Vol. 98, No. 2, 1981 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 30, 1981 Pages 541-547

P_{700} SENSITIZATION BY LOW CONCENTRATION OF DCMU IN ISOLATED PEA CHLOROPLASTS

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Received December 11,1980

Summary: Using steady-state relaxation spectrophotometric technique a P_{700} component (t 1/2 ∿20 ms) has been detected which was sensitized by low concentration (10^{-7}M) DCMU in isolated broken chloroplasts of pea. The relative quantum yield of electron flux through DCMU-sensitized P_{700} was similar to that with methyl viologen or NADP as terminal electron acceptor and water as electron donor. Kinetic analysis showed that a small fraction (10%) of the total P_{700} reaction centers was sensitized by low DCMU.

INTRODUCTION

P_{700}, the photochemical reaction center of the one of the two known photosystems in higher plants, has been shown to exist in three kinetic forms with half-times of ∿20 ms, ∿200 μs and ∿20 μs (1, 2 for review). The functional relationship of these components with various partial photoreactions is not known. Such information would be necessary to elucidate the role of these different kinetic forms of P_{700} in photosynthetic electron transport. In this communication a DCMU-sensitized P_{700} species with ∿20 ms half-time has been described which has been detected under steady-state conditions of illumination in isolated chloroplasts.

MATERIALS AND METHODS

Class II chloroplasts were isolated from dwarf peas (Greater Progress) as described earlier (3). Electron flux through P_{700} and its relaxation time were

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ABBREVIATIONS: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MV, methyl viologen; DCIP, 2,6-dichloroindophenol; NADP, Nicotiaamide adenine dinucleotide phosphate; ASC, Ascorbate.

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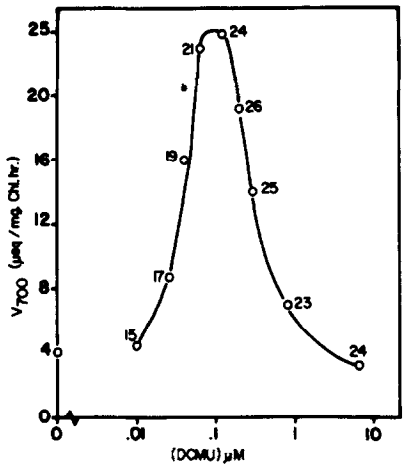

Fig. 1. Electron flux through P_{700} vs DCMU concentration. Chloroplasts were were suspended in 2.5 ml of 15 mM tricine at pH 7.5, 50 mM sucrose, 20 mM NaCl, 2.5 mM NH_4Cl. The chlorophyll concentration was 15 μg/ml. Actinic light was filtered through a broad-band interference filter transmitting between 530 and 645 nm. Intensity was 1.5 x 10^3 erg. $cm^{-2}sec^{-1}$. The extinction coefficient for rate calculation was 65 $mM^{-1}cm^{-1}$ (8). Modulation frequency was 39 rads/sec. The number against each point on the graph represent the relaxation time in msec.

monitored by steady-state relaxation spectrophotometer (4,5). The rate calculations are based on measurements of absorption changes and relaxation times observed at 700 nm. All measurements were made at low light intensities.

RESULTS AND DISCUSSION

Stimulation of electron flux through P_{700} by low DCMU:

In isolated broken chloroplasts suspended in a medium containing no added electron acceptors, electron flux through P_{700} (V_{700}) under weak actinic illumination was very low (Fig. 1). In presence of increasing concentration of DCMU the flux increased reaching an optimum value at 10^{-7} M DCMU, and then decreased to a value similar to that in absence of DCMU at 10^{-5} M DCMU. The increase by 10^{-7} M DCMU was 4-5 fold of the value obtained in the absence of DCMU. The dark relaxation time (inverse of the rate constant) did not change markedly with DCMU concentration, it increased from 15 msec at 10^{-8} M to 24 msec at 10^{-7} M and remained practically unchanged at higher concentrations. The observed stimulation of V_{700} by DCMU is unusual in the

Table I

Electron flux through P_{700} and its relaxation time with various additives.

