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EFFECTS OF α-BENZYL-α-BROMO-MALODINITRILE ON THE PRIMARY ELECTRON ACCEPTOR OF PHOTOSYSTEM II IN SPINACH CHLOROPLASTS

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SUMMARY

Reactions at the reducing side of Photosystem II in spinach chloroplasts are modified by α-benzyl-α-bromo-malodinitrile (BBMD).

On addition of 50 μM BBMD to chloroplasts the following phenomena can be observed: (1) electron flow to an acceptor like 2,6-dichlorophenolindophenol is partly deflected to electron flow to oxygen; (2) the electron flow to oxygen is carbonyl cyanide *m*-chlorophenylhydrazone sensitive but 3-(3,4-dichlorophenyl)-1,1-dimethylurea insensitive; (3) variable fluorescence is abolished but basal fluorescence is not altered; (4) a strong photobleaching of carotenoids is induced. BBMD seems a very efficient acceptor for electrons from the primary electron acceptor of Photosystem II, resulting in a BBMD-mediated electron transport from this primary acceptor to oxygen.

On pretreatment of chloroplasts with 50 μM BBMD the effects are different; (1) electron flow to 2,6-dichlorophenolindophenol, ferricyanide, or NADP is almost completely inhibited and is not restored by addition of artificial electron donors: (2) no electron flow to oxygen is observable unless BBMD again is added to reaction media; (3) no variable fluorescence is observable but basal fluorescence is not affected; (4) there is no photobleaching of carotenoids unless BBMD again is added; (5) no reduction of C-550 can be recorded. Pretreatment of chloroplasts with BBMD seems to induce an intense cycling of electrons around Photosystem II and only anew added BBMD can interrupt this cycling.

INTRODUCTION

Several compounds and treatments now are known to affect reactions at the Photosystem II part of the photosynthetic electron transport chain. At the oxidizing side of Photosystem II electron flow from water to Photosystem II is inhibited by treatment of chloroplasts with Tris[1], chaotropic agents[2], heat[3], and ultraviolet irradiation[3], or by addition of compounds like hydroxylamine[4], carbonyl cyanide

Abbreviations: BBMD, α-benzyl-α-bromo-malodinitrile; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

m-chlorophenylhydrazone (CCCP)[5], acetazolamide[6], salicylaldoxime[7], antimycin A[7], and azide[7]. At the reducing side of Photosystem II electron flow from Q (the primary electron acceptor[8] of Photosystem II) to the adjacent pool of electron acceptors is inhibited by addition of compounds like 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU)[9], *o*-phenanthrolin[5], simazin[5], piericidin A[7], ioxynil[7], broxynil[7], 2 *n*-nonyl-4-hydroxyquinoline-*N*-oxide[10], and *n*-butyl-3,5-diiodo-4-hydroxybenzoate[10].

In the present communication α-benzyl-α-bromo-malodinitrile (BBMD) is introduced as a compound reacting with the primary electron acceptor Q of Photosystem II. Two modes of effects can be distinguished: (a) on addition of BBMD to chloroplasts the compound behaves like an efficient acceptor for electrons from Q in a DCMU-insensitive way, (b) on pretreatment of chloroplasts with BBMD an intense cycling of electrons around Photosystem II seems to be induced.

MATERIALS AND METHODS

Once-washed chloroplasts were isolated from spinach leaves as described by Yamashita and Butler[1]. Photoreduction of NADP, 2,6-dichlorophenolindophenol (DCIP), or ferricyanide was measured as outlined before[11].

Fluorescence induction measurements were performed in a set-up in which the excitation light could be administered to the sample in a time shorter than 3 ms by using a rotary relay (Kuhnke, 24 V, 10.5 W) as a light shutter. The excitation light was in a wavelength band of 520–600 nm (limits of 1% of peak transmission) and had a total intensity of 450 $\mu W/cm^2$. The fluorescence emission was isolated with a RG-8 Schott filter, measured with a photomultiplier (EMI 9558 B), and registrated by an analog taperecorder (Philips Analog-7). Fluorescence emission spectra were measured in a Perkin–Elmer MPF-2 A spectrofluorimeter.

