The Effects of 3-(3,4-Dichlorophenyl)-1,1-dimethylurea on the Photosynthetic Oxygen Complex

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In the presence of trypsin and ferricyanide as external electron acceptor, lettuce chloroplasts are resistant to DCMU, showing that the inhibitory site of DCMU is only situated on the acceptor side of photosystem II. However, kinetic properties of the oxygen evolving complex are modified at non-saturating concentrations of DCMU. These changes are interpreted in terms of a model with two distinct charges separation systems on the same center: the auxiliary donor-acceptor system DQ_L implicated in the transitions $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$ would be much less affected by DCMU than the main donor-acceptor system YQ_H after the first flash.

Introduction

DCMU inhibits photosynthetic electron transport by interrupting electron flow at the reducing side of PS II [1]. This inhibition occurs at the level of a protein bound plastoquinone Q_B [2]. DCMU induces a decrease in the redox potential of Q_B [3], making the transfer of electrons from the primary acceptor Q or Q_A thermodynamically unfavorable [2, 3]. However, DCMU not only decreases the amount of centers able to evolve O_2, but apparently also changes the kinetic properties of the remaining active centers [4]; the turn-over time of the transition $S_2 \rightarrow S_3$ is strongly slowed down, but only after the first flash; the deactivation kinetics of the S_2 and S_3 states are also modified [5]; in the O_2 yield pattern, a relatively high second O_2 yield is characteristic of an incomplete DCMU inhibition (Fig. 2 and [6]). These changes could either come from a binding of DCMU on the oxygen evolving complex or from an indirect effect of the DCMU-binding on the acceptor side. The following experiments answer to these alternatives and clearly show that no direct DCMU-inhibition occurs on the donor side. The existence of an auxiliary donor-acceptor system DQ_L besides the main donor-acceptor system YQ_H in the same center explains the O_2 properties of the centers in the presence of a non-saturating concentration of DCMU (Q_L and Q_H are defined as in [7]). The slow rise in the saturation curve of the transition $S_2 \rightarrow S_3$ at high flash energy has revealed the existence of an auxiliary donor D, efficient for O_2 evolution by increasing slightly the quantum efficiency of $S_2 \rightarrow S_3$ [8].

Our experiments show that after a first flash given to dark-adapted chloroplasts, DCMU blocks the electron transfer from the primary acceptor Q_L associated with the donor D less than that from Q_H coupled to the main donor Y. This is in agreement with the idea that the high potential necessary to reduce Q_H prevents communication between Q_H and the pool in the presence of DCMU.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS II, photosystem II.

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Material and Methods

Fresh chloroplasts were prepared from market lettuce as in [4], and suspended in medium containing 0.4 M sucrose, 10 mM NaCl, 3 mM $MgCl_2$ and 50 mM N-tris(hydroxymethyl) methylglycine(TRICINE) buffered to pH 7.8. A rate electrode was used for O_2 flash yield measurements as previously described [4].

Results and Discussion

In order to detect an eventually DCMU-effect on the donor side of PS II, the chloroplasts were mildly treated with trypsin in order to remove the Q_B protein, allowing ferricyanide to accept electrons from the Q_A site [9]. Fig. 1 shows the O_2 patterns successively observed in chloroplasts: (1) without any addition, (2) after trypsin treatment and ferricyanide addition, (3) and finally after 10^{-5} M DCMU. No inhibition of the O_2 yields was observed in the presence of DCMU; the yields after many flashes in the series were even higher, indicating that electrons

