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SPECTRAL PROPERTIES OF SYSTEM I-DEFICIENT MUTANTS OF *CHLAMYDOMONAS REINHARDI*

POSSIBLE OCCURRENCE OF UPHILL ENERGY TRANSFER

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SUMMARY

Chlamydomonas reinhardi mutants lacking the *P*-700-chlorophyll *a*-protein complex CP1, lack as well the long wavelength forms of chlorophyll Ca 691 and Ca 704 normally present in the absorption spectrum at −196 °C.

These mutants do not display the 715 nm peak in the fluorescence emission spectrum at −196 °C.

Studies of the System II action spectrum of one such mutant show an increase in the optical cross-section of the System II centers around 685 nm. This is interpreted as an energy transfer from pigment System I to pigment System II occurring in the mutant.

INTRODUCTION

In a previous paper [1] it was shown that two mendelian mutants of *Chlamydomonas reinhardi* (F1 and F14) lacking *P*-700 were deficient in a chlorophyll-protein complex CP_1 (identical to the *P*-700-chlorophyll *a*-protein of Thornber [2]). Recently, it was shown that these two mutants were genetically independent (Girard, personal communication). Two other mendelian mutants, F10 and F12 are also missing the System I chlorophyll-protein complex CP1 (Chua, N. H. and Bennoun, P., unpublished data).

In this paper, we analyze some of the spectral characteristics of these System I-deficient mutants.

MATERIALS AND METHODS

(1) The wild-type 137C and four mutant strains (F1, F10, F12, F14) of *C. reinhardi* were grown in Tris/acetate/phosphate medium under low light intensity (200 lux). The mutants were derived from the wild-type strain (WT) by mutagenesis

with methyl methane sulfonate and selected as high fluorescence mutants [3].

(2) Low temperature absorption spectra were measured with the following device: A Millipore filter is maintained between two light pipes (Sovis) and placed in a Dewar containing liquid nitrogen. The biological material is spread on the Millipore filter. The close juxtaposition between the sample and the light pipes minimizes the light scattering effects. One of the light pipes receives monochromatic light (bandpath 0.2 nm) from a monochromator (Jobin Yvon HRS 1) fitted with a quartz iodin lamp (Osram, 100 W) powered with a constant-current power supply (Chauvin-Arnoux LB 1000). The incident beam is modulated at 20 Hz with a mechanical chopper. The transmitted light is collected by the second light pipe and measured with a photomultiplier RTC 1003. The signal of the photomultiplier is amplified with a lock-in amplifier (Tekelex TE 9700) and transmitted to a computer (Tektronik TEK 31 fitted with a DM 501 interface). The computer was programmed to start taking readings at a specified wavelength, to take readings at a specified interval and to take a specified number of readings. After subtraction of the absorption of the Millipore filter alone one obtains the absorption spectra of the algae spread on the filter.

(3) Fluorescence emission spectrum at low temperature were measured as described previously [4]. Corrections have been made for the response of the photomultiplier and the transmission of the monochromator using a standard lamp ISCO No. 413.

(4) Activation reaction of the oxygen precursors were measured with a modulated polarograph [5]. Action spectra of System II were obtained as described by Joliot et al. [6].

(5) Chlorophyll measurements were made according to Arnon [17].

RESULTS AND DISCUSSION

Fig. 1 shows the absorption spectrum at −196 °C of *Chlamydomonas* wild-type and mutant F14.

To permit comparison, these spectra were deconvoluted into gaussian components using the average parameters of French et al. [8]. The results are shown in Fig. 1 and are compiled in Table I. We have computed for each gaussian component of the spectrum the ratio of its weight in the mutant over that in the wild-type. We expect that only some of these components are affected in the mutant. We see that the relative weight of the different bands is comparable in the wild-type and the mutant except for the far red absorbing forms Ca 691 and Ca 704 which appear to be strongly deficient in the mutant. (This is also clear from Fig. 1). Taking into account this qualitative comparison of the spectra we have normalized the spectrum of the mutant so that the sum of all gaussian components except Ca 691 and Ca 704 is identical to that of the wild-type. We then see (Table I) that almost 80 % of the far red chlorophyll *a* forms Ca 691 and Ca 704 are missing, which corresponds to a loss of 14 % of the chlorophyll *a* in the mutant. This conclusion is in good agreement with the earlier observation that the CP1 complex is missing in the mutants: this complex contains specifically chlorophyll *a*.