Addition	V_{700} usec / mg.Chl.hr	T (msec)
0.25 mM NADP	24	21
0.2 mM MV	25	28
10^{-7} M DCMU	25	20
1 mM ASC + 10 μM DCIP + 5 μM DCMU + 0.2 mM MV	23	29
10^{-7} M DCMU + 0.2 mM MV	11	27

The basic reaction mixtures and other conditions as in Fig. 1. 30 μg/ml ferredoxin was added in case of NADP.

sense that DCMU is neither known to be a non-cyclic electron acceptor nor a cofactor of cyclic electron transport.

Comparison with MV and NADP mediated V_{700}:

V_{700} with 10^{-7} M DCMU was almost of identical magnitude to that obtained with NADP or MV as terminal electron acceptor and water or $DCIPH_2$ as electron donor under short wavelength illumination of limiting intensity (Table 1). These results indicated that the relative quantum yield of the flux with 10^{-7} M DCMU was similar to that with NADP or MV. It is to be noted that the fluxes were not additive when both MV and 10^{-7} M DCMU were present (Table 1). These two observations - similar quantum yields and non-additive nature of the fluxes, indicated that low DCMU was sensitizing a P_{700} system which competed for the same absorbed quanta with P_{700} system functioning in presence of MV. Moreover, from the observation that the flux in presence of MV and 10^{-7} M DCMU together was less (by 50%) than in presence of MV or 10^{-7} M DCMU alone, it appears that in presence of MV, 10^{-7} M DCMU acted as a usual PS II inhibitor. The rate of NADP reduction ($H_2O \rightarrow$ NADP) was inhibited by 10^{-7} M DCMU to a similar extent (by $\sim$ 50%) (Data not shown).

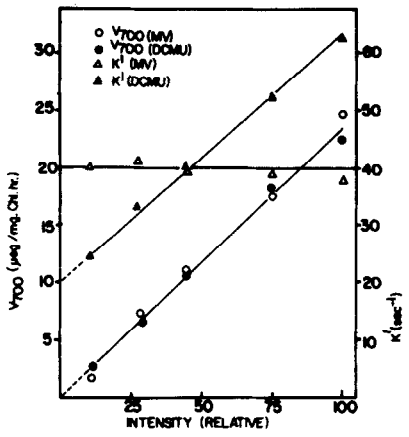

Fig. 2. V_{700} and observed rate constant (K^1) as a function of intensity. Reaction conditions as in Fig. 1, except that 0.2 mM MV or 10^{-7} M DCMU was added. Light intensity was adjusted by neutral density glass filters.

Kinetic analysis:

In presence of MV the flux was a linear function of the intensity of illumination, the rate constant, K, remaining unchanged (Fig. 2). This means that as the intensity increased, the number of P_{700} reaction centers participating in electron transport increased proportionally without altering the turnover rate. In DCMU-sensitized system K was not constant; it increased linearly with the same slope as that of the increase in flux. This indicated that the reaction centers were turning over faster with increasing intensity in a linear fashion, the number of participated reaction centers remaining unaltered. If the number of reaction centers participating would increase along with increase of K, one would expect a second order relation between flux and intensity. With this assumption, that in the DCMU system the number of reaction centers functioning did not change with intensity, it is possible to calculate the concentration of P_{700} sensitized by 10^{-7} M DCMU. The following analysis shows that only 10% of the total P_{700} was functioning in presence of 10^{-7} M DCMU.

