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STUDY OF THE PHOTOSYNTHETIC ELECTRON TRANSFER REACTIONS IN CHLOROPLASTS AND ALGAE WITH THE PLASTOQUINONE ANTAGONIST DIBROMOTHYMOQUINONE

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SUMMARY

1. As is known, low concentrations of the plastoquinone antagonist dibromothymoquinone partly inhibit the Hill reaction (with dichlorophenolindophenol or ferricyanide) and completely the electron transfer from water or NH_2OH to methylviologen. At high concentrations (where dibromothymoquinone serves as an electron acceptor), it was found that dibromothymoquinone also inhibits the System I-dependent electron transfer from reduced 2,6-dichlorophenolindophenol to methylviologen (less readily in uncoupled chloroplasts, suggesting two sites of reduced 2,6-dichlorophenolindophenol entry in the redox chain). This practically means that, by varying dibromothymoquinone concentration, one may have chloroplasts where each system is active independently of the other or where only System II remains efficient.

2. The concentrations of dibromothymoquinone for which the different inhibitions are seen depend on the chlorophyll concentration and the inhibition is not reversed by simple washing.

3. Dibromothymoquinone cancels completely the O_2 burst, a purely System II-dependent phenomenon, thought to measure the plastoquinone pool "A". The slope of the concentration curve of O_2-burst inhibition is less than that of the NH_2OH to methylviologen electron transfer, a System II+I reaction. This suggests that at low concentrations dibromothymoquinone acts on the plastoquinone molecules closest to photoreaction I oxidants and that the progressive increase of dibromothymoquinone concentration disconnects the plastoquinone pool nearer and nearer to Q, the primary electron acceptor of photoreaction II. Finally all plastoquinone is "out-circuit", and then dibromothymoquinone may react rather close to Q, an hypothesis supported by the fact that 3-(3,4-dichlorophenyl)-1,1-dimethylurea inhibits the Hill reaction less efficiently with dibromothymoquinone (at high concentration) than with 2,6-dichlorophenolindophenol or ferricyanide.

4. The flash-yield O_2 oscillation pattern is not changed by dibromothymoquinone; therefore, it does not act at the oxidizing side of the reaction center II.

Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone (dibromothymoquinone); DCIP, 2, 6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

5. The effect of dibromothymoquinone on the O_2 evolution by *Chlorella* cells (burst and steady-state photosynthesis) is similar to that reported above for chloroplasts.

INTRODUCTION

Some years ago, Trebst's group found that a quinone derivative, 2,5-dibromo-3-methyl-6-isopropyl-1,4-benzoquinone (dibromothymoquinone, DBMIB), stops the electron flow between the two photoacts, yet allows the functioning of a purely System II-dependent Hill reaction [1, 2]. Using this compound, it was possible to demonstrate that an ATP-forming site [3, 4] and a light-induced external proton consumption [5, 6] are associated with photoreaction II, and many papers were published with this compound as a tool for studying different aspects of the electron transport pathway and of the energy-conservation mechanism (see, for instance, refs 7–13).

More specifically, DBMIB being a quinone, it was supposed to act at the plastoquinone level [2], although the inhibition site was essentially proposed to be between it and cytochrome *f* [5, 7].

We were therefore interested to see how sensitive to DBMIB the pool A of oxidant revealed by the O_2 burst [14, 15] would be. This pool is situated immediately after the primary electron acceptor of photoreaction II, the "quencher" Q, and is thought to be all or part of the plastoquinone. (Indeed, only plastoquinone as electron acceptor may account for the relatively high concentration of A: approx. 1 for 40 chlorophylls, on the basis of 4 A for 1 O_2). It was also important to determine whether DBMIB could act at the oxidizing side of photoreaction II. This led us to undertake the present experiments.

METHODOLOGY

"Whole" chloroplasts were isolated from spinach leaves according to Nelson et al. [16], with the exception that Tris was used instead of Tricine. Also, instead of disrupting the chloroplasts, they were washed once in 0.01 M Tris–0.01 M NaCl buffer at pH 8, with or without 0.4 M sucrose.

