# Herbicide and Plastoquinone Binding to Photosystem II

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Z. Naturforsch. **39 c**, 393–396 (1984); received November 30, 1983

Photoaffinity Label, Plastoquinone Depletion, Photosystem II Particles, *Chlamydomonas*

In plastoquinone-depleted thylakoids "DCMU-type" herbicides generally have a lower binding affinity as compared to control thylakoids, with the exception of DCMU itself. Contrary, binding properties of phenolic herbicides are not affected by plastoquinone depletion. DCMU in plastoquinone-depleted thylakoids can be displaced from the membrane by various substituted 1,4-benzoquinones, including plastoquinone-1. Labeling patterns by azido-atrazine are compared for thylakoids, plastoquinone-depleted thylakoids, and different photosystem II preparations from spinach and *Chlamydomonas*. Azido-atrazine tags a 32–34 kDa protein, which seems to be different from another 32–34 kDa protein which is preferentially labeled by a plastoquinone-azide.

## Introduction

Herbicides which act as inhibitors of photosynthetic electron transport bind reversibly and non-covalently to proteins on the acceptor side of photosystem II. These binding proteins have recently been identified as the 32–34 kDa HBP for "DCMU-type" herbicides [1, 2], and as the photosystem II reaction center for phenolic herbicides [2, 3] by means of photoaffinity labels. As to the mechanism of herbicide action it is assumed that herbicide binding to the respective herbicide binding proteins prevents binding of plastoquinone from the "pool" and thus interrupts photosynthetic electron flow [4, 5]. However, the exact nature of herbicide/plastoquinone interaction is still unclear, especially the question whether herbicide and plastoquinone have identical binding sites.

In order to investigate this we have studied herbicide/quinone interaction and photoaffinity label binding in plastoquinone-depleted spinach thylakoids and in photosystem II preparations as well. Furthermore, direct identification of plastoquinone binding proteins was attempted by use of a plastoquinone-azide photoaffinity label [6], which has been resynthesized at a high specific activity.

*Abbreviations:* Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine; Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; HBP, herbicide binding protein; i-dinoseb, 2,4-dinitro-6-isobutylphenol; ioxynil, 4-hydroxy-3,5-diiodobenzonitrile; LDS, lithium dodecyl sulfate; metribuzin, 4-amino-6-*tert*-butyl-3-(methylthio)-1,2,4-triazin-5-(4H)-one; PAGE, polyacrylamide gel electrophoresis; PQAz, 2,3-dimethyl-5-{4′-[3-(4-azido-2-nitroanilino)-[2′,3′-$^3$H]-propionoxy]-*n*-butyl}-1,4-benzoquinone.

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0341-0382/84/0500-0393 $ 01.30/0

## Results and Discussion

### *1. Plastoquinone-depleted spinach thylakoids*

Radioactively labeled inhibitors can be displaced from the thylakoid membrane by another inhibitor [7]. Any attempts to displace an inhibitor by plastoquinone itself or the short chain analogue plastoquinone-1 have failed so far [8]. One reason for that may be the high endogenous plastoquinone content of the thylakoid membrane. We have, therefore, prepared plastoquinone-depleted thylakoids by means of *n*-hexane extraction [9]. Binding parameters for herbicides in plastoquinone-depleted thylakoids together with the control are summarized in Table I. As can be seen, the binding affinity for most "DCMU-type" herbicides in plastoquinone-depleted thylakoids is one order of magnitude less as compared to control thylakoids, whereas the number of binding sites is unchanged. DCMU itself is the only exception; its binding is not affected by *n*-hexane extraction (Table I). If plastoquinone-depleted thylakoids are labeled by azido-atrazine at a concentration of 10 nmol/mg Chl, no radioactivity in the 32–34 kDa HBP can be found (Fig. 1 B). At this concentration of azido-atrazine the 32–34 kDa HBP in normal thylakoids would be heavily tagged [2]. The 32–34 kDa HBP, however, is still present in plastoquinone-depleted thylakoids: if the labeling by azido-atrazine is performed prior to *n*-hexane