The photochemical reaction of P_{700} oxidation can be given by:

$$P + h\nu \underset{K}{\overset{I}{\rightleftharpoons}} q$$

where P and q represent the reduced and oxidized forms of P_{700}; I, the intensity of illumination and K the rate constant of dark relaxation of q to P. The net rate of formation of q is given by:

$$\frac{dq}{dt} = IQ - qK$$

where IQ = rate of formation of q, qK = rate of disappearance of q, and Q = $\alpha Q_i \frac{P}{P_T}$, α being the fraction of the absorbed intensity going into the DCMU sensitized system, Q_i being the intrinsic quantum yield of quanta absorbed by this system, and $P_T = p + q$, the total number of reaction centers sensitized by low DCMU.

Applying the steady-state condition ($\frac{dq}{dt} = o$) and rearranging the terms in above equations, one obtains:

$$q = \frac{I \alpha Q_i}{\frac{I \alpha Q_i}{P_T} + K}$$

Therefore the flux is given by:

$$qK = \frac{I \alpha Q_i K}{\frac{I \alpha Q_i}{P_T} + K}$$

Putting $Q_i = 1$, which is reasonable to assume, the apparent (i.e. observed) rate constant K^I from the above equation is given by:

$$K^I = \frac{I\alpha}{P_T} + K$$

The slope of the plot I vs K^I (Fig. 2) gives α/P_T, and the extrapolation of the straight line to zero intensity gives K. Thus K = 20 sec^{-1} (K scale is on the right side of the figure). Now taking the flux ($V_{700} = I\alpha$) at any intensity and the corresponding K^I, P_T can be calculated. Thus at 45% intensity K^I = 40 sec^{-1} and V_{700} = 21 μeq/mg Chl. hr. Substituting these values in the last equation, P_T comes out as 2.8 x 10^{-4} μeq/mg Chl i.e. 2.8 x 10^{-4} P_{700} reaction centers per mg Chl. This value is approximately 10% of the estimated

Table II

Effect of Mg^{++} on V_{700} and T in DCMU-sensitized P_{700}.

Addition	V_{700} $\frac{\mu sec}{mg\ Chl\ hr}$	T (msec)
Short wavelength		
0.25 mM NADP	23	20
NADP + 5 mM $MgCl_2$	4.5	25
10^{-7} M DCMU	23	23
DCMU + $MgCl_2$	12	26
Far red (696 nm)		
0.25 mM NADP	21	20
NADP + 5 mM $MgCl_2$	24	19
10^{-7} M DCMU	24	24
DCMU + $MgCl_2$	12	22

The intensities of the two kinds of illumination were adjusted to show same rate of NADP reduction in H_2O → NADP reaction. Other conditions as in Table I.

P_{700} concentration using one P_{700} per 400 chlorophylls (4). Thus a small fraction of the total P_{700} centers was sensitized by DCMU.

Effects of $MgCl_2$ on DCMU sensitized V_{700}:

Mg^{++} has been shown to affect V_{700} (ms component) under certain conditions. For instance, V_{700} in H_2O → NADP (or MV) reaction under short wavelength illumination would decrese by 80% when 5 mM Mg^{++} was added to chloroplasts originally suspended in low salt medium (6). Under far red illumination, However, Mg^{++} had no effect on V_{700}. Table 2 confirms these observations and compares the effects of Mg^{++} on the DCMU system. Two differences were noted: first, Mg^{++} inhibited DCMU-sensitized V_{700} under both kinds of illumination, and second, the inhibition under short wavelength illumination was only 50%. This suggests that DCMU-sensitized P_{700} was not identical to

P_{700} in $H_2O \rightarrow$ NADP reaction. As a side point, in $DCIPH_2 \rightarrow$ MV (or NADP) reaction, Mg^{++} inhibited V_{700} by 50% under both kinds of illumination (7).

The mechanism by which low DCMU sensitizes P_{700} is not known. Since DCMU is not expected to mediate a linear electron transport, it is likely to sensitize certain form of cyclic electron transport through P_{700}. An appropriate redox poising of the illuminated thylakoid membranes in presence of low DCMU may be responsible in sensitizing this cyclic P_{700} photosystem.

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