ON THE SITE OF ELECTRON DONATION TO THE PHOTOSYNTHETIC ELECTRON TRANSPORT CHAIN BY 1,5-DIPHENYLCARBAZIDE*

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Summary:

This communication reports on studies carried out with a mutant of Euglena gracilis unable to use water as an electron donor. It was found that: (1) DPC is a donor which acts very close to the reducing side of photosystem II (2) contrary to results published in the literature electron donation by DPC is not inhibited by PMA.

INTRODUCTION

Different approaches have been used in order to resolve the steps associated with the water side of photosystem II in photosynthesis[1]. These approaches include inhibition of the water side of photosystem II by the use of various chemical and physical means which block this region at different points[2]. The sequence of these steps can then be elucidated by determining the efficiency of various natural and artificial electron donors in restoring the light-induced photosynthetic electron transport and by studying the kinetics of fluorescence emitted by photosystem II[3-5]. Another approach which has been used for the same purpose, although not to the same extent as the first approach, is the

Abbreviations: Asc, ascorbate; chl, chlorophyll a + b; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPC, 1,5-diphenylcarbazide; DPCN, 1,5-diphenylcarbazone; HQ, hydroquinone; 102, mutant 102; $NADP^+$, nicotinamide adenine dinucleotide phosphate; PMA, phenylmercuric acetate; WT, wild type.

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study of algal species with induced mutations at the water side of photosystem II[6].

MATERIALS AND METHODS

Ferredoxin was prepared from swiss chard according to Losada and Arnon[7]. Euglena gracilis var. bacillaris mutant 102 was obtained as described by Shneyour and Avron[8]. Chloroplasts of E. gracilis were prepared as described previously[9]. Spinach and pea chloroplasts were prepared as described for lettuce[10]. Chlorophyll was determined according to Arnon[13]. Tris-treatment was carried out as described by Yamashita and Butler[3]. Chloroplasts were treated with PMA as described by Honeycutt and Krogmann[11]. Photo-induced reduction of DCIP and $NADP^+$, and DPCN disproportionation were followed spectrophotometrically as described by Shneyour and Avron[12]. The kinetics of fluorescence were measured at 25° and recorded on a Tektronix Type 564B storage oscilloscope connected to an EMI 9592B photomultiplier. The high voltage for the photomultiplier was supplied by the high voltage unit of an Aminco-Chance dual wavelength spectrophotometer. Blue actinic light at the intensity of 1.75×10^4 erg $cm^{-2}sec^{-1}$ was obtained by passing the light of a 500 W slide projector lamp through a Corning C.S. 4-96 glass filter. The photomultiplier was protected from the actinic light by a Schott & Gen., Mainz 665 R.G. cut-off filter (>665 nm).

RESULTS

The results presented in Table I show that only DPC supports photoinduced electron transfer associated with photosystem II in 102. The Asc + HQ couple does not restore the activity of photosystem II in this mutant while it donates electrons to WT chloroplasts that have been treated with tris. This result indicates that the point affected by the mutation is

	$H_2O \rightarrow NADP^+$	Asc+HQ→$NADP^+$	$H_2O \rightarrow$DCIP	DPC→DCIP	Asc+DCIP→NADP
	% of Control				
Wild Type	100	100	100	100	100
Wild Type Tris-Treated	31	65	11	150	85
Mutant 102	5	6	16	87	142

Table I - Comparison of some light-induced photosynthetic electron transfer reactions in isolated chloroplasts of E. gracilis.

The 100% rates were in μmoles electrons mg chl^{-1} $hour^{-1}$ as follows: $H_2O \rightarrow NADP^+$, 71; Asc+HQ→$NADP^+$, 54; $H_2O \rightarrow$DCIP, 125; DPC→DCIP, 90; Asc+DCIP→$NADP^+$, 54. The reaction mixture contained in a final vol. of 3 ml the following compounds in μmoles: For $H_2O \rightarrow NADP^+$, 150 phosphate pH 6.5; 30 NaCl; 0.5 $NADP^+$ and saturating amount of ferredoxin; For Asc+HQ→$NADP^+$, 20 Ascorbate and 0.3 HQ were added to the previous reaction mixture; For Asc+DCIP→$NADP^+$, 0.1 DCIP and 10^{-2} DCMU replaced the HQ; For $H_2O \rightarrow$DCIP the same as for $H_2O \rightarrow NADP^+$ except 0.1 DCIP replaced $NADP^+$ and no ferredoxin was added; For DPC→DCIP the same as for $H_2O \rightarrow$DCIP except 5 DPC were added. Chloroplasts equivalent to 30 μg chlorophyll were added to each reaction.