We have observed the same type of spectrum for three other mendelian mutants of *C. reinhardi*, F1, F10, F12 which are also deficient in CP1 (figure not shown). Similar results were obtained previously on *Scenedesmus* mutant No. 8 which also

TABLE I

Line 1 and 2: Relative weight of the gaussian components of the absorption spectra shown Fig. 1. Line 3: Ratio of the weight of each gaussian component, mutant over wild-type. Line 4: Normalization of the spectrum of the mutant assuming that the sum of the first six gaussian components (Cb 642 to Ca 683) is identical in the mutant and in the wild-type. Line 5: Percentage of variation of the weight of the different gaussian components in the mutant compared to wild-type, using the normalization of line 4.

	Cb 642	Cb 651	Ca 661	Ca 669	Ca 677	Ca 683	Ca 691	Ca 704	Total chlorophyll	Chorophyll *a*
(1) WT	49.2	133.3	164	200	215	121.5	91.5	25.5	1000	817.5
(2) F14	63.4	160	182	213.5	239	112	23.6	6	1000	776.1
(3) F14/WT	1.28	1.20	1.10	1.06	1.11	0.92	0.25	0.23	1	0.95
(4) F14N normalized	57	145	165	194	217	102	21.5	5.5	907	705
(5) $\frac{\text{F14N}-\text{WT}}{\text{WT}}$ %	+16	+8.8	+0.6	−3	+0.9	−16	−76	−78	−10	−14

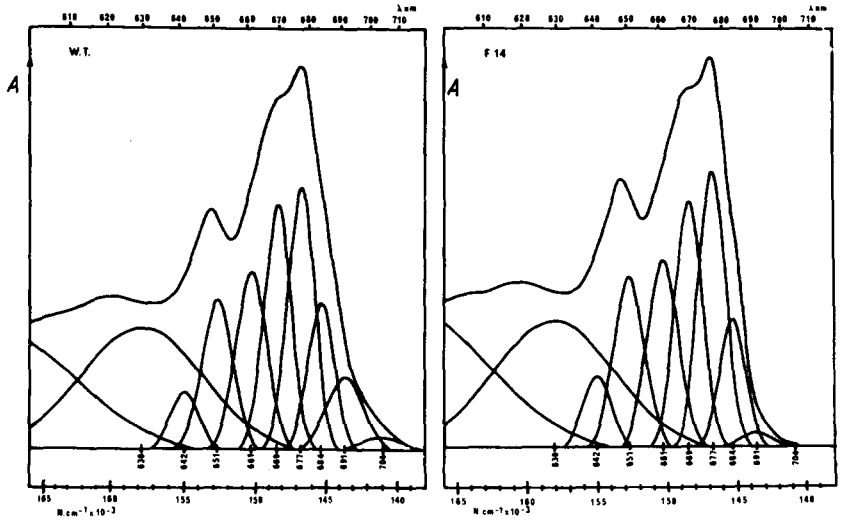

Fig. 1. Absorption spectra at −196 °C of *C. Reinhardi* wild-type and mutant F14. Deconvolution of the spectra into gaussian components is achieved using the following parameters. Peak wavelengths and half-widths of the different chlorophyll forms are (in nm) 592 : 47, 630 : 40, 642 : 10, 651 : 11, 661 : 11, 669 : 10, 677 : 10, 683 : 10, 691 : 15, 704 : 19. A deficiency of the 691 and 704 nm components is visible in the mutant.

lacks the complex CP1 [9–11].

In Table II, we have gathered together the values measured for the ratio chlorophyll *a*/chlorophyll *b* in the mutant strains and in the wild-type. We see that this ratio is lower in the mutants than in the wild-type: average values are, respectively, of 2 and 2.4. It is interesting to compare these data in relation with those of Table I. If we make the approximation that the decrease in the chlorophyll *a*/chlorophyll *b* ratio in the mutants is due to a deficiency in chlorophyll *a* we are led to the conclusion that 16 % of the chlorophyll *a* is missing in the mutants. This value agrees with the estimation given in Table I (14 %).

