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Photosystem II Core Phosphorylation Heterogeneity, Differential Herbicide Binding, and Regulation of Electron Transfer in Photosystem II Preparations from Spinach[1]

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ABSTRACT

The effect of photosystem II core phosphorylation on the secondary quinone acceptor of photosystem II (Q_B) domain environment was analyzed by comparative herbicide-binding studies with photosystem II preparations from spinach (*Spinacia oleracea* L.). It was found that phosphorylation reduces the binding affinity for most photosynthetic herbicides. The binding of synthetic quinones and of the electron acceptor 2,6-dichlorophenolindophenol is also reduced by photosystem II phosphorylation. Four photosystem II core populations isolated from membranes showed different extents of phosphorylation as well as different degrees of affinity for photosynthetic herbicides. These findings support the idea that heterogeneity of photosystem II observed in vivo could be, in part, due to phosphorylation.

The PSII reaction center comprises four proteins: D_1, D_2, Cyt b_{559}, and the *psb*I gene product (22). The D_1 and D_2 proteins are involved in the photochemistry at the reducing and oxidizing sides of PSII. Electron transfer on the acceptor side of PSII also involves two quinones, Q_A[2] and Q_B. Q_A is tightly associated with the D_2 protein and does not exchange with the PQ pool, whereas Q_B is bound at the Q_B site of the D_1 protein when in the semiquinone form but exchanges readily with the PQ pool in the fully oxidized or reduced form (13). PSII herbicides such as phenylurea, triazines, and phenolic compounds compete with PQ at its binding site and prevent oxidation of reduced Q_A (21, 25). Exogenous quinones, often employed as PSII electron acceptors, have been shown to compete with herbicides for binding. Moreover, quinones act as inhibitors of PSII electron transfer by binding to the same Q_B pocket and displacing the native PQ (28).

It has been suggested that phosphorylation of the PSII core leads to a decreased rate of PSII electron transfer at saturating light intensities (17–19). Further, it has been proposed that protein phosphorylation increases the ability of the Q_B pocket to bind herbicides (29), which may confer protection against photoinhibition (20). However, the inhibition of the Hill reaction by herbicides in phosphorylated membranes has been related to a secondary effect on the reduction of PQ rather than to an increased affinity for herbicides (17, 18). The effect of protein phosphorylation on the function of the Q_B site, therefore, is not clear.

The presence of heterogeneous PSII core populations has been recently reported (11). These PSII cores have been shown to have different capacities to bind dinoseb (9), suggesting that protein phosphorylation may reduce the ability of the Q_B pocket to bind herbicides.

The work presented here was designed to clarify the effect of phosphorylation on the herbicide-PQ binding domain and to distinguish the effects due to the reduction of the PQ pool. The results indicate that the activity of the most PSII-directed herbicides as well as of some synthetic quinones appears to be significantly reduced in phosphorylated membranes.

MATERIALS AND METHODS

Phosphorylation and Isolation of Membranes

Phosphorylation of spinach (*Spinacia oleracea* L.) thylakoids was performed as previously described (20) with a slight modification. Thylakoids were resuspended in the suspension buffer containing 50 mM Tricine (pH 7.5), 150 mM KCl, 15 mM NaCl, 5 mM $MgCl_2$, and 0.1 M sucrose. After the addition of 0.1 mM ATP, they were illuminated (50 μE m^{-2} s^{-1}) for 10 min. The phosphorylation profile of polypeptides was examined in thylakoids incubated in the presence of [γ-^{32}P]ATP (1 mCi, 3000 Ci/mmol) and 0.1 mM ATP. Control thylakoids were illuminated in the absence of ATP and $MgCl_2$, washed with the suspension buffer, dark-adapted for 2 h, and finally NaF was added to a concentration of 5 mM. PSII membranes were isolated (3), solubilized in 1% DM (0.5 mg of Chl mL^{-1}), and then applied to the cathode region of a flat-bed of granulated gel (9). The bed (2.5 × 22 cm) of Ultrodex (LKB) was prepared in 0.1 M glycine, 0.1% DM, and 2% ampholine carrier ampholytes (pH range 3.5–5.5) and left at 4°C in the dark. Isoelectrofocusing was carried out at a constant power of 3 W/tray for 16 h in an LKB Multiphor