Oxygen exchange was measured in a thermostated cuvette with a membrane-covered concentration-type oxygen electrode [15], of about 2 s response time. Saturating red light of approx. 40 mW cm^{-2} was obtained with a combination of a Schott, W. Germany, cut-off filter OG 550 and a Balzers, Liechtenstein, heat-reflecting B_1K_1 filter (half-transmission bandwidth from $\approx$ 550 to $\approx$ 710 nm). For the "microburst", O_2 evolved in short saturating flashes of white light (General Radio, U.S.A., Xenon "Strobotac"), a double-membrane rate electrode of Joliot's type [17] was used, but without modulated analytic light. The signals were differentiated and displayed on an oscilloscope screen. DBMIB (dissolved in methanol) and 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (dissolved in ethanol) were kindly provided by Dr Trebst and Dr Heytler, respectively.

RESULTS

Hill reaction

The effect of DBMIB on the O_2 evolution in the Hill reaction with ferricyanide and 2,6-dichlorophenolindophenol (DCIP), illustrated in Fig. 1, is similar to that reported by Böhme et al. [2], only for the inhibition seen at low concentration of inhibitor. Above a particular value of DBMIB, a "recovery" of activity is seen, and

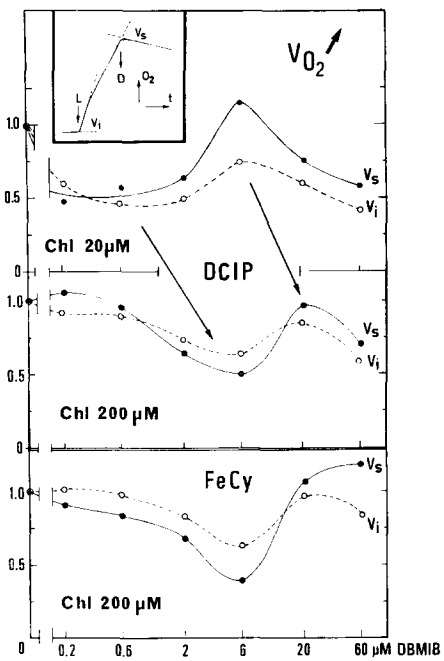

Fig. 1. Variation of the Hill reaction activity, measured by the rate of O_2 evolution, with ferricyanide (1 mM) or DCIP (30 μM for low chlorophyll concentration or 120 μM for high chlorophyll concentration) as a function of DBMIB concentration. Chloroplasts in sucrose–Tris–NaCl buffer, pH 8. Air, 16 °C. Saturating red light.

a further increase of DBMIB concentration leads to a new decline. As the recovery of activity is certainly due to the property of DBMIB being an electron carrier (see refs 10, 18 and below), the explanation of the final decline is more speculative. One may think of a slight membrane disorganization as a consequence of the presence of a high amount of the lipophilic quinone added (at 200 μM DBMIB, however, there is still an appreciable O_2 evolution). Similar results are obtained when measuring spectrophotometrically the Hill oxidant reduction, although the recovery of activity is less pronounced. This shows that the O_2 evolution is indeed due to the direct reduction of DBMIB alone (which in turn partly reduces the dye [10]) and not to parallel and independent reduction of the two reagents.

The general shape of the diagram with DCIP (or ferricyanide) is preserved when the chlorophyll concentration is increased, but the positions of the minimum and maximum are shifted towards higher DBMIB concentrations. Therefore, the

DBMIB/chlorophyll ratio seems more important than the absolute concentration of the quinone and this was indeed found by keeping DBMIB concentration constant and changing that of chlorophyll.

Electron transfer between photoreactions II and I and through photoreaction I alone

Two electron donors to methylviologen were used: hydroxylamine (but H_2O gave similar results), via photoreactions II and I, and ascorbate-reduced dichlorophenolindophenol (= $DCIPH_2$), via photoreaction I. In general, to destroy the O_2-evolving capability [19], chloroplasts were first incubated for 5 min at 5 °C in darkness in 10^{-2} M NH_2OH buffered at pH 7.5, then washed with buffer; on the other hand, the medium used for the experiments contained azide, which inhibits the remaining catalase. With this procedure, no light-induced O_2 evolution (due to H_2O splitting) or dark release of O_2 (due to H_2O_2 dismutation) could interfere with the net O_2 uptake which we wanted to measure.

The insert in Fig. 2 shows that the kinetics of this O_2 uptake starts with a fast initial rate, v_i, followed by a slower steady-state rate, v_s; addition of NH_4Cl enhances essentially v_s and this will be discussed below.