Table I. Binding constants $K_b$ and number of binding sites $x_t$ for "DCMU-type" and phenolic herbicides in control and plastoquinone-depleted thylakoids.

| Compound | Control | | Plastoquinone-depleted | |
|---|---|---|---|---|
| | $K_b$ (nM) | $x_t$ (nmol/mg Chl) | $K_b$ (nM) | $x_t$ (nmol/mg Chl) |
| A. "DCMU-type" | | | | |
| atrazine | 63 | 1.48 | 599 | 1.69 |
| metribuzin | 41 | 1.67 | 390 | 1.43 |
| phenisopham | 23 | 5.33 | 310 | 4.22 |
| DCMU | 34 | 2.36 | 31 | 1.60 |
| B. phenolic | | | | |
| 2-iodo-4-nitro-6-isobutylphenol | 18 | 2.75 | 25 | 3.50 |
| i-dinoseb | 69 | 1.45 | 40 | 0.97 |
| picric acid | 250 | 2.84 | 108 | 2.49 |
| ioxynil | 29 | 2.44 | 42 | 2.18 |

extraction, bound radioactivity in the 32 kDa region can be found (Fig. 1 A). These findings altogether support the idea of different but overlapping binding sites for various classes of "DCMU-type" herbicides at the 32–34 kDa HBP [10]. Furthermore, binding of herbicides to the 32–34 kDa HBP is strongly influenced by its environment: perturbations as exerted by *n*-hexane extraction, which in addition to plastoquinone also removes some lipids, results in a loss of affinity of some herbicides.

In contrast, binding of phenolic herbicides, which are directed against the photosystem II reaction center, is not affected by plastoquinone depletion (Table I).

Since DCMU-binding in plastoquinone-depleted thylakoids is not changed, displacement experiments with a variety of 1,4-benzoquinones have been performed [11]. Four examples are demonstrated in Fig. 2. The degree of displacement depends on the chemical nature of the quinone substituents: tetraiodo-benzoquinone is an effective, 3,5-di-*tert*-butyl-benzoquinone only a moderate displacer. In a quantitative structure-activity relationship, displacement activity of the 25 quinones tested so far could be correlated to their redox potential and their steric properties [11]. As Fig. 2 shows, also plastoquinone-1[5,6-dimethyl-3-(3-methyl-2-butenyl)-1,4-benzoquinone] can displace DCMU from the membrane. An in-depth analysis of the binding data at various concentrations of plastoquinone-1 and DCMU revealed a competitive interaction between both compounds. A binding constant $K_b = 51 \pm 19$ µM could be calculated for plastoquinone-1 [8]. It can be concluded, therefore,

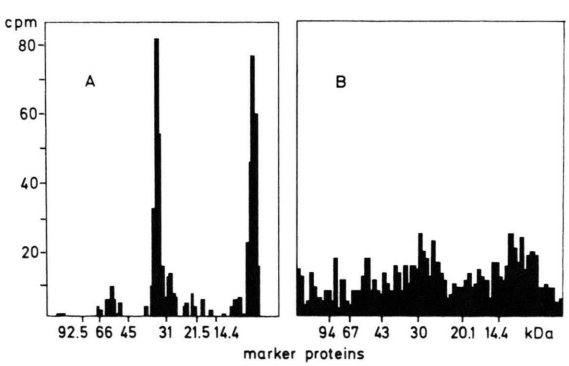


Fig. 1. Radioactivity distribution of plastoquinone-depleted thylakoids in an LDS-PAGE gel (11–15%) after labeling with azido-atrazine (10 nmol/mg Chl) before (A), and after plastoquinone depletion (B).