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[2] Abbreviations: Q_A, primary quinone acceptor of PSII; Q_B, secondary quinone acceptor of PSII; PQ, plastoquinone; DPC, diphenylcarbazide; DCPIP, 2,6-dichlorophenolindophenol; DPQ, decylplastoquinone; p-BzQ, *para*-benzoquinone; NAQ, 2-hydroxy-1,4-napthoquinone; DM, *n*-dodecyl β-D-maltoside.

apparatus (9, 11). PSII core populations separated in the granulated gel were eluted in a buffer containing 50 mM Mes (pH 6.5), 15 mM NaCl, 5 mM $MgCl_2$, and 0.1% DM, and then filtered.

Four Chl bands were identified and isolated (11). These were applied to a linear gradient (0–1 M) of sucrose made up in the above extraction buffer and centrifuged at 60,000*g* (Beckman L2 75B ultracentrifuge) for 12 h, under which conditions four distinct, differently phosphorylated PSII core populations were separated (11). The PSII core fractions were dialyzed against the same extraction buffer and concentrated by centrifuging down at 435,000*g* (Beckman TL100 ultracentrifuge) for 1 h at 4°C.

PQ Extraction

PQ was extracted in a heptane-isobutanol solvent system according to ref. 30.

Herbicide Binding Measurements

The binding experiments were performed as reported by Tischer and Strotmann (26) for PSII-enriched membranes and as reported by Giardi et al. (10) for isolated PSII preparations. The reaction buffer contained 20 mM Mes (pH 6.5), 15 mM NaCl, 5 mM $MgCl_2$, and 0.1 M sucrose. In addition, 0.1% DM was added for binding experiments of isolated PSII core preparations. Each reaction mixture contained 50 μg of Chl of PSII membranes, or 20 μg of Chl of isolated PSII core, and a range of concentrations (about 10) of indicated radiolabeled herbicide. Incubations were carried out in the dark at 4°C for 5 min, and then the mixture was centrifuged at 7,000*g* for 2 min in an Eppendorf centrifuge to separate supernatant containing free herbicide from the pellet containing bound herbicide (26). A loose pellet of isolated PSII core preparations was obtained by centrifugation at 430,000*g* for 1 h at 4°C (Beckman TL100 ultracentrifuge) according to ref. 10. For competition studies, nonradioactive quinone was added at known concentrations (0.2 or 0.5 mM) and radiolabeled-herbicide displacement was measured as described in ref. 26. Calculation of the dissociation constants for the binding of herbicide and quinone was obtained from double reciprocal plots of free versus bound herbicide as reported in ref. 26.

Chl, Cyt b_{559} Content, and Electron Transfer

These measurements were carried out as reported by Hipkins and Baker (15). Cyt was quantified spectrophotometrically in a Shimadzu UV-3000 from the dithionite-induced increase at 559 nm using an absorption coefficient of 15 cm^{-1} mM^{-1}. Electron transfer from DPC (150 μM) to DCPIP (100 μM) was measured spectrophotometrically at 600 nm with samples (Chl concentrations of 15 μg of Chl/mL) under 1200 $\mu E/m^{-2}$ s^{-1} illumination in a 1-cm optical path at 4°C.

SDS-PAGE and Immunoblotting

SDS-PAGE in the presence of 6 M urea was performed according to Barbato et al. (2) using a 12 to 18% linear acrylamide gradient. Densitometric analyses of Coomassie-stained gels were carried out using a Shimadzu CS 9000. For immunoblotting, the resolved proteins were transferred to a nitrocellulose filter and probed with antibodies. The immunocomplexes were detected using biotinylated anti-rabbit secondary antibody and extravidine-labeled alkaline phosphatase. Polyclonal antibodies against the main PSII core polypeptides have been previously described (2). Antibodies against 9-kD phosphoprotein were a kind gift from Prof. P. Boger (Konstanz Universität, Germany).

Radioactivity Measurements

Radioactivity was determined by scintillation counting using Optiphase Safe (LKB) as the cocktail. Counting efficiencies were determined by making similar samples containing radioactive standards. Particular attention was given to the measurements of efficiencies for colored quinone solutions. Counting was obtained in a Packard Tri-Carb 2200 CA liquid scintillation analyzer. Radiolabeled polypeptides were visualized by autoradiography of Coomassie-stained gels using hyperfilm TM MP (Amersham). The autoradiograms were scanned in a Shimadzu 9000 densitometer.