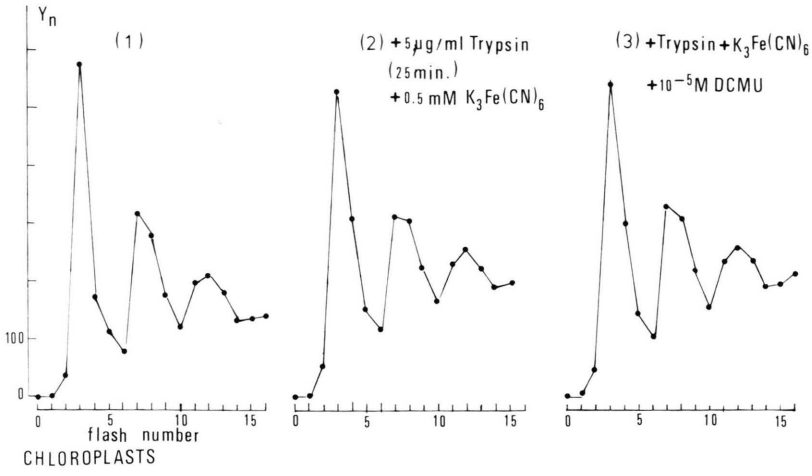

Fig. 1. O_2 flash yield as a function of flash number in dark adapted chloroplasts (stroboslave flashes, spacing: 400 ms) – (1) in untreated chloroplasts (2) in chloroplasts treated with 5 µg/ml trypsin (25 min) and 0.5 mM ferricyanide (3) finally, also with 10^{-5} M DCMU.

on the acceptor side were efficiently accepted. This result indicates that the changes observed in the properties of O_2 evolution at unsaturated concentration of DCMU [4] come from an indirect action of DCMU blocking on the acceptor side. The time during which the chloroplasts remain resistant to DCMU and trypsin depends on batches: it may be as long as one hour like the batch of Fig. 1, or as short as 15 minutes. After this DCMU-resistance time, the characteristic O_2 pattern with a high second O_2 yield may again be observed.

The high second O_2 yield in the pattern observed in the presence of DCMU (Fig. 2b) is not produced by double advancement of the S states because the strong sigmoidal curve expected in the case of two successive photochemical reactions is not observed in the saturation curve of the second O_2 yield (Y_2) as a function of the energy of the first flash (I_1) as in Fig. 2b. This is only explained by the presence of a large amount of non deactivated S_2 state centers in the dark preceding the flash sequence. The saturation curve $Y_2(I_1)$ in the presence of DCMU is qualitatively similar to the saturation curve $S_2 \rightarrow S_3$ measured without DCMU [10], characterized by a slow rise at the highest energy (100%) which is strictly saturating for the other transitions. However, quantitatively, in DCMU poisoned chloroplasts (5×10^{-6} M), the slope of this rise is much larger. Re-

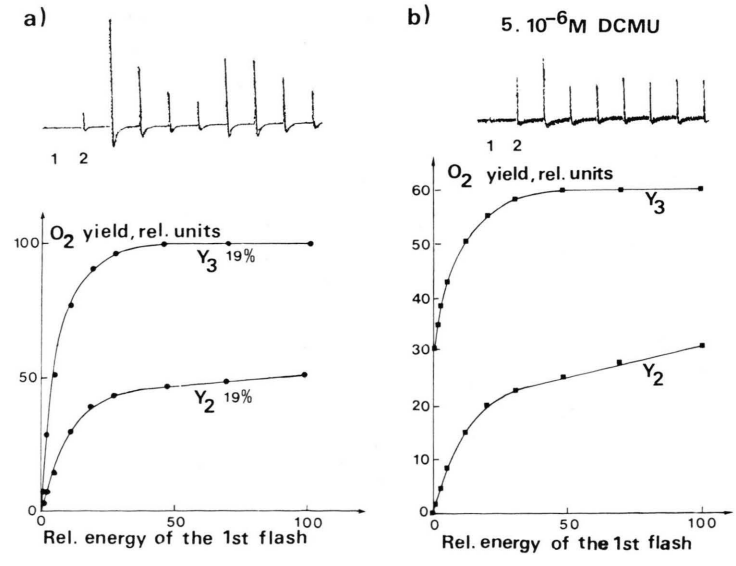

Fig. 2. a) In intact chloroplasts, O_2 yields evolved on the second (Y_2) and third (Y_3) non-saturating (19%) flashes as a function of the light intensity of the first flash. b) In 5×10^{-6} M DCMU poisoned chloroplasts, O_2 yields on the second (Y_2) and third (Y_3) high energy (100%) flashes as a function of the light intensity of the first flash; dark adapted (5 min) chloroplasts, spacing 400 ms. Upper part: O_2 flash yield as a function of flash number in untreated and DCMU poisoned chloroplasts (dark adapted (5 min) chloroplasts, high energy flashes, spacing 400 ms).

cently [8], we have suggested that this slow increase of the amount of S_3 state centers at high flash energy could originate from a photoreaction converting S_2 into S_3 using another donor than that (Y) involved in the main pathway: the auxiliary donor D. In Fig. 2, for a relative flash energy of 100%, 30% of photoreactions $S_2 \rightarrow S_3$ have used D in DCMU-treated chloroplasts instead of 10% in intact chloroplasts.