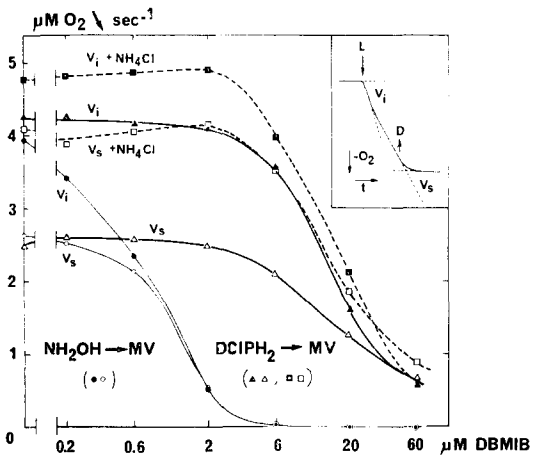

Fig. 2. Rate of the electron transfer from NH_2OH (Systems II+I) or reduced DCIP (System I) to methylviologen. Chloroplasts (pretreated with NH_2OH, see text) in sucrose–Tris–NaCl buffer, pH 8; 200 μM chlorophyll. Air, 16 °C. Saturating red light. NH_2OH, 10 mM; $DCIPH_2$, 50 μM DCIP+ 1 mM ascorbate; methylviologen (MV), 50 μM (with 500 μM sodium azide); NH_4Cl, 1 mM. For "$DCIPH_2 \rightarrow$ MV", 100 μM DCMU added (and NH_2OH absent).

It follows from the concentration curves marked "$NH_2OH \rightarrow$ MV" in Fig. 2 that, as is already well documented [1, 2], the redox chain between the two photoacts is totally blocked by DBMIB. When all O_2 uptake was inhibited, DBMIB was still reduced by photoreaction II, as we have checked spectrophotometrically; this confirms that reduced DBMIB cannot feed electrons to photoreaction I.

Fig. 2 shows also the inhibition of a purely System I-dependent reaction (curves marked "$DCIPH_2 \rightarrow$ MV"), a result contradicting the report of Böhme et al. [2]. However, the concentration required is about 10 times higher than that needed for the NH_2OH to methylviologen electron transfer chain. Although the degree of coupling

does not fundamentally change the result, it appears that the uncoupled chloroplasts are a little more "resistant" to DBMIB, suggesting that $DCIPH_2$ may enter the chain at more than one site, a proposal already made by Arntzen et al. [20], who used another quinone analog.

TABLE I

EFFECT OF LIGHT INTENSITY ON DBMIB INHIBITION OF COUPLED OR UNCOUPLED ELECTRON TRANSFER RATES OF CHLOROPLASTS

Chlorophyll, 200 μM in Tris–NaCl buffer, pH 8. Air, 16 °C. Light (550–710 nm) 40 $mW \cdot cm^{-2}$ at $I = 1.00$. Data in nM O_2 s^{-1}; Ferricyanide, 1 mM; $DCIPH_2$ = 1 mM ascorbate + 50 μM DCIP; methylviologen, 50 μM (+azide, 500 μM); DBMIB, 6 μM for ferricyanide and 20 μM for methylviologen. v_i, initial rate; v_s, steady-state rate.

	System II (+I) O_2 evolution: $H_2O \rightarrow$ ferricyanide				System I O_2 uptake: $DCIPH_2 \rightarrow$ methylviologen (+DCMU 50 μM)			
	No HN_4Cl		2 mM NH_4Cl		No HN_4Cl		2 mM NH_4Cl	
	v_i	v_s	v_i	v_s	v_i	v_s	v_i	v_s
I = 1.00								
No DBMIB	980	680	1370	1460	2160	1500	3920	3250
+DBMIB	650	370	610	370	340	170	1080	1190
Inhibition	0.34	0.46	0.55	0.75	0.84	0.89	0.72	0.63
I = 0.13								
No DBMIB	340	260	320	280	1730	1280	1760	1500
+DBMIB	220	190	210	150	280	210	280	240
Inhibition	0.35	0.27	0.34	0.46	0.84	0.84	0.84	0.84
$\frac{\text{Inhibition I = 1.00}}{\text{Inhibition I = 0.13}}$	1.0	1.7	1.6	1.6	1.0	1.1	0.9	0.8

Table I shows that, contrary to the Hill reaction with ferricyanide, which is known to be less inhibited in low- than in high-intensity light, the inhibition of the reduced DCIP to methylviologen electron transfer is essentially insensitive to the light intensity factor. That is, whereas the redox chains are pooled on the System II side [21, 22], they are apparently independent in System I.