Oxygen uptake was measured in a Gilson oxygraph with a collodion-coated Gilson platinum electrode. Oxygen concentration was assumed to be 0.26 μmole O_2 per ml at 25 °C. Side-illumination was as previously described[11]: intensity of actinic light at the centre of the reaction vessel was 10 mW/cm^2.

Cytochrome-559 and C-550 signals were recorded in an Aminco–Chance dual wavelength spectrophotometer. BBMD was dissolved in methanol and added as a 60% methanolic solution; final concentration of methanol in reaction media was 1–2% (v/v).

RESULTS

In Table I the effects of pretreatment of chloroplasts with Tris[1] in comparison with pretreatment with BBMD are shown. It may be concluded that: (1) pretreatment with 50 μM BBMD results in the same degree of inhibition as pretreatment with 0.8 M Tris; (2) NADP photoreduction in Tris-treated chloroplasts is reconstituted over 50% by adding artificial electron donors or donor couples, but reconstitution in BBMD-treated chloroplasts is very poor; (3) DCIP or ferricyanide photoreduction also is strongly inhibited by BBMD pretreatment and no relieve of inhibition is obtained on addition of artificial electron donors; (4) Photosystem I activity, manifested by electron flow from ascorbate–DCIP or ascorbate–*N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) to NADP in the presence of DCMU is hardly affected

TABLE I

CHLOROPLAST PRETREATMENT WITH 0.8 M TRIS OR 50 μM BBMD

Chloroplasts were pretreated with 0.8 M Tris[1] or with 50 μM BBMD during 10 min at 10 °C. The suspensions then were centrifuged[1] and the treated chloroplasts were resuspended in STN solution[1]. The reaction medium for NADP photoreduction contained (in μmoles per 3 ml): Tris (pH 8.0), 45; NaCl, 60; $MgCl_2$, 12; K_2HPO_4, 10; ADP, 2; NADP, 1; 17 nmoles ferredoxin and chloroplasts containing 40 μg chlorophyll. The reaction medium for DCIP or ferricyanide photoreduction contained (in μmoles per 3 ml): Tris (pH 7.4), 45; DCIP, 0.3 or ferricyanide, 3; and chloroplasts containing 40 μg chlorophyll. Electron donors when added (in μmoles per 3 ml): ascorbate, 2; hydroquinone, 1.2; *p*-phenylenediamine, 0.1; 1,5-diphenylcarbazide, 1.5; NH_2OH, 75. When ascorbate–DCIP or ascorbate–TMPD was used as electron donor couple 10 μmoles ascorbate and 1 μmole DCIP or 1.5 μmoles TMPD were added together with 0.03 μmole DCMU.

Electron transport type	*Pretreatment*	*Reduction of acceptor (μmoles/mg chlorophyll/h)*
$H_2O \rightarrow$ NADP	None	130
$H_2O \rightarrow$ NADP	Tris	18
Ascorbate–hydroquinone $\rightarrow$ NADP	Tris	72
1,5-Diphenylcarbazide $\rightarrow$ NADP	Tris	69
$H_2O \rightarrow$ NADP	BBMD	13
Ascorbate–hydroquinone $\rightarrow$ NADP	BBMD	22
Ascorbate–*p*-phenylenediamine $\rightarrow$ NADP	BBMD	15
1,5-Diphenylcarbazide $\rightarrow$ NADP	BBMD	14
$H_2O \rightarrow$ DCIP	None	198
$H_2O \rightarrow$ DCIP	BBMD	9
1,5-Diphenylcarbazide $\rightarrow$ DCIP	BBMD	14
$NH_2OH \rightarrow$ DCIP	BBMD	22
$H_2O \rightarrow$ ferricyanide	None	200
$H_2O \rightarrow$ ferricyanide	BBMD	33
Ascorbate–DCIP $\rightarrow$ NADP	None	65
Ascorbate–DCIP $\rightarrow$ NADP	BBMD	48
Ascorbate–TMPD $\rightarrow$ NADP	None	228
Ascorbate–TMPD $\rightarrow$ NADP	BBMD	217

by BBMD treatment. The effects of BBMD could be DCMU-like but as shown in Table II, contrary to DCMU which benefits variable fluorescence, variable fluorescence is almost completely eliminated on pretreatment of chloroplasts with BBMD or on addition of BBMD to chloroplasts. Addition of DCMU or DCMU *plus* an artificial electron donor couple does not change this inhibition of variable fluorescence.

