Herbicide Cross-Resistance and Mutations of the *psb*A Gene in *Chlamydomonas reinhardtii*

Günter F. Wildner, Ursula Heisterkamp, and Achim Trebst

Biochemie der Pflanzen, Ruhr-Universität Bochum, Postfach 102148, D-4630 Bochum 1, Bundesrepublik Deutschland

Z. Naturforsch. **45c**, 1142–1150 (1990); received July 2/October 23, 1990

Dedicated to Professor André Pirson on the occasion of his 80th birthday

Chlamydomonas reinhardtii, Herbicide Resistance, Photosynthesis, *psb*A Gene, Q_B-Protein

The resistance against herbicides (inhibitors of the electron accepting side) of photosystem II originates from mutations of the *psb*A gene, coding for the D1 or Q_B binding protein. Each of the five mutants used in the present study had single base changes in the *psb*A gene that resulted in a unique amino acid substitution at a different residue of the D1 protein (Val-219, Ala-251, Phe-255, Ser-264, Leu-275). The differences of the I_{50}-values in the electron transfer reaction of H_2O to dichlorophenolindophenol were used to analyze the correlation of these amino acid residue changes to their impact on the binding of diverse chemical classes of inhibitors. The binding domains on the D1 protein of the inhibitors overlap, but are nevertheless distinct. Even minor changes in the chemical structure of the inhibitors resulted in changes of the resistance toward a specific amino acid residue.

A pattern of response of the inhibitors to the amino acid substitutions evolves that allows easy differentiation between the groups of PS II inhibitors. Two principally different response curves emerge for two different families of PS II inhibitors, that had been proposed earlier already on functional studies with wild types. But the response pattern of newly described inhibitors, like ketonitrile and quinolone derivatives, in the mutants allows to group them with confidence and in turn generalize the phenol type family. In none of the five mutants, studied here, is there a marked cross-resistance to the new phenol type inhibitors. Just the opposite, in several cases, there is negative cross-resistance (or supersensitivity). Because of this response pattern this group of inhibitors cannot be oriented with certainty in the three dimensional folding model of the D1 protein.

Introduction

Resistance against herbicides which inhibit the acceptor side of photosystem II can be traced to a change in the primary target. This has been studied in higher plants [1], in unicellular algae, like *Chlamydomonas reinhardtii* [2–5] or *Euglena gracilis* [6] and in cyanobacteria [7, 8]. The understanding of the molecular basis of herbicide resistance has been improved by biochemical, biophysical and by genetic methods comparing resistant and susceptible biotypes; this information has also greatly influenced the knowledge on the structure and function of photosystem II (reviewed in [9–11]). Several mutants resistant to photosystem II herbicides have been correlated with mutations in the *psb*A gene coding for the herbicide binding protein (or Q_B-protein, D1 polypeptide or *psb*A gene product). This protein binds the secondary electron acceptor plastoquinone Q_B [12] and the electron transport inhibitors, among them herbicides, which compete with oxidized Q_B for the binding site of the protein [13–15] and interrupt the electron flow from the photosystem II to the plastoquinone pool.

The specific binding of the inhibitors to the Q_B-protein was established with the photoaffinity labelling technique using azido-triazine [16, 17]. Later, azido-triazinone [18] and azido-monuron [19] and recently azido-ketonitrile derivatives [20] – all inhibitors in this study – were employed.

The large variety of inhibitory compounds are grouped into classes according to chemical structures with common chemical essential elements (see [10]). *p*-Alkylanilides, *p*-alkylthioanilides, amino-triazinones, s-triazines, biscarbamates, chlorinated phenylureas, cyclopropane-carboxamides, certain diphenylethers, pyrazolones, pyridazinones, pyrimidinones, thiadiazolones, triazinones, carbamates, and uracils, have to be accommodated and bound on the same herbicide binding protein. This D-1 protein, with an approx. molecu-

Reprint requests to Prof. Dr. Trebst.

Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/90/1100–1142 $ 01.30/0

lar mass of 32 kDa, has five hydrophobic membrane spans in its amino acid chain, of which only two, helix IV and V and their connecting loop totalling about 60 amino acid residues are directly involved in the electron transfer reactions at the Q_B site [9–11, 21]. Together with the highly homologous D-2 or Q_A binding protein the D-1 protein forms also the reaction center of PS II.

The early attempt to find a common structural element in those compounds was successful, and the proposed sp^2-hybrid $-C-NH-$ seemed to be the essential element for inhibition (reviewed many times, for example by [22, 23]). Then a second group of phenolic derivatives that also efficiently inhibit photosystem II at the same functional site, but could not be accommodated among the classical herbicides was characterized [23]. Therefore a model with overlapping binding sites in a common binding domain was proposed with the suggestion that certain amino acid residues were involved in one, but not another herbicide binding [23, 24]. The DNA sequence of herbicide-resistant mutants provided the identity of such amino acids involved in herbicide and from there in Q_B binding. The use of mutants of resistant biotypes and their analysis by determination of the electron transport activities or of binding studies therefore allow conclusions about alterations of the binding domains and the contribution of the individual amino acids in the D-1 protein to the total binding affinity of a herbicide. The unicellular green algae, *C. reinhardtii,* is especially advantageous for these studies. A series of mutants has been collected and characterized by sequence analysis of the *psb*A gene [5, 25, 26]. The high homology between this protein of *C. reinhardtii* and the same protein of higher plants permits the generalization of the description of the binding area. The mutants are used for grouping the inhibitory substances now by attachment to the binding niche rather than merely by structural chemical elements, as done in the past.