Oxygen burst

As said in the introduction, one of the main purpose of this study was to see how the O_2 burst may be affected by DBMIB. Fig. 3 shows that, contrary to what was expected [7, 10, 12, 18], the O_2 burst is inhibited by DBMIB, whatever is its redox state. Ascorbate, used to reduce DBMIB, has almost no effect on the pool A of the control sample (as indicated in the legend of this figure and always verified by O_2 burst or fluorescence measurements). Thus, unless ascorbate cannot reach A, a very unlikely situation, the following sequence of redox potentials may be written: E_m plastoquinone (in vivo) $< E_m$ ascorbate $< E_m$ DBMIB, and therefore DBMIB cannot reduce A. If high concentrations of oxidized DBMIB are used, there is a "recovery"

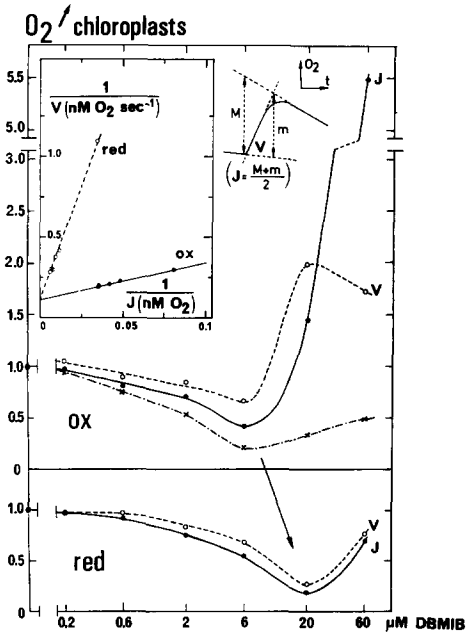

Fig. 3. Inhibition of the O_2 burst of chloroplasts by oxidized (ox) or reduced (red) DBMIB (different chloroplasts samples for the two cases). Chlorophyll 200 μM in sucrose–Tris–NaCl buffer, pH 8. Air, 16 °C. Saturating red light. Top right, schematic diagram indicating the two parameters used: the amount J (mean of maximum M and minimum m estimates) and the rate V. $\times - \cdot - \times$, J measured/(J burst + J DBMIB theoretical) (see text). Insert, straight-line relationships between V^{-1} and J^{-1} obtained from the V and J points shown on concentration curves (for DBMIB $\leqq$ maximum inhibition). The "red" series was made under N_2 in presence of 1 mM ascorbate (which has no effect on the control chloroplasts: for five experiments, the mean values were J (nM O_2) = 2145 without and 2040 with ascorbate, V (nM O_2 s^{-1}) = 410 without and 395 with ascorbate).

seen (similar to that reported in Fig. 1 for the Hill reaction), due to the substitution of DBMIB for plastoquinone as an electron acceptor for the reductant formed by photoreaction I (cf. ref. 10). The slight recovery seen with the highest concentration of reduced DBMIB is probably due to its limited reoxidation during the short time between the ending up of the bubbling of N_2 in the reaction cuvette and the introduction of the oxygen electrode. Anyway, it is clear that, provided the electron-acceptor property of DBMIB is abolished, one may expect a complete inhibition of the burst. An indication of this is given by the dash-and-point line joining the crosses in Fig. 3. This curve was obtained by plotting the ratio of the measured quantity of O_2 evolved over that of the control burst plus amount of O_2 expected from the DBMIB present. (The following reaction being assumed: $4H_2O + 2DBMIB \rightarrow 2DBMIBH_2 + O_2 + 2H_2O$). This curve shows that DBMIB starts to be an appreciable electron acceptor only when almost all plastoquinone is "out of circuit". The asymptote of the sloping-up part of the curve corresponds to about half of the expected value on the basis of the above equation: therefore, either only part of DBMIB is reacting, or the reduced DBMIB is reoxidized, as it is known [10], but without appreciable accumulation of hydrogen peroxide in the present conditions (if this were the case, a net O_2 uptake should have occurred after the initial emission, which was not observed).