Herbicides and Acceptors

[^{14}C-ring]ter-Butryne (9.8 Ci/mol) (4-ethylamino-2-methyl-thio-6-ter-butylamino-1,3,5-triazine) and [^{14}C-ring]chlorbromurone (8.6 Ci/mol) (3-(3′-chloro-4′-bromophenyl)-1,1-dimethylurea) were a kind gift from Ciba-Geigy; [^{14}C-ring] ioxynil (9.5 Ci/mol) (3,5-diidio-4-hydroxybenzonitrile) was a gift from May and Baker Ltd. (2′,3′-3H)i-Dinoseb (490 Ci/mol) (2,4-dinitro-6-isobutylphenol) was a kind gift from Dr. W. Oettmeier of the Ruhr Universität (Bochum, Germany). DCPIP and DPQ were purchased from Sigma; p-BzQ and NAQ were from Aldrich.

RESULTS

Herbicide Binding to Isolated PSII Cores

The heterogeneous nature of PSII core populations was previously revealed by isoelectrofocusing of solubilized PSII particles, and each PSII population was demonstrated to be phosphorylated to different extents (see Fig. 1 and ref. 11). Four PSII core complexes (referred to as complexes *a*, *b*, *c*, and *d*) were found with their characteristic polypeptide composition (CP47, CP43, D_2, D_1, and Cyt b_{559} plus the *psb*H plastidial gene product) (Fig. 1B). The polypeptide composition of the complexes was tested using polyclonal antibodies against the main PSII polypeptides previously described (2); because the 9-kD phosphoprotein was not stained by Coomassie blue (Fig. 1), its presence was revealed using polyclonal antibodies (data not shown).

The four PSII core populations were isolated from [^{32}P]-ATP spinach thylakoids and further analyzed for the relative distribution of radioactivity in each polypeptide (Fig. 1C and Table I). Complex *a* showed little phosphorylation of D_1 and D_2, whereas the 9-kD phosphoprotein was the most abundantly phosphorylated, confirming our previous results (11). The complex with the more acidic isoelectric point, i.e. complex *d*, showed the highest content of radioactivity, localized

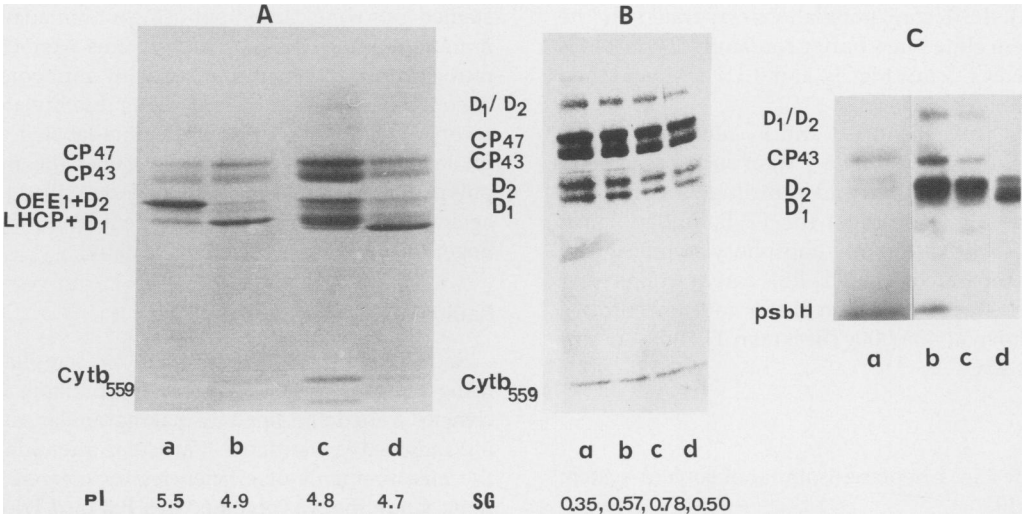

Figure 1. Analysis of the PSII core populations *a* through *d*. A, SDS-PAGE analysis of the four green bands obtained from isoelectrofocusing; pI, isoelectric points of the bands. B, SDS-PAGE analysis of the four PSII core complexes obtained by sucrose gradient ultracentrifugation of the bands from isoelectrofocusing; SG, position of each complex in the sucrose gradient expressed as molarity of sucrose. C, Autoradiography of the SDS-PAGE analysis. SDS-PAGE and autoradiography analyses derive from independent experiments, whereas quantitation of the incorporated radioactivity in the polypeptides of each individual lane is given in Table I.