Materials and Methods

Mutants of *C. reinhardtii* (MZ-1, MZ-2, and MZ-4) were collected after treatment of the cells with 5-fluoro-2′-deoxyuridine and mutagenesis with methanesulfonic acid ethyl ester and selected against metribuzin [4, 27]. The cells (5×10^4 cells/ml) were grown in presence of 1 mM 5-fluoro-2′-deoxyuridine for 5 days and then kept for 24 h in the stationary growth phase. The collected cells were washed with 0.1 M phosphate buffer, pH 6.9, and incubated in light (9.5 klx) with 0.27 M methanesulfonic acid ethyl ester for 1 h. After the mutagenesis the cells were washed several times, plated on minimal medium plates supplemented with 10^{-5} M metribuzin, and kept at 20° in light (6 klx) for two weeks. The strains Ar 207 and Dr 2 were originally isolated by Galloway and Mets [28] and were kindly provided by Dr. J.-D. Rochaix, Geneva. The sequence analysis of the *psb*A gene of the MZ-mutants was performed either by sequencing the DNA or the m-RNA [26]. The sequencing data for the mutants Ar 207 and Dr 2 were published earlier [25], but we have sequenced them again to confirm the previous results. Mutations in Ser 264 and Leu 275 in *C. reinhardtii,* like MZ-1 and MZ-4, have been found and sequenced previously [5, 11, 28], but were selected by DCMU or bromacil tolerance, whereas ours were selected *via* metribuzin tolerance.

The mutant strains and the wild type strains were grown under identical conditions mixotrophically (5% CO_2 in air and 18 mM acetate) in light (8 klx). The cells were harvested in the exponential growth phase and washed with 20 mM Hepes-NaOH buffer, pH 7.0, containing 50 mM KCl and 2.5 mM $MgCl_2$. The cells were resuspended in 20 mM Hepes-NaOH buffer, pH 7.5, containing 0.3 M sorbitol, 5 mM $MgCl_2$, 1 mM $MnCl_2$ and 2 mM KCl (chlorophyll content of 0.3 mg/ml). They were broken by Yeda press treatment (500 psi) and this step was repeated five times. The resulting thylakoids were collected by centrifugation and resuspended in the same buffer. The I_{50}-values were determined in the uncoupled electron transfer reaction H_2O to DCPIP with equal amounts of chlorophyll in all assays [29]. Typical rates of the uncoupled system were 500 μmoles/h/mg chlorophyll. The thylakoids were preincubated with the inhibitors for 5 min in the dark.

Results

The mutants of *C. reinhardtii* (MZ-1, MZ-2, and MZ-4) were selected against 10^{-5} M metribuzin and screened for photosynthetic competence. They were compared with two mutant strains of *C. reinhardtii,* Ar 207 and Dr 2, that were originally iso-

lated by Galloway and Mets [28] by selection for resistance to atrazine and DCMU. Cells in the exponential growth phase were harvested and, after cell breakage by Yeda press treatment, the thylakoids were isolated by differential centrifugation. The photometric assays were carried out with equal amounts of chlorophyll in the reaction mixture. The following mutants were assayed: MZ-1 (Ser 264 to Ala), MZ-2 (Ala 251 to Val), MZ-4 (Leu 275 to Phe), Ar 207 (Phe 255 to Tyr) and Dr 2 (Val 219 to Ile). The inhibitor molecules tested were chosen either by their structural elements or by their marked difference in inhibitor or tolerance pattern. The cross-resistance of the following series of compounds has been compared:

Triazine (atrazine), triazinone (metribuzin), urea (DCMU) and thiazolyliden-ketonitriles (2-phenylthiazolyl-3-hydroxy-4-phenylbutenonitrile) (Fig. 1);
substituted triazines (Fig. 2);
substituted urea derivatives (Fig. 3);
substituted triazinones (Fig. 4);
"phenolic" derivatives (dinoseb, ioxynil, bromonitrothymol, tetrabromo-4-hydroxypyridine) compared with cyanoacrylate (2-cyano-3-ethyl-4-(4-chlorbenzyl)-aminoacrylate ethoxyethylester (Fig. 5);
3-bromo-2,6-di-trifluoromethyl-4-hydroxyquinoline compared with bromonitrothymol and atrazine (Fig. 6).

