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DEACTIVATION OF OXYGEN PRECURSORS IN PRESENCE OF 3(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA AND PHENYLURETHANE

B. BOUGES-BOCQUET, P. BENNOUN and J. TABOURY

Institut de Biologie Physico-Chimique, 13, rue Pierre et Marie Curie, 75005 Paris (France)

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SUMMARY

Non-saturating concentrations of 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and phenylurethane affect the deactivation reactions of O_2 precursor states.

1. In *Chlorella*, S_3 deactivation is slowed whereas S_2 deactivation is accelerated.

These data show that a center blocked by an inhibiting molecule can still produce oxygen, if in State S_3, but cannot if in State S_2.

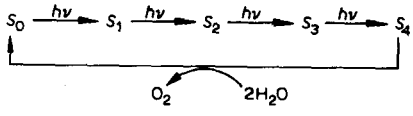
Furthermore, Q^-, the primary electron acceptor of System II, is the substrate for S_3 deactivation. This deactivation implies a back electron transfer from the pool of acceptor A to Q.

2. In spinach chloroplasts, DCMU accelerates both S_2 and S_3 deactivation.

However, these different effects of action of DCMU in *Chlorella* and spinach chloroplasts explain the discrepancy between the results of Duysens (1972, *Proc. 2nd Int. Congr. Photosynth. Res.*, pp. 19–25) and Rosenberg, Sahu and Bigat (1972, *Biophys. J.* 12, 839–850).

INTRODUCTION

According to the model of Kok *et al.*[1], four consecutive photochemical reactions per System II center are necessary for the production of one molecule of oxygen:



S states differ by the number of oxidizing equivalents stored. The dark steps occurring between two photochemical reactions are not shown in this scheme.

States S_0 and S_1 are stable in the dark whereas States S_2 and S_3 deactivate to S_1 and S_0[2,3].

The pool of electron acceptor, A, located between the two photosystems, and Q, the primary acceptor of System II, are two possible substrates of the deactivation reactions[4]. It was, therefore, of interest to study the deactivation reactions in the presence of inhibitors which block the electron transfer between Q and A. The effects

Abbreviation: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea.

of 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and phenylurethane are reported in the present study.

MATERIALS AND METHODS

Chlorella pyrenoidosa are grown on Knop medium with Arnon's trace elements A5 and B6. Before use, cells are suspended in 0.05 M phosphate buffer (pH 6.4) containing 0.1 M KCl. Spinach chloroplasts are prepared according to the method of Avron[5] and are suspended in 0.05 M Tris–HCl buffer (pH 7.5) containing 0.01 M NaCl, 0.1 M KCl, 0.4 M saccharose, 9 g/l serum albumin, 0.2 mM $NADP^+$ and 1 μM ferredoxin.

Oxygen evolved by a flash is measured by the polarographic method described by Joliot *et al.*[6,7]. We used a flash lamp (General Radio Strobotron: duration 3 μs at one-third peak intensity). The fluorescence apparatus has been described by Bennoun[8].

Kinetics of deactivation

Dark adapted *Chlorella* or spinach chloroplasts are in State S_1 or S_0. Preillumination with two saturating flashes transforms the centers in State S_1 to State S_3. A detecting flash of saturating intensity is given at time t after the second preilluminating flash. The amount of oxygen evolved after the detecting flash is proportional to the number of centers still in State S_3 at time t ($S_3(t)$).

After only one preilluminating flash, centers in State S_1 are transformed to State S_2. Two detecting flashes separated by 180 ms are given at time t after the preillumination. The amount of oxygen evolved after the last flash is proportional to the number of centers that were still in the S_2 state at time t ($S_2(t)$).

RESULTS AND DISCUSSION

(1) *Kinetics of deactivation of S_2 and S_3 in Chlorella*

The deactivation of S_3 in *Chlorella* in the presence of non-saturating concentrations of DCMU is decreased whereas the deactivation of S_2 is accelerated. Comparable data are observed in presence of phenylurethane. However, for the same level of inhibition of oxygen evolution, phenylurethane induces the largest modification of the deactivation pattern (Fig. 1).

When deactivation is studied following non-saturating flashes which excite only 25% of the centers, no change in the deactivation pattern is observed (Fig. 1a, black crosses). The modification of the deactivation pattern observed in the presence of inhibitors is not correlated to the fact that a reduced number of centers are reacting.

The slowing of S_3 deactivation and the acceleration of S_2 deactivation are observed when active centers are blocked by either DCMU, orthophenanthroline (not presented on the figures), or phenylurethane. All of these compounds are known to inhibit electron transfer between Q and A[9,10].

