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The action of 3-(3,4-dichlorophenyl)-1,1-dimethylurea on the water-splitting enzyme system Y of photosynthesis

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SUMMARY

The effect of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) on the relative average oxygen yield per flash, $\varphi(t_d)$ as a function of the time t_d between the flashes has been investigated in spinach chloroplasts. It was found, that at $2 \cdot 10^{-7}$ M DCMU – where about 80% of the system II electron transport chains are blocked – the relative average oxygen yield per flash, $\varphi(t_d)$, decreases with increasing time t_d. This effect shows that DCMU not only acts as an inhibitor of the reducing side of system II, but in addition accelerates the decay of the holes stored in the water-splitting enzyme system Y of photosynthesis.

The most widely used inhibitor in the present photosynthesis research is the phenyl urea derivative, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which blocks the electron transport of System II (for reviews see refs 1,2). It is now generally accepted that DCMU interrupts the electron transport between the primary electron acceptor of System II and the plastoquinone pool[3–5]. An additional reversible inhibitory effect of DCMU on the System II centers has been reported by Bennoun and Li[6].

Recently the site of DCMU action has been questioned by Rosenberg *et al.*[7] who claimed, on the basis of oxygen measurements, that DCMU was acting on the oxidizing side of System II. However, the results of Rosenberg *et al.* are not only understandable in the light of a DCMU-caused inhibitory effect on the oxidizing side of Photosystem II, but also

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

by an accelerating effect on the decay of the holes trapped in the water-splitting enzyme system Y (this effect has previously been called the ADRY effect, see ref. 11). DCMU resembles, in its molecular architecture, the well known agents which accelerate the deactivation reactions of the water-splitting enzyme system Y[8] and which were found to destabilize the higher trapped hole accumulation states of the water-splitting enzyme system Y (refs 9, 10). Hence, the question arises, whether DCMU exerts an acceleration of the deactivation reactions of the water-splitting enzyme system Y in addition to its well known inhibitory effect. In order to clarify this question, the relative average oxygen yield per flash, $\varphi(t_d)$, as a function of the time t_d between the flashes has been determined, which is a measure of the acceleration of the deactivation reactions of the water-splitting enzyme system Y[11].

Chloroplasts were prepared according to the method of Winget *et al.*[12]. The experimental details for the determination of $\varphi(t_d)$ have been described elsewhere[11]; the reaction mixture is given in Fig. 1.

In Fig. 1 the relative average oxygen yield per flash, $\varphi(t_d)$ as a function of the time t_d between the flashes in the absence and in the presence of $2 \cdot 10^{-7}$ M DCMU is depicted. At this DCMU concentration the average oxygen yield per flash at the reference time t_d = 100 ms (see ref. 11) is diminished to 20% in comparison to the corresponding value for DCMU-free chloroplast suspensions. This indicated an inhibitory effect of 80%. However, besides the well known inhibitory effect, a clear accelerating effect is observed as indicated by the decrease of $\varphi(t_d)$ with increasing time t_d in a time range, where the influence of the natural deactivation reactions[13,14] on the water-splitting enzyme system Y can be neglected[15].

Hence, this result leads to the conclusion, that DCMU not only acts on the reducing side of Photosystem II, but also on the oxidizing site *via* an effect similar to that caused by the well-known agents which accelerate the deactivation reactions of the water-splitting enzyme system Y. (see refs 9, 10) leading to the destabilization of the higher trapped hole accumulation states.

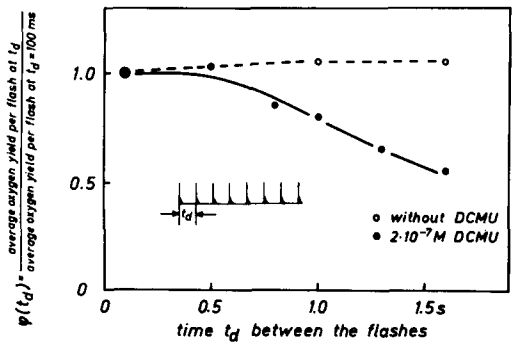

Fig. 1. Relative average oxygen yield per flash $\varphi(t_d)$ as a function of the time t_d between the flashes in the absence (○) and in the presence of $2 \cdot 10^{-7}$ M DCMU (●). The reaction mixture contained: chloroplasts ($5 \cdot 10^{-5}$ M chlorophyll), 10^{-4} M $K_3[Fe(CN)_6]$ + 10^{-4} M $K_4[Fe(CN)_6]$ as electron acceptor, $2 \cdot 10^{-3}$ M $MgCl_2$, 10^{-2} M KCl, $2 \cdot 10^{-2}$ M morpholinoethanesulfonate–NaOH buffer, pH 6,5. Excitation, white flashes, duration approx. 20 μs, saturating intensity. Temperature, 21 °C.

In comparison to the strongest agents which accelerate the deactivation reactions of the water-splitting enzyme system Y (2-anilinothiophene derivatives, see refs 8–11) DCMU exerts such an effect of only moderate power. Furthermore, the DCMU-induced acceleration of the deactivation reactions of the water-splitting enzyme system Y arises in a concentration range, where already a strong inhibition of the System II electron transport occurs. Hence, a more pronounced acceleration of the deactivation reactions of the water-splitting enzyme system Y, which would be expected for higher DCMU concentrations[16], is masked by the blockage of System II electron transport.