The least square fitting method [8] applied to the O_2 yield pattern in DCMU-treated chloroplasts (Fig. 2b) leads to the conclusion that there exists only an important miss on one transition (S state miss distribution: 0, 0, 0.9, 0); this result rigorously agrees with the S state saturation curves in Fig. 2: the 100% energy flashes saturated all the transitions except the $S_2 \rightarrow S_3$, where the large miss decreases continuously with increasing light [8].

We have interpreted these results with the following ideas:

1) Each center has two different charge separation systems: the main YQ_H and the auxiliary DQ_L where two primary acceptors of different midpoint potentials Q_H and Q_L [7] are associated with the two different donors Y and D.

2) Without any contribution of the auxiliary donor D, the quantum efficiency of the transition $S_2 \rightarrow S_3$ is low, $\leqq 0.5$ in intact chloroplasts [8].

The large miss with only the system YQ_H on the transition $S_2 \rightarrow S_3$ could be due to a conformation change of the O_2-evolving protein (a rotation, as shown in Fig. 3), in order to accumulate in the S_2 state the second water molecule necessary to prepare the O_2 formation. In the active S_2 state, Y is bound to the free site receiving the positive charge in the transition $S_2 \rightarrow S_3$. In the inactive S_2 state corresponding to the protein conformation of the transition $S_1 \rightarrow S_2$, Y is in front of an already occupied site. In this last configuration, the auxiliary donor D is in front of a free site so that it can give a positive charge to perform the $S_2 \rightarrow S_3$ transition when Y is in an inactive state or position. The quantum efficiency of oxidation of donor D in the S_2 state is > 10 times smaller than that of Y. This could be due to the fact that this oxidation is a

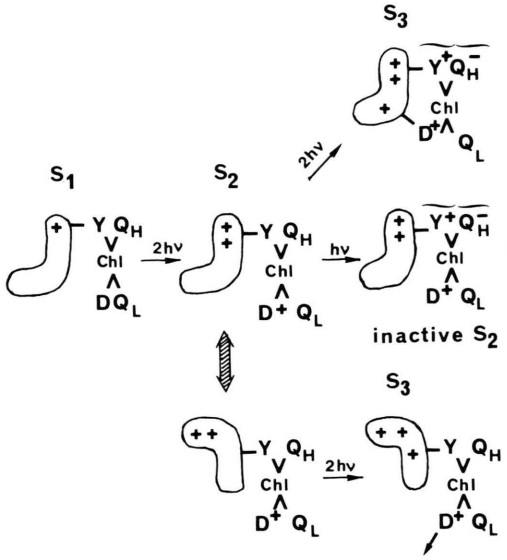

Fig. 3. Proposed model explaining the rôle of the donors Y, D, and acceptors Q_H, Q_L, especially in a sequence of high energy flashes. In the S_2 state, the O_2 evolving protein switches continuously between the two states: active and inactive. Chl: chlorophyll.

second oxidation, $D^+ \rightarrow D^{++}$, after the first oxidation observed in the transition $S_1 \rightarrow S_2$ by several experiments: fluorescence [11] absorbance changes near 300 nm [12], EPR [13]. The donor D is a likely candidate for the Signal II species [14–16]; especially D^+ could correspond to Signal II slow, the decay of which is sufficiently slow ($t\frac{1}{2} = 1$ hour) to preclude an essential rôle in water oxidation [14]. The formation of the radical giving rise to Signal II slow is not inhibited by DCMU [14].

The faster rise of Y_2 (I_1) in Fig. 2 shows that 5×10^{-6} M DCMU inhibits less the conversion $S_2 \rightarrow S_3$ by the intermediate of DQ_L than that by the main system YQ_H. For this reason, the slow component of the turn-over time of $S_2 \rightarrow S_3$ at intermediate concentration of DCMU after a first flash and not after the following flashes [4] corresponds to a DQ_L reaction. The strong miss on $S_2 \rightarrow S_3$ with DCMU (0.9 instead of 0.5 in intact chloroplasts) proves that DCMU blocks selectively the transition $S_2 \rightarrow S_3$ by interrupting the electron transfer from Q_H and less from Q_L especially after the first flash.

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