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THE PHOTOSYNTHETIC ELECTRON TRANSPORT CHAIN OF A MUTANT STRAIN OF *CHLAMYDOMONAS REINHARDI* LACKING P700 ACTIVITY

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SUMMARY

Components and reactions of the photosynthetic electron transport chain were investigated in a mutant strain of the unicellular green alga *Chlamydomonas reinhardi* which is virtually devoid of the System I reaction center pigment, P700. The plastocyanin and ferredoxin isolated from this mutant strain are both qualitatively and quantitatively indistinguishable from that isolated from the wild-type strain. Cytochromes with absorption maxima at 553 and 559 nm cannot be oxidized by far-red light in the mutant strain, but they are reduced by red light. The $Fe(CN)_6^{3-}$–Hill reaction in the mutant strain is about 50 % of that of wild type at high light intensities; however, at low light levels, it is not significantly different from the rate of wild type. These results are interpreted to indicate that P700 is not so closely involved or complexed with adjacent electron carriers or with the reaction center of System II that destruction of P700 necessarily leads to alteration of these other components of the electron transport chain. It is suggested that the Hill reaction data can be explained by the existence of two separate sites for photoreduction of $Fe(CN)_6^{3-}$ in wild type, whereas only one remains operative in the mutant strain.

INTRODUCTION

It has been previously reported that *acetate-80a* (*ac-80a*), a mutant strain of the unicellular green alga *Chlamydomonas reinhardi*, is virtually devoid of the photosynthetic System I reaction center pigment, P700, and that despite such a deficiency this strain is able to oxidize water and reduce high-potential Hill oxidants in a reaction coupled to phosphorylation. However, this mutant strain is unable to carry out phenazine methosulfate (PMS)-catalyzed cyclic phosphorylation or to reduce $NADP^+$, either in the Hill reaction or with electrons donated by reduced 2,6-dichlorophenolindophenol (DCIP)[1]. It has also been observed that *ac-80a* lacks the so-called "fast" ESR signal (D. TEICHLER-ZALLEN AND A. L. GIVAN, unpublished work) which is believed to reflect P700 oxidation[2,3].

It has been postulated that reaction center pigment may be a membrane-bound or critically oriented component which functions in a complex with other components

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

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of the photosynthetic electron transport chain[4–6]. In addition, because the Hill reaction rate of P700-less strains has been reported to be significantly less than that of wild-type strains[1,2,7], it has been suggested that a disorganization (perhaps of pigments or membrane components) causing or resulting from loss of P700 activity may affect the efficiency of Photosystem II (ref. 7). Because interpretation of the role of P700 and of the structural and functional relationships between components of the light and dark reactions of the electron transport chain depends critically on the validity of such hypotheses, experiments were undertaken to analyze the electron transport of the mutant strain, *ac-80a*, in some detail.

METHODS

Cells of both wild type (strain 137c) and *ac-80a* were cultured in liquid acetate medium under 4000 lux continuous illumination and harvested as previously described[8]. The Hill reaction and cytochrome redox changes were measured in chloroplast fragments prepared by ultrasonic disintegration[9]. The fragments were centrifuged and then resuspended in 10 mM potassium phosphate buffer (pH 7.0) containing 20 mM KCl and 2.5 mM $MgCl_2$. Chloroplast fragments prepared in this way show virtually no phosphorylation coupled to photosynthetic electron flow[10].

Absorbance changes resulting from oxidation and reduction of cytochromes were measured with an Aminco-Chance double-beam spectrophotometer and an actinic light source as described elsewhere[11]. Methylviologen and biopterin were obtained from K and K Rare Chemicals.

Plastocyanin and ferredoxin were extracted and partially purified by the methods of GORMAN AND LEVINE[12,13]. The amounts of plastocyanin and ferredoxin present were calculated using published extinction coefficients[14,15]. Absorption spectra were measured on a Cary Model-14 recording spectrophotometer.

The $Fe(CN)_6^{3-}$–Hill reaction was followed titrimetrically[1]. Illumination at 56000 lux was provided by means of a Sylvania "Sun Gun". Light intensity was varied by the use of calibrated neutral density screens purchased from Perforated Products, Inc., Brookline, Mass. For lowest light intensities (less than 1.2 % of the light source), the screens were used in combination with one of two Corning neutral density filters (0.3 or 0.6 density).