Mehler[12] discovered oxygen as the ultimate acceptor for reducing equivalents produced photochemically by chloroplasts. This Mehler reaction originates in electrons transported from water *via* Photosystem II and Photosystem I to a low potential, autoxidizable acceptor such as methyl viologen. The reduced acceptor is reoxidized by air to produce H_2O_2. Davenport[13] circumvented dismutation of the

TABLE II

THE EFFECTS OF BBMD ON CHLOROPLAST FLUORESCENCE

The reaction medium contained (in μmoles per 3 ml): sucrose, 1200; Tris (pH 7.8), 150; $MgCl_2$, 12; NaCl, 30; and chloroplasts containing 10 μg chlorophyll. When added: DCMU, 0.03; ascorbate, 2; hydroquinone, 1.2.

Expt	*Chloroplast type*	*Fluorescence (arbitrary units)*		
		F_0	F_v	F_t/F_0
A	Control	29	38	2.31
	50 μM BBMD pretreated	30	2	1.06
	50 μM BBMD pretreated+DCMU	33	3	1.09
	Control+50 μM BBMD added	31	2	1.06
B	Control	24	45	2.88
	Control+50 μM BBMD added+DCMU	27	4	1.15
	Control+50 μM BBMD added+DCMU+ +ascorbate-hydroquinone	22	2	1.09

TABLE III

BBMD-MEDIATED OXYGEN UPTAKE IN THE PRESENCE OF DCMU

Reaction medium (1.5 ml) contained: Tris (pH 7.4), 22.5 μmoles; DCMU, 0.015 μmole; catalase (Sigma C-100), 25 units; ethanol, 0.17% (v/v) final concn; chloroplasts containing 40 μg chlorophyll. When Photosystem I particles[14] were used the reaction medium contained moreover 1 μmole ascorbate, 0.1 μmole DCIP, 0.09 mg plastocyanin, and only 2 μg chlorophyll were used. Abbreviation: DPC, 1,5-diphenylcarbazide.

Preparation	*Additions (μmoles)*	*Oxygen uptake (μatoms/mg chlorophyll/h)*
Control chloroplasts	—	12
	0.15 BBMD	68
Tris-washed chloroplasts	—	12
	1.5 DPC	12
	1.5 DPC+0.03 BBMD	68
	1.5 DPC+0.06 BBMD	83
	1.5 DPC+0.09 BBMD	98
	1.5 DPC+0.12 BBMD	105
	1.5 DPC+0.15 BBMD	116
	1.5 DPC+0.15 BBMD+0.15 CCCP	54
	1.5 DPC+0.15 BBMD (no DCMU)	123
	No DPC+0.15 BBMD	12
Photosystem I particles	—	1400
	0.15 BBMD	1380
	0.1 methyl viologen	9660

H_2O_2 formed by adding catalase *plus* ethanol, resulting in the following reactions:

$$4\ H_2O \xrightarrow{\text{Light}} 4\ H + 4\ OH \quad (1)$$

$$4\ OH \longrightarrow 2\ H_2O + O_2 \quad (2)$$

$$4\ H + 4\ \text{methyl viologen} \xrightarrow{\text{Photosystem II+I}} 4\ \text{reduced methyl viologen} \quad (3)$$

$$4\ \text{Reduced methyl viologen} + 2\ O_2 \longrightarrow 4\ \text{methyl viologen} + 2\ H_2O_2 \quad (4)$$

$$2\ H_2O_2 + 2\ C_2H_5OH \xrightarrow{\text{Catalase}} 4\ H_2O + 2\ CH_3CHO \quad (5)$$

