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# BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF HERBICIDE-RESISTANT MUTANTS OF THE UNICELLULAR CYANOBACTERIUM, *ANACYSTIS NIDULANS* R2

SUSAN S. GOLDEN * and LOUIS A. SHERMAN **

*Division of Biological Sciences, 110 Tucker Hall, University of Missouri, Columbia, MO 65211 (U.S.A.)*

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**Two herbicide-resistant mutants of the unicellular cyanobacterium, *Anacystis nidulans* R2, were obtained by mutagenesis with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. These mutants, *A. nidulans* R2D1 and R2D2, were selected by growth of mutagenized cells in the presence of $10^{-6}$ M and $10^{-5}$ M 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU), respectively. Both were found to be cross-resistant to 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine (atrazine) and 2-*n*-heptyl-4-hydroxyquinoline-*n*-oxide (HQNO) by measurement of Photosystem II activity in the presence of the inhibitors. The DCMU-resistance trait from each mutant was transferred to a wild-type genetic background by DNA-mediated transformation of *A. nidulans* cells. The two resulting transformants, *A. nidulans* R2D1-X1 and R2D2-X1, were similar to the original mutants with respect to DCMU- and HQNO-resistance. However, both exhibited increased sensitivity to atrazine relative to the mutants from which they were derived. Polyacrylamide gel electrophoretic analysis revealed that the mutants and transformants were deficient in a 34 kDa, surface-exposed polypeptide which was present in the wild-type strain; the transformants exhibited a new polypeptide of 35.5 kDa which was also highly surface-exposed.**

## Introduction

The herbicides DCMU and atrazine inhibit photosynthetic electron flow by blocking electron transport between the primary Photosystem II acceptor, Q, and the secondary acceptor, B [1,2]. DCMU, a urea class inhibitor, and atrazine, a triazine, have similar physiological effects. Binding experiments have shown that the two agents bind competitively to the same site on the membrane [3]; DCMU has been shown to displace the plastoquinone cofactor of B (Robinson, H., and Crofts, A.R., personal communication). However, the binding site contains unique contact points for each of the herbicides, as mutations which block binding of one of the agents may or may not affect action of the other. Thylakoids from atrazine-resistant pigweed are as sensitive to DCMU as those of the atrazine-sensitive biotype [4], but a DCMU-resistant mutant of *Chlamydomonas reinhardii* exhibits some cross-resistance to atrazine [5]. Atrazine cross-resistance has also been reported in DCMU-resistant mutants of the unicellular cyanobacterium, *Aphanocapsa* 6714 [6].

The binding site of atrazine has been identified by photoaffinity labeling of thylakoid membranes

* Present address: Department of Biophysics and Theoretical Biology, University of Chicago, Chicago, IL 60637, U.S.A.
** To whom correspondence should be addressed.

Abbreviations: nitrosoguanidine, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine; DCMU, 3-(3,4-dichlorophyl)-1,1-dimethyl urea; atrazine, 2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine; HQNO, 2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide; DCBQ, 2,6-dichloro-*p*-benzoquinone.

with a [$^{14}$C]azido derivative of atrazine [7]. Upon ultraviolet irradiation, azido-atrazine becomes cross-linked to a 32 kDa thylakoid membrane polypeptide which is thought to be the apoprotein component of the Photosystem II acceptor, B [2]. Additionally, DCMU sensitivity is lost when a 32 kDa polypeptide is cleaved from thylakoids by trypsin treatment [8,9].

HQNO is another inhibitor of electron transport which acts between Photosystem I and Photosystem II [10,11]. Binding-competition experiments, fluorescence data, and trypsin treatment have shown that the binding site for HQNO differs from that of DCMU and atrazine [10]. However, cross-resistance to HQNO of atrazine-resistant pigweed has been reported [10].