mainly on D_1. This incorporation level was about 40 times that seen with the complex that was least acidic, complex *a* (Table I). The relative distribution of phosphorylated PSII core populations is dependent upon the light conditions of the tissue prior to membrane extraction (see ref. 11). After incubation in complete darkness, at least two PSII core populations were observed (Table II). It was previously observed that PSII core complexes in the more acidic region of the isoelectrofocusing bed did not retain bound dinoseb (9). The possibility of an artifactual association of the herbicide with PSII core due either to the different length of the electrophoretic run or the affinity of the free herbicide for the electrodes was considered in the present study. For this reason and for the purpose of quantifying the differences in herbicide-binding affinity, the core complexes were extracted from the bed and tested for their ability to bind photosynthetic herbicides (Fig. 2 and Table III) as previously reported for isolated PSII preparations (10).

The affinity of binding to triazines was found to be much lower with PSII particles compared with the intact mem-

Table I. *Relative Phosphorylation Index of Individual Polypeptides in PSII Core Populations*

PSII core populations were isolated from thylakoids radiolabeled with [^{32}P]ATP as described in "Materials and Methods" and analyzed by SDS-PAGE/autoradiography. The values, in arbitrary units, were obtained by dividing the area of the peaks of the single polypeptides from densitometric measurement of an autoradiograph by the total area of the polypeptides in the corresponding Coomassie-stained SDS-polyacrylamide gel. Ratios represent: (1) specific radioactivity into D_1 and D_2 proteins (D_1/D_2); (2) specific radioactivity relative to D_1 for each complex. The polypeptides were assigned as follows: 55 kD, D_1/D_2 heterodimer; 43 kD, CP43; 34 kD, D_2; 32 kD, D_1; 9 kD, *psb*H plastidial gene product. SE is approximately 11%.

Complex	Polypeptides					Ratio	
	55 kD	43 kD	34 kD	32 kD	9 kD	1	2
a	1	5	3	2	30	0.7	1
b	1	5	16	18	4	1.1	9
c	1	6	25	33	0	1.3	16
d	0	1	40	82	0	2.0	41

Table II. *Relative Chl Distribution of PSII Core Populations in Thylakoids Adapted to Light or Dark Conditions and Electron Transfer Inhibition by Herbicide in PSII Core Populations*

Thylakoids were isolated from light-adapted plants and immediately solubilized for the isolation of PSII particles (4). Phosphorylation of these thylakoids (see "Materials and Methods") before the isolation of PSII particles gave similar results. Also, thylakoids were isolated from dark-adapted plants (12 h of incubation in the dark) in a room with indirect green light (less than 5 μE m^{-2} s^{-1}) and incubated in the dark for an additional 2 h. Electron transfer rates were measured from DPC (150 μM) to DCPIP (100 μM). The activity is represented as μmol DCPIP reduced per mg of Chl h^{-1}. Percent inhibition represents inhibition observed in the presence of 10 μM DCMU. nd, Not determined. The values for Chl distribution are an average of four independent experiments. SE is approximately 7%. The values for electron transfer are an average of 10 independent experiments. SE is approximately 16%.

Complex	Percent Chl Distribution		Electron Transfer	
	Light	Dark	Control	Percent inhibition
a	24	40	230	100
b	42	43	120	20
c	6	13	140	38
d	28	4	20	nd

