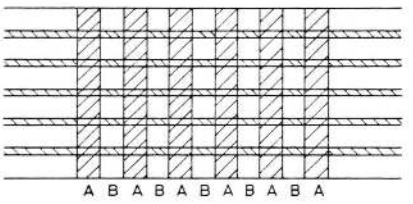


Abb. 3. Schematischer Aufbau der Membranmatrix der peritrophischen Membran von *Calliphora* (Aufsicht), wie er sich ohne Berücksichtigung von Adsorption an der Oberfläche des Membranschlauches zur Zeit ergibt. A = vermutlich Mucopolysaccharidschicht (Glucose-^{14}C-Einbau, ^{210}Pb-Austausch), Querbanden ca. 30—40 μ, Längsbanden ca. 5—1— μ. B = vermutlich Proteinschicht (Acetat-^{14}C-, Phosphat-^{32}P-Austausch), Querbanden ca. 30—40 μ.

oberfläche kann darüber hinaus nicht völlig ausgeschlossen werden. Die ca. 160 μ breiten Obereinheiten der ^{210}Pb-Autoradiographien lassen sich z. B. durch Bindung an periodisch alternierende Oberflächenstrukturen mit SH-Gruppen deuten.

Ein Membrantyp, der alternierend aus Anionen- und Kationenaustauscherschichten aufgebaut ist, die sog. Mosaikmembran, wird seit langem in der Literatur theoretisch diskutiert [7], da diese Membran überaus interessante Eigenschaften haben muß.

Bei Anwendung der linearen Ansätze der Thermodynamik irreversibler Prozesse läßt sich nämlich für diesen Membrantyp zeigen, daß er neben einer hohen Salzpermeabilität eine negative Osmose zeigt, d. h. daß der Reflexionskoeffizient negativ ist. Darüber hinaus findet als Folge des negativen Reflexionskoeffizienten bei der Filtration von verdünnten Salzlösungen durch eine solche Membran eine Konzentrierung statt, ein Effekt, der bisher noch nicht nachgewiesen werden konnte. Um deshalb eindeutig zu entscheiden, ob hier eine Mosaikmembran vorliegt, muß der Reflexionskoeffizient bekannt sein, dessen Bestimmung mit der von uns an anderer Stelle beschriebenen Methode [8] möglich ist, wenn es gelingt, den Membranschlauch ohne Beschädigung zu durchströmen.

[7] O. KEDEM u. A. KATCHALSKY, Trans. Faraday Soc. **59**, 1931 [1963].

[8] U. ZIMMERMANN u .E. STEUDLE, Z. Naturforsch. **25 b**, 500 [1970].

On a new inhibitor of photosynthetic electron-transport in isolated chloroplasts

A. TREBST and E. HARTH

Abteilung für Biologie, Ruhr-Universität Bochum

and W. DRABER

Farbenfabriken Bayer AG, Forschungszentrum, Wuppertal-Elberfeld

(Z. Naturforsch. **25 b**, 1157—1159 [1970]; eingegangen am 9. Juni 1970)

A halogenated benzoquinone has been found to inhibit the photosynthetic electron transport system in isolated chloroplasts. $2 \cdot 10^{-6}$ M of dibromo-thymoquinone inhibit the Hill-reaction with NADP, methylviologen or anthraquinone to 100%, but do not effect the photoreduction of NADP at the expense of an artificial electron donor. The Hill-reaction with ferricyanide is inhibited even at the high concentration of $2 \cdot 10^{-3}$ M of dibromo-thymoquinone to only 60%. The remaining reduction in the presence of the inhibitor reflects the rate of ferricyanide reduction by photosystem II. It is concluded that the inhibition of electron transport by the quinone occurs between photosystem I and II and close to or at the functional site of plastoquinone.

Numerous compounds have been found to inhibit photosynthetic electrontransport from water to NADP in chloroplasts in the region of photosystem II like for example DCMU * (l. c. [1]). Only antimycin A and heptyl-hydroxy-quinoline-oxyde (HOQNO) have been reportet to affect cyclic systems driven by photosystem I [2]. We wish to report on the inhibition of non cyclic electron flow

Reprints request to Prof. Dr. A. TREBST, Ruhr-Univ. Bochum, Institut f. Biochemie d. Pflanzen, *D-4630 Bochum-Querenburg*, Postfach 2148.

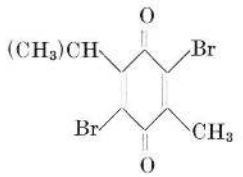
* Abbreviation used: AQ = Anthraquinone-2-sulfonate, DAD = Diaminodurene. DBMIB = Dibromo-methyl-isopropyl-*p*-benzoquinone = Dibromo-thymoquinone, DCMU = Dichloro-phenyl-dimethyl-urea, MV = Methylviologen.

[1] See summaries in: Prog. in Photosynthesis Research, Ed. H. METZNER, METZNER, Tübingen 1969.