The I_{50}-values were determined from a concentration series and are given in Table I and II. The ratios of the values of the I_{50}-values for the mutants (R for resistant) to the I_{50}-values for the wild type (S for sensitive) are presented in Fig. 1–6. The type of blotting of the differences should reflect the role of specific amino acid residues in the binding niche in their contribution to total binding affinity. In the figures the ratio of the I_{50}-values resistant/sensitive for the inhibitors in the five mutants are plotted against a X-axis with arbitrary spacing of the 5 mutants. By this a response pattern of each inhibitor evolves which turns out to be quite indicative for the type of inhibitor. Already a quick glance spots the phenol type inhibitors with a very flat curve, often above the zero line because of their increased sensitivity in the mutants as against the up and downs of the "classical" inhibitors. But also these can be grouped according to

Table II. pI_{50}-changes of triazine derivatives in WT-cells (Ser at position 264) and mutant cells (Ala at position 264) in *C. reinhardtii*.

Derivatives	pI_{50} Ser	Ala	ΔpI_{50} Ser − Ala
N-iprop., N-ethyl	6.7	4.5	2.2
N-iprop., N-iprop.	6.15	4.75	1.4
N-ethyl, N-ethyl	6.05	3.6	2.45

pI_{50} = negative logarithm of the I_{50}-values.

Table I. The effect of aminoacid substitutions in the D1 protein on inhibitor efficiency on PS II.

Compound	Wildtyp	pI_{50}-value Mutants with an aminoacid substitution at V219I	A251V	F255Y	S264A	L275F
atrazine	6.7	6.4	5.3	5.5	4.5	6.7
metribuzine	7.3	6	4.6	7.5	3.6	5.9
metamitron	5.5	5.4	3.4	6	4	4.7
diuron	7.7	6.2	6.8	7.8	5.4	7
metabenzthiazuron	6.5	4.7	4.4	7	5.1	6.4
benzthiazuron	6.2	5	5	6.3	4.5	5.7
cyanoacrylate	8	7.7	6.8	6.4	6.5	8
ioxynil	6.1	4.4	4.7	5.7	6.4	6.8
dinoseb	5.8	4.9	5.3	5.3	6.5	5.3
bromonitrothymol	7.3	7	6.4	7.5	7.7	7.1
ketonitrile	7.8	8	8.1	8	8	7.7
tetrabromopyridinol	6.7	6.3	6.3	6.6	6.5	6.4

ketonitrile, 2-phenylthiazol-3-hydroxy-4-phenyl-butenonitrile;
cyanoacrylate, 2-cyano-3-ethyl-3-(4-chlor-benzyl)-aminoacrylate ethoxyethylester;
quinoline, 3-bromo-2,6-di-trifluoromethyl-4-hydroxyquinoline.

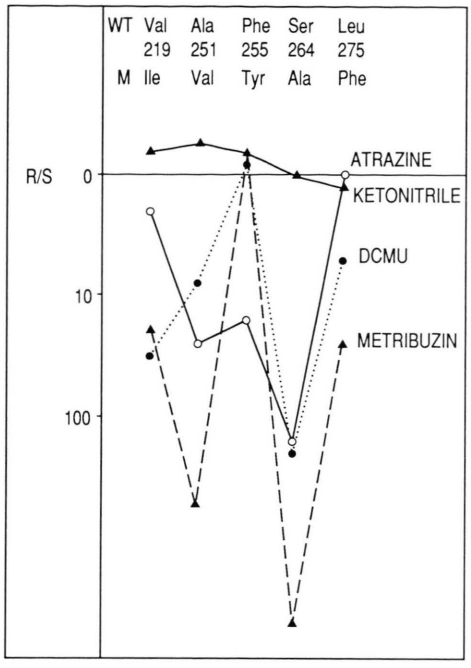

Fig. 1. *psb*A mutants and their resistance against triazine derivatives, triazinone, urea, and thiazolyliden-ketonitrile herbicides. The I_{50}-value of the mutant cell(R) was divided by the I_{50}-value of the wild type cells(S) and the R/S-values for each mutant and for each inhibitor were compared: atrazine (○—○), metribuzin (▲---▲), DCMU (●···●), 2-phenyl-thiazolyl-3-hydroxy-4-phenylbutenonitrile (▲—▲).

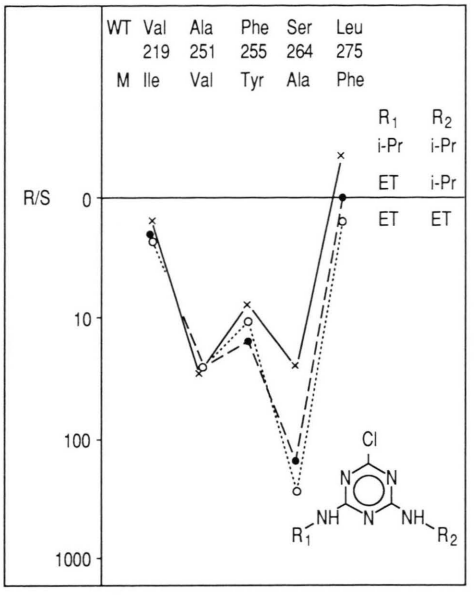

Fig. 2. The impact of the substitution of triazines on the resistance. The R/S-values for each mutant were compared for atrazine (●---●), N-ethyl (○···○) and N-isopropyl (×—×) derivatives of triazines.