The interactions between various components of the photosynthetic apparatus can be studied with herbicide-resistant mutants and inhibitors which affect related but distinct aspects of photosynthetic electron transport. We have induced mutations which confer resistance to DCMU in the unicellular cyanobacterium, *A. nidulans* R2. This organism is highly transformable by exogenously added DNA, and recent progress has been made in use of this property for genetic study [12–14]. A combination of genetic, biochemical, and biophysical techniques has allowed extensive characterization of the defect responsible for DCMU resistance in these mutants. The DCMU-resistant strains were cross-resistant to atrazine and HQNO and exhibited an alteration in a 34 kDa thylakoid membrane protein.

Portions of this work were presented at the 1982 Annual Meeting of the American Society of Plant Physiologists [15].

## Materials and Methods

*A. nidulans* R2 was originally characterized by Grigorieva and Shestakov [16] and was obtained from G. van Arkel (Utrecht, The Netherlands). This strain was grown in liquid BG-11 medium or on plates of BG-11 agar [17] as previously described [18].

Mutagenesis of *A. nidulans* was performed by a modification [18] of the technique described by Adelberg et al. [19]. Nitrosoguanidine was used as a mutagen at a final concentration of 50 $\mu$g/ml, which resulted in survival of 10% of the treated cells. Aliquots of mutagenized cells were selected in $10^{-6}$ M or $10^{-5}$ M DCMU (DuPont) for resistance to that agent. 3% of wild-type *A. nidulans* cells survived treatment with $10^{-7}$ M DCMU, whereas no cells survived $10^{-6}$ M or higher concentrations of DCMU. A single colony of each culture, obtained by growth on BG-11 agar in the presence of $10^{-6}$ M or $10^{-5}$ M DCMU, was chosen for photosynthetic characterization; these mutants were assigned the designations *A. nidulans* R2D1 and R2D2, respectively.

Total nucleic acid was prepared from 500 ml late-logarithmic phase cultures of *A. nidulans* mutants by published procedures [20]. Cells were collected by centrifugation and resuspended in 20 ml of 25% sucrose/50 mM Tris/100 mM EDTA (pH 8.0). Glass beads were added to a packed volume of 5 ml, and the cells were disrupted by vortex mixing for 5 min. The ruptured cell supernatant was removed from the settled beads, and extracted with phenol and chloroform. The nucleic acid was collected by precipitation with ethanol and resuspended in 10 mM Tris/1.0 mM EDTA (pH 8.0).

Mid-logarithmic growth phase *A. nidulans* cells were prepared for transformaton by washing once in 10 mM NaCl and resuspending at $5 \cdot 10^8$ cells/ml in fresh BG-11 medium. Mutant total nucleic acid (containing chromosomal DNA, sheared to approx. 15 kb in size, and rRNA) was added to 300-$\mu$l aliquots of cells to a final concentration of 1–2 $\mu$g/ml. The transformation mixtures were continually agitated during a 15-h incubation in darkness at 30°C. Agar plates (containing 40 ml of 1.5% agar in BG-11) were inoculated with 100 $\mu$l of cells, and incubated for 6 h under standard lighting conditions [18]. The agar was then partially lifted with a sterile spatula and 400 $\mu$l of a $10^{-4}$ M or $10^{-3}$ M solution of DCMU was dispensed underneath. The agar slab was replaced, and the plates were incubated for 5–7 days prior to removal of transformed (DCMU-resistant) colonies. Six colonies were obtained following transformation with *A nidulans* R2D1 nucleic acid and greater than 450 were obtained using that from *A. nidulans* R2D2. (Subsequent experiments with donor DNA which had been purified free of RNA by CsCl buoyant density

centrifugation showed that the frequency of transformation was approx. 1000 transformants per microgram of DNA from either donor strain). A colony transformed by DNA from each mutant (designated R2D1-X1 or R2D2-X1) was selected for photosynthetic characterization.

Mutants *A. nidulans* R2D1 and R2D2 were maintained in growth medium supplemented with the concentration of DCMU in which each was initially selected. However, both mutants and corresponding transformants were later maintained in $10^{-6}$ M DCMU, because the strains did not differ significantly in their sensitivities to the compound, and *A. nidulans* R2D2 (and R2D2-X1) had faster growth rates at the lower concentration.