It is reasonable to assume that the 15 % of chlorophyll *a* missing in the mutants consists of those chlorophyll molecules attached to the chlorophyll-protein complex CP1. It is rather satisfactory to observe that this complex contains the long wavelength-absorbing forms of chlorophyll *a* Ca 691 and Ca 704 together with *P*-700 as energy transfer from these pigments to *P*-700 wil]be favoured.

TABLE II

Ratio chlorophyll *a*/chlorophyll *b* measured in the mutants and in the wild-type.

Chlorophyll *a*/chlorophyll *b*	WT	F1	F10	F12	F14
	2.4	1.91	2.01	–	–
	2.43	1.92	–	2.15	1.86
	2.32	1.88	1.95	–	2.05
Average	2.4±0.25		2±0.25		

One can estimate that the 15 % of chlorophyll *a* associated with CP1 correspond roughly to 50 chlorophyll molecules per System I unit. We point out that this is the actual size of the bacterial antenna [12]. One could speculate whether a link exists in the course of evolution between the bacterial photosystem and the CP1 of higher plants. Also recent results by Diner (personal communication) show that the System II antenna of *Cyanidium* mutant IIIC (lacking phycobilisoms) is made of 40–50 chlorophyll *a* molecules.

In Fig. 2, we have drawn the difference spectrum wild-type minus F14 using the normalization given Table I. This can be considered as an in vivo spectrum of CP1. It is clear that two components are present in this spectrum.

In Fig. 3, we show the fluorescence emission spectra of wild-type and F14 at −196 °C. One observes an obvious deficiency of the 715 nm emission peak in the mutant. We conclude that this emission originates from the long wavelength-absorbing forms of chlorophyll *a* present in CP1. This is in agreement with earlier data showing that the far red emission peak of fluorescence is related to the far red-absorbing form of chlorophyll *a* [13–16]. However, this result is opposite to that observed in *Scenedesmus* mutant No. 8 in which the far red emission peak is quite pronounced [10–16].

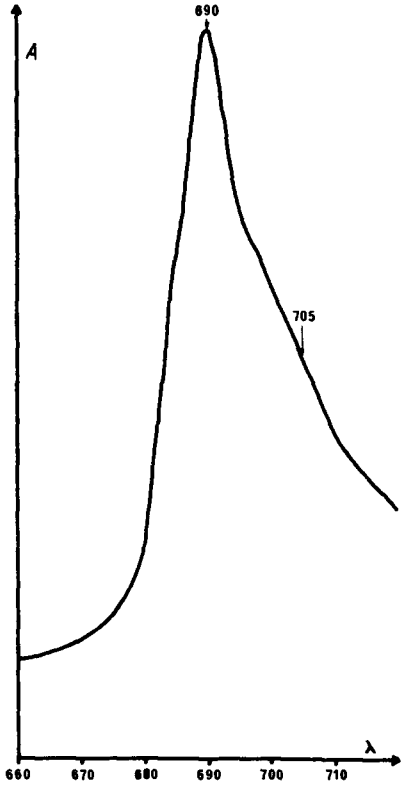

Fig. 2. Difference between absorption spectra of Fig. 1 wild-type minus mutant F14. The difference has been drawn by using the normalization of the F14 spectrum as indicated Table I, line 4.

