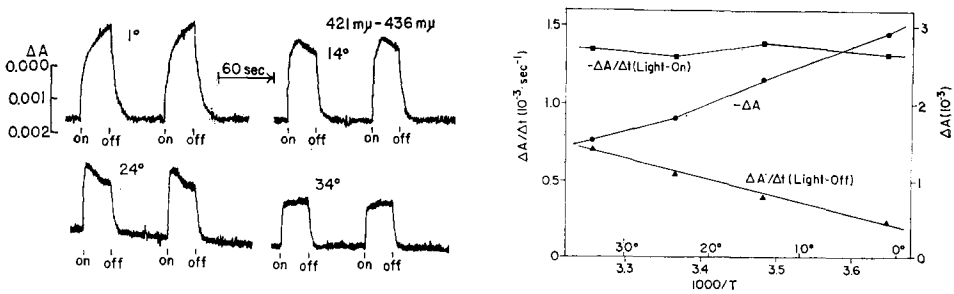

Fig. 6. Effect of temperature on light-induced cytochrome oxidation of *Porphyra yezoensis*. Double-beam spectrophotometer traces (421–436 mμ). Excitation light, 685 mμ, $1.80 \cdot 10^{15}$ quanta/cm²/sec; thalli, two layers; temperatures as indicated.

Fig. 7. Effect of temperature on steady-state change, "light-on" and "light-off" rates of light-induced cytochrome oxidation in *Porphyra yezoensis*. Double-beam spectrophotometric measurements (421–436 mμ). ●, illuminated *minus* dark steady-state change (*minus* sign); ■, initial "light-on" rate (*minus* sign); ▲, initial "light-off" rate. Other details same as in Fig. 6.

cytochrome oxidation (in this temperature range) and the increase of the reduction rate at higher temperatures.

Interaction of two photochemical systems through dark electron transfer

The effect of background illumination on the rapid kinetics of cytochrome *f* is shown in Table II, where the maximum absorbance change and the half-time of cytochrome reduction are indicated. Continuous background illumination of 575 mμ was given to excite phycoerythrin associated to system II (see Fig. 3 of ref. 3 for absorption spectrum of cells). Pulsed excitation light of 694 mμ was used to activate photochemical system I.

TABLE II

EFFECT OF BACKGROUND ILLUMINATION AND BIMU AND CMU ON LASER-INDUCED CYTOCHROME OXIDATION

Rapid single-beam spectrophotometric measurements. *Porphyridium cruentum*, chlorophyll *a*, 2.26 μg/ml; path length, 3.2 mm. Excitation light, 694 mμ, $8.68 \cdot 10^{15}$ quanta/cm^2; duration, approx. 20 nsec. Background illumination, 575 mμ, continuous.

575-mμ background illumination (10^{14} *quanta/cm^2 per sec*)	*Inhibitor* (μM)	$\Delta A_{422\ m\mu} \times 10^3$	$\tau_{1/2}$ *off* (*msec*)
0.00		1.91	9.27
0.08		1.87	8.90
0.20		1.98	7.82
0.44		1.90	7.45
0.85		2.04	6.13
1.50		1.88	6.01
0.00	BIMU (75)	1.87	12.8
0.85	BIMU (75)	1.79	14.5
1.50	BIMU (75)	1.44	13.1
0.00	CMU (75)	2.06	12.2
1.50	CMU (75)	1.56	13.1

In the absence of the system II inhibitors, the half-time became shorter with the increasing background illumination, from 9 msec to 6 msec in this set of experiments. This probably means the direct "pull" by system II in the time range of milliseconds. In other words, it means the presence of system I–system II interaction through dark electron transfer in the msec time range. In the presence of 5-bromo-

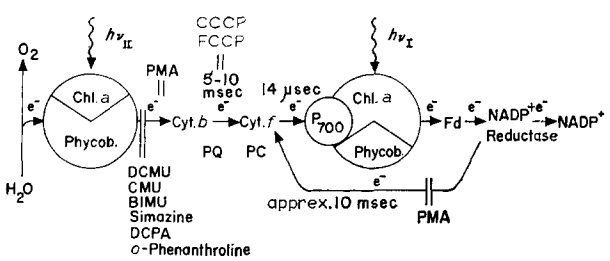

Fig. 8. Scheme for electron transfer in Porphyridium and Porphyra. DCMU, 3(3′,4′-dichlorophenyl)-1,1-dimethylurea; DCPA, 3,4-dichloropropionanilide; Fd, ferredoxin; PC, plastocyanin; PQ, plastoquinone; PMA, phenylmercuric acetate; Simazine, 2-chloro-4,6-bis-(ethylamino)-1,3,5-triazine.