Fluorescence kinetics assays were performed as previously described [21], using culture samples normalized to 2 μg/ml chlorophyll. Various concentrations of DCMU were added to some samples during the dark adaptation period.

Photosystem II electron transport was assayed as oxygen production during transfer of electrons from $H_2O$ to DCBQ [21]. Assays were performed on cells or membranes normalized to 5 μg chlorophyll per ml, and ethanolic solutions of the compounds were added to appropriate concentrations. A Labsource QH150 fiber optic illuminator was used to supply saturating, cool white light.

Oxygen-evolving, photosynthetic membranes with attached phycobilisomes were isolated by the procedure of Gantt et al. [22]. Cells were harvested and resuspended in buffer 1 (0.5 M sucrose/0.5 M phosphate/0.3 M citrate (pH 6.8)) at 350 μg Chl/ml. The cells were then passed twice through a chilled French pressure cell at 20000 lb/inch. After removal of cell debris, the supernatant was centrifuged at 15000 rpm for 15 min to yield a membrane pellet. These membranes demonstrated high rates of $O_2$ evolution and were stable to within 80% of maximum for at least 6 h. Data from absorption spectra were used to estimate the chlorophyll and phycocyanin contents of the samples with the equations of Arnon et al. [23].

Radiolabeling of membrane polypeptides with $H_2^{35}SO_4$ in vivo, or by lactoperoxidase-catalyzed iodination ($^{125}I$), were carried out as previously described [24]. Membranes were prepared by the procedure of Guikema and Sherman [24], except that spheroplast washes were supplemented with 150 mM KCl to remove lysozyme prior to osmotic rupture of the cells.

Electrophoretic separation of membrane polypeotides was performed on 10–20% polyacrylamide gels in the presence of lithium dodecyl sulfate as previously described [24]. Samples were normalized to 200000 cpm of radiolabeled material for autoradiography.

## Results

### *Mutant isolation and characterization*

Two mutants of *A. nidulans* were isolated which were resistant to the photosynthetic electron transport inhibitor, DCMU. These mutants, *A. nidulans* R2D1 and R2D2, were selected by growth of

TABLE I

RELATIVE PIGMENT COMPOSITION OF *A. NIDULANS* R2 AND DCMU-RESISTANT STRAINS

| Strain | Chl (mutant) [a] / Chl (wild-type) | | Chl/Phy [a] (nmol/nmol) | |
|---|---|---|---|---|
| | intact cells | membranes [b] | intact cells | membranes [b] |
| Wild-type | 1.0 ±0.05 | 1.0 ±0.05 | 1.96±0.10 | 1.96±0.10 |
| R2D1 | 0.90±0.08 | 0.67±0.08 | 1.92±0.16 | 1.70±0.16 |
| R2D1-X1 | 0.98±0.07 | 0.87±0.07 | 1.95±0.12 | 1.70±0.15 |
| R2D2 | 0.92±0.08 | 0.75±0.08 | 2.00±0.12 | 1.86±0.16 |
| R2D2-X1 | 0.96±0.10 | 0.83±0.10 | 1.90±0.08 | 1.60±0.05 |

[a] Relative amount of chlorophyll per $10^8$ cells. Chlorophyll and phycocyanin (Phy) concentrations were calculated from absorbances at 620 and 678 nm using the equations of Arnon et al. [23]. Each value is an average of six independent measurements ±S.D.

[b] Membranes prepared by French pressure cell breakage in buffer 1 (see Materials and Methods).

nitrosoguanidine-mutagenized cells in $10^{-6}$ M and $10^{-5}$ M DCMU, respectively. Both mutants and the wild-type strain had a doubling time of approx. 15 h in the absence of DCMU. *A. nidulans* R2D1 and R2D2 cultures doubled in cell number every 15–20 h during logarithmic phase growth in the presence of $10^{-6}$ M DCMU, whereas the wild-type cells failed to divide during 120 h of incubation in DCMU at this concentration.