Chlorophyll was determined by the method of ARNON[16].

RESULTS

Wild-type *C. reinhardi* chloroplast fragments show an increase in absorbance at both 553 and 559 nm when illuminated with 650-nm light. Conversely, 720-nm light brings about a decrease in absorbance at these two wavelengths. LEVINE AND GORMAN[11] have concluded that these light-induced absorbance changes correspond to changes in the redox state of cytochromes 553 (probably cytochrome *f*) and 559 (a *b*-type cytochrome), both of which are reduced by 650-nm light and oxidized by 720-nm light. In chloroplast fragments of *ac-80a*, light of 650 nm caused a reduction of both cytochromes, but 720-nm light was inactive. Recordings from the double-beam spectrophotometer which illustrate these absorbance changes are shown in Figs. 1 and 2. Neither 1 mM biopterin nor methylviologen restored the activity of far-red light in

ac-80a preparations. Clearly, neither cytochrome can be photooxidized in this strain, although both can be photoreduced.

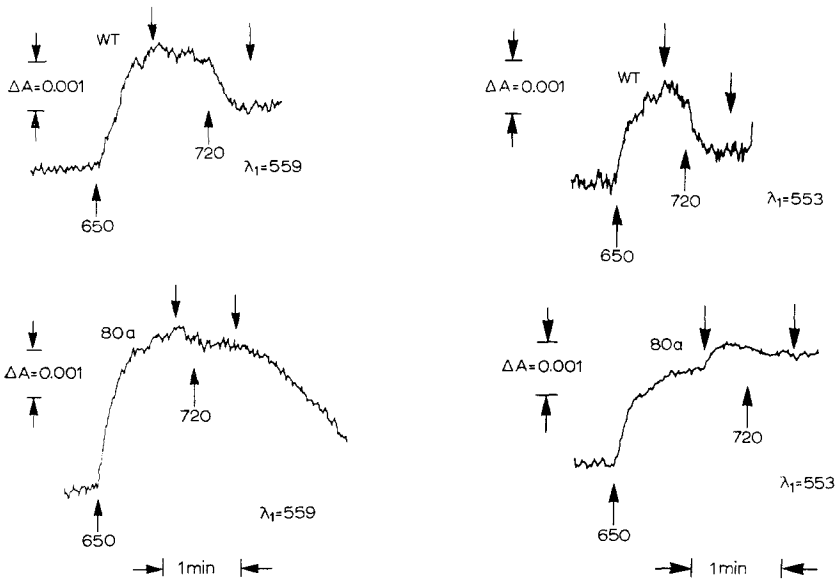

Fig. 1. Light-induced oxidation and reduction of cytochrome 559 in *ac-80a* and wild-type chloroplast fragments. Arrows pointing upward signify that actinic light of the indicated wavelength was turned on; downward arrows signify light off. The measuring beam was set at 559 nm and the reference beam at 542 nm. An increase in absorbance corresponds to a reduction of a cytochrome. The concentration of chlorophyll in the cuvette was 0.1 mg per ml. The path length was 1 cm.

Fig. 2. Light-induced oxidation and reduction of cytochrome 553 in *ac-80a* and wild-type chloroplast fragments. Conditions and figure notations are the same as described for Fig. 1, except that the measuring beam was at 553 nm.

Whereas absorbance changes *in vivo* resulting from changes in redox levels of plastocyanin and ferredoxin have not yet been observed in *C. reinhardi*, both compounds can be isolated from this alga and quantitative estimates of their content can be made[12,13]. The amounts of these two compounds isolated from both wild-type and *ac-80a* strains of *C. reinhardi* are indicated in Table I. Their absorption spectra in partially purified form were recorded and show no detectable differences between the two strains. Ferredoxin and plastocyanin appear to be both quantitatively and qualitatively the same in normal and mutant strains.