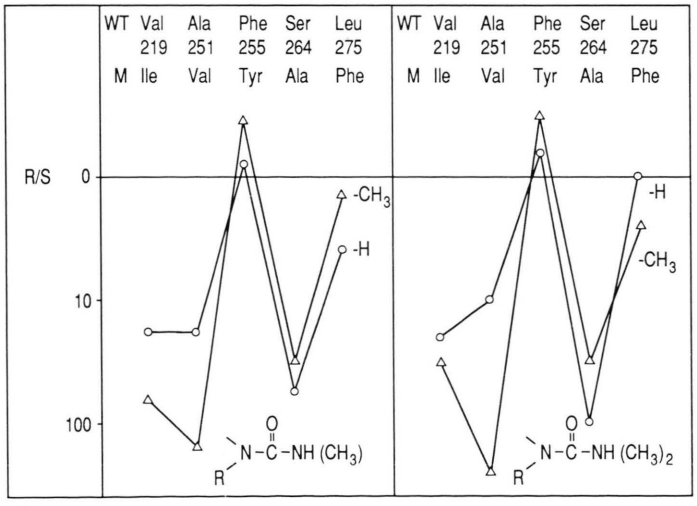

Fig. 3. The impact of the substitution of urea derivatives on the resistance. The R/S-values for each mutant were compared for methabenzthiazuron (△—△) and benzthiazuron (○—○) (left side) and their -N$(CH_3)_2$-derivatives (right side).

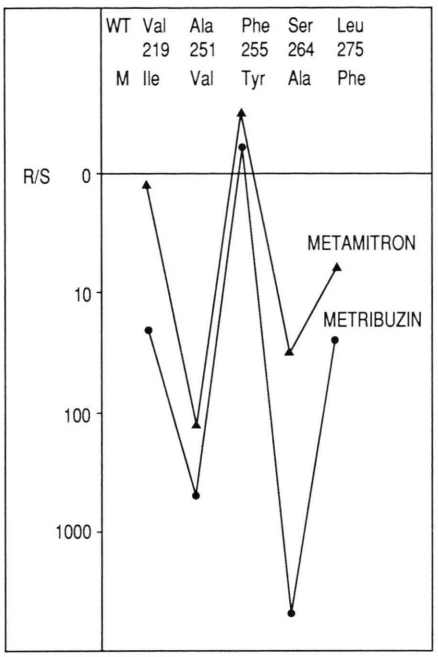

Fig. 4. The impact of the substitution of triazinones on the resistance. The R/S-values for each mutant and metribuzin (●—●) and metamitron (▲—▲) were compared.

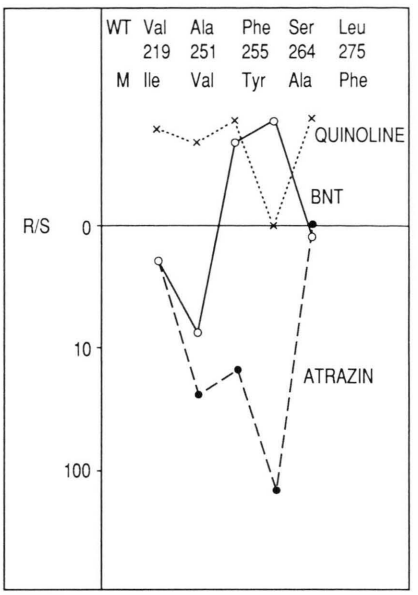

Fig. 6. *psb*A mutants and their resistance against a quinoline derivative grouped among the phenol-type inhibitors. (●---●) atrazine, (○—○) bromonitrothymol, (×···×) 3-bromo-2,6-di-trifluoromethyl-4-hydroxy-quinoline.

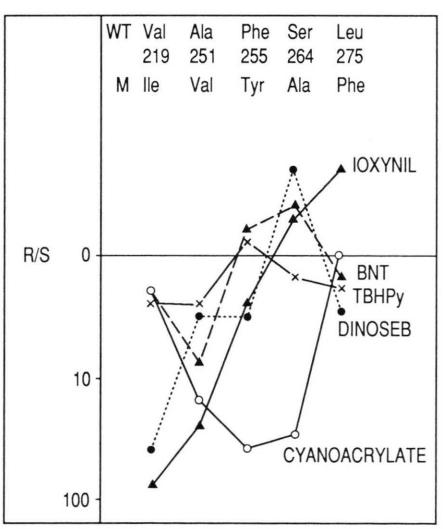

Fig. 5. *psb*A mutants and their resistance against phenolic derivatives and a cyanoacrylate. The R/S-values for each mutant and the following substances were compared: ioxynil (▲—▲), dinoseb (●···●), bromonitrothymol (▲---▲), tetrabromo-4-hydroxypyridine (×—×), and 2-cyano-3-ethyl-3(4-chlorbenzyl)-amino-acrylate ethoxy ethylester (○—○).

