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SYNTHETIC QUINONES INFLUENCING HERBICIDE BINDING AND PHOTOSYSTEM II ELECTRON TRANSPORT

THE EFFECTS OF TRIAZINE-RESISTANCE ON QUINONE BINDING PROPERTIES IN THYLAKOID MEMBRANES

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We have analyzed the binding of synthetic quinones and herbicides which inhibit electron transport at the acceptor side of Photosystem II (PS II) of the photosynthetic electron-transport chain in thylakoid membranes. These data show that quinones and PS II-directed herbicides compete for binding to a common binding environment within a PS II region which functions as the Q^-/PQ oxidoreductase. We observed that (1) synthetic quinones cause a parallel inhibition of electron transport and [^{14}C]herbicide displacement, and (2) herbicide binding is affected both by the fully oxidized and fully reduced form of a quinone. Quinone function and inhibitor binding were also investigated in thylakoids isolated from triazine-resistant weed biotypes. We conclude the following. (1) The affinity of the secondary accepting quinone, B, is decreased in resistant thylakoids. (2) The observation that the equilibrium concentration of reduced Q after transferring one electron to the acceptor side of PS II is increased in resistant as compared to susceptible chloroplasts may be explained both by a decrease in the affinity of PQ for the herbicide/quinone binding environment, and by a decrease of the midpoint redox potential of the B/B^- couple. (3) The binding environment regulating quinone and herbicide affinity may be divided roughly into two domains; we suggest that the domain regulating quinone head-group binding is little changed in resistant membranes, whereas the domain-regulating quinone side-group binding (and atrazine) is altered. This results in increased inhibitory activity of tetrachloro-*p*-benzoquinone and phenolic herbicides, which are hypothesized to utilize the quinone head-group domain. The two domains appear to be spatially overlapping because efficient atrazine displacement by tetrachloro-*p*-benzoquinone is observed.

Introduction

In recent years, the conceptualization of electron-transfer steps involving the plastoquinones on the electron-acceptor side of Photosystem II (PS II) has increasingly focused upon the protein components of the membrane which determine the properties of 'bound quinones'. An emerging concept is that specific PS II proteins contain peptide regions which create two PQ binding sites – one for the primary electron accepting plastoquinone (Q) and another for the secondary two-electron accepting quinone (B). The quinone at the primary

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Abbreviations: PS II (I), Photosystem II (I); Chl, Chlorophyll; BQ, Benzoquinone; Tricine, *N*-tris(hydroxymethyl)methylglycine; DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone.

electron acceptor site does not exchange rapidly with the plastoquinone (PQ) pool, whereas the secondary acceptor exchanges readily when in the fully oxidized or reduced form [1]. The protein complex which creates this binding site for the secondary quinone acceptor may be designated as Q^-/PQ oxidoreductase. To be consistent with the previous literature, the PQ bound to the Q^-/PQ oxidoreductase is designated as B (or R) to differentiate its properties from those of the free PQ pool.

An understanding of the function of the quinones acting at the acceptor side of PS II was initially developed, based upon evidence obtained by fluorescence and absorption change measurements [2–4]. Further information has also been obtained by studying the mode of action of PS II-directed herbicides. PS II herbicides such as diuron, atrazine and ioxynil were suggested to replace B from its binding site, thus preventing oxidation of Q^- by $B^{(-)}$ [1]. Indeed, a competitive binding interaction between a synthetic quinone, 6-azido-5-decyl-2,3-dimethoxy-*p*-benzoquinone, and atrazine or ioxynil has been shown [5]. We have concluded that atrazine and ioxynil do not require identical peptide determinants in the herbicide/quinone binding environment as does PQ [5]. In related studies, Oettmeier et al. [6] have reported that several substituted benzoquinones act as inhibitors of PS II electron transport. More recently, Pfister et al. [7] showed an inhibition of PS II electron flow by substituted naphthoquinones; it was suggested that the naphthoquinones bound to a slightly different site than herbicides because 2-bromo-3-methyl-1,4-naphthoquinone was found to compete poorly with atrazine [7]. We have further investigated these interactions between synthetic quinones, plastoquinone, and herbicides in an effort at further clarification of their binding domains.

