3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA (DCMU) INHIBITION OF SYSTEM II AND LIGHT-INDUCED REGULATORY CHANGES IN ENERGY TRANSFER EFFICIENCY

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ABSTRACT Oxygen pulses produced in *Chlorella* by a xenon flash of 15 μsec half-width were measured by means of a rapid oxygen polarograph. Under appropriate conditions the height of the pulse caused by a saturating flash was a measure of the number of active reaction centers in system II. In pigment state II, caused by illumination during several minutes with light II, the number of active centers II was the same as in pigment state I. Oxygen pulses produced by about half-saturating flashes were diminished by about 7–10% in state II, showing that the fluorescence decrease in light II was at least partly caused by a decrease in energy transfer to reaction center II. After addition of 3(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), only the first flash produced oxygen which gives additional support for the hypothesis that DCMU inhibits between Q and sytem I.

INTRODUCTION

The experiments described in this paper will be discussed in terms of the following model (Duysens and Sweers, 1963). Light energy absorbed by or transferred to chlorophyll a_2 molecules (of photosystem II) is transferred via a number of chlorophyll a_2 molecules to a specialized chlorophyll a molecule C in a reaction center. The molecule C forms a complex ZCQ with an electron donor Z and an electron acceptor Q. The absorption and fluorescence spectra of C do not differ too much from those of the light-harvesting chlorophyll a_2. Only the fluorescence yield of the complex ZCQ must be much lower than that of the light-harvesting chlorophyll a in order to make efficient trapping possible.

The excitation energy is rapidly used for the transfer of an electron from Z to Q:

$$ZCQ + h\nu_2 \rightarrow ZC^*Q \rightarrow Z^+CQ^-. \quad (1)$$

The ratio of the number of chlorophyll a_2 molecules to that of reaction centers II is of the order of 200, which is of the same order as the number of steps taken to reach the reaction center (Duysens, 1964; Knox, 1968).

Since the chlorophyll a_2 fluorescence increases upon reduction of Q by illumination with light II, or by addition of the reductant dithionite, and decreases upon oxidation by light I, it has been concluded (Duysens and Sweers, 1963) that $Z^{(+)}C^*Q^-$ has a relatively long lifetime and is thus able to transfer the excitation back to the bulk of chlorophyll a_2 which causes an appreciable increase in fluorescence yield. It should be kept in mind that whatever the state of the complex, at least 99.5% of the fluorescence is emitted by the light-harvesting chlorophyll, and less than $\frac{1}{200}$ by the reaction center.

Before there was good evidence about the existence of such complexes Rabinowitch (1951) discussed in a general way possible properties of the various forms of the complexes, especially the fluorescence yields. He pointed out that nothing could be said a priori about the fluorescence yield of the forms Z^+CQ, ZCQ^-, and Z^+CQ^-, since the oxidized and reduced forms of Z and Q may quench or "dequench" the fluorescence yield of C (e.g., by speeding up or slowing down internal conversion). Furthermore the fluorescence yield of the light-harvesting chlorophyll may be influenced by conformational and other changes in the thylakoid membrane in which the pigments are imbedded.

It should be kept in mind that such possible complications make part of the conclusions in this paper somewhat uncertain; however, we will discuss the experiments in terms of one model which appears most plausible, and which can be extended, modified, or rejected by further evidence.

Even if the primary reaction would be, which seems plausible, a rapid electron transfer

$$ZC^*Q \rightarrow ZC^+Q^-, \tag{2}$$

followed by

$$ZC^+Q^- \rightarrow Z^+CQ^-, \tag{3}$$

we would not be able to state for certain that the fluorescence yield of Z^+CQ is small. The reason is that the charge on Z may change the conformation of the complex so that the reaction $Z^+CQ + h\nu_2 \rightarrow Z^+C^+Q^-$ cannot occur. Thus the fluorescence yield of Z^+CQ might be higher than that of ZCQ. The experiments reported in this paper have been done to check and possibly extend conclusions based on the model discussed.