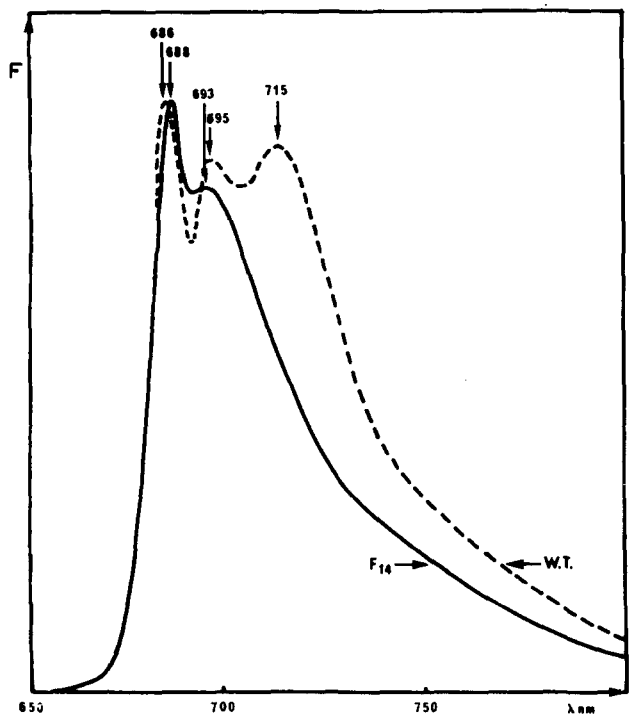

Fig. 3. Low temperature (−196 °C) fluorescence emission spectra of *C. Reinhardi*. Wild-type and mutant F14, normalized at 687 nm.

The activation reaction of the oxygen precursors is a photochemical process. The half-time of this reaction is inversely proportional to the efficient optical cross-section of the System II centers [17]. We have measured this reaction for both wild-type and mutant F14 at various wavelengths. As shown in Fig. 4, the half-time of the activation reaction is shorter in the mutant. It is interesting to compare the data obtained at two wavelengths for which the absorption of the algae is low. In that case, the results will not be altered by screening effects. By comparing 560 and 700 nm, one observes that the decrease of the half-time of the kinetics in the mutant is twice as important in the far red than it is in the green part of the spectrum. Furthermore effects of comparable size are observed at 560 and 655 nm. We can therefore conclude that the efficient optical cross-section of the System II centers is specifically enlarged at longer wavelengths in the mutant.

Several hypotheses can be put forward in order to account for this observation:

(1) One can make the ad hoc assumption that additional System II pigments are present in the mutant. Though this cannot be disproved easily it seems very unlikely.

(2) One might imagine that some System II centers are blocked in a non-quenching state in the mutant. Taking into account energy transfer between System II units, this could enlarge the efficient optical cross-section of the active centers. However, this effect should not be wavelength dependent.

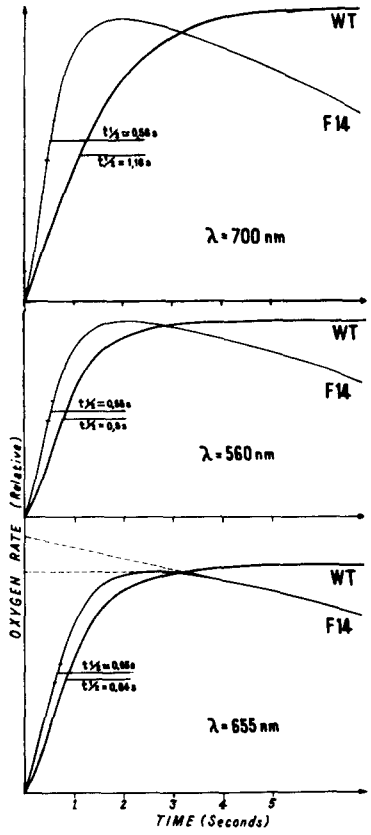

Fig. 4. Activation reaction of the oxygen precursors in dark-adapted wild-type and F14 at various wavelengths. The absence of active System I centers in F14 leads to a decrease of the oxygen rate in the light. In order to estimate the half-time of the activation phase in the mutant we have taken as a maximum oxygen rate two possible extrapolations as indicated in the lower part of the figure (dotted lines). The half-times indicated on the figures correspond to the average value of these two measurements. Half-times corresponding to each extrapolation are indicated in the rise curve by two horizontal bars.

(3) The simplest hypothesis is to assume that the modification observed at the System II level is a consequence of the primary effect of the mutation which is the absence of the System I protein complex CP1.

The interpretation that we propose is the following: Energy absorbed by the major chlorophyll form of System I (absorbing around 685 nm) is normally transferred to the Ca 691 and Ca 704 holochromes which act as an energy trap. In that case, transfer of excitation from pigment System I to pigment System II is not favoured. In the case of the mutant, the System I trap is missing. It is therefore reasonable to assume that energy transfer from System I to System II becomes substantial.