The close relationships between herbicide and quinone binding postulated above is supported by measurements with triazine-resistant plant biotypes. The triazine-resistance is caused by a change in the environment of the atrazine-binding domain resulting in a loss of atrazine affinity [8,9]. The activity of most other PS II-directed herbicides also appears to be changed in triazine-resistant weeds [10]. Furthermore, the Chl *a* fluorescence induction curve of thylakoids from resistant biotypes is different from that obtained with susceptible thylakoids [8], indicating that electron transport at the acceptor side of PS II is affected by the change in the atrazine-binding domain. The kinetics of Q^- decay have also changed dramatically in resistant compared to susceptible thylakoids [11].

Materials and Methods

Pea thylakoids were isolated as described elsewhere [5]. Thylakoids were prepared from 2-month-old, greenhouse-grown atrazine-resistant and susceptible pigweed (*Amaranthus hybridus* L., seed collections from Whatcon County, Washington, U.S.A.) analogously. [^{14}C]Ioxynil and [^{14}C]atrazine binding and electron transport measurements were carried out as described earlier [5]. Binding experiments were performed under very dim light. The instrumentation used for measurements of fluorescence induction kinetics was as previously described [5]. For all experiments, thylakoids were suspended in 25 mM Tricine/NaOH/10 mM NaCl/5 mM $MgCl_2$/0.1 M sorbitol (pH 7.8). For electron-transport measurements, 5 mM NH_4Cl was added. Quinone stock solutions in methanol or ethanol were prepared freshly before use.

TetraCl-*p*-BQH_2, 2,6-diCl-*p*-BQ, 2,5-diCl-*p*-BQ, quinhydrone and 2,5-dimethyl-*p*-BQ were purchased from Eastman Kodak Chemical, Rochester, NY; 2-OH-1,4-naphthoquinone, 5-OH-1,4-naphthoquinone and tetramethyl-*p*-BQ from Sigma, St. Louis, MO; anthraquinone-2,6-disulfonate and 2,5-diOH-*p*-BQ from Aldrich, Milwaukee, WI; *p*-BQ, 3,6-diCl-2,5-diOH-*p*-BQ and pentabromophenol from Fluka, Switzerland. Further purification by recrystallization or chromatography did not change the effects on herbicide binding and electron transport described in this paper.

Results and Discussion

Action of synthetic quinones

Herbicide binding in the presence of added quinones. We tested a number of quinones for their ability to replace [^{14}C]atrazine from its binding site. It was observed that *p*-BQ, 5-hydroxy-1,4-

TABLE I

EFFECT OF SYNTHETIC QUINONES ON BINDING OF [^{14}C]ATRAZINE AND [^{14}C]IOXYNIL TO THYLAKOID MEMBRANES

$K_{b,0}$ is the herbicide binding constant in the absence of synthetic quinones. The quinone binding constants have been calculated from equations described in Ref. 13.

[^{14}C]ATRAZINE

Quinone added	Concn. (mM)	K_b^{atr} (nM)	$K_b^{atr}/K_{b,0}^{atr}$	$K_b^{quinone}$ (μM)
None	–	55	1.0	–
2-Hydroxy-1,4-naphthoquinone	0.5	138	2.5	300
2,3-Dimethoxy-5,6-methylenedioxy-*p*-benzoquinone	0.5	78	1.4	850
2,5-Dichloro-*p*-benzoquinone	0.5	120	2.2	400
2,6-Dichloro-*p*-benzoquinone	0.25	190	3.4	100
2,5-Dichloro-3,6-dimethoxy-*p*-benzoquinone	0.25	110	2.0	250

[^{14}C]IOXYNIL

Quinone added	Concn. (mM)	K_b^{iox} (nM)	$K_b^{iox}/K_{b,0}^{iox}$	$K_b^{quinone}$ (μM)
None	–	6.9	1.0	–
2-Hydroxy-1,4-naphthoquinone	0.5	20.2	2.9	250
2,5-Dichloro-*p*-benzoquinone	0.5	15.3	2.2	400
2,6-Dichloro-*p*-benzoquinone	0.5	20.4	3.0	125