METHODS

A thin layer of *Chlorella* cells was deposited on a stationary horizontal platinum electrode. The other electrode was a cylindrical silver one surrounding the platinum electrode. The voltage between the electrodes was 0.7 v. The algae were covered by a GE "Nucleopore" membrane with holes of 0.5 μ. This membrane permits rapid diffusion of inhibitors in contrast to a cellophane membrane. The cells were illuminated with saturating flashes of about 16

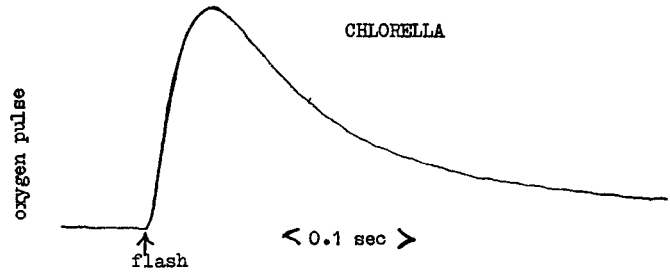

FIGURE 1 Recording of the current of an oxygen polarograph, caused by illumination with a 15 μsec xenon flash of a thin layer of *Chlorella* deposited on a horizontal platinum electrode. The current is proportional to the rate of oxygen diffusion to the electrode.

μsec half-width. A recording of the electrode current after a flash is given in Fig. 1. The current increases to 70% of the maximum in 0.02 sec, and decreases slowly. The shape of the curve is not markedly influenced by the recording system, which has a frequency response of several hundred hertz. For low intensity flashes the value at the maximum of the curve was proportional to the light intensity, and the curves had "similar shapes"; that is, the curves could be transformed into each other by multiplying by a constant factor. This indicates that the value of the maximum can be taken as proportional to the amount of oxygen produced by the flash. The current starts increasing within a millisecond or less after the flash and the 0.02 sec rise time may be caused largely by diffusion of oxygen through the thin algal layer. The relatively slow decrease of a half-time of 0.1 sec is presumably caused by back diffusion of oxygen from a distance. Only one turnover of Q is to be expected by a 16 μsec flash, since the reoxidation of Q^- requires about 200 μsec (Zankel and Kok, 1970). Thus the value of the maximum of the oxygen pulse in a saturating flash may be assumed to be proportional to the number of active reaction centers, if the Z is in the steady state, that is, the concentrations of the Z^{n+}'s (n = 0, 1, 2, 3) are equal (see below), and if Q is in the oxidized state.

RESULTS AND DISCUSSION

In Fig. 2 is shown an experiment which was performed by L. Vorst. A number of saturating flashes is given, then a period of darkness of about 29 sec, then again a number of flashes. The algal species used was *Chlorella vulgaris*. The oxygen pulses after the period of darkness show a fourfold periodicity; the first, fifth, etc., pulses are smaller than the others. This periodicity has been studied by Joliot et al. (1969) and Kok et al. (1970), and was explained by the latter authors by the hypothesis that at each reaction center four oxidizing equivalents, each produced by one quantum, are needed in order to produce one oxygen molecule. In the redox states Z^{n+} (n = 0, 1, 2, 3), each quantum exciting C causes the reaction $Z^{n+} \rightarrow Z^{(n+1)+}$. The concentration of Z^{4+} is, in the dark, negligible because Z^{4+} reacts rapidly with water: $Z^{4+} + 2H_2O \rightarrow Z + O_2 + 4H^+$. The concentrations of the Z^{n+}'s after a dark period are reflected in the periodic responses. After some time the variations in the oxygen pulses damp out and a "steady state" results in which the Z^{n+}'s have equal concentrations.

After a new sequence of flashes a dark period of equal length is given but at the beginning of a second dark period of 29 sec the inhibitor of photoreaction II,

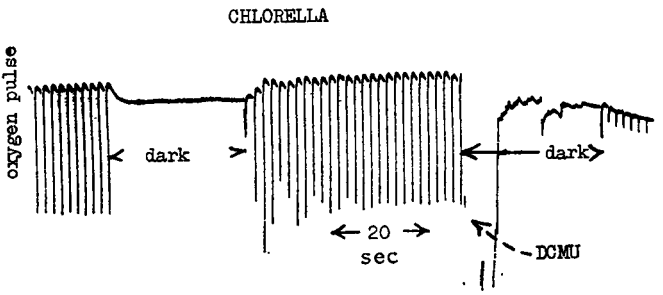

FIGURE 2 Recording of current produced by oxygen pulses in a series of xenon flashes spaced by two dark periods of 29 sec. At the beginning of the second dark period DCMU is added to a concentration of 10 μM. After DCMU addition only the first pulse yields oxygen (see text). In this recording only the AC component of the current is recorded in order to reduce disturbances caused by the addition of DCMU.