between the points of electron donation to the water side of photosystem II by Asc + HQ and by DPC and that the latter point is closer to the reducing side of photosystem II. This situation is illustrated in Fig. 1. Measurements of the kinetics of light-induced fluorescence emission by chloroplasts of 102 (Fig. 2c,2d) as compared with fluorescence emission by chloroplasts of WT (Fig. 2a,2b) support the data presented in Fig. 1. It is assumed that the kinetics of fluorescence rise during illumination of chloroplasts from an initial level F_o to a final level F_∞ are dependent on the existence of "Q" the primary electron acceptor of photosystem II and reflect changes in its redox state[14,15]. From Fig. 2d it is clear that F_∞ of the mutant is lower than that of the

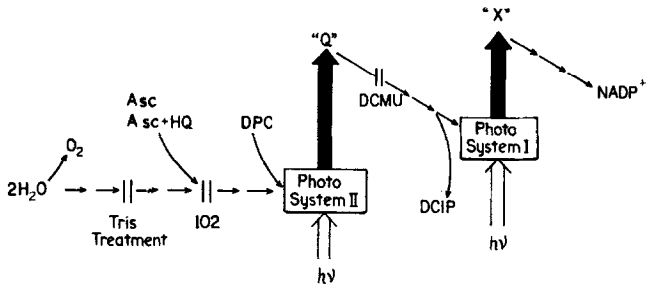

Fig. 1. Location of the block at the water side of the photosynthetic electron transport chain of 102 and the point of electron donation by DPC.

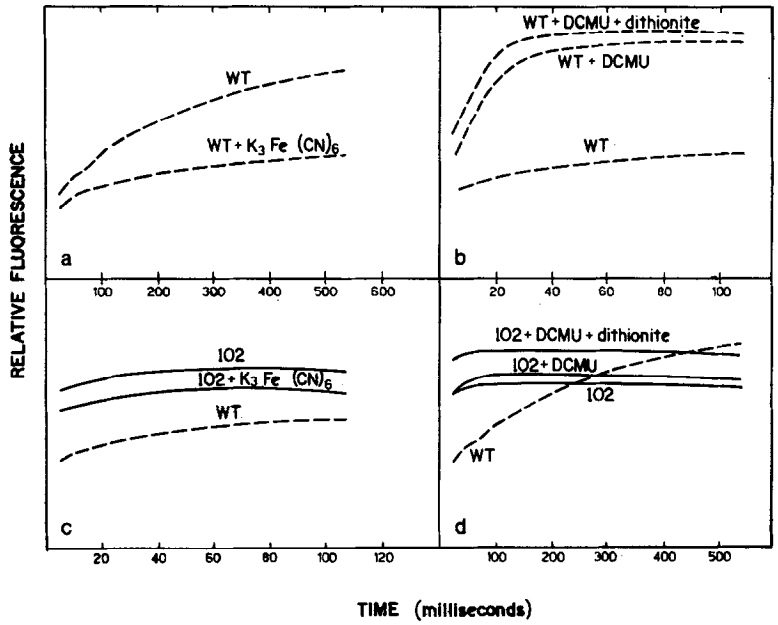

Fig. 2. The kinetics of light-induced fluorescence emission by chloroplasts of Euglena isolated from cells of the wild type and 102. $7.5 \cdot 10^{-3}$ µmoles DCMU, 1.5 µmoles $K_3Fe(CN)_6$ or a few grains of sodium dithionite, were added where indicated. The reaction mixture contained 500 µmoles sucrose, 250 µmoles NaCl, 125 µmoles Tris HCl at pH 8.0 and chloroplasts equivalent to 5 µg chlorophyll in a final volume of 2.5 ml.