According to these reactions the transport of 4 μequiv results in the uptake of 1 μmole oxygen or 2 μequiv transported results in the uptake of 1 μatom oxygen. It can easily be seen that when artificial electron donors replace water the reaction starts at Eqn 3 and 1 μequiv transported results in uptake of 1 μatom oxygen. In Table III a "short-chain Mehler reaction" is shown on addition of BBMD to chloroplasts. It can be concluded that: (1) the reaction is mediated only by Photosystem II for DCMU is present throughout; (2) at 0.15 μmole BBMD oxygen uptake in Tris-washed chloroplasts with 1,5-diphenylcarbazide as electron donor is almost double to that in control chloroplasts with water as electron donor (in Tris-washed chloroplasts water is not completely eliminated as electron donor); (3) reactions are CCCP sensitive in accordance with an inhibitory site of CCCP between the acceptor Y for artificial electron donors and Photosystem II[15]; (4) omitting DCMU does not alter the rate of oxygen uptake; (5) BBMD cannot replace methyl viologen regarding the strong stimulation of Photosystem I-mediated electron flow from ascorbate-DCIP to oxygen. In Fig. 1 is illustrated that the rate of oxygen uptake in the presence of BBMD with intermittent illumination is constant over a long period and completely dependent on light.

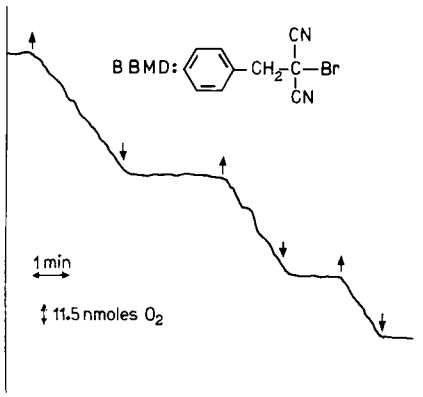

Fig. 1. BBMD-mediated oxygen uptake by chloroplasts in the presence of DCMU. The reaction medium was outlined in Table III. Upward arrows mean light on, downward arrows mean light off.

Photosystem II-mediated oxygen uptake is dependent on the presence of BBMD in reaction media, since with BBMD-pretreated chloroplasts this phenomenon could not be recorded (Table IV). On addition of BBMD to BBMD-pretreated chloroplasts oxygen uptake is restored and the rate is equal to that in control chloro-

TABLE IV

THE EFFECTS OF BBMD ON DCIP REDUCTION AND OXYGEN UPTAKE

The reaction media for DCIP reduction and oxygen uptake were described in Tables I and III, respectively. The Photosystem II particles were obtained by 1.3% digitonin treatment of the chloroplasts: the fraction precipitating at $10000 \times g$ after $1000 \times g$ centrifugation was used.

Expt	*Preparation*	*Additions* (*μmoles*)	*DCIP reduction* (*μmoles/mg chlorophyll per h*)	*Oxygen uptake* (*μatoms/mg chlorophyll per h*)
A	Control chloroplasts	—	286	12
		0.15 BBMD	216	72
B	50 μM BBMD-pretreated chloroplasts	—	11	12
		0.15 BBMD	9	70
C	Tris-washed chloroplasts	—	7	18
		1.5 1,5-diphenylcarbazide	203	24
		1.5 1,5-diphenylcarbazide +0.15 BBMD	143	126
D	Photosystem II particles	—	56	—
		0.15 BBMD	21	30

plasts with added BBMD (Expts A and B, Table IV). However, DCIP reduction is not restored in BBMD-pretreated chloroplasts on addition of BBMD. From the results in Table IV it needs to be emphasized that on addition of BBMD to control chloroplasts, Tris-treated chloroplasts, or Photosystem II particles a strict correlation exists between the decrease in the rate of DCIP reduction and the rate of oxygen uptake originating from BBMD addition. In Expt A the decrease in the rate of DCIP reduction on addition of BBMD amounts to 70 μmoles/mg chlorophyll per h. As DCIP is a two-electron acceptor the decrease in electron flow to DCIP is 70×2 μequiv/mg chlorophyll per h. As water is the electron donor 2 μequiv transported might result in the uptake of 1 μatom oxygen and the decrease of 70×2 μequiv is found back in an uptake rate of 72 μatoms oxygen per mg chlorophyll per h. In Expt C with an artificial electron donor (1 μequiv transported might result in an uptake of 1 μatom oxygen) the decrease in the rate of DCIP reduction on BBMD addition amounts to 60 (or 120 μequiv), and the rate of oxygen uptake now is 126. In Expt D the decrease in DCIP reduction is 35, water is the electron donor, and an oxygen uptake rate of 30 is recorded. Yamashita *et al.*[16] described photobleaching of carotenoids in spinach chloroplasts on addition of an effective electron acceptor (ferricyanide) or in the presence of compounds like CCCP, which inhibit electron transport from water to Photosystem II. An intense photobleaching of carotenoids could be recorded on addition of BBMD to chloroplasts (Table V). In BBMD-pretreated chloroplasts there was no photobleaching of carotenoids, addition of CCCP to these chloroplasts had no effect, but by adding BBMD the photobleaching was reconstituted.