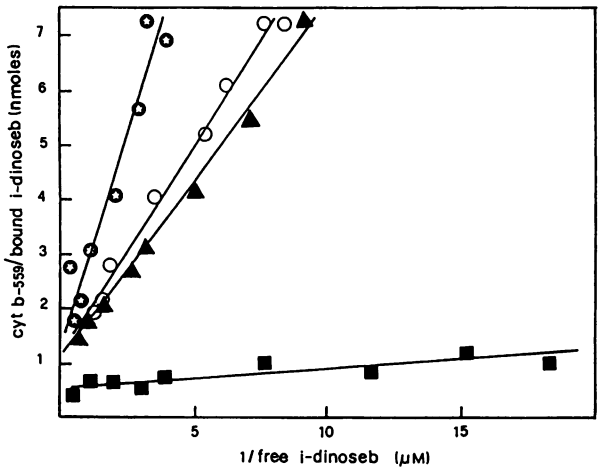

Figure 2. Double reciprocal plots for the binding of i-dinoseb to differently phosphorylated PSII core populations. PSII core populations were isolated from thylakoids of light-adapted plants and binding experiments were performed as described in "Materials and Methods" according to Giardi et al. (10). PSII core *a*, ■; PSII core *b*, O; PSII core *c*, ▲; PSII core *d*, stars.

branes (Table III and see, for comparison, refs. 9, 10, and 26). Furthermore, it was observed that the different PSII cores show (Table II and Fig. 2) different abilities to bind photosynthetic herbicides. An increased level of phosphorylation on D_1 and D_2 in the complex corresponded to a decreased affinity of the same complex for binding herbicides, particularly dinoseb. For instance, the number of sites for dinoseb per Cyt b_{559} increased significantly in complex *a*, which contained highly phosphorylated 9-kD protein. These data suggest that this protein may be directly linked to the binding of this herbicide, but further evidence needs to be obtained to confirm this (Table III).

Our observations concerning the differences in the ability of phosphorylated core populations to bind herbicides are consistent with the observations on the different degrees of electron transfer inhibition measured on the cores in the presence of DCMU (Table II). Lower concentrations of DPC (150 μM) and DCPIP (100 μM) were used because at higher concentrations inhibition due to the herbicide was reversed (data not shown). Under these conditions, the most phosphorylated core *d* was not able to transfer electrons to DCPIP (Table II).

Herbicide Binding to PSII Particles

An approach to investigate if protein phosphorylation affects the herbicide-binding site was to extract the secondary PQ acceptor molecule from the Q_B site. The PQ at the Q_B site was selectively removed by heptane-isobutanol extraction of thylakoids without disturbing significantly the Q_A site and the functioning of these membranes as described (30). Table IV shows the dissociation constants for the binding of some herbicides (representative of the four classes of herbicides, namely triazines, phenylurea, hydroxybenzonitriles, and phenolic compounds) to phosphorylated and nonphosphorylated PSII particles and to those PSII particles from which PQ had been extracted. Control thylakoids were illuminated in the absence of ATP and $MgCl_2$ to keep them from being phosphorylated; however, the solution contained 150 mM KCl to keep the membranes stacked. The thylakoids were then dark adapted for 2 h. This procedure allowed us to exclude the effects due to photoinhibition of PSII.

The results in Table IV show that the membrane phosphorylation resulted in slight but systematic increases in the dissociation constants for the binding of most photosynthetic herbicides, indicating a reduced herbicide affinity. This modification in herbicide affinity was more evident in samples from which PQ had been extracted. The results were more striking for the phenolic herbicides. These data are consistent with the observations that the introduction of negatively charged groups into the proteinaceous fold affects mainly the binding of nucleophilic compounds such as the phenolic herbicides (27). The membranes after extraction showed a decreased number of Chls per binding site, indicating that the PQ extraction procedure probably results in reduction in the binding of Chl. Chlorbromurone herbicide was found to have a significantly different stoichiometry with the PSII reaction center compared with the other herbicides (Table IV).

Competition Studies of Herbicides with Quinones and DCPIP in PSII Particles

It was of interest to investigate whether added quinones are able to displace herbicides in isolated PSII particles and

Table III. *Dissociation Constants for the Binding of Differently Phosphorylated PSII Cores*

Herbicide binding experiments were performed according to Giardi et al. (10) and the dissociation constants (K_d) were calculated from double reciprocal plots of free versus bound herbicide according to Tischer and Strotmann (26). Each value given was obtained from three replicates.