naphthoquinone (5-OH-1,4-naphthoquinone), 2,5-dimethyl-*p*-BQ, tetramethyl-*p*-BQ, anthraquinone-1,5-disulfonate, anthraquinone-2,6-disulfonate, 2,5-dihydroxy-*p*-BQ (2,5-diOH-*p*-BQ) and 3,6-diCl-2,5-diOH-*p*-BQ (all added at 0.5 mM concentration) did not increase [^{14}C]atrazine binding to any significant extent. However, 2,5-diCl-*p*-BQ, 2,6-diCl-*p*-BQ, 2-OH-1,4-naphthoquinone, 2,5-diCl-3,6-dimethyl-*p*-BQ and 2,3-dimethoxy-5,6-methylenedioxy-*p*-BQ were able to increase the [^{14}C]atrazine binding constant, K_b (the concentration of free herbicide necessary to occupy 50% of the herbicide binding sites), by a factor of 2 or 3 when added in concentrations of less than 1 mM (Table I). All these quinones interacted with [^{14}C]atrazine similarly; they changed the atrazine binding constant without changing the number of [^{14}C]atrazine binding sites (1 per approx. 370 Chl molecules) (data not shown). We therefore propose a competitive interaction between these synthetic quinones and atrazine for binding to the herbicide/quinone binding domain. Three quinones were tested for their ability to replace [^{14}C]ioxynil: 2-OH-1,4-naphthoquinone, 2,5-diCl-*p*-BQ and 2,6-diCl-*p*-BQ. These quinones replaced [^{14}C]ioxynil competitively with approximately the same efficiency as that with which [^{14}C]atrazine was replaced (Table I). We observed that 2-OH-1,4-naphthoquinone acted as an inhibitor of Q^- oxidation in approximately the same concentration as we used for [^{14}C]herbicide displacement (Fig. 1): the high fluorescence yield observed shortly after the beginning of illumination (the *I* level at approx. 100 ms) indicates a partial inhibi-

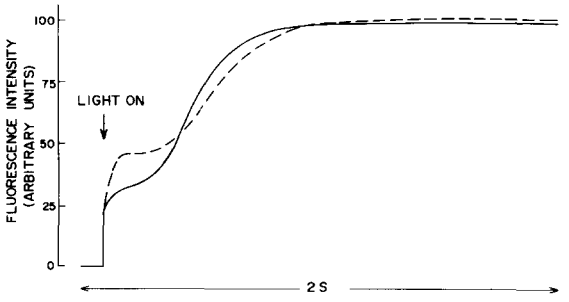

Fig. 1. Chl *a* fluorescence induction curve of pea thylakoids in the presence (- - - - -) and the absence (———) of 0.5 mM 2-hydroxy-1,4-naphthoquinone. Both samples contained also $5 \cdot 10^{-7}$ M DBMIB/2 mM sodium ascorbate (to reduce DBMIB) to eliminate effects of PS I electron acceptance by the synthetic quinone (10 μg Chl/ml).

tion of Q^- oxidation. Thus, we suggest that substituted naphthoquinones have the same binding domain as the PS II herbicides and thus compete for binding. This is in contrast to a suggestion that atrazine and a naphthoquinone may not compete for binding [7]. Relatively high concentrations of these synthetic quinones have to be used in order to observe a herbicide displacement. We found a substituted quinone with higher activity in tetrachloro-*p*-benzoquinone (Cl_4-*p*-BQ).

Action of tetrachloro-p-benzoquinone (Cl_4-p-BQ). It is known that Cl_4-*p*-BQ in the reduced form is a good donor to PS I [12]. However, Cl_4-*p*-BQ has also been reported [6] to be a good inhibitor of whole-chain photosynthetic electron transport. The pI_{50} (the negative logarithm of the molar concentration necessary for 50% inhibition of electron transport) was found to be 4.90, and inhibition at a site before PQH_2 oxidation was proposed [6]. In order to determine the site of Cl_4-*p*-BQ inhibition more precisely, we monitored Chl *a* fluorescence induction in the presence of 250 μM Cl_4-*p*-BQ, which blocks electron transport completely. We used the reduced form of Cl_4-*p*-BQ because Cl_4-*p*-BQ itself is a very potent fluorescence quencher, and is rather water-insoluble. Fig. 2 indicates that Cl_4-*p*-BQ inhibits the reoxidation of Q^- by $B^{(-)}$. The slight decrease in fluorescence yield in the presence of Cl_4-*p*-BQH_2 may be due to the oxidation of a small part of Cl_4-*p*-BQH_2 by thylakoid components. We suggest that Cl_4-*p*-BQH_2 is able to replace B from the quinone binding domain of the Q^-/PQ oxidoreductase. To test this hypothesis, the affinity of the [^{14}C]labelled herbicides ioxynil and atrazine was determined in the presence and absence of Cl_4-*p*-BQH_2 (Fig. 3). As can be seen in this figure, 5 μM Cl_4-*p*-BQH_2 is able to increase the binding constant (K_b) of both atrazine and ioxynil by a factor of 3. K_b is the negative reciprocal of the intercept of the binding curve with the abscissa. However, the number of Chl molecules per binding site (given by the intercept of the binding curve with the ordinate multiplied by 10^6 divided by the molecular weight of Chl) does not change upon addition of Cl_4-*p*-BQH_2. This implies that the interaction between Cl_4-*p*-BQH_2 and atrazine or ioxynil is competitive, and