[2] Z. GROMET-ELHANAN, in: Prog. in Photosynthesis Research, **Vol. III**, p. 1197, Ed. H. METZNER, Verlag C. Lichtenstern, München 1969.

in isolated chloroplasts by a substituted benzoquinone, which blocks electrontransport between the two light reactions.

Since WARBURG[3] described the photoreduction of *p*-benzoquinone by chloroplasts, numerous substituted benzoquinones have been tested as electron acceptors in Hill-reactions[4]. Whereas alkyl substituted *p*-benzoquinones are photoreduced[4], halogene substituted benzoquinones appear to be inhibitors of photosynthetic electron transport. Of the compounds we have tried so far, 2,5-Dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) is the best inhibitor.



This compound was obtained in 50% yield by bromination of thymoquinone in water (20 °C, 3 days) and recrystallisation from methanol/ethanol. (M.p. 74° (Lit.[5]: 73.5°) NMR-spectrum (in $CDCl_3$, TMS as internal standard, ppm) : 1.32 (doublet, τ = 7 Hz, 2 CH_2); 2.26 (1 CH_3); 3.50 (multiplet, 1 CH)). DBMIB inhibits the photoreduction of anthraquinone-sulfonate and of methylviologen by isolated chloroplasts as shown in Fig. 1.

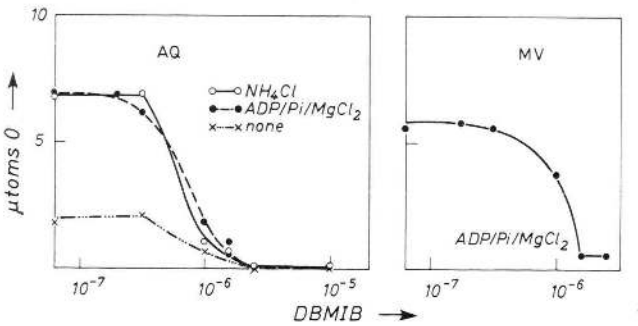


Fig. 1. The inhibition of photoreduction of anthraquinone and of methylviologen by dibromo-methyl-isopropyl-benzoquinone (DBMIB). (Washed broken chloroplasts from spinach with 0.2 mg chlorophyll were illuminated for 10 min in air in 3 ml tris puffer pH=8.0 and 0.1 µmole anthraquinone-2-sulfonate or methylviologen. Either 10 µmole of ADP and inorganic phosphate + 5 µmol of $MgCl_2$ or $2 \cdot 10^{-3}$ M NH_4Cl were added where indicated. The oxygen uptake in the presence of 10^{-3} M KCN was taken as the rate of electron transport.)

[3] O. WARBURG u. W. LÜTTGENS, Naturwissenschaften **38**, 301 [1944].

[4] A. TREBST u. H. ECK, Z. Naturforsch. **16 b**, 44 [1961].

The inhibition of these photoreductions of an electron acceptor of photosystem I at the expense of water is independent of the phosphorylating conditions. The not coupled and coupled systems as well as the system uncoupled by ammoniumchloride are inhibited to the same extent (Fig. 1). DBMIB also inhibits photosynthetic NADP reduction with water as electrondonor, shown in Fig. 2. 50% inhibition is obtained at a concentration of $8 \cdot 10^{-7}$ and 100% inhibition at $2 \cdot 10^{-6}$ M. The photoreduction of NADP at the expense of an artificial electrondonor DAD/ascorbat, however, is not affected even at high concentrations (10^{-5}) of the inhibitor (Fig. 2).

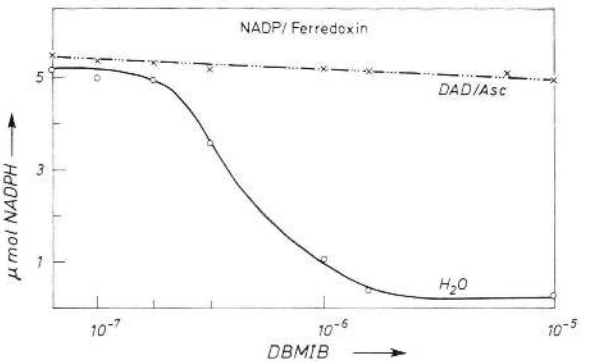


Fig. 2. The influence of dibromo-methyl-isopropyl-benzoquinone (DBMIB) on the rate of NADP reduction at the expense of water or DAD/ascorbate. (General conditions as the coupled system in Fig. 1, except that the reaction was run in nitrogen. 5 mμmole of ferredoxin were added and 0.2 μmole of DAD and 20 μmole ascorbate where indicated.)