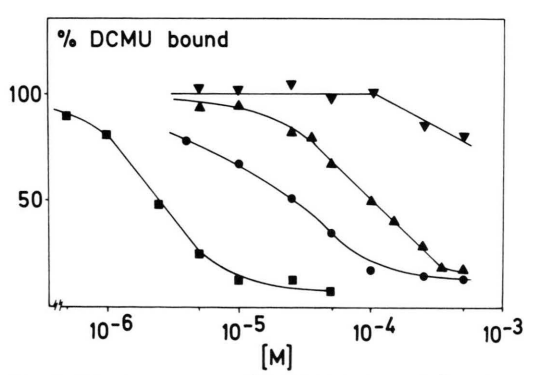


Fig. 2. Displacement of [$^3$H]DCMU ($5 \times 10^{-9}$ M) in plastoquinone-depleted thylakoids by tetraiodo (■—■), 5,6-dibromo-3-*tert*-butyl (●—●), 5,6-dimethyl-3-(3-methyl-2-butenyl) (▲—▲), and 3,5-di-*tert*-butyl-1,4-benzoquinone (▼—▼).

that herbicide binding indeed influences plastoquinone binding at the thylakoid membrane.

### *2. Photosystem II preparations*

A photosystem II preparation from spinach thylakoids as obtained by Triton treatment according to the procedure by Berthold *et al.* [12] retains an active oxygen evolving system. In this preparation, photosynthetic electron transport in the system water to dichlorophenolindophenol is sensitive both to "DCMU-type" and phenolic herbicides [13, 14]. Consequently, after labeling with azido-atrazine radioactivity is found in the 32–34 kDa HBP and after labeling with azido-dinoseb in the photosystem II reaction center proteins [13, 14].

A photosystem II particle from the alga *Chlamydomonas reinhardtii* according to Diner and Wollman [15] does not possess an intact water splitting enzyme system. In this preparation, photosynthetic electron transport is insensitive toward "DCMU-type" herbicides, but still sensitive toward phenolic herbicides (system diphenylcarbazide to dichlorophenolindophenol) [2]. Labeling with azido-atrazine reveals no radioactivity within the 32–34 kDa molecular weight range, where HBP migrates in the gel (Fig. 3 A). The only radioactivity detected is due to some unspecific labeling of lipids and free pigments (right side, Fig. 3 A). This further supports the notion as stressed above that an intact environment of the 32–34 kDa HBP is necessary to facilitate herbicide binding. In contrast, azido-dinoseb still tags the photosystem II reaction center [2, 14].

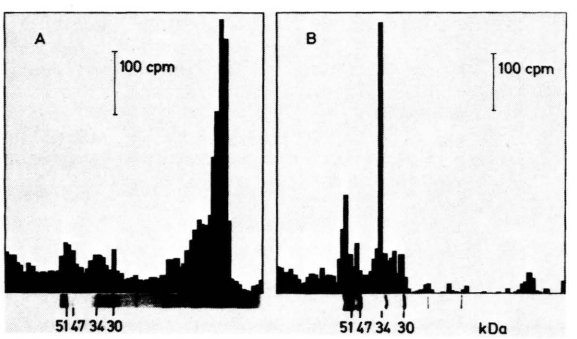


Fig. 3. Photographs and radioactivity scans of LDS-PAGE gels (11–15%) of photosystem II particles from *Chlamydomonas* labeled either by azido-atrazine (50 nmol/mg Chl) (A) or isolated from [$^{14}$C]acetate grown algae (B).

The lack of radioactivity in the 32–34 kDa molecular weight range within the gel after labeling with azido-atrazine, however, does not indicate that the 32–34 kDa HBP is missing in this preparation. Presently, it is assumed that the "rapidly turning over" or "photogene" 32–34 kDa protein [16], and the 32–34 kDa HBP are identical. If *Chlamydomonas* was grown in a medium containing [$^{14}$C]acetate, about 20% of the radioactivity was incorporated into thylakoids after 20 min growth in the light. Gel electrophoresis and scanning revealed that most of the radioactivity was incorporated into a poorly staining 32–34 kDa protein. Furthermore, after preparation of photosystem II particles from these $^{14}$C enriched thylakoids, it could be demonstrated that the most prominent radioactivity peak is located within the 32–34 kDa molecular weight region (Fig. 3 B).