(2) *Turnover of the inhibiting molecules in Chlorella*

For a given level of inhibition, the amplitude of the slow phase of S_3 deactivation (and the acceleration of S_2 as well) is more pronounced in the presence of

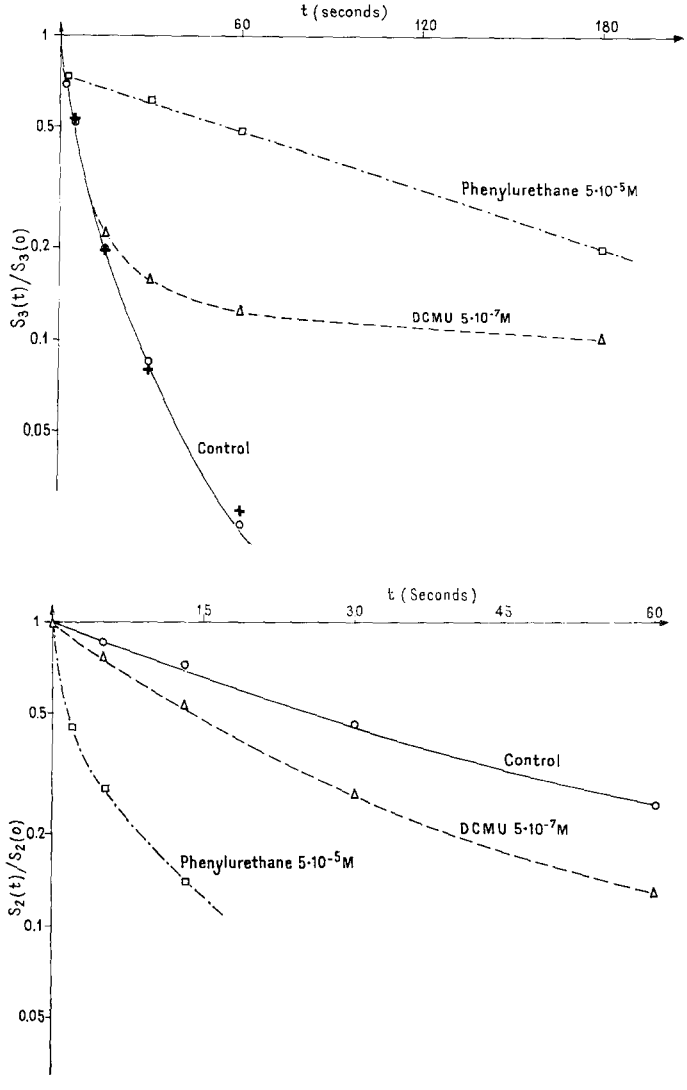


Fig. 1. Deactivation of S_3 (a) and S_2 (b) in *Chlorella* (log scale). ○—○, control. △—△, $5 \cdot 10^{-7}$ M DCMU. The inhibition ratio of oxygen evolution was 75%. □—□, $5 \cdot 10^{-5}$ M phenylurethane. The inhibition ratio of oxygen evolution was 75%. +—+, with non-saturating flashes which excite only 25% of the centers.

phenylurethane than in the presence of DCMU. Both compounds inhibit electron transfer between Q and A, but it is interesting to note that their rates of turnover are not the same.

This conclusion results from the following experiment. The maximum level of fluorescence attained in the light in the presence of inhibitors like DCMU, does not return to the minimum level in the dark if hydroxylamine is also present[8]. However, when non-saturating concentrations of DCMU (or phenylurethane) are used, Q^- re-oxidize very rapidly, in the non-inhibited centers, because of the very rapid transfer

from Q to A. In the inhibited centers, the turnover of the DCMU (or phenylurethane) molecules allows some electron transfer from Q to A to take place and a slow decrease of the fluorescence yield is observed in the dark. Fig. 2 shows that this decrease of the fluorescence yield in the dark is 20 times faster in the presence of phenylurethane than in the presence of DCMU.

The difference in turnover times is also apparent from the pattern of oxygen evolution. Dark-adapted *Chlorella*, subjected to a sequence of short saturating flashes show damped oscillations of oxygen yield, of period 4[1,12].

The damping of the oscillations suggests that some centers do not react during a saturating flash. A partial inhibition of the oxygen evolution will not increase the damping, if the inhibited centers are the same for all the flashes of the sequence. Kok *et al.*[1] showed that DCMU induces no additional damping which means that the turnover of DCMU molecules is long compared to the time between two flashes (300 ms). On the other hand, in the presence of phenylurethane, the damping is increased indicating a turnover time of the same order as the time between two flashes.