The physical properties of the mutants were very similar to those of the wild type. Analysis of absorption spectra of intact cells demonstrated that the ratio of chlorophyll to phycocyanin was virtually unchanged in all of the strains studied (Table I). The chlorophyll content per cell of the mutants and the subsequent transformants were within 90% of the wild-type level (Table I), and the cellular protein content was identical for all strains (data not shown). However, the pigment content of the mutant photosynthetic membranes differed from that of the wild type (Table I). The mutant membranes had a depressed chlorophyll content and a somewhat lower chlorophyll-to-phycocyanin ratio. The changes were small but reproducible, and demonstrated that the mutants are somewhat more susceptible to alteration during membrane preparation.

*Photosystem II electron transport assays*

The sensitivity of Photosystem II activity to DCMU was assessed for wild-type *A. nidulans* and the mutant strains by measuring photosynthetic electron transport between water and the electron acceptor, DCBQ. These measurements were performed on intact cells and on isolated $O_2$-evolving membranes with very similar results. This finding indicated that the resistance of the mutants to DCMU was not due to a defect in permeability to the compound. The effect of DCMU on membranes from wild-type and mutant cells is shown in Fig. 1. The concentration of DCMU which exhibited electron transport by 50% ($I_{50}$) was $(1.0 \pm 0.1) \cdot 10^{-7}$ M for wild-type *A. nidulans*. Both mutants evolved oxygen at that DCMU concentration at greater than 85% of their control rates in the absence of DCMU. The $I_{50}$ values for the mutants were $10 \pm 1$ μM and $12 \pm 1$ μM for R2D1 and R2D2, respectively. Thus, the mutant membranes demonstrated an approx. 100-fold increase

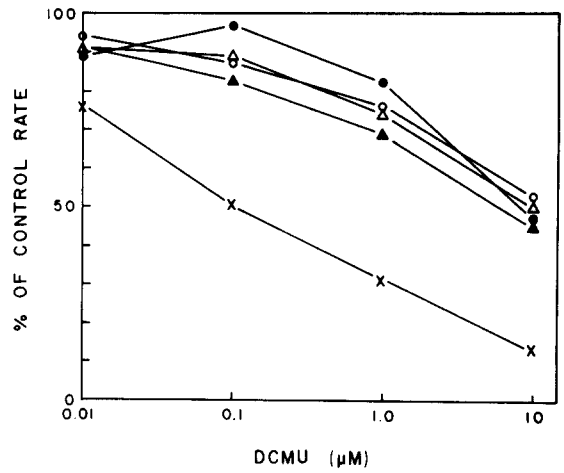


Fig. 1. Effect of DCMU on Photosystem II electron transport in membranes of wild-type and DCMU-resistant strains of *A. nidulans* R2. Photosystem II activity was assayed as $O_2$ evolution during transfer of electrons from $H_2O$ to DCBQ, and various concentrations of DCMU were added to samples. The level of oxygen production at each point was divided by that of a control to which no DCMU was added. In all cases, control rates of $O_2$ production were 250–320 μmol/h per mg chlorophyll. The curves are averages of six experiments for the wild-type and three experiments for each mutant. The standard error for each point was less than 5%. X, wild type *A. nidulans*; △, R2D1; ▲, R2D1-X1; ○, R2D2; ●, R2D2-X1.