3-isopropyl-6-methyluracil (BIMU) or 3(4'-chlorophenyl)-1,1-dimethylurea (CMU), both specific inhibitors of photochemical system II (refs. 1–3), the half-time for reduction increased to about 13 msec, indicating the absence of the system I–system II interaction. When BIMU or CMU was added, the background illumination of 575 mμ did not affect the reduction time of cytochrome oxidized by system I pulse.

DISCUSSION

An *f*-type cytochrome (Porphyra-cytochrome-553) was purified by KATOH[9] and by YAKUSHIJI *et al.*[10] from the red alga *Porphyra tenera*. Physicochemical properties of the protein were determined[11] and its reactivity was also measured[12]. A similar cytochrome can also be partially purified from *Porphyridium cruentum*.

The existence of *b*-type cytochrome in the photosynthetic apparatus of green plants was first demonstrated by HILL AND SCARISBRICK[13], HILL[14] and HILL AND BONNER[15]. Although there are several papers on the function of cytochrome *b* in the photosynthetic electron-transport chain of green plants[16–22], the role of this protein in photosynthesis is not fully understood. Isolation of *b*-type cytochrome from red algae has been tried unsuccessfully, but recently E. YAKUSHIJI (personal communication) isolated a *b*-type cytochrome from Porphyra.

From the analyses of the time course of the "light-off" changes, slow dark reduction of cytochrome *b* was indicated in Porphyridium cells and CCP-treated Porphyra cells. In the CCP-treated Porphyridium cells, the reduction rate of cytochrome *f* became higher, so that the steady-state level cytochrome oxidation solely consisted of the cytochrome *b* change. The effects of CCCP or FCCP indicate that these substances interact with the energy-transfer system between cytochrome *f* and cytochrome *b*. The action of CCCP on electron transfer in chloroplasts has also been reported by DE KIEWIET, HALL AND JENNER[23].

The very rapid oxidation of the *f*-type cytochrome in Porphyridium and Porphyra is an indication of the close association of the cytochrome *f* and the photochemical reaction center in photochemical system I. Even in the glutaraldehyde-fixed cells of Porphyridium, the laser-induced cytochrome *f* oxidation was as rapid as in the untreated cells, although the cytochrome reduction after the flash was much slower in the glutaraldehyde-fixed cells[24].

The fast reduction of cytochrome *f* is explained by the reduction through a reducing system produced by photochemical system I, along with the reducing power originating from photochemical system II. This is indicated by the rapid recycling of cytochrome *f* when only pigment system I was excited. Also, when system I was activated an apparent increase of quantum yield of cytochrome oxidation was observed in the presence of phenylmercuric acetate, which blocks the rapid cytochrome *f* reduction[25]. The comparison of the dark reduction time in the presence of background system-II-excitation light, with and without the system II-inhibitor BIMU or CMU (13 msec and 6 msec) (Table II), should correspond to the sizes of cyclic electron-transfer flux (around system I) and the sum of non-cyclic (between systems I and II) and cyclic electron-transfer fluxes. A high activity of NADPH–cytochrome-553 reductase was reported in cell-free extracts of Porphyra[12]. The function of ferredoxin–$NADP^+$ reductase as the NADPH–cytochrome *f* reductase was discussed by ZANETTI AND FORTI[26].

From the data shown here and other data, we constructed a scheme which represents the electron transfer of Porphyridium and Porphyra (Fig. 8). From the analysis of the time course of the "light-off" change, slow dark reduction of cytochrome *b* in the presence of CCCP or FCCP was indicated. Increase of the reduction rate of cytochrome *f* was observed in the presence of CCP. For the rapid dark reduction of cytochrome *f* and cytochrome *b*, the reduction by a reduced compound(s) produced by photochemical system I is a major path. The half-oxidation time for cytochrome *f* by system I is 14–15 μsec. The reaction between cytochrome *f* and cytochrome *b* has the half-change time of 5–10 msec. The reduction of cytochrome *f* by the cyclic electron flow around system I has the reaction time of about 10 msec. It was also demonstrated that the interaction of system I and system II takes place within 10 msec.

A somewhat longer value of interaction time of two photochemical systems was found in Chlorella and chloroplasts by WITT and coworkers[4,5]. There, the use of longer flash and the higher intensities to saturate all the electron carrier pools may have made the value a little larger than in our case. This type of analysis, extended further, will give us an opportunity to find the pool sizes of electron carriers as well as reaction rate constants.