Thus, the faster the turnover of the inhibiting molecules, the more pronounced the slow phase of S_3 deactivation and the acceleration of S_2 deactivation.

(3) *Interpretation of the mode of action of DCMU and phenylurethane in Chlorella*

When the two preilluminating flashes are given, the centers to which no inhibiting molecules are bound, reach State S_3. When inhibiting molecules get bound to these centers before they deactivate but after the electrons are transferred from Q to A, they become stabilized as long as the inhibitor is bound. Deactivation of State S_3 is thus slowed because an electron transfer from A to Q is required, Q being the substrate of the reaction.

When bound to an inhibitor molecule, a center in State S_2 will be unable to produce oxygen, if we assume that only one photochemical reaction is possible in

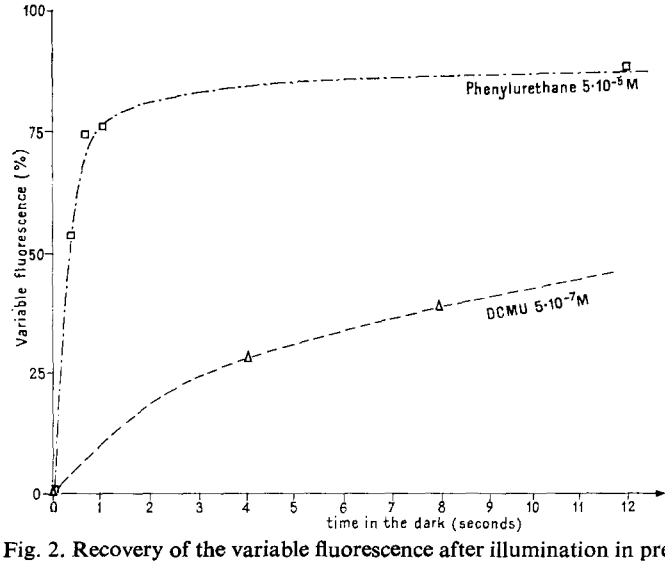
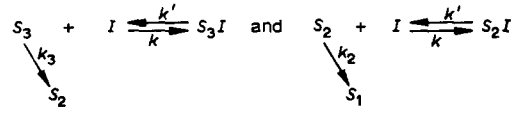


Fig. 2. Recovery of the variable fluorescence after illumination in presence of $5 \cdot 10^{-5}$ M hydroxylamine, on *Chlorella*. △—△, $5 \cdot 10^{-7}$ M DCMU. □—□, $5 \cdot 10^{-5}$ M phenylurethane.

State S_2 in the presence of the inhibitor. This explains the apparent acceleration of S_2 deactivation.

This can be summarized by the following scheme:



The reactions $S_3 \xrightarrow{k_3} S_2$ and $S_2 \xrightarrow{k_2} S_1$ are the deactivation in absence of inhibitor. I represents an inhibiting molecule. Oxygen can be evolved from State S_3I after a flash whereas no oxygen can be produced from State S_2I.

The rate of reactions $S+I \underset{k}{\overset{k'}{\rightleftarrows}} SI$ may be calculated out of two of the four following experimental results. (a) The slope of S_2 deactivation. (b) The amplitude of the fast phase of S_3 deactivation. (c) The slope of the slow phase of S_3 deactivation. (d) The inhibition ratio $SI/(S+SI)$ (inhibition ratio of oxygen in stationary conditions).

The results calculated by these different characteristics are coherent. The half-time of the reaction $S+I \rightarrow SI$ is 24 s for $5 \cdot 10^{-7}$ M DCMU, 1.5 s for $5 \cdot 10^{-5}$ M phenylurethane. The half-time for the reaction $SI \rightarrow S+I$ is 82 s for $5 \cdot 10^{-7}$ M DCMU, 6 s for $5 \cdot 10^{-5}$ M phenylurethane.

The turnover deduced from fluorescence measurement is faster for both DCMU and phenylurethane. However, firstly, the fluorescence is not linearly correlated to the concentration of Q^-. Secondly, the experimental conditions are different (hydroxylamine is present).

For the theoretical curves (Fig. 3), we assumed exponential deactivation for S_2 and S_3, to simplify the calculation, and verified the relation:

$$k[I]+k'+k_2 \gg \frac{k'k_2}{k[I]+k'+k_2}$$

The curves obtained with this scheme fit qualitatively with the experimental results (Fig. 1), so it is likely that, on *Chlorella*, in this concentration range, DCMU has only one site of action.