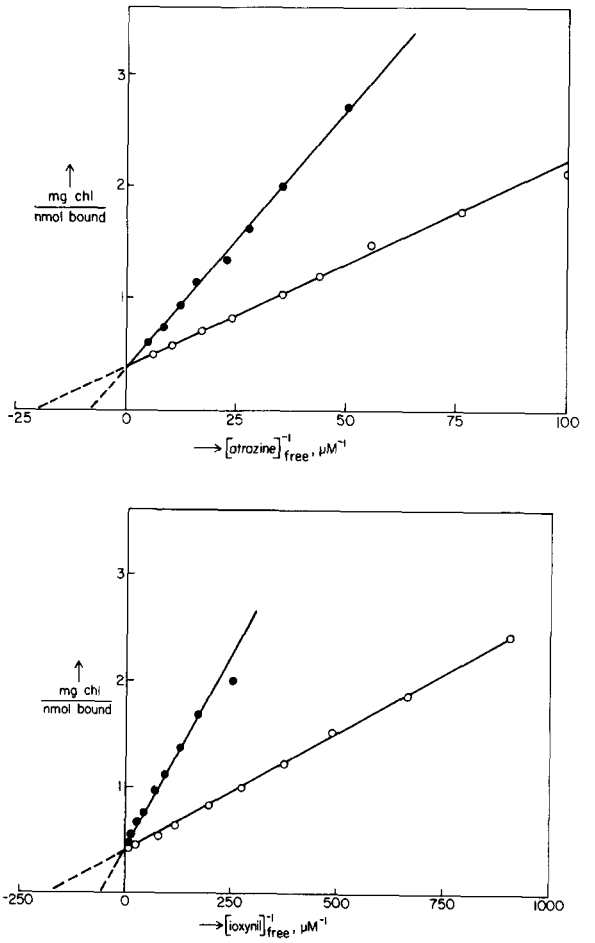

Fig. 3. Double-reciprocal [^{14}C]atrazine (a) and [^{14}C]ioxynil (b) binding curve in the absence (○) and the presence (●) of 5 μM Cl_4-*p*-BQH_2 using pea thylakoids.

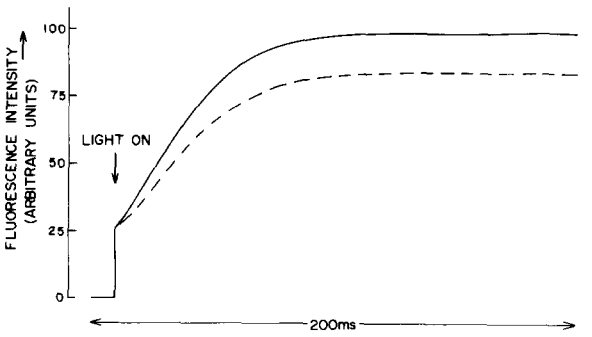

Fig. 2. Chl *a* fluorescence induction curve of pea thylakoids in the presence of 10 μM diuron (———) or of 250 μM Cl_4-*p*-BQH_2 (- - - - -) (10 μg Chl/ml).

suggests that the quinone 'head' of the molecule can interfere with herbicide interaction with the binding site. From equations derived elsewhere [13], we calculated the binding constant of Cl_4-*p*-BQH_2 to be 3 μM.