DCMU, is added to a concentration of 5×10^{-5} M. The deflections in this dark period are disturbances on the electrode by the addition of DCMU. Fig. 2 shows that only the first flash causes oxygen evolution. Oxygen evolution in the other flashes is inhibited. The remaining deflections are disturbances of the flash. The experiment was repeated many times also with shorter dark periods. Always only the first pulse was not reduced or only slightly reduced; the other oxygen pulses were partly or completely inhibited. If partly inhibited, the ratios of the pulses after the first one were the same as those after the first pulse after a dark period without inhibitor. The simplest and most plausible interpretation of this experiment is that DCMU prevents the reoxidation of Q by the oxidant A, presumably plastoquinone (Amesz, 1964), and thus only the first excitation of C is active.

Within 10 sec after the flash, whether in the presence or absence of DCMU, the fluorescence yield of chlorophyl a_2 and thus the concentration of Q^- has dropped to a rather low value (Bennoun, 1970), so that even in the presence of DCMU we may expect that oxygen pulses will be caused by flashes spaced at 10 sec. In the absence of DCMU with flashes spaced at 10 sec appreciable oxygen pulses occur, but we found that in the presence of 5×10^{-5} M DCMU the oxygen pulses in the steady state virtually were reduced to zero. This can be explained by the hypothesis that Q^-, in the absence of DCMU, reacts most rapidly with A, but in its presence cannot react with A and reacts back with Z^{3+} or another of the Z^{n+}'s, thus preventing accumulation of Z^{3+}. This interpretation is in accordance with the observation (Bertsch et al., 1963; Clayton, 1969; Bennoun, 1970) that in the presence of DCMU, luminescence, presumably resulting from the back reaction of Z^{n+} with Q^- (Bennoun, 1970), still occurs.

A long known phenomenon is the slow decrease in chlorophyll a_2 fluorescence yield in algae, after the rapid increase in fluorescence yield due to reduction of Q upon onset of illumination. This decrease takes place in a time of a few minutes and was shown to occur only in light II. It was interpreted as a decrease in activity of

system II (Duysens and Sweers, 1963). The system II activity could be restored in about 10 sec by light I (or in some species of algae by a longer period of darkness). This restoration of activity also occurred in the presence of DCMU by excitation of system I (Duysens and Talens, 1969). Thus the changes between pigment states are not produced by electron transfer between Q^- and system I. Pigment state I appeared to be produced by the accumulation of one or more components between Q and system I in the oxidized state, pigment state II by accumulation in the reduced state. The inactivation of system II was originally attributed to an inactivation of Q by a transition of the form Q into a photoinactive form Q' which quenched the fluorescence (Duysens and Sweers, 1963; Duysens and Talens, 1969). Bonaventura and Myers (1969) concluded from parallel measurements of oxygen rate and fluorescence yields that in prolonged light II the photosynthetic activity of system II decreased (in *Chlorella* by 9%) and that of system I increased. They proposed the hypothesis that changes in activities were caused by a change in distribution of the absorbed energy over the two systems, rather than by a decrease in activity of reaction center II.

The following experiments, reported earlier (Duysens, 1969, 1970), show that indeed the reaction center II is not inactivated in light II. These experiments were performed by A. S. Kwak and Th. E. van der Schatte Olivier. *Chlorella pyrenoidosa* was used. Within the precision of measurement of about 1%, the oxygen pulse produced by a saturating xenon flash of 16 μsec half-width was found to be the same in the two states which were caused by prolonged illumination with light I and II, respectively. Bonaventura and Myers have called these states light I and light II states, but since the shift to the light I state also occurs in darkness (Duysens and Sweers, 1963) and, in the presence of DCMU also is caused by light II (Duysens and Talens, 1969), the terms pigment states I and II are preferable (Duysens, 1969, 1970).