The action of phenylmercuric acetate on cyclic and non-cyclic electron transfer, indicated in the scheme, was reported previously[25]. Similar, but slightly different effect of phenylmercuric acetate has been published by VREDENBERG AND DUYSENS[27].

The interaction of two photochemical systems through dark reactions was also analyzed by the delayed light emission from Porphyridium cells under different modes of excitation and physiological conditions. Reversal of early processes of photosynthetic energy conversion and the feedback of dark reactions (electron transfer and phosphorylation) to the activation of photochemical reaction center can be demonstrated. The details of the data concerning these subjects will be published in the near future.

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REFERENCES

1 L. N. M. DUYSENS, J. AMESZ AND B. M. KAMP, *Nature*, 190 (1961) 510.
2 L. N. M. DUYSENS AND J. AMESZ, *Biochim. Biophys. Acta*, 64 (1962) 243.
3 M. NISHIMURA AND A. TAKAMIYA, *Biochim. Biophys. Acta*, 120 (1966) 45.
4 H. T. WITT, B. RUMBERG, P. SCHMIDT-MENDE, U. SIGGEL, B. SKERRA, J. VATER AND J. WEIKARD, *Angew. Chem.*, 77 (1965) 821.
5 H. T. WITT, G. DÖRING, B. RUMBERG, P. SCHMIDT-MENDE, U. SIGGEL AND H. H. STIEHL, *Energy Conversion by the Photosynthetic Apparatus, Brookhaven Symp. Biol.*, 19 (1967) 161.
6 R. F. JONES, H. L. SPEER AND W. KURY, *Physiol. Plantarum*, 16 (1963) 636.
7 D. DEVAULT AND B. CHANCE, *Biophys. J.*, 6 (1966) 825.
8 G. MACKINNEY, *J. Biol. Chem.*, 140 (1941) 315.

9 S. KATOH, *J. Biochem. Tokyo*, 46 (1959) 629.
10 E. YAKUSHIJI, Y. SUGIMURA, I. SEKUZU, I. MORIKAWA AND K. OKUNUKI, *Nature*, 185 (1960) 105.
11 S. KATOH, *Plant Cell Physiol. Tokyo*, 1 (1960) 91.
12 S. KATOH, *Plant Cell Physiol. Tokyo*, 1 (1959) 29.
13 R. HILL AND R. SCARISBRICK, *New Phytologist*, 50 (1951) 98.
14 R. HILL, *Nature*, 174 (1954) 501.
15 R. HILL AND W. D. BONNER, JR., in W. D. MCELROY AND B. GLASS, *Light and Life*, Johns Hopkins Press, Baltimore, Md., 1961, p. 424.
16 B. CHANCE AND R. SAGER, *Plant Physiol.*, 32 (1957) 548.
17 B. CHANCE, H. SCHLEYER AND V. LEGALLAIS, *Studies on Microalgae and Photosynthetic Bacteria, Japan. Soc. Plant Physiol.*, (1963) 337.
18 J. M. OLSON AND R. M. SMILLIE, in B. KOK AND A. T. JAGENDORF, *Photosynthetic Mechanisms of Green Plants*, Natl. Acad. Sci.–Natl. Res. Council, Washington, D.C., 1963, p. 56.
19 B. RUMBERG, *Biochim. Biophys. Acta*, 102 (1965) 354.
20 G. HIND AND J. M. OLSON, *Energy Conversion by the Photosynthetic Apparatus, Brookhaven Symp. Biol.*, 19 (1967) 188.
21 R. P. LEVINE AND D. S. GORMAN, *Plant Physiol.*, 41 (1966) 1293.
22 W. A. CRAMER AND W. L. BUTLER, *Biochim. Biophys. Acta*, 143 (1967) 332.
23 D. Y. DE KIEWIET, D. O. HALL AND E. L. JENNER, *Biochim. Biophys. Acta*, 109 (1965) 284.
24 M. NISHIMURA, *Comparative Biophysics and Biochemistry of Photosynthesis, Hakone, 1967*, Univ. of Tokyo Press, Tokyo, 1968, p. 196.
25 M. NISHIMURA, *Energy Conversion by the Photosynthetic Apparatus, Brookhaven Symp. Biol.*, 19 (1967) 132.
26 G. ZANETTI AND G. FORTI, *J. Biol. Chem.*, 241 (1966) 279.
27 W. J. VREDENBERG AND L. N. M. DUYSENS, *Biochim. Biophys. Acta*, 94 (1965) 355.