Effect of the quinone redox state on herbicide binding

The next question is whether the redox state of the added synthetic quinone affected the competition for herbicide binding. The affinity of the oxidized synthetic quinone for the binding site may be different from that of the reduced form of this quinone. We measured [^{14}C]atrazine binding in the presence of fully oxidized and reduced Cl_4-*p*-BQ, and also checked the redox dependence of the 2,5-diCl-*p*-BQ and the 2,6-diCl-*p*-BQ binding. Cl_4-*p*-BQH_2 was oxidized by addition of 0.25 mM ferricyanide, 2,5-diCl-*p*-BQ and 2,6-diCl-*p*-BQ were reduced by addition of 2.5 mM sodium ascorbate. It was confirmed by Chl *a* fluorescence yield measurements that these treatments were able to oxidize or reduce the quinones. The quinones were allowed to incubate for no longer than 1 min with the thylakoid suspension because some quinones, especially 2,6-diCl-*p*-BQ, were found to be rather unstable in the aqueous and slightly alkaline buffer. The results are shown in Fig. 4. The redox state does not appear to affect the affinity for the binding site in the case of Cl_4-*p*-BQ. However, 2,6-diCl-*p*-BQ is a better atrazine replacer in the oxidized than in the reduced form, whereas the oxidized form of 2,5-diCl-*p*-BQ appears to have a slightly higher affinity for the quinone binding site near Q than its reduced form. [^{14}C]Ioxynil replacement by these oxidized and reduced quinones gives qualitatively identical results (data not shown). Thus, for the synthetic quinones tested, the difference in affinity of the quinone and the corresponding quinol is less than an order of magnitude.

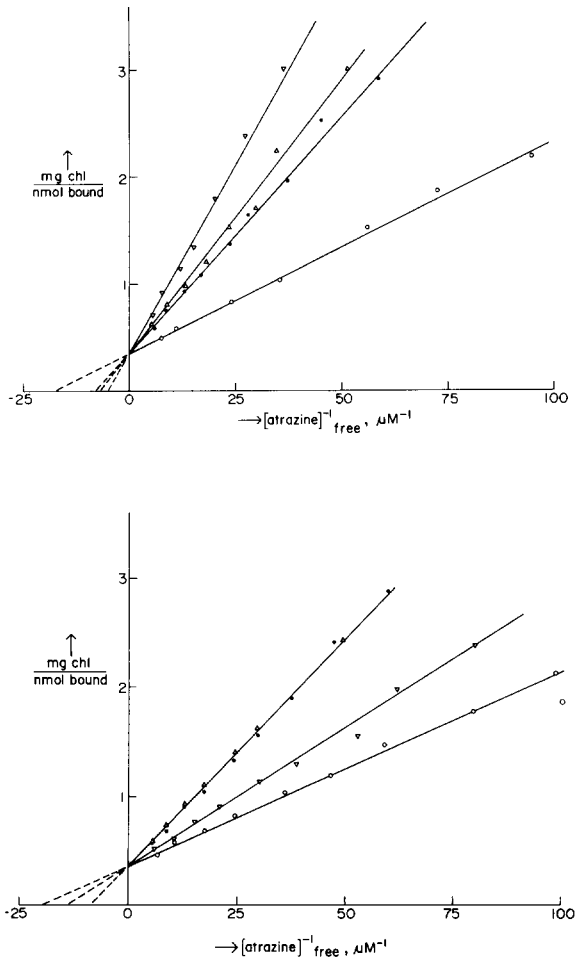

Fig. 4. Double-reciprocal plot of [^{14}C]atrazine binding to pea thylakoids (a) in the presence of 0.25 mM ferricyanide and (b) with 2.5 mM sodium ascorbate. ○, control; ●, +3 μM Cl_4-*p*-BQH_2; △, +0.5 mM 2,5-Cl_2-*p*-BQ, ▽, +0.25 mM 2,6-Cl_2-*p*-BQ. Quinone incubation for 1 min.

PS II electron transport at saturating light intensity can be fully (more than 95%) inhibited by addition of large amounts of these quinones (data not shown). This implies that no significant Q^- oxidation by a quinone occurs when a chlorinated *p*-BQ is bound to the herbicide/quinone binding environment. This may indicate that the midpoint redox potential of the synthetic quinone/semiquinone couple is much lower than that of the Q/Q^- couple, perhaps due to a lack of internal stabilization of the chlorinated semiquinone anion. Another possibility is that the synthetic quinone does not bind to the quinone/herbicide binding environment in the same fashion as the native PQ, due to steric differences between the chlorinated *p*-BQ and the PQ molecule. In this case, the chlorinated *p*-BQ might not be bound with proper steric configuration to oxidize Q^-. Until now, we have no evidence to prefer one explanation to the other.