the response pattern. The combination of inhibitors in the figures were chosen either on a chemical basis (Fig. 2, 3, 4) or for accentuation of their differential behaviour pattern (Fig. 1, 5 and 6). It should be kept in mind that the mutants were selected for by the acquired metribuzin resistance (MZ 1–4) or acquired atrazine and DCMU resistance (Ar 207 and Dr-2). Therefore, the former mutants show a marked resistance towards metribuzin, and the latter for atrazine or DCMU, respectively. In general, resistance to atrazine and metribuzin run parallel, except for mutant strain Ar 207 (Phe 255 to Tyr) where no metribuzin resistance is acquired (Fig. 1). On the other hand, the substitution of Leu by Phe has little effect on atrazine sensitivity, but does change metribuzin (compare Fig. 1 and 2). There is resistance to a N-alkyl substituted urea derivative = benzthiazuron in this mutant (Fig. 1). This is even more clearly demonstrated in Fig. 3, where a series of N-alkylated ureas is compared. This possibly indicates an interaction of the tyrosine 255 with the

side chain of the ureas, not possible in the N-alkylated derivatives. A comparison of urea derivatives of the type $X-NR-CO-NHCH_3$ and of $X-NR-CO(CH_3)_2$ (Fig. 3) shows that the most pronounced effect on the I_{50}-value occurs at the amino acid at position 251. A change from -H to $-CH_3$ results in a large shift of the I_{50}-value, indicating that the compounds with the bulkier methyl group are more poorly accommodated in the binding niche when the Ala residue is replaced by Val. The opposite can be observed with the change of the residue Ser-264 to Ala. The better fitting H-derivatives are more drastically reduced in their I_{50}-values at this position than the methyl derivatives.

The importance of the type of the substitution in the series of related compounds is indicated in Fig. 2, in which the influence of triazine substituents on acquired resistance is compared. The symmetrical di-isopropyl-substituted triazine shows the least cross-resistance. The symmetrical di-ethyl derivatives the most (see also Table II).

The data for the substitutions of triazinones and their impact on I_{50}-changes are presented in Fig. 4. The triazinones metribuzin and metamitron showed the following characteristic changes: the substitution of Val-219 by Ile had no effect on resistance to metamitron, but caused a 20-fold lower I_{50}-value in presence of metribuzin. This indicates that the binding niche of the latter compound also includes this residue of helix IV. The two compounds are severely decreased in their I_{50}-values at position 251, especially in the case of metribuzin. The change of Phe-255 to Tyr, however, improved the binding of both compounds to the Q_B-protein. The most drastic differences between metribuzin and metamitron was observed at Ser 264 to Ala where the I_{50} for metribuzin was reduced 5000-fold, and metamitron was only reduced 30-fold. The drop of the I_{50}-value of metribuzin due to the change of the residue Leu-275 to Phe demonstrates that the binding area of this compound includes elements of both helix IV and helix V.

The serine 264 substitution has the strongest influence in its effect on the inhibitor sensitivity. But even among closely related compounds the effect might be small (metamitron) or large (metribuzin) = Fig. 4. The impact of the substitution of Ser 264 on DCMU or atrazine sensitivity is also markedly different in different organisms. As already noted [30, 31], there is little influence on DCMU sensitivity in the higher plants, but there is in *E. gracilis* [32] and *A. nidulans* [8] and, as reported here, also in *C. reinhardtii* (Table I).

The most interesting phenomenon is the quite different behaviour of phenol type inhibitors in the mutants when compared with inhibitors of the classical urea/triazine type family, as immediately seen in the response pattern in the figures. In some mutants there is even a marked increase in sensitivity (Fig. 5 and 6). This behaviour *i.e.* supersensitivity or negative cross-resistance strongly suggests that the binding behaviour of this type of inhibitors is quite different from that of the urea/triazine family. This lack of response in D1 protein mutants has been noted before for some simple phenols, then available, like dinoseb and ioxynil [33–36]. By now further compounds in this phenoltype class have been described – where the chemical nature is not so immediately obvious, like in the ketonitriles [20] and the pyridones [37] and quinolones [38] – which now allows to generalize about this group. Their behavior in the mutants, discussed here, – their pattern of response – appears now to be a particular telling indication of their difference to the "classical" PS II inhibitors. A ioxynil tolerance has been ascribed by Creuzet *et al.* [39] to an Asn 266 substitution in the D1 protein. But also the Val 219 substitution (Table I) changes ioxynil sensitivity, but not necessarily the phenol type compounds in general. This shows that a response pattern or curve is more indicative than a single value. And there are, of course, also specific interactions among the phenols.