(4) *Kinetics of deactivation of S_2 and S_3 in spinach chloroplasts*

In the presence of non-saturating concentrations of DCMU, spinach chloroplasts display a faster deactivation for both S_2 and S_3 (Fig. 4). This is in agreement with recent experiments of Renger (personal communication). The acceleration of both S_2 and S_3 deactivation in DCMU-treated spinach chloroplasts remains an open question. It can be noticed that this effect is different from those induced by the other Adry agents studied by Renger *et al.*[13]: the other Adry agents block the recovery of the variable fluorescence in presence of DCMU, while a back reaction takes place when DCMU alone is present.

(5) *Oxygen evolved in presence of saturating amount of DCMU*

Using preilluminated chlorella, Duysens[14] observed some oxygen evolution induced by a flash given just after addition of saturating amount of DCMU. Rosenberg

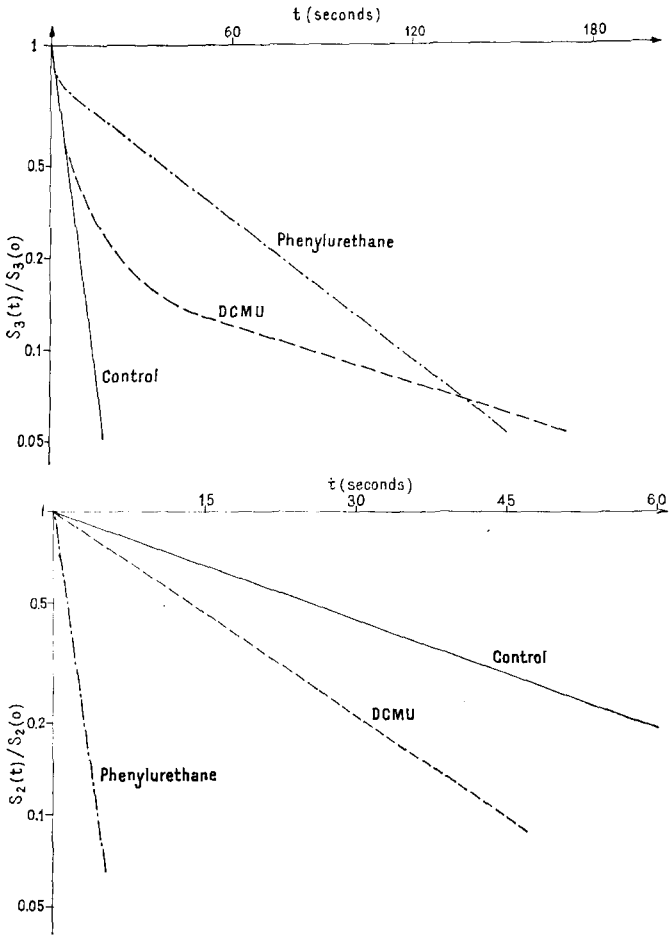


Fig. 3. Theoretical curves for deactivation of *Chlorella*.

$$\text{(a)}\quad \frac{S_3(t)}{S_3(0)} = \frac{k_3}{k[I]+k'+k_3}\, e^{-(k[I]+k'+k_3)t} + \frac{k[I]+k'}{k[I]+k'+k_3}\, e^{-\left(\frac{k'k_3}{k[I]+k'+k_3}\right)t}.$$

$$\text{(b)}\quad \frac{S_2(t)}{S_2(0)} = e^{-(k[I]+k'+k_2)t}.$$

The k values are depicted from Fig. 1. $k_3 = 7.5 \cdot 10^{-2}\ s^{-1}$. $k_2 = 1.2 \cdot 10^{-2}\ s^{-1}$. Control, $k[I] = k' = 0$ DCMU, $k[I] = 1.2 \cdot 10^{-2}\ s^{-1}$ and $k' = 3.8 \cdot 10^{-3}\ s^{-1}$. Phenylurethane, $k[I] = 2 \cdot 10^{-1}\ s^{-1}$ and $k' = 5 \cdot 10^{-2}\ s^{-1}$.

et al.[15], performing the same type of experiment with spinach chloroplasts, could observe no oxygen evolution.

Our data allow the clarification of the reason of these contradictory reports. DCMU stabilizes the S_3 state in *Chlorella* so that oxygen can be evolved 29 s after preillumination and the addition of saturating concentration of DCMU (Duysens[14] and Fig. 1).