The rate V/amount J ratio changes with the DBMIB concentration. It reaches a maximum when maximum inhibition is attained. This variation of the V/J ratio corresponds to the existence of a non-linear relationship between these two parameters. Let us consider that the relation of Joliot and Joliot [23] between the rate of O_2 evolution and the concentration E of open centers II may be applied to the case of the O_2 burst (i.e. A, which is measured by J, is in equilibrium with E).
The basic equation is:

$$V = k^* \frac{E/E_M}{1-p+pE/E_M}$$

where k^* is the product of the photoreaction II quantum yield by the number of photons absorbed by System II and p is the probability of exciton transfer from a closed unit II to another unit II. E_M is the total concentration of open, E, and closed, E', centers: $E_M = E+E'$.
The equilibrium is $E'+A \rightleftharpoons A'+E$, with the constant $K = (E)(A')/(E')(A)$. (The sum of the concentrations of oxidized, A, and reduced, A', molecules is constant: $A_M = A+A'$). Replacing E/E_M in the above equation and taking the reciprocal of the rate, a new equation may be derived ($A_M/A = J_M/J$):

$$\frac{1}{V} = \frac{p+(K-1)}{k^*K} + \frac{(1-p)J_M}{k^*K} \cdot \frac{1}{J}$$

That is, the reciprocal of the rate should vary linearly with the reciprocal of the amount: see the insert in Fig. 3, where the experimental points of V and J are those obtained for concentrations of DBMIB below that for which the"recovery" is seen. Since the intersection of the straight lines with the ordinate is identical in the two shown examples, and this was reasonably confirmed at other times, it means that $[p+(K-1)/k^*K]$ did not change, i.e. p and K were constant (since k^* is kept invariable for the given experiments). Only the slope is changed, that is the J_M values were different in these specific experiments. (Indeed, this is a rather variable quantity, since for 22 experiments, it was found a chlorophyll/J molecular ratio of about 120 ± 60.) Above the concentrations of oxidized DBMIB giving the recovery, a divergence from the linearity is observed (as may be expected from the creation of artificial traps [24]).

The final conclusion is therefore that the DBMIB inhibiting effect is at the plastoquinone level or between plastoquinone and photoreaction II, and not between plastoquinone and photoreaction I. To be more precise about this inhibition site, the investigation was moved a step closer to photoreaction II, as illustrated in the next paragraph.

Flash-yield O_2 evolution ("microburst")

As shown by Joliot's [25] and Kok's [26] groups, the amount, Y, of O_2 evolved per flash presents a damped oscillation with a period of four if a series of regularly spaced short saturating flashes is given. The oscillation would represent the necessity of accumulating four positive charges for evolving one O_2 molecule, hence Kok's S_0 to S_4 numbered states, and the damping would be related to the existence of some "misses" α (lack of conversion of one state to the next), and "double hits" β (meaning that one state moves two steps forward). With the help of Mrs Marie-José

Delrieu, the effect of DBMIB on Y oscillations was investigated. The results were submitted to a computer working with a model [27] which supposes equal α on the last two steps and equal β (if any) only on the first two.

Fig. 4 illustrates the results (increase of DBMIB concentration was made by successive additions, with about 10 min dark interval between the measurements). It is clear that there is no appreciable effect of DBMIB on the oscillations; a strong damping is observed only when high concentrations of reduced DBMIB are present. The progressive increase of DBMIB concentration leads to some decrease of the

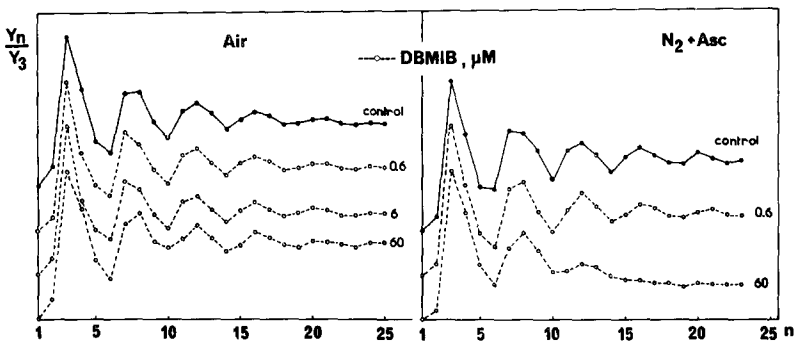

Fig. 4. Effect of oxidized and reduced DBMIB on "microburst" oscillations (amount Y_n of O_2 evolved as a function of the flash number n) with chloroplasts in sucrose–Tris–NaCl buffer, pH 8 (a thin layer on the platinum cathode of a double membrane rate electrode [17]). Air, 22 °C. Saturating white light flashes ($\approx$ 10 μs at 10 % of peak intensity) given at 300 ms dark intervals. Y_3 is taken for reference (= 1), Y_1 = 0 in all cases. The computed "misses" α and "double hits" β (see text) are for the control, α = 0.235 and β = 0.075; for 0.6 μM DBMIB, α = 0.230 (ox) or 0.265 (red) and β = 0.075 (ox) or 0.040 (red); for 60 μM DBMIB, α = 0.260 (ox) or 0.305 (red) and β = 0.055 (ox) or 0.005 (red).

absolute Y values: it is likely that DBMIB replaces plastoquinone as an oxidant of reduced Q, but less effectively. With reduced DBMIB, at a high enough concentration for disconnecting all plastoquinone from Q, photoreduced Q cannot be reoxidized any more: hence the strong damping observed (some O_2 is still evolved, because in the aerobic conditions, a reoxidation of reduced DBMIB cannot be entirely avoided).