Analysis of triazine-resistant thylakoids

Effects of triazine resistance on electron transport. It has been proposed that the midpoint redox potential of the B/B^- couple is decreased in triazine-resistant compared to triazine-susceptible plants [11]. This means that the equilibrium $Q^- \cdot B \leftrightharpoons Q \cdot B^-$ is shifted to the left in triazine-resistant plants, assuming that the Q/Q^- midpoint redox potential is not significantly changed in resistant plants. Chl *a* fluorescence induction data of triazine-resistant and -susceptible thylakoids (Fig. 5) can be explained by this hypothesis. The curves, comparable to data published earlier [8], indicate a fast accumulation of Q^- in resistant thylakoids (high *I*-level) followed by rather normal fluorescence induction kinetics. It was checked by decreasing the light intensity that the $Q^- \rightarrow B^{(-)}$ reaction was not rate-limiting. In the case of a shifted equilibrium in resistant thylakoids, indeed a higher initial steady-state Q^- concentration and a slow filling of the PQ pool are expected. Results obtained with an unmodulated Joliot-type O_2 electrode can also be explained by the hypothesis of a changed B/B^- midpoint redox potential. In resistant thylakoids, the damping of the oscillation in O_2 production was found to be much higher than in susceptible thylakoids [14] regardless of the flash frequency (0.4–4 Hz) (Vermaas, W. and Renger, G., unpublished observations), indicating an increased Q^- concentration. At this time we cannot speculate on changes in midpoint redox potential of the B^-/B^{2-} couple, because the fully reduced B is assumed to be readily released from the binding site and replaced by an oxidized PQ molecule [1]. No real equilibration between $Q^- \cdot B^-$ and $Q \cdot B^{2-}$ may occur. It should be noted that we do not exclude protonation of B^- and B^{2-} on a short time-scale; in this paper we do not distinguish between B^-/B^{2-} and BH/BH_2.

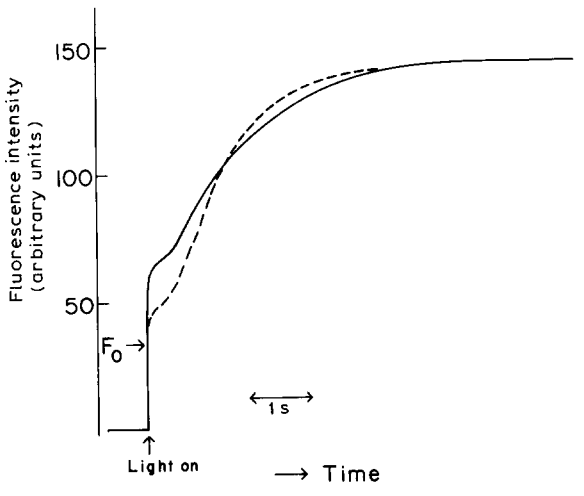

Fig. 5. Chl *a* fluorescence induction curves of thylakoids isolated from triazine-resistant (———) and triazine-susceptible (- - - - - -) pigweed (10 μg Chl/ml). F_0 is initial fluorescence intensity. The curves were normalized to an equal maximal fluorescence yield, since this was slightly higher in the resistant than in the susceptible thylakoids.

Another possibility to explain the increased steady-state Q^- concentration is a decrease in the affinity of B. This affinity is expected to be lower in resistant thylakoids because the Q^- decay in the sub-millisecond to millisecond range after the first saturating flash after dark adaptation (which may represent the rate of the $Q^- \cdot B \rightarrow Q \cdot B^-$ reaction) is slower than the Q^- decay after the second saturating flash (representing the rate of the $Q^- \cdot B^- \rightarrow Q \cdot B^{2-}$ reaction) in resistant thylakoids [11]. B^- is assumed to be tightly bound to the binding site [1], and thus, in principle, fast Q^- oxidation is possible. B, however, is mobile [1] and can oxidize Q^- only when bound. In triazine-susceptible thylakoids, $Q^- \cdot B \rightarrow Q \cdot B^-$ transfer is faster than $Q^- \cdot B^- \rightarrow Q \cdot B^{2-}$ electron transport [15]. It should be kept in mind that in resistant thylakoids also the Q^- oxidation by B^- is slower than in susceptible thylakoids, probably indicating a change in the spatial arrangement of B.