Herbicide	K_d of PSII Core Populations			
	a	*b*	*c*	*d*
			nM	
Chlorbromurone	70 ± 13	250 ± 23	180 ± 20	400 ± 35
Sites/Cyt b_{559}	0.9 ± 0.3	1.1 ± 0.1	1.3 ± 0.2	0.8 ± 0.2
i-Dinoseb	94 ± 13	700 ± 61	400 ± 36	1000 ± 77
Sites/Cyt b_{559}	1.9 ± 0.1	0.8 ± 0.3	0.9 ± 0.3	0.9 ± 0.3
Ioxynil	40 ± 6.5	180 ± 26	130 ± 17	600 ± 58
Sites/Cyt b_{559}	1.1 ± 0.1	0.7 ± 0.2	0.9 ± 0.2	0.8 ± 0.3

whether protein phosphorylation of proteins affect their competition with herbicides. We tested some synthetic quinones that were previously shown to replace efficiently PSII herbicides and the native PQ bound to the Q_B pocket (6, 7, 28, 30). The affinities of herbicides and quinones for the binding site(s) differ by a factor of more than 100. Therefore, relatively high concentrations of synthetic quinones had to be used to observe herbicide displacement (28). Figure 3 shows that DPQ is able to displace the herbicide chlorbromurone from PSII preparations from both phosphorylated and nonphosphorylated membranes. The displacement seems to be slightly more effective in nonphosphorylated membranes (Fig. 3). The number of Chl molecules per binding site in the presence of the quinone did not change, indicating that in the PSII membranes the interaction between herbicides and quinones is apparently competitive (Fig. 3), as was shown previously for whole thylakoid membranes (26, 28).

Table IV shows the constants for the binding of electron acceptors calculated from radiolabeled-herbicide displacement as previously reported (26). All of these experimental measurements indicated that the functioning of the Q_B pocket in quinone binding is somewhat reduced by protein phosphorylation. Like the quinones, DCPIP, the indophenol compound used in the present study as an acceptor in electron transfer measurements, showed reduced affinity for phosphorylated membranes (Table IV).

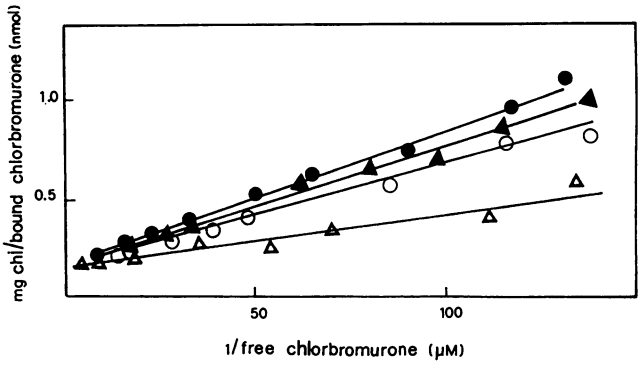

Figure 3. Effect of unlabeled DPQ on the binding of radiolabeled chlorbromurone to isolated PSII particles. Triangles represent nonphosphorylated membranes and circles represent phosphorylated membranes. Open symbols represent data obtained in the absence of DPQ, and closed symbols represent data obtained in the presence of DPQ (0.5 mM). Competition between radiolabeled herbicide and unlabeled DPQ was performed according to ref. 26 (see "Materials and Methods" for details).

DISCUSSION

Although it is known that some PSII core proteins are phosphorylated by a protein kinase(s), the function of this phosphorylation process remains unknown. Some effects that have been attributed to the phosphorylation of the PSII core polypeptides include an inhibition of light-saturated PSII electron transfer (18, 19), a destabilization of the anionic semiquinone Q_B^-, and an accumulation of Q_A^- (17). Recently, the existence of PSII core populations that differ in the extent of phosphorylation of their polypeptides has been described (9, 11). It was possible to convert the cores from one form to another in the dark; however, this conversion was never complete because at least two differently phosphorylated populations were always detected (see Table II and ref. 11). Incompleteness of this interconversion is also supported by the recent observations of Elich et al. (8) that in vivo dephosphorylation of PSII core proteins appears to be a relatively slow process, with a half-time of hours. One of the interesting results of this study was the observation that the differently phosphorylated PSII core populations show different degrees

Table IV. *Dissociation Constants for the Binding of Herbicides and Electron Transfer Acceptors to PSII Particles*

PSII particles were isolated from phosphorylated (Phos) and nonphosphorylated (Nonphos) thylakoids as described in "Materials and Methods." PQ was extracted from particles by a heptane-isobutanol extraction (30). K_d values (nM) for herbicide binding were calculated from double reciprocal plots of free versus bound herbicide according to Tischer and Strotmann (26). K_d values (μM) for electron acceptor binding were obtained by competition of unlabeled compounds (0.2 and 0.5 mM) with radiolabeled chlorbromurone according to Tischer and Strotmann (26). Chl concentration was 50 μg/mL. Each value given was obtained from three replicates.