Four phenolic compounds (dinoseb, ioxynil, bromonitrothymol, tetrabromo-4-hydroxypyridine) are compared with a member of the urea/triazine family, a cyanoacrylate (2-cyano-3-ethyl-3-[4-chlorbenzyl]-aminoacrylate ethoxyethylester), in Fig. 5. Two subgroups of phenolic compounds can be distinguished by their interaction with Val-219. Ioxynil and dinoseb show a drastic change in the mutant, whereas bromonitrothymol and tetrabromo-4-hydroxypyridine reveal only a minor change in their I_{50}-value. The residue change Ala-251 to Val significantly reduces the binding of ioxynil, whereas the other compounds are not as strongly affected. The residue changes at Phe-255, Ser-264 and Leu-275 did not contribute to resistance.

The cyanoacrylate pattern as such is already very interesting because no other compound of the same urea/triazine group shows such a severe effect of the change Phe-255 to Tyr. Neither urea-, nor the triazinone-class herbicides are affected, and only a moderate change (20-fold) was observed with the triazines. Changes of amino acid residues at the helices Val-219 and Leu-275 were not involved in the occurrence of resistance to the cyanoacrylate.

Although the ketonitrile derivatives share some chemical elements, to the cyanoacrylates, the response of the two inhibitors in the mutants is quite different (Fig. 5 and Table I). In the case of 2-phenylthiazolyl-3-hydroxy-4-phenylbutenonitrile, none of the amino acid residue alterations showed a decrease in the I_{50}-values. This observation is very interesting, since it implies that this compound binds differently in the domain on the Q_B-protein [see 20] and/or in addition also on the Q_A-protein (D-2 protein, *psb*D gene product). The extreme in no-response to the mutations in the *psb*A gene is 3-bromo-2,6-di-trifluoromethyl-4-hydroxyquinoline (Fig. 6). This compound shows no tolerance at all in any of the mutants and actually increases its potency in four of them. The group of quinoline inhibitors has recently been described by Draber *et al.* [38] and shown to belong to the phenol type compounds.

Discussion

The amino acid substitutions in herbicide tolerant mutants had been instrumental for the proposal of a three dimensional folding of the D-1 protein and for the Q_B-binding site in particular [9, 10]. The folding model was based on the homology of PS II on the bacterial reaction center, whose structure was solved by X-ray analysis [40]. The mutants indicated amino acids of the D 1 protein involved in the Q_B site located at the end of transmembrane helix IV and in a relatively short sequence as of a parallel helix to the beginning of transmembrane helix V. Although Tyr 237 is now also implicated in the Q_B site [41, 42], the sequence between helix IV and the parallel helix, much longer in the D-1 protein than in the equivalent L subunit of the bacteria, is not of direct importance for herbicide binding. The folding model now accepted, the attempt is now to get further information from the mutants on the dimensions in the Q_B site by analyzing the cross-resistance. One can correlate the amino acid residue exchanged in the mutant with the chemical substituents of the inhibitors, whose inhibitory efficiency has been altered. The data from the cross-tolerance were plotted here against an arbitrary scale with the 5 mutants as this yielded a response pattern, typical for the different chemical classes of inhibitors. The data have been used to refine the folding model of the D 1 protein and the orientation of the inhibitors in their binding site, as reported on elsewhere [43, 44].

According to the model the residue Val-219 is above His-215 which binds Q_B and the central Fe of the reaction center complex. The change from Val to the bulkier side chain of Ile is not enough to cause a drastic decrease of the I_{50}-value for triazines and triazinones, but it does for the urea derivatives and for ioxynil and i-dinoseb, but almost no effect on two other phenol compounds, BNT and tetrabromo-4-hydroxypyridine and even an increase in potency for a ketonitrile and a quinoline derivative. This can be explained by the structure of the compounds. The first two have an unsubstituted metaposition to the phenol group, whereas the latter both have substituents (-Br and -methyl). It seems likely that the substituted phenols do not contact to helix IV, but that the meta-unsubstituted phenols can extend far enough. The general question concerning the interpretation of the data focuses on the problem of whether steric hindrance is sufficient to explain changes of the I_{50}-values. It has been demonstrated that the binding of halogenated nitrophenols is better described by steric parameters than by electronic factors or even lipophilicity [23]. In a recent study [45] with the mutant Dr 2 only a moderate resistance against DCMU and metribuzin was also observed, whereas with atrazine a R/S value of 2–4.5 could be measured.

The change from Ala-251 to Val has a similar steric effect to the binding of the inhibitors, with the strongest impact on the binding of metribuzin and metamitron, and to a lesser extent on atrazine and ioxynil. The other phenolic compounds show smaller changes (less than a factor of 10), in contrast to ioxynil. Again the ketonitrile and the quinoline derivatives increase their inhibitory potency as compared to the wild type. This substitu-

tion also has a marked effect on photosynthetic efficiency and on the donor side of photosystem II, indicating possibly major conformational changes [26].