TABLE II

ELECTRON TRANSPORT INHIBITOR CONCENTRATIONS CAUSING 50% INHIBITION OF PHOTOSYSTEM II ACTIVITY IN WILD-TYPE AND DCMU-RESISTANT STRAINS OF *A. NIDULANS* R2

Intact cells of each strain were tested for PS II activity using an herbicide concentration series and the concentration resulting in 50% imhibition of oxygen evolution ($I_{50}$) was determined. Atrazine $I_{50}$ values were obtained from two experiments, and the HQNO value was obtained from a single HQNO concentration series; $n$ = number of experimental repetitions yielding identical results for the determination of the DCMU $I_{50}$. Samples were adjusted to 5 μg/ml chlorophyll for all assays.

| *A. nidulans* strain | $I_{50}$ (μM) | | |
|---|---|---|---|
| | DCMU | atrazine | HQNO |
| R2 | 0.05 ($n = 4$) | 0.2, 0.4 | 3.5 |
| R2D1 | 20 ($n = 3$) | 25, 34 | 20 |
| R2D1-X1 | 20 ($n = 3$) | 10, 15 | 15 |
| R2D2 | 20 ($n = 3$) | 25, 30 | 20 |
| R2D2-X1 | 20 ($n = 3$) | 2.5, 6.0 | 20 |

in resistance to DCMU. Comparable results were obtained with intact cells as shown in Table II. Interestingly, the $I_{50}$ for the wild-type cell was lower than for membranes, whereas the $I_{50}$ for the mutants was somewhat higher. This once again indicates that the mutants respond somewhat differently in the membrane preparation procedure. Nonetheless, qualitatively similar results between the two techniques allowed us to conclude that measurements on intact cells was adequate for routine screening of resistance to various drugs.

Similar assays were performed with intact cells of these strains to determine sensitivities to the compounds atrazine and HQNO. The $I_{50}$ values of the mutants for atrazine and HQNO were 100-fold and 7-fold higher, respectively, than those of the wild-type strain (Table II).

*Transfer of DCMU resistance by genetic transformation of wild-type cells*

The DCMU-resistance marker of each mutant was transferred to a wild-type genetic background to exclude possible secondary mutations from influencing biophysical and biochemical characteristics. Chromosomal DNA was prepared from each mutant and used as donor DNA for the transformation of *A. nidulans* R2 cells to DCMU resistance. A transformed colony from each reaction was selected for further characterization (see Materials and Methods). These clones were designated *A. nidulans* R2D1-X1 and R2D2-X1, originating from transformation by DNA from *A. nidulans* R2D1 and R2D2, respectively.

The Photosystem II electron transport sensitivities of the transformants to DCMU, atrazine and HQNO were assessed as for the original mutants (Fig. 1, Tables I and II). The DCMU $I_{50}$ values for membranes of R2D1-X1 and R2D2-X1 were $8 \pm 1$ $\mu$M and $10 \pm 1$ $\mu$M, respectively. Both values were somewhat lower than those of the original mutants (Fig. 1), although the $I_{50}$ values for intact cells were identical (Table II). The slight rise in rates for R2D2-X1 between $10^{-8}$ M and $10^{-7}$ M DCMU was entirely reproducible (Fig. 1). The transformants showed resistance to HQNO that was similar to that of the original mutants. However, the $I_{50}$ values of atrazine for both transformants were lower than those of the two original mutants (Table II). The transformant *A. nidulans* R2D1-X1 was approx. 2-fold more sensitive to atrazine than the mutant from which it was derived, but was still 50-fold more resistant than the wild type. The resistance level in *A. nidulans* R2D2-X1 dropped 5–10-fold relative to R2D2, but was still significantly more resistant than wild type ($I_{50}$ ratio of 12–15).

*Chlorophyll fluorescence induction kinetics*

Chlorophyll fluorescence induction kinetics experiments were performed with the wild-type strain and the four DCMU resistant derivatives, in the presence of various concentration of DCMU (Fig. 2). The yield of chlorophyll variable fluorescence in the wild-type strain increased in the presence of