Whereas these and previous[1] results demonstrate the existence and quality of various electron carriers and reactions in *ac-80a*, it was of further interest to investigate the efficiency of electron transport, specifically of the Hill reaction, in this strain. Figs. 3 and 4 illustrate the effect of light intensity on the $Fe(CN)_6^{3-}$–Hill reaction in chloroplast fragments of wild type and *ac-80a*. At low light intensities reaction rates in both strains are similar and the efficiency of *ac-80a* is not significantly different from that of wild type (Fig. 3). This can be seen more clearly in Fig. 4 where the results are plotted as rate *versus* efficiency. Regression lines drawn through the data indicate that, whereas *ac-80a* has about half the maximum Hill reaction rate of wild type at infinitely high light intensities, both lines extrapolate to virtually

identical points on the abscissa. The relative quantum yields at infinitely low light intensities are equivalent.

TABLE I

FERREDOXIN AND PLASTOCYANIN CONTENT OF *ac-80a* AND WILD-TYPE CELLS OF *C. reinhardi*

C. reinhardi	*Ferredoxin* (*moles per 1000 moles chlorophyll*)	*Plastocyanin* (*atoms copper per 1000 moles chlorophyll*)
ac-80a	3.0	1.2
Wild type	2.6	0.8

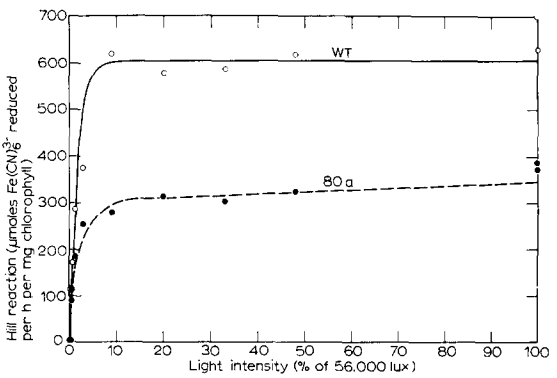

Fig. 3. The effect of varied light intensity on the $Fe(CN)_6^{3-}$–Hill reaction in wild-type and *ac-80a* chloroplast fragments. The reaction mixture (2.0 ml) contained chloroplast fragments equivalent to 15–25 μg of chlorophyll, and the following in μmoles: KCl, 40; $MgCl_2$, 5; KH_2PO_4, 2; $K_3Fe(CN)_6$, 2. The pH was adjusted to 7.0 with KOH before the addition of the chloroplast fragments. The reaction mixture was maintained at 25°.

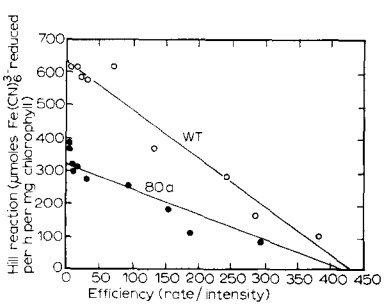

Fig. 4. The $Fe(CN)_6^{3-}$–Hill reaction rate plotted against the efficiency of light of different intensities in producing $Fe(CN)_6^{3-}$ reduction. The data are the same as those plotted in Fig. 3. The efficiency is given in arbitrary units. The lines were calculated by the method of the least squares.

DISCUSSION

The photooxidation of cytochrome *f* proceeds even at very low temperature[17,18]. DE VAULT AND CHANCE[4], in reference to work with the photosynthetic bacterium

Chromatium, hypothesized that the light-induced electron transfer between a cytochrome and its oxidant must occur without translocation of molecules and across very short distances. The possibility of chlorophyll and a cytochrome existing in some sort of complexed form was suggested by BUTLER[5] to explain the long-wavelength absorption band of C700. If such a complex does exist between a cytochrome and the reaction center chlorophyll responsible for its photooxidation, it is apparent that in *ac-80a* breaking the connection between cytochrome 553 and its oxidant does not appear to alter the ability of this cytochrome to interact with its reductant.

However, there is some doubt about this close association between cytochrome *f* and P700. Studies on detergent-treated spinach chloroplasts[19,20] and on *C. reinhardi* mutants[8,11] have suggested that plastocyanin lies between cytochrome 553 and P700. KOK *et al.*[6] suggested that plastocyanin is complexed with P700. The finding that plastocyanin is normal in *ac-80a* indicates that at least the synthesis of this compound and the form in which it is isolated from cells are not appreciably altered by a mutation which destroys P700 activity. Likewise the loss of P700 activity does not appear to have any effect on ferredoxin, a compound which acts on the reducing side of System I (ref. 21).