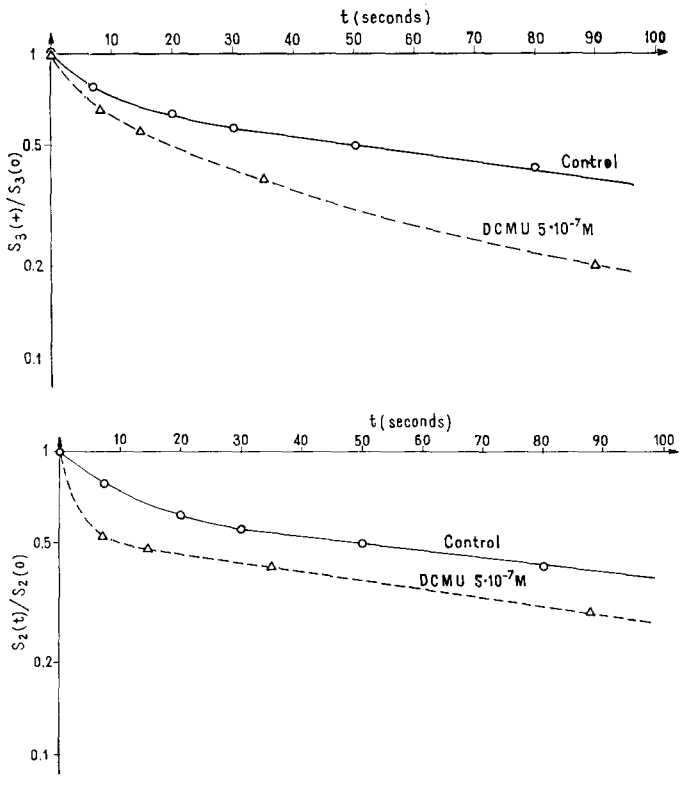


Fig. 4. Deactivation of S_3 (a) and S_2 (b) in spinach chloroplasts (log scale). o—o, control. △—△, $5 \cdot 10^{-7}$ M DCMU. The inhibition ratio of oxygen evolution was 50%.

On the other hand, S_3 disappears rapidly in the same conditions for spinach chloroplasts. We observed at high inhibition level (95%) that complete deactivation of S_3 is achieved within 10 s (not shown on Fig. 2). Therefore, no oxygen can be evolved 10 s after preillumination and addition of saturating concentration of DCMU, in the conditions of Rosenberg *et al.*[15].

The interpretation of our results confirms Duysens's conclusions: a center blocked by an inhibiting molecule can still produce oxygen, if in State S_3, but cannot, if in State S_2.

REFERENCES

1 Kok, B., Forbush, B. and McGloin, M. (1970) *Photochem. Photobiol.* 11, 457–475
2 Joliot, P., Joliot, A., Bouges, B. and Barbieri, G. (1971) *Photochem. Photobiol.* 14, 287–305
3 Forbush, B., Kok, B. and McGloin, M. (1971) *Photochem. Photobiol.* 14, 307–321
4 Lemasson, C. and Barbieri, G. (1972) *Proc. 2nd Int. Congr. Photosynth. Res.*, pp. 107–113
5 Avron, M. (1960) *Biochim. Biophys. Acta* 40, 257–272
6 Joliot, P. and Joliot, A. (1968) *Biochim. Biophys. Acta* 153, 625–634
7 Joliot, P., Barbieri, G. and Chabaud, R. (1969) *Photochem. Photobiol.* 10, 309–329
8 Bennoun, P. (1971) Thèse de Doctorat d'Etat, Paris

9 Duysens, L. N. M. and Sweers, H. (1963) in *Studies on microalgae and Photosynthetic bacteria*, p. 353, Univ. of Tokyo Press
10 Zweig, G., Tamas, I. and Greenberg, E. (1963) *Biochim. Biophys. Acta* 66, 196–205
11 Bennoun, P. (1970) *Biochim. Biophys. Acta* 216, 357–363
12 Joliot, P., Barbieri, G. and Chabaud, R. (1969) *Photochem. Photobiol.* 10, 309–329
13 Renger, G., Bouges-Bocquet, B. and Delosme, R (1973) *Biochim Biophys Acta* 292, 796–807
14 Duysens, L. N. M. (1972) *Proc. 2nd Int. Congr. Photosynth. Res.*, pp. 19–25
15 Rosenberg, J. L., Sahu, S. and Bigat, T. K. (1972) *Biophys J.* 12, 839–850