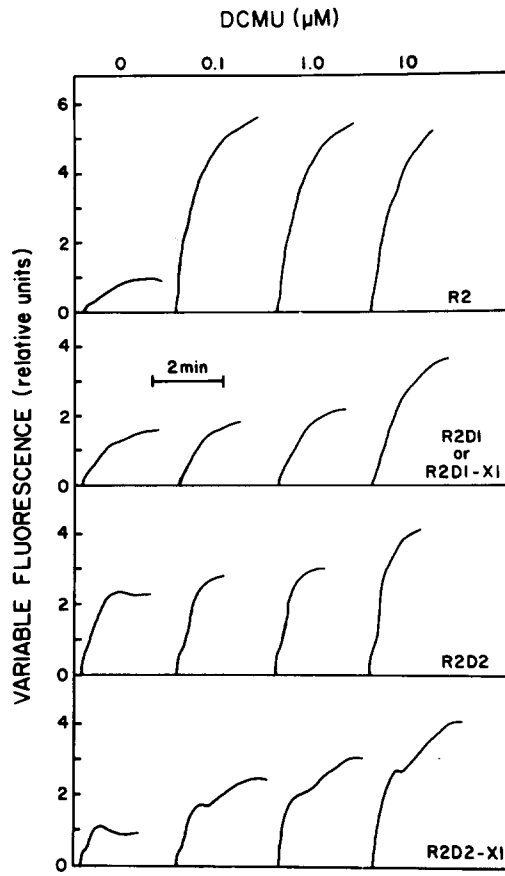


Fig. 2. Effect of DCMU on fluorescence induction kinetics in wild-type and DCMU resistant strains of *A. nidulans* R2. Fluorescence induction kinetics were obtained in the absence of DCMU and in the presence of various concentrations of the inhibitor. The variable portion of each trace is plotted in relative units.

$10^{-7}$ M DCMU. By contrast, the fluorescence of the resistant mutants *A. nidulans* R2D1, R2D1-X1 and R2D2 were unaffected by concentrations below $1 \cdot 10^{-5}$ M DCMU. No difference was detectable between *A. nidulans* R2D1 and the corresponding transformant, R2D1-X1. However, the fluorescence pattern of the transformant, *A. nidulans* R2D2-X1, differed from that of the other strains. A biphasic curve of variable fluorescence was observed, and the variable fluorescence maximum increased steadily with increasing concentrations of DCMU.

*Polyacrylamide gel eletrophoretic analysis of membrane polypeptides*

Cultures of each of the five strains were incubated with $H_2{}^{35}SO_4$ for labeling of proteins, and membranes were prepared from each. The DCMU-resistant strains were grown both in the presence and absence of $10^{-6}$ M DCMU for this analysis. Membranes were solubilized in the presence of lithium dodecyl sulfate and subjected to polyacrylamide gel electrophoresis, and polypeptides were detected by autoradiography (Fig. 3). A major difference in the protein patterns was visible in the region of 30–40 kDa apparent molecular weight. The mutants *A. nidulans* R2D1 and R2D2 had a reduced level of a major polypeptide species of approx. 34 kDa, which was present in the wild-type strain (Band 48, [24]). The transformants *A. nidulans* R2D1-X1 and R2D2-X1 also had reduced levels of the 34 kDa band, and in