The replacement of the Phe-255 by Tyr causes a strong decrease of the I_{50}-value with the cyanoacrylate derivatives and with the triazines, but not with others. It was suggested that the Phe-255 group may be involved in Q_B-binding by interacting with those herbicides with an aromatic ring system [21]. Since Tyr can replace Phe in this function, it was not surprising that the replacement had only minimal effects on most herbicides. Actually, there is even an increase in binding with some of the compounds, as the OH-group may form additional bonds. The increase of DCMU sensitivity in this mutant was already noticed by Galloway and Mets [28] when they first identified this mutant. This has been studied in more detail by Ohad and Hirschberg *et al.* in *Synecocystis* [46] mutants and shall not be discussed further here.

The most dramatic changes of I_{50}-values were observed with the alteration of Ser-264 to Ala. This substitution causes not only resistance to many of the inhibitors, but also a drastic change in the electron transfer kinetic [47]. It has been discussed that Ser itself plays a major role in Q_B-binding [31]. The equivalent serine 223 in the L-subunit of the bacterial system is shown directly by X-ray structures to be involved in terbutryn- and Q_B-binding [48]. This serine in the L-subunit is also changed in a bacterial terbutryn mutant. The more intriguing result is that the serine substitution induces no tolerance to any of the phenol type inhibitors, such as BNT, hydroxypyridines, ketonitriles and quinolines. Actually, there is even an increase in inhibitor sensitivity. This supports the notion that the phenol-type inhibitors are pushed away from the OH-group of serine towards His-215 [21]. The differences in the resistance pattern between mutants with substitutions for the Ser-264 residue of higher plants [30, 31, 49], of green algae [5, 27, 32] and of cyanobacteria [8, 50] are well known [50–53].

The residue Leu 275 is part of Helix V and is replaced by Phe in the mutant. This amino acid faces the central core and is very close to His-272 which is also involved in the binding of the central Fe atom in the reaction center complex. The highest I_{50}-change due to this substitution was observed with metribuzin, followed by DCMU and metamitron. With this change the cells were not resistant to atrazine nor to the phenolic derivatives which shows particularly clearly that triazines on the one hand and urea derivatives and triazinones on the other hand bind to differently extended niches. Indeed the triazines interact with the free electron pair of the ring nitrogen, whereas the others possibly with their carbonyl group with the polypeptide backbone extending much less into the hydrophobic core.

Acknowledgements

The authors are indebted to Bayer AG (Monheim), CIBY-GEIGY AG (Basel), Schering AG (Berlin), ICI (Jealott's Hill Research Station) and to Dr. Phillips (Canberra) for the compounds tested in this study. The financial support by the Deutsche Forschungsgemeinschaft is gratefully acknowledged.