The affinity decrease of quinones in triazine-resistant thylakoids is not likely to be by more than an order of magnitude. Preliminary results indicate that PQ-1 and 6-azido-5-decyl-2,3-dimethoxy-*p*-BQ are not much less effective in herbicide displacement compared to susceptible thylakoids (Vermaas, W. and Renger, G., unpublished observations).

In principle, the effects of reduced quinone affinity and of shifted midpoint redox potential of the B/B^- couple on the equilibrium Q^- concentration can be easily calculated. Considering only the equilibria shown in Eqn. 1:

$$Q^- + PQ \overset{K_1}{\leftrightharpoons} Q^- \cdot B \overset{K_2}{\leftrightharpoons} Q \cdot B^- \qquad (1)$$

it can be derived that the fraction F of semiquinone-containing centers that have a reduced Q depends on [PQ], K_1 and K_2 as:

$$F = \frac{[Q^-]+[Q^-\cdot B]}{[Q^-]+[Q^-\cdot B]+[Q\cdot B^-]} = 1 - \frac{K_1K_2}{[PQ]^{-1}+K_1+K_1K_2} \quad (2)$$

From this equation it is clear that both K_1 and K_2 influence F, but at this moment it is impossible to distinguish between a change in K_1 or in K_2 in triazine-resistant as compared to triazine-susceptible thylakoids.

Action of electron transport inhibitors in triazine-resistant thylakoids. The sensitivity of the *p*-BQ Hill reaction for Cl_4-*p*-BQH_2 in triazine-resistant and -susceptible thylakoids is shown in Fig. 6. The I_{50}-values were $8.5 \cdot 10^{-7}$ and $1.9 \cdot 10^{-5}$ M, respectively. Thus, the triazine-resistant thylakoids are approx. 20-times more susceptible to inhibition by Cl_4-*p*-BQH_2 than the triazine-susceptible ones. A similar ratio was obtained when methylviologen was used as electron acceptor (data not shown). This reversed resistance for inhibitors in triazine-resistant thylakoids, although quantitatively less, has been reported for phenolic herbicides [10,16].

The [^{14}C]ioxynil affinity is somewhat less in triazine-resistant than in -susceptible thylakoids [13] (Table II) (however, see also Ref. 16). The influence of Cl_4-*p*-BQH_2 addition on [^{14}C]ioxynil binding in resistant and susceptible thylakoids was investigated (Table II). In order to prevent possible redox potential changes in the system, 0.25 mM quinhydron was added. Addition of quinhydrone itself did not change the ioxynil binding. From the data in Table II we calculated the K_b of Cl_4-*p*-BQH_2 to be 0.4 μM in resistant and 3 μM in susceptible thylakoids. Thus, Cl_4-*p*-BQH_2 is a much more potent displacer of PQ and ioxynil in resistant than in susceptible thylakoids. We explain the higher Cl_4-*p*-BQH_2 activity and lower B affinity as follows. If the affinity of PQ binding determinants other than the quinone head group decreases dramatically, whereas the quinone head group affinity increases somewhat or does not change considerably, the net result is a lower affin-

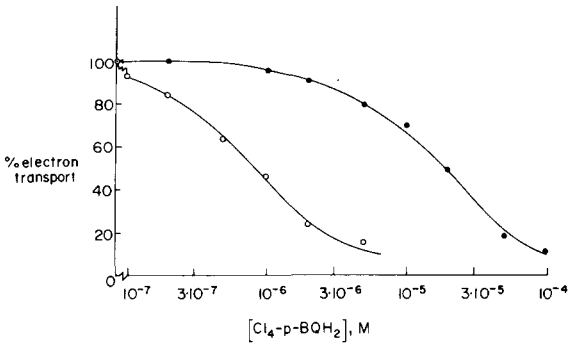

Fig. 6. Inhibition curve of the *p*-benzoquinone Hill reaction with Cl_4-*p*-BQH_2 in triazine-susceptible (●) and -resistant (○) pigweed thylakoids. The *p*-benzoquinone concentration was 0.25 mM. For the experiment shown, the control Hill reaction rate was 148 μmol O_2/mg Chl per h for susceptible and 128 μmol O_2/mg Chl per h for resistant thylakoids. The light intensity was saturating.