To avoid the "Joliot periodicity" the experiments were done under the steady state of the Z's. In state I a background of light I was used, and in state II, immediately after switching off the light II needed for producing state II, a short period of illumination with light I was given, sufficient to secure oxidation of all Q^-, but too short to cause a back transition to state I. In half-saturating flashes the oxygen pulses were 7–10% smaller in pigment state II than in pigment state I, showing that fewer quanta became available in the reaction centers in state II. Extending Bonaventura and Myers' interpretation, the hypothesis was proposed (Duysens, 1969, 1970) that the pigment state I to II transition is caused by a change in the thylakoid membrane by which the pigments of the two reaction centers are moved closer towards each other. This causes a decrease in the fluorescence yield (mainly from system II) and an increase in system I trapping. This hypothesis is supported by the observation that in *Chlorella* the fluorescence yield increase in the xenon flash adjusted to a just saturating intensity for oxygen pulses is about 10% higher in pigment state I than in pigment state II.

Independently analogous conclusions were drawn by Murata (1969) concerning decreases in chlorophyll a_2 fluorescence in spinach chloroplasts. In Murata's chloroplast preparation the decrease in maximum fluorescence yield could not be brought about by illumination with light II, but rather by decreasing the concentration of magnesium or other ions, or by producing a so-called "high energy state" (see also Wraight and Crofts, 1970). These two changes in chloroplasts presumably have different causes. In vivo a high energy state may be brought about by illumination with light I, but in vivo the effect of illumination with light I is a higher maximum fluorescence yield (pigment state I) and not a smaller one as in chloroplasts.

It is thus quite possible that the shifts of the pigment systems occurring in intact cells have a different cause from those observed in chloroplasts, although conformational changes and changes of charged ions on the membrane (Vredenberg, 1970) or of the membrane potential may play a role in both chloroplasts and intact cells. (See recent reviews by Myers, 1971; Govindjee and Papageorgiou, 1971.)

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REFERENCES

AMESZ, J. 1964. *Biochim. Biophys. Acta.* **79**:257.

BENNOUN, P. 1970. *Biochim. Biophys. Acta.* **216**:357.

BERTSCH, W. F., J. B. DAVIDSON, and J. R. AZZI. 1963. *Natl. Acad. Sci. Natl. Res. Counc. Publ.* **1145**: 701.

BONAVENTURA, C., and J. MYERS. 1969. *Biochim. Biophys. Acta.* **189**:366.

CLAYTON, R. K. 1969. *Biophys. J.* **9**:60.

DUYSENS, L. N. M. 1964. *Prog. Biophys. Mol. Biol.* **14**:1.

DUYSENS, L. N. M. 1969. Structure, Function and Control Mechanism in Photosynthetic Organelles. Gordon Research Conference. Plymouth, N.H.

DUYSENS, L. N. M. 1970. International Conference on the Photosynthetic Unit, Gatlinburg, Tenn. (Abstr. A4).

DUYSENS, L. N. M., and H. E. SWEERS. 1963. *In* Studies on Microalgae and Photosynthetic Bacteria. Special issue of *Plant Cell Physiol.* Japanese Society of Plant Physiologists, editors. University of Tokyo Press, Tokyo. 353.

DUYSENS, L. N. M., and A. TALENS. 1969. *In* Progress in Photosynthesis Research. H. Metzner, editor. Laupp'sche Buchhandlung, Tübingen, W. Germany. **2**:1073.

GOVINDJEE and G. PAPAGEORGIOU. 1971. *Photophysiology.* **6**:1.

JOLIOT, P., G. BARBIERI, and R. CHABAUD. 1969. *Photochem. Photobiol.* **10**:309.

KNOX, R. S. 1968. *J. Theor. Biol.* **21**:244.

KOK, B., B. FORBUSH, and M. MCGLOIN. 1970. *Photochem. Photobiol.* **11**:457.

MURATA, N. 1969. *Biochim. Biophys. Acta.* **189**:171.

MYERS, J. 1971. *Annu. Rev. Plant Physiol.* **22**:289.

RABINOWITCH, E. I. 1951. Photosynthesis and Related Processes. Interscience Publishers Inc., New York. 2(Pt. 1):823.

VREDENBERG, W. J. 1970. *Biochim. Biophys. Acta.* **223**:230.

WRAIGHT, C. A., and A. R. CROFTS. 1970. *Eur. J. Biochem.* **17**:319.

ZANKEL, K. L., and B. KOK. 1970. International Conference on the Photosynthetic Unit, Gatlinburg, Tenn. (Abstr. B3).