From these experiments, it is concluded that DBMIB does not affect the oxidizing side of photoreaction II (water splitting) but acts rather close to Q.

DCMU inhibition of DBMIB reduction

The above statement is supported by the lower sensitivity to DCMU of the DBMIB reduction compared to that of ferricyanide or of DCIP, as indicated in Table II (cf. ref. 10). This table also indicates that the ferricyanide reduction in the presence of DBMIB behaves as the DBMIB reduction alone and not as that of ferricyanide alone, and this was expected since DBMIB serves as a reductant for ferricyanide. It should be noted that this partial resistance of the DBMIB reduction to DCMU was observed if the DBMIB/chlorophyll ratio was high, and that with lower DBMIB concentration, the inhibition is finally complete. Also, the order of addition of DCMU and DBMIB plays a role: the inhibition by DCMU is slightly lower if DBMIB is added first, suggesting a competition for the same site.

TABLE II

EFFECT OF DCMU ON HILL REACTIONS WITH DBMIB AND OTHER OXIDANTS IN CHLOROPLASTS

Chlorophyll, 100 μM in Tris–NaCl buffer, pH 8. Air, 16 °C. Saturating light (550–710 nm), 40 mW cm^{-2}. Data in nM O_2 s^{-1}. Different experiments for ferricyanide and DCIP, benzoquinone, and DBMIB+ferricyanide, respectively, and, for DBMIB, mean value of the three corresponding experiments.

	Ferricyanide (400 μM)	DCIP (200 μM)	Benzoquinone (200 μM)	DBMIB (200 μM)	DBMIB+ferricyanide (200+400 μM)
Control	395	420	270	195	180
0.2 μM DCMU	105	165	105	115	105
2 μM DCMU	10	35	20	90	50
$\frac{0.2\ \mu M\ DCMU}{control}$	0.27	0.39	0.30	0.59	0.58
$\frac{2\ \mu M\ DCMU}{control}$	0.03	0.08	0.07	0.46	0.27

O_2 evolution by algae

The effect of DBMIB on *Chlorella* cells is comparable to that on chloroplasts. The steady-state photosynthesis, which implies a fully functional redox chain, is strongly inhibited (decrease of the rate and even more dramatic increase of the induction time). The O_2 burst is inhibited also, but, as with chloroplasts, a recovery is seen with high concentrations of DBMIB (thus, contrary to the statements of Gimmler and Avron [28], DBMIB is an electron acceptor in algae). These results are illustrated in Fig. 5.

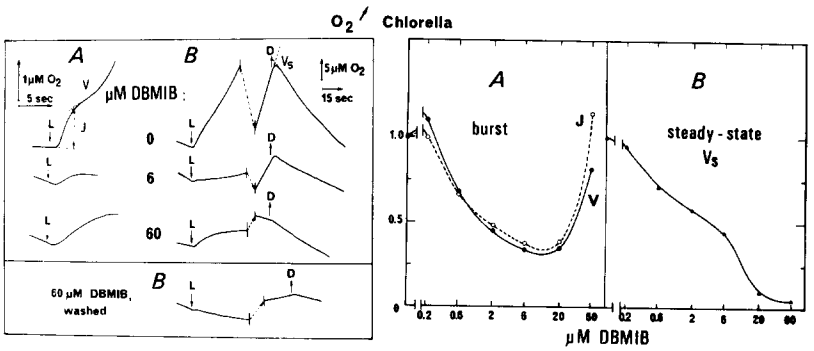

Fig. 5. Effect of DBMIB on oxygen evolution by algae. *Chorella* sp. in the mineral culture medium pH 6.8, ≈ 200 μM chlorophyll. Air, 17 °C. Saturating red light. Left hand. Top 3 horizontal sections: (A) (left) = recording at high sensitivity and fast speed in order to measure the amount (J) and the rate (V) of the O_2 burst; (B) (right) = recording, at lower sensitivity and slower speed, of the beginning and the end of a 3 min illumination period for measuring the steady-state photosynthesis rate (v_s). The lag time between the onset (L) of illumination and the starting of O_2 evolution is due to the response time of the electrode (≈ 1.5 s). Bottom section: Chlorella incubated for approx. 5 min in darkness at ≈ 21 °C with 60 μM oxidized DBMIB, then twice washed; different experiment as above, but the controls (DBMIB 0 or 60 μM present in the medium) are almost identical to the upper traces.