Fig. 5 shows the action spectrum of oxygen evolution (pure System II spectrum) for both wild-type and the mutant. These spectra were obtained using a polarograph in which modulated light of various wavelengths is superimposed on a continuous far red background light. As the modulated polarograph gives only relative

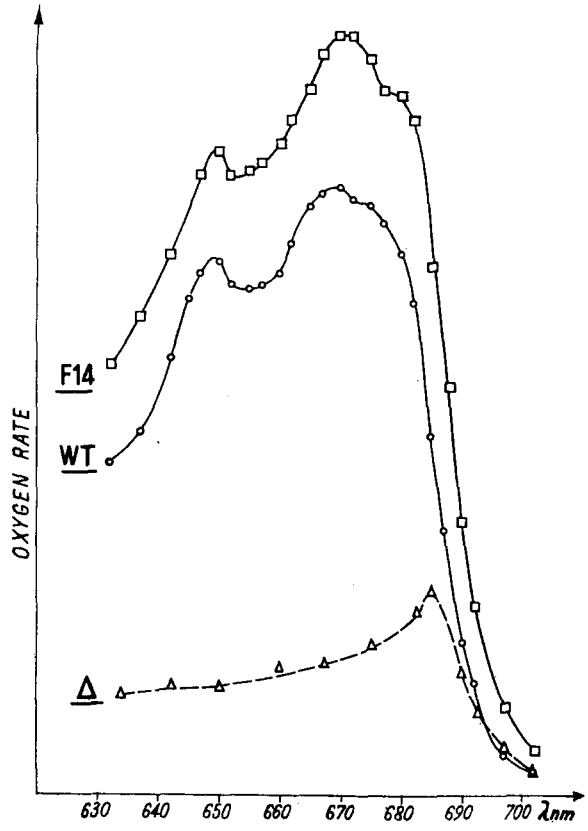

Fig. 5. Action spectrum of Photosystem II in *C. Reinhardi* wild-type and mutant F14. A continuous far red (700 nm) light is used to maintain a constant concentration of active System II centers. A weak modulated beam of variable wavelength gives rise to the modulated oxygen rate indicated in the figure.

measurements, we have used the data of Fig. 4 in order to calibrate one spectrum with respect to the other. This calibration permits a measurement of the difference spectrum, mutant minus wild-type. The difference between the two spectra shows only one peak at 685 nm. This result is very consistent with the hypothesis of an energy transfer from System I to System II occurring in the mutant. From the data of Fig. 5, we can estimate that the average increase of the optical cross-section of System II centers in the mutant is roughly 25 % between 630 and 700 nm. This value is a rough estimation of the percentage of excitation absorbed by System I which can be transferred to System II in the mutant.

This conclusion implies that not all the System I antenna is missing in the mutant. This was also clear from the above estimation that only 15 % of the chlorophyll *a* is missing in the mutants.

The occurrence of an energy transfer from pigment System I to pigment System II in the mutant leads to question whether such a transfer could also occur in the wild-type. As shown Fig. 5, a shoulder does exist in the System II action spectrum of the wild-type around 680–685 nm. This could either be interpreted as the presence

of a particular System II holochrome or as the existence of an energy transfer from the System I antenna. More information is needed to clarify this point. The possible occurrence of uphill energy transfer has been considered previously in the case of *Rhodopseudomonas* sp [18].

We point out in Fig. 5 that, in one case (F14) both *P*-700 and Ca 691-Ca 704 are missing, and that in the other case (WT) Ca 691-Ca 704 is present but *P*-700 is oxidized because of the far red background light (intensity of the far red beam is such that one photon is absorbed by System I center every 50 ms). If *P*-700 were the actual trap of System I, one should not expect important differences between the two cases, *P*-700 missing or *P*-700 oxidized, unless we admit that $P\text{-}700^+$ could still be a quencher. It is more likely that the System I trap consists of the far red holochromes Ca 691 and Ca 704. When these holochromes are present, the energy absorbed by the major form of chlorophyll of System I (absorbing around 685 nm) is preferentially transferred to Ca 691 and Ca 704. When these holochromes are absent, transfer to the System II pigments becomes more probable.

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