TABLE V

EFFECTS OF BBMD ON PHOTOBLEACHING OF CAROTENOIDS

Chloroplasts were suspended in STN solution[1] at 100 μg chlorophyll per 3 ml. Side illumination was the same as for DCIP reduction.

Preparation	*Additions (μmoles)*	*Decrease in $A_{492\ nm}$ per min*
Control chloroplasts	—	0.002
	0.15 BBMD	0.540
50 μM BBMD-pretreated chloroplasts	—	0.002
	0.03 CCCP	0.004
	0.15 BBMD	0.142

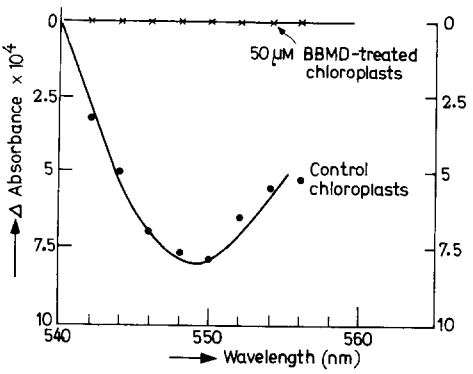

Fig. 2. The effects of BBMD on photoreduction of C-550. The reaction medium was exactly that described by Knaff and Arnon[19].

TABLE VI

LIGHT-INDUCED OXIDATION OF CYTOCHROME-559 AT ROOM TEMPERATURE

The reaction medium was as described by Knaff and Arnon[19]; 100 μg chlorophyll/ml were used. The 560 nm signal *minus* the 540 nm signal was recorded; actinic light intensity was 10 mW/cm^2 (650–700 nm).

Preparation	*Oxidation rate (nmoles/mg chlorophyll per min)*	*Extent (nmoles)*
Control chloroplasts	6.0	0.41
+50 μM CCCP	33.0	0.65
50 μM BBMD-pretreated chloroplasts	4.1	0.16
+50 μM CCCP	18.7	0.26

Knaff and Arnon[17] described a new photoreactive chloroplast component, designated C-550, which shows a reversible decrease of absorbance with a maximum at 550 nm. Erixon and Butler[18] suggested C-550 to be equivalent to Q, and it seemed interesting to trace the effects of BBMD on C-550 photoreduction. As shown in Fig. 2 no photoreduction of C-550 is recorded after BBMD pretreatment of chloroplasts. The role of cytochrome-559 in electron transport reactions around Photosystem II is uncertain[20]. The effects of BBMD on photooxidation of cytochrome-559 at room temperature, as described by Hiller *et al.*[20], are listed in Table VI. In accordance with these authors the oxidation rate of cytochrome-559 in control chloroplasts was quite low, the rate was strongly stimulated by addition of CCCP. In BBMD-pretreated chloroplasts the oxidation rate as well as the extent were lower than in control chloroplasts; stimulation was observable on addition of CCCP.

DISCUSSION

The modification of chloroplast photoreactions by BBMD can be divided into two kinds of effects, depending on addition of the compound to chloroplasts or pretreatment of chloroplasts with the compound.