TABLE II

[^{14}C]IOXYNIL BINDING CONSTANTS IN TRIAZINE-SUSCEPTIBLE (S) AND -RESISTANT (R) PIGWEED THYLAKOIDS AS A FUNCTION OF THE Cl_4-*p*-BQH_2 CONCENTRATION

All samples contained 0.25 mM quinhydrone. $K_{b,0}^{iox}$ is the binding constant of ioxynil in the absence of Cl_4-*p*-BQH_2.

Susceptible/ resistant	Cl_4-*p*-BQH_2 concentration (μM)	K_b^{iox} (nM)	$K_b^{iox}/K_{b,0}^{iox}$	Chl molecules per ioxynil binding site
S	0	6.4	1.0	430
S	1	8.0	1.5	430
S	5	17.5	2.2	430
S	25	59	9.2	430
R	0	12.5	1.0	470
R	1	53	4.2	470
R	5	143	11.4	470
R	25	430	35	470

ity of PQ without a loss in quinone-group affinity. In this case molecules, which lack the other PQ binding determinants but still retain the quinone head group, such as Cl_4-*p*-BQH_2, can better compete with PQ and are better inhibitors of electron transport. The fact that atrazine affinity is strongly decreased may mean that the atrazine binding determinants are located mainly in the same domain as the PQ determinants different from the quinone head group. However, the atrazine molecule and the quinone head group seem to partially occupy the same space because atrazine and Cl_4-*p*-BQ compete for binding to the herbicide/quinone binding environment.

Phenolic herbicides are more active in triazine-resistant thylakoids [10,16], although this may depend on the source of the plant material (cf. Ref. 17). This may suggest that the quinone head group determinants and part of the phenolic herbicide binding determinants are identical or very closely related, which is not surprising because of the close structural analogy between the reduced quinone and most phenolic herbicides. Ioxynil (4-hydroxy-3,5-diiodobenzonitrile), however, which contains a phenol group, does not seem to fit in the phenolic herbicide group. The affinity of ioxynil is lower in resistant than in susceptible thylakoids (Table II). Therefore, it may be better to refer to ioxynil as a 'nitrile-type' herbicide rather than as a 'phenolic' one. It is possible that the binding determinants for DCMU-type and for phenolic herbicides are located on separate proteins as hypothesized by Oettmeier et al. [18]. This would indicate that the quinone head group and DCMU-type herbicides also would interact with non-identical proteins.

The hypothesis that the binding determinants of phenolic herbicides and the quinone head group of PQ may be identical, whereas atrazine or diuron-type herbicides have other binding determinants, is strengthened by observations on herbicide/quinone interactions at the site of PQH_2 oxidation by the Rieske Fe-S center, cytochrome *f* and/or cytochrome *b*-563. High concentrations (up to $2 \cdot 10^{-4}$ M) of diuron or atrazine do not significantly inhibit electron transport from reduced tetramethyl-*p*-BQ (durohydroquinone) to methylviologen (a reaction which monitors electron transport from PQH_2 to the acceptor side of PS I [19,20]). However, the phenolic herbicide dinoseb is a relatively good inhibitor ($I_{50} = 10^{-4}$ M) of this electron transport, and pentabromophenol inhibits this electron transport even better ($I_{50} = 6 \cdot 10^{-5}$ M). Electron transport from $TMPDH_2$ (reduced *N,N,N',N'*-tetramethyl-*p*-phenylenediamine) to methylviologen, which monitors electron transport from cytochrome *f* or plastocyanine to the acceptor side of PS I [21] is insensitive to these concentrations of dinoseb and pentabromophenol (data not shown). Thus, the quinone binding region near or at the Rieske Fe-S center also seems to bind phenoles, whereas atrazine and diuron that have no quinone head group determinants are not bound.

In conclusion, some synthetic quinones are found to be efficient replacers of PS II herbicides and the native PQ bound to the herbicide/quinone binding environment, especially in triazine-resistant thylakoids. We suggest that in triazine-resistant thylakoids the binding environment of B is changed, resulting in a changed affinity of B for its binding site in the Q^-/PQ oxidoreductase enzyme complex, and possibly in a changed B/B^- midpoint potential. The activity of herbicides in these resistant thylakoids depends on the affinity of their binding determinants compared to the average affinity of the PQ binding determinants.

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