Irreversibility of DBMIB inhibition

The bottom of the left part of Fig. 5 shows that the inhibition by DBMIB of the burst and of the steady-state photosynthesis cannot be reversed by simple washing. In addition, the comparison of the recording with 60 μM DBMIB either present in the medium or washed out confirms that the "recovery" seen with unwashed cells does not exist as such, but is due to the superimposition of a DBMIB-dependent O_2 evolution to an almost complete inhibition of the burst by bound DBMIB.

With chloroplasts, a similar irreversibility is observed for the Hill reaction and the NH_2OH or reduced DCIP to methylviologen electron transfer. For instance, with 6 μM DBMIB, 43 % of inhibition of the ferricyanide-dependent O_2 evolution was obtained in unwashed chloroplasts and 40 % in washed.

DISCUSSION

To allow quantitative comparisons, all experiments were generally carried out in identical conditions such as chlorophyll concentration, light intensity, temperature, gas phase, etc.

(1) Before discussing the main results, we would like to make a general comment. In the absence of an uncoupler or of phosphorylating conditions, many redox reactions, if measured in the first seconds of illumination, start with a high initial rate v_i compared to the following steady-state rate v_s: see Table I (for $I = 1.00$) and Fig. 2. Because of its limited stimulation by an uncoupler, v_i may represent the rate of a process not yet limited a great deal by a coupling mechanism: thus the v_i/v_s ratio is representative of the degree of coupling of the chloroplasts. It results also that an uncoupler will stimulate v_s more than v_i, and that an inhibitor such as DBMIB would decrease the uncoupled rate more than the coupled (i.e. it would limit the stimulating effect of the uncoupler, since it slows down the redox reaction). For the DBMIB-dependent O_2 evolution, $v_i \approx v_s$, and it is known that the sensitivity of this reaction is rather limited [6] or even non-existent [9].

(2) The inhibition of a System I-dependent reaction, such as the electron transfer from reduced DCIP to methylviologen, suggests that a quinone-related factor may participate in this process. One may recall, in that respect, the proposal made by Trebst [29] of the existence of plastoquinone sites within each photosystem. This possibility is being presently investigated.

(3) The slope of the inhibition curve of the burst is less than that of the NH_2OH to methylviologen redox chain, probably because the DBMIB action involves several stages. At very low concentrations, it may even enhance, by a slight rearrangement of the plastoquinone pool, possibly disturbed during the chloroplast isolation, the O_2 burst: although this is not clearly shown in the specific experiment of Fig. 3, this stimulation is sometimes around 20 %. An increase of its concentration causes DBMIB to start to disconnect from each other the plastoquinone molecules which are close to the oxidizing side of photoreaction I (at the inner membrane face): this would account for the strong inhibition of the electron transfer from photoreaction II to photoreaction I and certainly explains why the DBMIB inhibition site was generally proposed to be between plastoquinone and cytochrome *f* [7, 10, 12, 18]; however, no very important effect on the burst would be observed, the other plastoquinone molecules being still reducible by photoreaction II. A further increase of the DBMIB

concentration would lessen the number of plastoquinone molecules connected to photoreaction II and, finally, all the plastoquinone pool would be "out-circuited"; DBMIB would then derive (at the outer membrane face), for its unique benefit, all the electrons coming from Q, since it is no more in competition with plastoquinone for this reduction. The resulting closeness between DBMIB and Q may account for the limited sensitivity to DCMU reported above.

This action of DBMIB close to photoreaction II does not directly affect the functioning of the reaction centers and their interrelation, as evidenced by the constancy of the flash-yield O_2 oscillations and that of the dependency of the rate V vs. the amount J of the burst. At high concentration, however, oxidized DBMIB may create artificial traps and reduced DBMIB would prevent the electron removal from reduced Q, that favors the back reaction of reduced Q with the photoreaction II generated oxidant. It would result in the observed (Fig. 4) augmentation of the misses α, diminution of the double hits β, and increase of the damping (cf. Radmer's and Kok's experiments [30] on the influence of the redox state of A on the O_2 oscillations).