[1] D. J. Kyle, Photochem. Photobiol. **41,** 106–116 (1985).
[2] J. C. McBride, A. C. McBride, and R. K. Togasaki, Plant & Cell Physiol., Special Issue **3,** 239–241 (1977).
[3] R. E. Galloway and L. Mets, Plant Physiol. **70,** 1673–1677 (1982).
[4] H. Janatkova and G. F. Wildner, Biochim. Biophys. Acta **682,** 227–233 (1982).
[5] J. M. Erickson, M. Rahire, P. Bennoun, P. Delepelaire, B. Diner, and J.-D. Rochaix, Proc. Natl. Acad. Sci. U.S.A. **81,** 3617–3621 (1984).
[6] U. Johanningmeier and R. B. Hallick, Curr. Genet. **12,** 465–470 (1987).
[7] C. Astier, C. Vernotte, M. Der Vatanian M, and F. Joset-Esparellier, Plant Cell Physiol. **20,** 1501–1510 (1979).
[8] S. S. Golden and L. A. Sherman, Biochim. Biophys. Acta **764,** 239–245 (1984).
[9] A. Trebst, Z. Naturforsch. **41c,** 240–245 (1986).
[10] A. Trebst and W. Draber, Photosyn. Res. **10,** 381–392 (1986).
[11] J.-D. Rochaix and J. Erickson, Trends Biochem. Sci. **13,** 56–59 (1988).
[12] B. R. Velthuys and J. Amesz, Biochim. Biophys. Acta **723,** 266–275 (1974).
[13] B. R. Velthuys, FEBS Lett. **126,** 277–281 (1981).
[14] J. Lavergne, Biochim. Biophys. Acta **682,** 345–353 (1982).
[15] C. J. Arntzen, K. Pfister, and K. E. Steinback, in: Herbicide Resistance in Plants (H. M. LeBaron and J. Gressel, eds.), pp. 185–214, John Wiley, New York 1982.
[16] G. Gardner, Science **211,** 937–940 (1981).
[17] K. Pfister, K. E. Steinback, G. Gardner, and C. J. Arntzen, Proc. Natl. Acad. Sci. U.S.A. **78,** 981–985 (1981).
[18] W. Oettmeier, K. Masson, H.-J. Soll, and W. Draber, Biochim. Biophys. Acta **767,** 590–595 (1984).
[19] A. Boschetti, M. Tellenbach, and A. Gerber, Biochim. Biophys. Acta **810,** 12–19 (1985).
[20] U. Bühmann, E. C. Herrmann, C. Kötter, A. Trebst, B. Depka, and H. Wietoska, Z. Naturforsch. **42c,** 704–712 (1987).
[21] A. Trebst, Z. Naturforsch. **42c,** 742 750 (1987).
[22] K. H. Büchel, Pestic. Sci. **3,** 89–110 (1972).
[23] A. Trebst and W. Draber, in: Advances in Pesticide Science, Part 2 (H. Geissbühler, ed.), pp. 223–234, Pergamon Press, Oxford and New York 1979.
[24] K. Pfister and C. J. Arntzen, Z. Naturforsch. **34c,** 996–1009 (1979).
[25] J. M. Erickson, M. Rahire, J.-D Rochaix, and L. Mets, Science **228,** 204–207 (1985).
[26] U. Johanningmeier, U. Bodner, and G. F. Wildner, FEBS Lett. **211,** 221–224 (1987).
[27] N. Pucheu, W. Oettmeier, U. Heisterkamp, K. Masson, and G. F. Wildner, Z. Naturforsch. **39c,** 437–439 (1984).
[28] R. E. Galloway and L. J. Mets, Plant Physiol. **74,** 469–474 (1984).
[29] N. Pucheu and G. F. Wildner, Z. Naturforsch. **39c,** 434–436 (1984).
[30] C. J. Arntzen, C. L. Ditto, and P. E. Brewer, Proc. Natl. Acad. Sci. U.S.A. **76,** 278–282 (1979).
[31] J. Hirschberg, A. Bleecker, D. J. Kyle, L. McIntosh, and C. J. Arntzen, Z. Naturforsch. **39c,** 412–420 (1984).
[32] D. Laval-Martin, D. Grizeau, and R. Calvayrac, Plant Sci. Lett. **29,** 155–167 (1983).
[33] K. Pfister and C. J. Arntzen, Z. Naturforsch. **34c,** 996–1009 (1979).
[34] W. Oettmeier, K. Masson, C. Fedtke, J. Konze, and R. R. Schmidt, Pestic. Biochem. Physiol. **18,** 357–367 (1982).
[35] A. Thiel and P. Böger, Pestic. Biochem. Physiol. **25,** 270–278 (1984).
[36] J. Durner, A. Thiel, and P. Böger, Z. Naturforsch. **41c,** 881–884 (1986).
[37] A. Trebst, B. Depka, S. M. Ridley, and A. F. Hawkins, Z. Naturforsch. **40c,** 391–399 (1985).
[38] W. Draber, B. Pittel, and A. Trebst, in: Probing Bioactive Mechanisms (P. S. Magee, D. R. Henry, and J. H. Block, eds.), ACS Symposium Series No. 413, pp. 215–228, Washington 1990.
[39] S. Creuzet, G. Ajlani, C. Vernotte, and C. Astier, Z. Naturforsch. **45c,** 436–440 (1990).
[40] J. Deisenhofer, O. Epp, K. Miki, R. Huber, and H. Michel, J. Mol. Biol. **180,** 385–398 (1984).
[41] A. Trebst, B. Depka, B. Kraft, and U. Johanningmeier, Photosynthesis Res. **18,** 163–177 (1988).
[42] R. Dostatni, H. E. Meyer, and W. Oettmeier, FEBS Lett. **239,** 207–210 (1988).
[43] K. G. Tietjen, J. F. Kluth, R. Andree, M. Haug, M. Linding, K. H. Mueller, H. J. Wroblowsky, and A. Trebst, Pestic. Sci. (in press).
[44] A. Trebst, J. F. Kluth, K. G. Tietjen, and W. Draber, Proc. 7th Int. Congr. Pestic. Chem., VCH Press, Weinheim (in press).
[45] P. Haworth and K. E. Steinback, Plant Physiol. **83,** 1027–1031 (1987).
[46] N. Ohad and J. Hirschberg, Photosyn. Res. **23,** 73–79 (1990).
[47] J. Bowes, A. R. Crofts, and C. J. Arntzen, Arch. Biochem. Biophys. **200,** 303–308 (1980).
[48] H. Michel, O. Epp, and J. Deisenhofer, EMBO J. **5,** 2445–2451 (1986).
[49] J. Gasquez and G. Barralis, C. R. Acad. Sc. Paris **288,** 1391–1393 (1979).
[50] I. Ohad, I. Pecker, and J. Hirschberg, in: Progress in Photosynthesis Research (J. Biggins, ed.), **Vol. III,** pp. 807–810, Martinus Nijhoff Publishers, Dordrecht 1987.
[51] J. Hirschberg and L. McIntosh, Science **222,** 1346–1349 (1983).
[52] M. Tellenbach, A. Gerber, and A. Boschetti, FEBS Lett. **158,** 147–150 (1983).
[53] S. Golden and R. Haselkorn, Science **229,** 1104–1107 (1985).