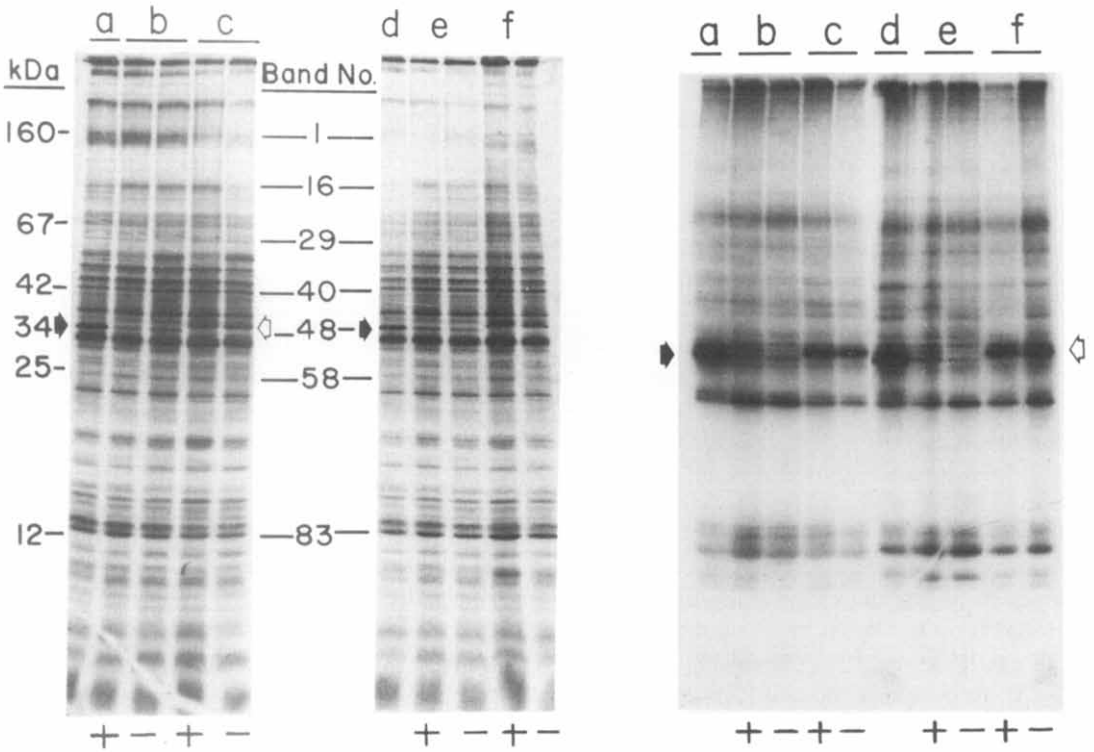


Fig. 3. Polyacrylamide gel electrophoresis of $^{35}S$-labeled membrane polypeptides. Membranes from cultures grown in the presence of $^{35}S$ and in the presence (lanes marked +) or absence (lanes marked −) of $10^{-6}$ M DCMU were solubilized with lithium dodecyl sulfate and subjected to electrophoresis on a 10–20% polyacrylamide gradient gel. Lanes a and d, *A. nidulans* R2; lanes marked b, R2D2; lanes marked c, R2D2-X1; lanes marked e, R2D1; lanes marked f, R2D1-X1.

Fig. 4. Polyacrylamide gel electrophoresis of $^{125}I$-labeled membrane polypeptides from wild-type and DCMU-resistant strains. Membranes from wild-type and DCMU-resistant cultures grown in the presence (lanes marked +) or absence (lanes marked −) of $10^{-6}$ M DCMU were labeled with $^{125}I$ by lactoperoxidase-catalyzed iodination to identify surface-exposed polypeptides. Labeled membranes were solubilized in lithium dodecyl sulfate and subjected to electrophoresis on 10–20% polyacrylamide gradient gels. Lanes a and d, *A. nidulans* R2; lanes marked b, R2D2; lanes marked c, R2D2-X1, lanes marked e, R2D1; lanes marked f, R2D1-X1.

addition exhibited a new band at 35.5 kDa. These polypeptides were also detectable by staining with Coomassie brilliant blue or silver (data not shown). Subsequent experiments suggest that there are at least two 34 kDa species present in the wild-type band; one of these is completely missing in the DCMU-resistant strains (Bricker, T., Golden, S. and Sherman, L., unpublished data). Only minor variations in band intensities were observed in DCMU-resistant strains between preparations from cells grown in the presence and absence of DCMU.

Externally-exposed membrane polypeptides were radioactively labeled with $^{125}I$ by lactoperoxidase-catalyzed iodination. Electrophoretic separation of polypeptides identified the 34 kDa wild-type band and 35.5 kDa transformant bands as major radioactively labeled species (Fig. 4). The patterns from the original mutants exhibited iodinated bands at 34 kDa and 35.5 kDa, but not of the intensity seen in the transformant and wild-type lanes.