The effect of DBMIB on photosynthetic ferricyanide reduction is shown in Fig. 3. This Hill-reaction is also affected by the inhibitor. However, whereas 100% inhibition of electrontransport from water to anthraquinone, methylviologen or NADP

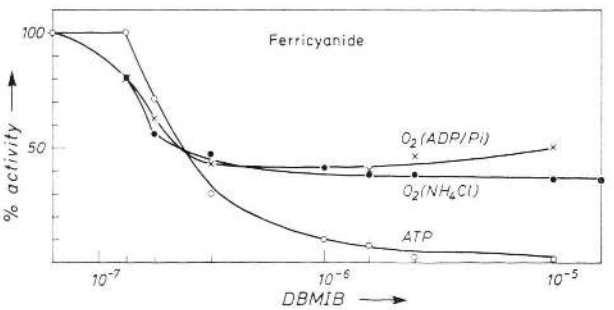


Fig. 3. The influence of dibromo-methyl-isopropyl-benzoquinone (DBMIB) on electrontransport and coupled ATP formation in the Hill-reaction with ferricyanide in the presence of phosphorylating system (ADP/Pi/Mg) or of an uncoupler (NH_4Cl). (General conditions as in Fig. 2.)

is obtained at $2 \cdot 10^{-6}$ M the reduction of ferricyanide is only inhibited to about 60%, independent of whether the system is coupled or uncoupled. High concentrations of the quinone (up to $2 \cdot 10^{-5}$ M) do not increase the inhibition of the ferricyanide system and 40% of the electron flow remain uninhibited. Whereas electrontransport proceeds with a diminished rate at concentrations of $2 \cdot 10^{-6}$ M DBMIB and above, the ATP formation coupled to ferricyanide reduction is lost at these concentrations. The inhibition of electron flow in the ferricyanide system down to 40% of the original activity and of coupled ATP-formation to 100% is obtained at the concentration which starts to impair electrontransport in the anthraquinone, methylviologen and NADP systems (when water is the electron donor).

As in the anthraquinone system (Fig. 1) the coupled and uncoupled ferricyanide Hill-reaction is inhibited to the same extent i. e. the inhibition of the coupled system is not reversed by adding an uncoupler (Fig. 3). The % inhibition of the not coupled ferricyanide system (i. e. in the absence of ADP/Pi/Mg) is less then in the coupled system (Table 1). Therefore the final rate of electrontransport from water to ferricyanide in the presence of 10^{-6} M or more DBMIB is the same in all three systems, whether coupled, not coupled or uncoupled. This excludes that DBMIB acts as an uncoupler or inhibitor of ATP formation. The electrontransport rate in the presence of DBMIB is still sensitive to DCMU (Table 1).

additions	μ atoms oxygen evolved	
	coupled system	not coupled system
none	5.0	2.5
$2 \cdot 10^{-6}$ M DMBIB	2.0	2.0
$2 \cdot 10^{-6}$ M DBMIB + 10^{-5} M DCMU	0.	0

Table 1. Inhibition of the ferricyanide system by dibromomethyl-isopropyl-benzoquinone in the presence or absence of ADP/Pi/Mg and the effect of DCMU. (Conditions as in Fig. 3.)

The different characteristics of the inhibition in the ferricyanide photoreduction vs. the other photoreductions by DBMIB distinguishes this inhibitor from the known inhibitors of photosystem II. Whereas for example DCMU inhibits all Hill-reactions to the same extent, DBMIB inhibits the Hill-reaction with ferricyanide only partly. The ferricyanide photoreduction in the presence of DBMIB is not longer coupled to ATP formation and shows no control by the phosphorylating system. We interpret these results that the site of inhibition of electron flow by DBMIB is different from the site of DCMU and is located closer to photosystem I. We suggest that DBMIB is an inhibitor of electrontransport between photosystem I and II and therefore inhibits all photoreductions which require both light reactions. The photoreduction of ferricyanide is only partly inhibited, since ferricyanide may also be reduced by photosystem II. The different rates of reduction of ferricyanide by photosystems I and II respectively are reflected in the rate of reduction before and after addition of DBMIB. It has been reported before [6] that the photoreduction of ferricyanide may require only photosystem II but it was obvious also that the Hill-reaction with ferricyanide shows properties common to all Hill-reactions. The new inhibitor permits to distinguish between photoreductions by photosystem II alone and those requiring both light reactions.

From the structure of the inhibitor it is tempting to speculate that DBMIB is an antagonist of plastoquinone. The site of inhibition of DBMIB would be in agreement with the functional site of plastoquinone in the photosynthetic electrontransport chain. Further evidence for this will be presented somewhere else. ZWEIG et al. [7] have claimed that also dichloro-naphthoquinone may be inhibiting close to plastoquinone.

[5] E. CARSTANJEN, I. prakt. Chem. **3**, 50 [1871].

[6] M. AVRON and G. BEN-HAYYIM, in: Prog. in Photosynthesis Research, **Vol. III**, p. 1185, Ed. H. METZNER, Verlag C. Lichtenstern, München 1969.

[7] G. ZWEIG, J. E. HITT, and D. H. CHO, in: Prog. in Photosynthesis Research, **Vol. III**, p. 1728, Ed. H. METZNER, Verlag C. Lichtenstern, München 1969.