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

Marie-José Delrieu
Laboratoire de Photosynthèse, C.N.R.S., 91190 Gif-sur-Yvette (France)


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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.

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

Reprint requests to M.-J. Delrieu.

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Introduction

DCMU inhibits photosynthetic electron transport by interrupting electron flow at the reducing side of PS II [1]. This inhibition occurs at a 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.

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...
on the acceptor side were efficiently accepted. This result indicates that the changes observed in the properties of O₂ 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 O₂ pattern with a high second O₂ yield may again be observed.

The high second O₂ 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 O₂ yield (Y₂) as a function of the energy of the first flash (I₁) as in Fig. 2b. This is only explained by the presence of a large amount of non deactivated S₂ state centers in the dark preceding the flash sequence. The saturation curve Y₂ (I₁) in the presence of DCMU is qualitatively similar to the saturation curve S₂ → S₃ 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-
cently [8], we have suggested that this slow increase of the amount of S1 state centers at high flash energy could originate from a photoreaction converting S2 into S3 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 S2 → S3 have used D in DCMU-treated chloroplasts instead of 10% in intact chloroplasts.

The least square fitting method [8] applied to the O2 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 S2 → S3, 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 YQH and the auxiliary DQL where two primary acceptors of different midpoint potentials QH and QL [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 S2 → S3 is low, ≈ 0.5 in intact chloroplasts [8].

The large miss with only the system YQH on the transition S2 → S3 could be due to a conformation change of the O2-evolving protein (a rotation, as shown in Fig. 3), in order to accumulate in the S2 state the second water molecule necessary to prepare the O2 formation. In the active S2 state, Y is bound to the free site receiving the positive charge in the transition S2 → S3. In the inactive S2 state corresponding to the protein conformation of the transition S1 → S2, 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 S2 → S3 transition when Y is in an inactive state or position. The quantum efficiency of oxidation of donor D in the S2 state is > 10 times smaller than that of Y. This could be due to the fact that this oxidation is a second oxidation, D+ → D++, after the first oxidation observed in the transition S1 → S2 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 (τ1/2 = 1 hour) to preclude an essential role 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 Y2 (Ii) in Fig. 2 shows that 5 × 10−6 M DCMU inhibits less the conversion S2 → S3 by the intermediate of DQL than that by the main system YQH. For this reason, the slow component of the turn-over time of S2 → S3 at intermediate concentration of DCMU after a first flash and not after the following flashes [4] corresponds to a DQL reaction. The strong miss on S2 → S3 with DCMU (0.9 instead of 0.5 in intact chloroplasts) proves that DCMU blocks selectively the transition S2 → S3 by interrupting the electron transfer from QH and less from QL especially after the first flash.