## Discussion

This report has been concerned with the isolation and preliminary characterization of two DCMU-resistant mutants of *A. nidulans* R2. Although the two mutants have similar characteristics in many respects, they appear to be non-identical. We have demonstrated that the lesions affect a protein of the photosynthetic apparatus and that the trait can serve as a marker for genetic transformation of *A. nidulans* recipient cells. The transfer of the mutant trait to a wild-type genetic background allows the construction of strains that probably contain only a single lesion. The ability to transform cells with this marker has taken on added significance based on recent results. We have demonstrated by Southern hybridization analysis that *A. nidulans* R2 possesses at least three discrete sequences that are homologous to a gene [25] which codes for the chloroplast herbicide-binding protein (Golden and Sherman, unpublished data). Similar results have been reported by Curtis, S. and Haselkorn, R. (unpublished data) in the filamentous cyanobacterium, *Anabaena* 7120. The reason for multiple copies of the presumptive herbicide-binding protein in these organisms is not clear, and a study of the expression of the different genes under various conditions is needed. The homology between the chloroplast and cyanobacterial genes suggests that it may be possible to transfer chloroplast genes to *A. nidulans* by genetic transformation in a manner similar to that reported here.

The major membrane polypeptide alteration which was detectable in all four DCMU-resistant strains occurred in the 34–36 kDa region. This is within the range of molecular weights which has been reported for the chloroplast herbicide-binding protein [7,26,27]. The transformed strains showed a major polypeptide band at 35.5 kDa which is not present in the original mutants. Since both the 34 kDa and 35.5 kDa species have large externally exposed domains (Fig. 4), it is possible that a secondary lesion could cause the 35.5 kDa protein to be released during preparation of membranes from *A. nidulans* R2D1 and R2D2. This possibility is currently being studied.

The identification of the 34 kDa and 35.5 kDa membrane polypeptides is under investigation at this time. Preliminary data have indicated that the 35.5 kDa polypeptide present in *A. nidulans* R2D2-X1 is an altered form of the wild-type 34 kDa species. Cross-linking of the atrazine photoaffinity label, [$^{14}$C]azidoatrazine, to thylakoid preparations of *A. nidulans* will indicate whether either, or both, of these species are atrazine-binding proteins.

The cross-resistance of DCMU-resistant mutants of *A. nidulans* to atrazine and HQNO indicates a relationship between the actions of these agents on the cyanobacterial thylakoid membrane. DCMU and atrazine have been shown by competition experiments to bind overlapping sites in higher-plant chloroplasts, and a 32 kDa polypeptide has been implicated in the binding of both agents [7–9]. The variation of atrazine resistance level between *A. nidulans* R2D2 and R2D2-X1 supports the distinction of contact sites between DCMU and atrazine binding. Removal of secondary background mutations did not alter the DCMU resistance of the strain, but decreased its resistance to atrazine. This may be due to a slight alteration in membrane topology which changed the accessibility of the atrazine binding site, or the spatial relationship to other membrane compo-

nents involved in electron transport.

Barton et al. [10] have shown that HQNO interacts with the reducing side of Photosystem II, but at a site which is distinct from that of DCMU and atrazine. An atrazine-resistant biotype of pigweed, like the DCMU-resistant *A. nidulans* mutants discussed here, also showed a high level of resistance to HQNO [10]. It is unlikely that resistance to HQNO was induced as an independent mutation in *A. nidulans*, because transformants of wild-type cells which had received the DCMU-resistance marker also exhibited HQNO resistance. These results imply that there is a binding site for HQNO on the reducing side of Photosystem II that is separate from, but close to, the DCMU-binding site. Oettmeier et al. [28] have proposed a model for herbicide binding that includes a second protein ($M_r = 41\,000$) which has been shown to bind the phenolic inhibitor, dinoseb. Although it is not known at the present time if dinoseb and HQNO bind to the same protein, these mutants may be useful for making such a determination.

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