Addition of BBMD to chloroplasts

We suggest BBMD being an effective electron acceptor prior to the reduction site of DCIP and prior to the DCMU-sensitive site, *i.e.* BBMD is an acceptor for electrons from Q. This suggestion is based on the following facts. One might think of photo-destructive effects of BBMD on Photosystem II or Q. This seems improbable because the rate of oxygen uptake (DCMU insensitive, CCCP sensitive, and donor dependent) on addition of BBMD remains the same over a long period and is strictly light dependent (Fig. 1). Moreover, variable fluorescence is abolished on addition of BBMD but basal fluorescence remains exactly the same (Table II) and the shape of the fluorescence emission spectra is not altered. Furthermore the turnover rate of electrons by Photosystem II (and Q) remains unaltered on addition of BBMD (Table IV). In Results it was calculated that with control chloroplasts as well as with Tris-treated chloroplasts and Photosystem II particles the electron transport rate to DCIP in the absence of BBMD was equal to the sum of the electron transport rates to oxygen and to DCIP (the latter in a DCMU-sensitive way) in the presence of BBMD. These observations are not explainable by destructive effects or by effects of BBMD on Photosystem II itself. The concept of BBMD being a very effective electron acceptor is further substantiated by the strong photobleaching of carotenoids on addition of BBMD (Table V). BBMD-mediated electron flow to oxygen is not affected by addition of DCIP, *i.e.* electrons arriving at Q preferentially flow to BBMD. At saturating amounts of DCIP it was observed that at increasing concentrations of added BBMD the decrease in the rate of electron flow to DCIP always was equal to the increase in the rate of BBMD-mediated electron flow to oxygen (measured in the presence of DCMU). It seems there is no competition for electrons from Q between BBMD and DCIP, but electron flow to DCIP is just superposed on electron flow to BBMD. Thus BBMD is a far more effective acceptor than DCIP, part of the electron flow is directed obligatory to BBMD and the rest of the turnover capacity is used for electron flow to DCIP. The relative slow rate of

electron flow to oxygen *via* BBMD may be caused by a slow terminal reaction with oxygen.

Pretreatment with BBMD

We suggest this pretreatment results in a strong cycling of electrons around Photosystem II. On pretreatment of chloroplasts with BBMD, contrary to the effects with added BBMD, no oxygen uptake nor photobleaching of carotenoids nor branched electron transport (to oxygen and to DCIP) can be observed. Electron flow to acceptors like NADP, DCIP, or ferricyanide is almost completely abolished after pretreatment (Table I), and also variable fluorescence is eliminated (Table II). Oxygen uptake in pretreated chloroplasts could be restored by adding again BBMD to reaction media, but DCIP reduction did not reappear (Table IV). The oxygen uptake rate in pretreated chloroplasts on addition of BBMD is equal to that in control chloroplasts with BBMD added (Table IV), thus the electron transport chain from water to Q seems not to be affected by the pretreatment. The elimination of electron flow to DCIP after pretreatment is not caused by extraction or destruction of a factor, required for electron transport from Q to DCIP. In that case Q should be reduced during illumination and variable fluorescence should be observed. However, no variable fluorescence is recorded in pretreated chloroplasts (Table II). In our concept of cycling there should be a very fast reaction between Q^- and Z^+ (the primary electron donor of photosystem II[21]). When Q is equivalent to C-550 (ref. 18) the photoreduction of C-550 should be inhibited owing to a fast reoxidation by Z^+. After BBMD pretreatment of chloroplasts no photoreduction of C-550 was observable (Fig. 2). Further support for the lack of accumulation of Q^- comes from preliminary investigations on the effects of BBMD on delayed fluorescence[21]. Delayed fluorescence was completely inhibited by BBMD. According to Lavorel[21] some Q^- must be present for delayed fluorescence; in accordance with our concept BBMD prevents the reduced state of Q by very fast reoxidation. This cycling of electrons around Photosystem II seems to be interrupted by the effective electron acceptor BBMD, as shown by the reconstitution of electron flow to oxygen and of photobleaching of carotenoids (Table V). Acceptors like DCIP, NADP, or ferricyanide are not able to interrupt this cycling. Addition of BBMD to chloroplasts as well as pretreatment of chloroplasts with the compound give rise to conversion of hydroquinone-reducible high-potential cytochrome-559 to lower potential forms (ascorbate- or dithionite-reducible forms). Several treatments[22] or compounds[23] attacking electron transport around Photosystem II cause this conversion. It is not known in how far this is specifically related to inhibition of electron transport or might be a secondary effect owing to structural modifications. Hiller *et al.*[20] suggested cytochrome-559 to be located on a side path from Photosystem II, but with a possible additional link to Photosystem I. Therefore, the effects of BBMD on cytochrome-559 photooxidation at room temperature (Table VI) are difficult to interpret. The rate as well as the extent of photooxidation of cytochrome-559 are decreased by BBMD-treatment and it might be suggested that part of the photooxidation of cytochrome-559, *i.e.* that part originating in electron flow through Photosystem II, is eliminated by BBMD treatment.