WT while the kinetics of fluorescence rise is much faster. In contrast to its influence on the fluorescence by WT chloroplasts (Fig. 2b), DCMU does not significantly raise the fluorescent yield of chloroplasts of 102. While the addition of the oxidant

$K_3Fe(CN)_6$ does not result in a large decrease in the fluorescent yield of 102 (Fig. 2c), the addition of a strong reductant like sodium dithionite increases it to a larger extent and brings it to F_∞ shown by WT chloroplasts (Fig. 2b,2d). This result indicates that "Q" is mainly in an oxidized state in chloroplasts of the mutant, a fact which supports the location of the block in the photosynthetic electron transfer of the mutant at the site proposed in Fig. 1[14-17].

The point affected by mutation in the photosynthetic electron transfer chain of 102 is different from the one on the water side of photosystem II described in a mutant of Chlamydomonas reinhardi[6]. The latter mutant shows photosynthetic characteristics very similar to those described for tris-treated or heat-treated chloroplasts[3-5]. Chloroplasts of 102 are in many respects similar to KCl-treated chloroplasts[18].

Recently[11], the donation of electrons by DPC to the photosynthetic electron transport chain[12,19] was reported by Honeycutt and Krogmann to be located at a point closer to the reaction site for water than the one which I propose. The location of the site proposed by these authors for electron donation to photosystem II was based on the inhibition of photosystem II by PMA and the inability of DPC to restore photosystem II activity. It was therefore decided to reinvestigate the effect of DPC on photosystem II inhibition by PMA. The results obtained using DPC to restore the photosynthetic electron transport inhibited by PMA treatment are presented in Table II. In these experiments DPC restored a significant part of the photosystem II associated DCMU-sensitive electron transport in both pea and spinach chloroplasts. As it is known that DPC forms a complex with Hg^{++} ions[20], this could be the cause of the inhibition of photosystem II when

Table II - The effect of DPC on DCIP reduction by pea and spinach chloroplasts treated with PMA.

Type of Chloroplasts	Additions	Rate of DCIP Reduction (μmoles mg chl^{-1} hour^{-1})
Untreated pea chloroplasts	none	70
PMA-treated pea chloroplasts	none	14
	10 μm DCMU	0
	100 μm DPC	33
	200 μm DPC	41
	400 μm DPC	45
	200 μm DPC + 10 μm DCMU	12
Untreated spinach chloroplasts	none	98
PMA-treated spinach chloroplasts	none	9
	100 μm DPC	30
	200 μm DPC	48
	400 μm DPC	46
	200 μm DPC + 10 μm DCMU	18

The reaction mixture contained in a total volume of 1 ml the following compounds in μmoles: phosphate at pH 8.0, 9; $MgCl_2$, 0.9; sorbitol, 27; DCIP, 20 and chloroplasts equivalent to 5 μg chlorophyll.

Table III - The effect of PMA treatment on DPCN reaction in pea chloroplasts.

Type of Chloroplast	Additions	Rate of DPCN Disappearance (μmoles μg chl^{-1} hour^{-1})
Untreated pea chloroplasts	100 μm DPCN	932
	200 μm DPCN	668
PMA-treated pea chloroplasts	100 μm DPCN	23
	200 μm DPCN	598

The reaction mixture is the same as that given in Table II except that DCIP was omitted.

DPC was used as an electron donor. Thus the effect of PMA on the disproportionation of DPCN[12] was studied, since like DPC, DPCN forms a complex with Hg^{++} ions[20]. This reaction should not be affected by PMA unless an inhibition results from the formation of a complex between DPCN and the Hg^{++} ions since only components of photosystem I that are rather insensitive to PMA are involved[11]. The results presented in Table III show clearly that this reaction is even more sensitive to PMA treatment than the photoreduction of DCIP from water. Nevertheless, it could be restored by using higher concentrations of DPCN. As PMA-treated chloroplasts most probably contain bound Hg^{++} ions it seems that the inhibition of both reactions might be explained by the formation of an inactive complex between DPC or DPCN and the Hg^{++} ions bound to the chloroplast. The ability of higher concentrations of DPCN and to a lesser extent those of DPC to overcome the effect of PMA treatment supports this assumption.

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