### *3. Plastoquinone-azide photoaffinity labeling*

We have recently synthesized a plastoquinone-derived photoaffinity label PQAz [6]. By this, in isolated thylakoid cytochrome $b_6/f$-complex the 23.5 kDa cytochrome $b_b$ and the 20 kDa Rieske Fe S protein could be identified as plastoquinone binding proteins [6]. However, the specific activity of PQAz was too low to allow for identification of plastoquinone binding proteins in a thylakoid preparation. PQAz was, therefore, resynthesized at a specific activity of 289 mCi/mmol. As we have reported recently, the high specific activity PQAz in thylakoids predominantly labels a protein in the 32 kDa molecular weight range [17]. Since several proteins comigrate in this molecular weight range [18], it was of special interest to ascertain whether the proteins which are tagged by azido-atrazine or PQAz, respectively, are identical or not. Fig. 4 A demonstrates the labeling pattern of thylakoids by 2 nmol/mg Chl PQAz in an LDS-PAGE gel (11–15%). Note that besides a 32 kDa protein also the light harvesting Chl *a/b* protein complex is heavily tagged.

Fig. 4 B compares the radioactivity distribution of thylakoids labeled either by azido-atrazine (10 nmol/mg Chl) or PQAz (2 nmol/mg Chl) run in adjacent lanes of a 9–14% LDS-PAGE gel. Unfortunately, in this particular experiment for some unknown reason the radioactivity in the light harvesting Chl *a/b* complex (3) is higher as compared to the 32 kDa

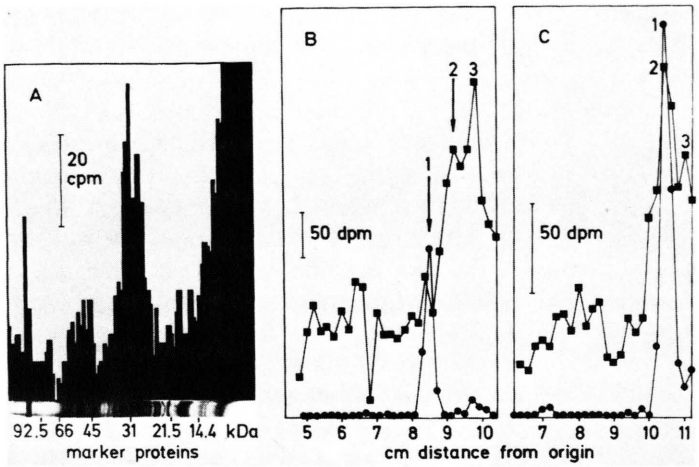


Fig. 4. A) Photograph and radioactivity scan of a LDS-PAGE gel (11–15%) of thylakoids labeled by PQAz (2 nmol/mg Chl). B, C). Superimposed parts of LDS-PAGE gels (9–14%) of thylakoids run in adjacent lanes and labeled either by 10 nmol/mg Chl azido-atrazine (●—●) or 2 nmol/mg Chl PQAz (■—■) in the absence (B) or presence (C) of 4 M urea. 1. Azido-atrazine binding protein; 2. PQAz binding protein; 3. Light harvesting chlorophyll *a*/*b* protein.

protein (2). We note that the two proteins labeled either by azido-atrazine (1) or PQAz (2) clearly migrate at different $R_f$-values (0.55 and 0.59, respectively). Contrary, $R_f$-values are identical if 4 M urea is included into the gel (Fig. 4C). This may indicate that the two proteins being tagged by azido-atrazine or PQAz, respectively, are indeed different. Another explanation might be that attachment of the quinone moiety, which originates from PQAz, as compared to the attachment of the triazine moiety which originates from azido-atrazine, to an identical protein differently affects its mobility in the gel. Further work has to be performed to clarify this question.

*Acknowledgements*

This work was supported by Deutsche Forschungsgemeinschaft.

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