	K_d		Ratio	Chl/Site	
	Phos	Nonphos	Phos/nonphos	Phos	Nonphos
t-Butryne	60 ± 4.1	45 ± 2.6	1.3	260 ± 20	254 ± 13
PQ-free	27 ± 2.9	16 ± 1.3	1.7	206 ± 16	200 ± 20
Chlorbromurone	37 ± 6.3	20 ± 2.6	1.8	150 ± 16	151 ± 19
PQ-free	19 ± 1.9	10 ± 2.2	1.9	127 ± 18	134 ± 18
Ioxynil	33 ± 2.3	18 ± 1.7	1.8	253 ± 24	247 ± 31
PQ-free	19 ± 4.3	9 ± 1.5	2.1	205 ± 30	199 ± 25
i-Dinoseb	33 ± 4.0	16 ± 2.6	2.0	208 ± 15	272 ± 50
PQ-free	27 ± 3.1	12 ± 1.1	2.2	165 ± 33	170 ± 42
NAQ	544 ± 51	270 ± 22	2.0	215 ± 29	282 ± 41
p-BzQ	620 ± 23	379 ± 31	1.6	199 ± 64	261 ± 37
DCPIP	325 ± 18	190 ± 16	1.7	222 ± 46	201 ± 25

of affinity for herbicides. Comparative studies using PSII preparations revealed that when the extent of phosphorylation on D_1 and D_2 increases, the Q_B-binding activity is affected. Further, the magnitude of this phenomenon measured in PSII membranes is consistent with the modification and the interconversion (about 30% after 2 h of dark incubation) observed for PSII core populations.

The existence of PSII cores with different behavior with herbicides and quinones strongly supports the idea that phosphorylation on PSII core polypeptides explains, in part, the heterogeneity of PSII observed when these inhibitors are used. The supporting evidence for the existence of PSII heterogeneity comes, for the most part, from interpretations of spectroscopic measurements in the presence of herbicides or quinones (5, 12, 16, 23, 24). A certain heterogeneity seems to derive from chemically different PSII, called PSIIα and PSIIβ, respectively located in grana or stroma regions of the thylakoids (5, 16, 23). PSIIα itself seems heterogeneous, giving rise to at least three phases in Chl fluorescence induction curves in DCMU-treated PSII particles and some populations with different antenna size (1, 16). Moreover, through several approaches it has been demonstrated that Q_B is not reduced by some PSII centers (5, 24). Heterogeneity is a difficult and controversial field, and it is not easy to judge the relationship that our findings may have to the PSII heterogeneity observed in vivo. Further work is needed to clarify these relationships.

We observed that, in accordance with other reports (18, 19), protein phosphorylation results in a lower rate of PSII electron transfer from DPC to DCPIP, and such an inhibition seems to be correlated with our finding of a reduced DCPIP affinity in phosphorylated membranes and the consequent inability of this acceptor to oxidize the PSII when D_1 and D_2 are highly phosphorylated, as they are in core complex *d*. Our results are at variance with the conclusions reported by Harrison and Allen (14) that the Q_B pocket is not modified by dephosphorylation with an alkaline phosphatase. However, some of our preliminary data (unpublished results) indicate that the exogenous phosphatase acts at a different level than endogenous phosphatase(s).

The following conclusions can be drawn. Phosphorylation of PSII core complexes reduces the ability of herbicides, quinones, and other artificial electron acceptors to bind to the Q_B site, and this correlates with PSII centers being less efficient in electron transfer. The reduction of quinone-binding affinity in phosphorylated membranes suggests that when increased light energy causes the reduction of the PQ pool and activates the kinase, bound PQ leaves its site, possibly also as a consequence of Q_B pocket modification.

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