Previous reports on *ac-80a*[1] and on the Scenedesmus mutant strain which lacks P700[2,7] have indicated that P700 is necessary for full Hill reaction capacity; without P700, oxidants of high potential are reduced at only 50–85 % of wild-type rates. Two explanations can be offered for this observation. KOK AND DATKO[7] suggested that System II in a P700-less mutant might in fact be less efficient than System II in normal cells. It can be imagined that loss of P700 might lead to or result from a structural rearrangement which could in turn affect the activity of System II or that P700 might itself play some role in the photochemistry of System II. Alternatively, the loss of Hill activity in a P700-less mutant strain could be explained by the hypothesis of two sites for the reduction of Hill oxidants. Early work[22,23] indicated that $Fe(CN)_6^{3-}$ and DCIP reduction had System II action spectra. However, KOK *et al.*[24] and KE[25] have more recently shown that DCIP reduction occurs with two distinct phases: a fast phase, which is insensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and absent in the Scenedesmus mutant strain that lacks P700, and a slower phase, which is inhibited by DCMU and is present in the Scenedesmus mutant strain. The conclusion from the data of KOK *et al.* and of KE is that DCIP can be reduced either by System I or by System II. Another line of experimentation which supports this conclusion has come from studies of Emerson enhancement, a phenomenon which results from cooperation between the System I and System II light reactions. GOVINDJEE *et al.*[26] reported enhancement of benzoquinone reduction; and BISHOP AND WHITTINGHAM[27] and WHITTINGHAM AND BISHOP[28] found enhancement with $Fe(CN)_6^{3-}$ reduction. The recent work by GOVINDJEE AND BAZZAZ[29] has attempted to specify the conditions under which enhancement of the Hill reaction can be observed. In $Fe(CN)_6^{3-}$ reduction, enhancement was seen only at high light intensities. It appears that at low light levels, most of the reduction of $Fe(CN)_6^{3-}$ occurs at a site between the two systems, and enhancement is not observed. (This may explain the various reports of inability to detect enhancement of the Hill reaction[30–32].)

The light-intensity response of the Hill reaction in *ac-80a* can be similarly explained. Since the efficiency of the Hill reaction at low light levels in the mutant strain is fully as high as that in wild type, the implication is that deficient P700

activity does not affect the efficiency of System II and that System II alone is responsible for the $Fe(CN)_6^{3-}$–Hill reaction at these intensities. KOK AND DATKO[7] report for the Scenedesmus mutant strain that, even at low light intensities, the Hill reaction in the mutant strain does not equal that in wild type. However, they did find that, at low intensity, the difference between the rates of the two strains was considerably less distinct than at high.

The Hill reaction of *ac-80a* reaches saturation at a lower light intensity than wild type so that the maximum rate attained by wild type is about double the maximum rate of *ac-80a*. Although KOK AND DATKO[7] felt that this lowered saturation rate in the Scenedesmus mutant strain might be a result of a P700–System II interaction that also lowered the quantum yield, there exists another interpretation. GOVINDJEE AND BAZZAZ[29] found enhancement of the Hill reaction only at high light intensities; it is also at high intensities that *ac-80a* cannot compete with wild type in attaining high rates. The simplest explanation of this phenomenon is that the mutant strain lacks the P700 required for passing electrons beyond System I to a second site for $Fe(CN)_6^{3-}$ reduction when the first site becomes saturated. At low light levels when only the System II light reaction is active in reducing the Hill reagent, *ac-80a* is quite as successful as wild type. Thus, it is unnecessary to postulate that P700 has any influence on System II.

The data presented here do not rule out the alternative possibility that the mutation destroying P700, while not affecting the light reaction of System II (and therefore not affecting its quantum efficiency), does affect in some way an electron transport step prior to the first site for reduction of $Fe(CN)_6^{3-}$. In this interpretation as in the previous one, the mutant Hill reaction would be affected only at high light intensities.

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