Possible effects of BBMD on other chloroplast components were examined with cytochrome *f*, plastocyanin, ferredoxin, and ferredoxin–NADP reductase,

purified from chloroplasts[24]. Using the spectral characteristics of the former three compounds and diaphorase activity of the latter compound[24] no effects of BBMD were recorded.

In conclusion it may be stated that BBMD interferes with the primary electron acceptor Q or C-550 of Photosystem II. As nothing is known about the nature of Q or C-550, it is not even known if it is a chemical compound, it is difficult to speculate about the mechanism of action of BBMD on Q or C-550. We might think of a nucleophilic attack or formation of a charge-transfer complex, as suggested for 3,5-di-*tert*-butyl-4-hydroxybenzylidene-malononitrile[25], an inhibitor of respiratory-chain phosphorylation.

Further investigation is in progress with structural derivatives of BBMD.

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REFERENCES

1 Yamashita, T. and Butler, W. L. (1969) *Plant Physiol.* 44, 435–438
2 Lozier, R., Baginsky, M. and Butler, W. L. (1971) *Photochem. Photobiol.* 14, 323–328
3 Yamashita, T. and Butler, W. L. (1968) *Plant Physiol.* 43, 2037–2040
4 Katoh, S., Ikegami, I. and Takamiya, A. (1970) *Arch. Biochem. Biophys.* 141, 207–218
5 Itoh, M., Yamashita, K., Nishi, T., Konishi, K. and Shibata, K. (1969) *Biochim. Biophys. Acta* 180, 509–519
6 Swader, J. A. and Jacobson, B. S. (1972) *Phytochemistry* 11, 65–70
7 Katoh, S. (1972) *Plant Cell Physiol.* 13, 273–286
8 Duysens, L. N. M. and Sweers, H. E. (1963) in *Studies on Microalgae and Photosynthetic Bacteria*, pp. 353–372, Univ. of Tokyo Press
9 Wessels, J. S. C. and van der Veen, R. (1956) *Biochim. Biophys. Acta* 19, 548–549
10 Avron, M. and Shavit, N. (1965) *Biochim. Biophys. Acta* 109, 317–331
11 Brandon, P. C. (1971) *Proc. 2nd Int. Congr. Photosynth. Res. Stresa (Italy)*, Vol. 2, pp. 1015–1022
12 Mehler, A. H. (1951) *Arch. Biochem. Biophys.* 33, 65–76
13 Davenport, H. E. (1963) *Proc. R. Soc. London B* 157, 332–345
14 Wessels, J. S. C. (1969) *Progress in Photosynthesis Research* (Metzner, H., ed.), Vol. 1, pp. 128–136, Univ. of Tübingen
15 Kimimura, M., Katoh, S., Ikegami, I. and Takamiya, A. (1971) *Biochim. Biophys. Acta* 234, 92–102
16 Yamashita, K., Konishi, K., Itoh, M. and Shibata, K. (1969) *Biochim. Biophys. Acta* 172, 511–524
17 Knaff, D. B. and Arnon, D. I. (1969) *Proc. Natl. Acad. Sci. U.S.* 63, 963–969
18 Erixon, K. and Butler, W. L. (1971) *Biochim. Biophys. Acta* 234, 381–389
19 Knaff, D. B. and Arnon, D. I. (1971) *Biochim. Biophys. Acta* 226, 400–408
20 Hiller, R. G., Anderson, J. M. and Boardman, N. K. (1971) *Biochim. Biophys. Acta* 245, 439–452
21 Lavorel, J. (1971) *Photochem. Photobiol.* 14, 261–275
22 Wada, K. and Arnon, D. I. (1971) *Proc. Natl. Acad. Sci. U.S.* 68, 3064–3068
23 Cramer, W. A. and Böhme, H. (1972) *Biochim. Biophys. Acta* 256, 358–369
24 Borchert, M. T. and Wessels, J. S. C. (1970) *Biochim. Biophys. Acta* 197, 78–83
25 Muraoka, S. and Terada, H. (1972) *Biochim. Biophys. Acta* 275, 271–275