Because in the presence of DCMU a back reaction takes place in System II between the primary electron donor and acceptor, respectively, one can conclude, that the reduced primary electron acceptor of System II can also act as electron source for the acceleration of the deactivation reactions of the water-splitting enzyme system Y induced by DCMU. This has not been found for typical agents which accelerate the deactivation reactions of the water-splitting enzyme system Y, like carbonylcyanidephenylhydrazones or 2-anilinothiophenes[10]. Hence, in comparison to the above-mentioned agents, the acceleration of the deactivation reactions of the water-splitting enzyme system Y effect of DCMU differs in its mechanism with respect to the electron source for the discharge of S_2 and S_3.

In light of the acceleration of the deactivation reactions of the water-splitting enzyme system Y by DCMU the results of Rosenberg *et al.*[7] are readily explainable. In the dark time after the injection of DCMU into the chloroplast suspension, which has been preilluminated by two 1.2 ms flashes, thereby generating the trapped hole higher accumulation states S_2 and S_3, the acceleration of the deactivation reactions of the water-splitting enzyme system Y exerted by DCMU leads to a practically complete discharge of these states, so that the third flash (fired approx. 10 s after the DCMU injection) cannot evolve oxygen.

The results presented and discussed above seem to be in contradiction to Duysens[17] who found an oxygen evolution in *Chlorella* in the first flash after DCMU injection (preillumination by a flash sequence) and a dark time of > 20 s. However, as has been pointed out in ref. 10 the acceleration of the deactivation reactions of the water-splitting enzyme system Y for 2-anilinothiophenes is only observed in chloroplasts, whereas in algae a stabilization of the higher trapped hole accumulation states occurs. A similar ambivalent acceleration of the deactivation reactions of the water-splitting enzyme system Y (in respect to algae and chloroplasts, respectively) cannot be excluded for DCMU, thereby providing an explanation of Duysens results.

The acceleration of the deactivation reactions of the water-splitting enzyme system Y by DCMU brings serious problems for all investigations, where DCMU is used as an inhibitor of System II electron transport and this property interferes with the phenomena under investigation. This is the case, if reactions of the higher trapped hole accumulation steates are involved as was shown for the above-mentioned oxygen measurements of Rosenberg *et al.*[7]. Another example is the delayed light luminescence[18,19].

The delayed light emission has been shown to be dependent on the activation

state of the water-splitting enzyme system Y (refs 19, 20). Hence, in delayed light experiments, where DCMU is used, two effects, the inhibitory effect as well as the acceleration of the deactivation reactions of the water-splitting enzyme system Y should be taken in to account for the interpretation of the experimental data. Recently it has been found, the the acceleration of the deactivation reactions of the water-splitting enzyme system Y by 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene leads to a significant decrease of the delayed light intensity at times > 10 ms (ref. 10). However, because the reaction pathway of the DCMU-induced acceleration of the deactivation reactions of the water-splitting enzyme system Y seems to be different in comparison to the 2-(3-chloro-4-trifluoromethyl)anilino-3,5-dinitrothiophene 2 s induced cycle, further experiments are required to clarify the influence of the DCMU-induced acceleration of S_2 and S_3 on the delayed light emission.

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REFERENCES

1 Witt, H.T. (1971) *Q. Rev. Biophys.* 4, 365–477
2 Avron, M. (1967) in *Current Topics in Bioenergetics* (Sanadi, D.R., ed.), Vol. II, pp. 1–22, Academic Press, New York
3 Duysens, L.M.N. and Sweers, H.E. (1963) *Microalgae and Photosynthetic Bacteria*, pp. 353–372, University of Tokyo Press, Tokyo
4 Joliot, P. (1965) *Biochim. Biophys. Acta* 102, 116–134
5 Forbush, B. and Kok, B. (1968) *Biochim. Biophys. Acta* 162, 243–253
6 Bennoun, P. and Li, Y.S. (1973) *Biochim. Biophys. Acta* 292, 162–168
7 Rosenberg, J.L., Sahu, S. and Bigat, T.K. (1972) *Biophys. J.* 12, 839–850
8 Renger, G. (1973) *J. Bioenerg.*, in the press
9 Renger, G. (1972) *Eur. J. Biochem.* 27, 259–269
10 Renger, G., Bouges-Bocquet, B. and Delosme, R. (1973) *Biochim. Biophys. Acta* 292, 796–807
11 Renger, G. (1972) *Biochim. Biophys. Acta* 256, 428–439
12 Winget, G.D., Izawa, S. and Good, N.E. (1965) *Biochem. Biophys. Res. Commun.* 21, 438–443
13 Joliot, P. (1966) *Brookhaven Symp. Biol.* 19, 418–433
14 Lemasson, C. and Barbieri, G. (1971) *Biochim. Biophys. Acta* 245, 386–397
15 Renger, G. (1972) in *Proc. 2nd Int. Congr. Photosynthesis Res. Stresa, 1971* (Forti, G., Avron, M. and Melandri, A., eds), Vol. 1, pp. 53–60, Dr. W. Junk N.V., The Hague
16 Renger, G. (1973) *Biochim. Biophys. Acta*, in the press
17 Duysens, L.M.N. (1972) in *Proc. 2nd Int. Congr. Photosynthesis Res. Stresa, 1971* (Forti, G., Avron, M. and Melandri, A., eds), Vol. 1, pp. 19–25, Dr. W. Junk N.V., The Hague
18 Lavorel, J. (1971) *Photochem. Photobiol.* 14, 261–275
19 Joliot, P., Joliot, A., Bouges, B. and Barbieri, G. (1971) *Photochem. Photobiol.* 14, 287–305
20 Zankel, K.L. (1971) *Biochim. Biophys. Acta* 245, 373–385