(4) The appropriate amount of DBMIB for observing a given action depends to a certain extent on the chlorophyll quantity present (see Fig. 1). That is, at least a part of DBMIB binds strongly to the membranes. This is illustrated by the irreversibility of the inhibition after eliminating the excess of DBMIB from treated chloroplasts or algae (Fig. 5).

Two practical consequences derive from this last point and from the comparison of Figs 1 and 2. Depending on the DBMIB concentration, it is possible to obtain chloroplasts where both photoreactions are functionally independent of each other or where only photoreaction II is efficient. On the other hand, it is easy to suppress, by simple washing, the external DBMIB reduction and the concomitant oxygen evolution which may interfere with a particular redox reaction studied.

A final conclusion concerns the site of DBMIB action. The possibility of disconnecting progressively the plastoquinone pool from the reaction center II makes DBMIB a useful tool for investigating the specific role of plastoquinone in the photosynthetic mechanism.

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REFERENCES

1 Trebst, A., Harth, E. and Draber, W. (1970) Z. Naturforsch. 25b, 1157–1159
2 Böhme, H., Reimer, S. and Trebst, A. (1971) Z. Naturforsch. 26b, 341–352
3 Trebst, A. and Reimer, S. (1973) Biochim. Biophys. Acta 305, 129–139
4 Izawa, S., Gould, J. M., Ort, D. R., Felker, P. and Good, N. E. (1973) Biochim. Biophys. Acta 305, 119–128
5 Gould, J. M. and Izawa, S. (1973) Biochim. Biophys. Acta 314, 211–223
6 Kouchkovsky, Y. de (1974) C. R. Acad. Sci. France 278D, 667–670
7 Trebst, A. and Reimer, S. (1973) Biochim. Biophys. Acta 325, 546–557

8 Trebst, A. and Reimer, S. (1973) Z. Naturforsch. 28c, 710–716
9 Gould, J. M. and Ort, D. R. (1973) Biochim. Biophys. Acta 325, 157–166
10 Gould, J. M. and Izawa, S. (1973) Eur. J. Biochem. 37, 185–192
11 Forti, G. and Rosa, L. (1971) FEBS Lett. 18, 55–58
12 Böhme, H. and Cramer, W. A. (1971) FEBS Lett. 15, 349–354
13 Böhme, H. and Cramer, W. A. (1972) Biochim. Biophys. Acta 283, 302–315
14 Joliot, P. (1961) J. Chim. Phys. 58, 570–595
15 Kouchkovsky, Y. de (1963) Physiol. Vég. 1, 15–76
16 Nelson, N., Drechsler, Z. and Neumann, J. (1970) J. Biol. Chem. 245, 143–151
17 Joliot, P. and Joliot, A. (1968) Biochim. Biophys. Acta 153, 625–634
18 Lozier, R. H. and Butler, W. L. (1972) FEBS Lett. 26, 161–164
19 Cheniae, G. M. and Martin, I. F. (1973) Biochim. Biophys. Acta 197, 219–239
20 Arntzen, C. J., Neumann, J. and Dilley, R. A. (1971) Bioenergetics 2, 73–83
21 Stiehl, H. H. and Witt, H. T. (1969) Z. Naturforsch. 24b, 1588–1598
22 Delrieu, M.-J. and Kouchkovsky, Y. de (1971) Biochim. Biophys. Acta 226, 409–421
23 Joliot, A. and Joliot, P. (1964) C. R. Acad. Sci. France 258, 4622–4625
24 Kouchkovsky, Y. (1974) Biochim. Biophys. Acta, in the press
25 Joliot, P., Barbieri, G. and Chabaud, R. (1969) Photochem. Photobiol. 10, 309–329
26 Kok, B., Forbush, B. and McGloin, M. (1970) Photochem. Photobiol. 11, 457–475
27 Etienne, A.-L. (1974) Thèse, Université de Paris-Sud (Orsay)
28 Gimmler, H. and Avron, M. (1972) in Proc. 2nd Int. Congress on Photosynthesis Research (For G., Avron, M. and Melandri, A., eds), Vol. I, pp. 789–800, Dr. W. Junk, The Hague
29 Trebst, A. (1963) Proc. R. Soc. 157B, 355–366
30 Radmer, R. and Kok, B. (1973) Biochim. Biophys. Acta 314, 28–41