Molecular Modelling Studies on the Binding of Phenylurea Inhibitors to the D 1 Protein of Photosystem II

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A hypothetical molecular model of part of the D 1 protein of photosystem II, based on the analogous portion of the L subunit of the *Rhodopseudomonas viridis* reaction centre, has been used to study the binding of an extended hydrophobic phenylurea inhibitor (N,N-dimethylcarbamoyl)4-amino-4′-chloro-*trans*-stilbene) (**I**) to the Q_B site. The inhibitor was fitted by eye into a cleft in the site, and a limited part of the inhibitor/D 1 complex was energy minimized. The gross orientation of the inhibitor placed the dimethylurea moiety towards the predicted binding domain of the plastoquinone head group, and the stilbene moiety directed along the quinone isoprenoid side chain binding domain, suggesting a similar pathway of approach of the two molecules from the membrane into the binding site. Binding interactions of the inhibitor included hydrogen bonds to the side chain hydroxyl of ser 264 and the peptide carbonyl group of ala 251, with the side chain hydroxyl of ser 268 as an alternative ligand. Numerous hydrophobic contacts were also possible. Although phenylureas do not bind to reaction centres of *Rp. viridis*, many of the binding interactions to D 1 could also be detected in *Rp. viridis*. However, the β-CH_2 and δ-CO_2^- groups of glu 212 in *Rp. viridis* are located in the corresponding region of D 1 occupied by the dimethylurea moiety of the inhibitor in our model of its binding to D 1. This may explain why diuron (DCMU) does not bind to *Rp. viridis* reaction centres.

Introduction

Numerous biochemical and biophysical studies indicate that many classes of herbicide which block electron transport through photosystem II (PS II) bind in competition with plastoquinone at the so-called Q_B site on PS II and hence block oxidation of the reduced primary quinone Q_A^- by Q_B [1–3]. There is considerable interest in the specific-binding interactions of these herbicides since it could lead to predictions for novel inhibitors.

The availability of a high resolution crystal structure for the photochemical reaction centres of *Rhodopseudomonas viridis* [4, 5] and *Rhodobacter sphaeroides* [6, 7] has provided a considerable impetus to molecular modelling of the photosystem II reaction centre. The realization that the L and M subunits of the bacterial reaction centre showed regions of homology with the D 1 and D 2 subunits of PS II lead to the suggestion that the latter formed the reaction centre core [8, 9]. This has since been confirmed following the isolation and characterization of a PS II reaction centre consisting of D 1, D 2, cytochrome b_{559} and the *psb*I gene product [10–13].

Photoaffinity-labelling studies using azidoatrazine, a derivative of the PS II herbicide atrazine, indicated that this herbicide binds to D 1 [14]. A number of other photoaffinity-labelling herbicide derivatives have since been shown to label the same protein [15, 16]. Furthermore, a number of mutants resistant to PS II herbicides mapped to a localized region of the D 1 protein (summarized in [17–19]) and specific amino acids labelled by photoaffinity-labelling herbicides were located in the same region [20–22]. These data suggested that the Q_B site in PS II is located on the D 1 protein.

Atomic coordinates for the hypothetical model of the herbicide/Q_B binding domain of D 1, in Brookhaven Protein Database format, are available on floppy disc from Antony Crofts.

Reprint requests to John Bowyer.

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Comparison of the amino acid sequences of D1 and the L subunit, and a knowledge of the tertiary structure of the L subunit have enabled an approximate folding pattern for D1 in the membrane to be predicted [9]. This folding predicts that D1 has five hydrophobic membrane-spanning alpha-helical segments (A–E), a model which has been confirmed by antibody-binding experiments [23]. The Q_B and herbicide-binding domain is located in the region comprising the stromal ends of helices D and E and the stromally exposed connecting loop (approximately residues phe 211 to leu 275). Within this region there are a number of conserved residues between D1 and the L subunit (see Fig. 1 and Table I). Furthermore, a segment of alpha helix within the loop connecting helices D and E in the L subunit (the de helix) can also be identified in D1 [9, 17].

The binding of the triazine herbicide terbutryne to *Rp. viridis* and *Rb. sphaeroides* reaction centres has been solved by X-ray crystallography [5]. It seems highly likely that triazines bind in a similar fashion to D1. Unfortunately, most other classes of PS II herbicide do not bind to bacterial reaction centres [31, 32], so that, in the absence of a crystal structure for PS II, indirect methods must be used to predict the binding environment of these herbicides. One approach is to use molecular-modelling techniques to create a hypothetical model of the herbicide-binding region of D1 based on the *Rp. viridis* structure. This model may then be used to obtain a best fit for the herbicides of interest. We have used this approach to study the binding of phenylurea herbicides, but have also made some observations pertinent to the binding of triazines and phenolic compounds.

Spinach	D1	209	S L F S A M H G S L V T S S L I R E T T
Synechococcus	D1	209	S L F S A M H G S L V T S S L V R E T T
Rp. viridis	L	184	A M A L G L H G G L I L S V A N P G D G
			.....D.....|
Spinach	D1	229	- - E N E S A N E G Y R F G Q E E E T Y
Synechococcus	D1	229	- - E T E S Q N Y G Y K F G Q E E E T Y
Rp. viridis	L	204	- - - - - - - D K V K T A E H - - - - -
Spinach	D1	247	- N I V A A H G Y F G R L I F Q Y A S F
Synechococcus	D1	247	- N I V A A H G Y F G R L I F Q Y A S F
Rp. viridis	L	212	- - - - - E N Q Y F R D V V - - G Y S I
			|.....de.....|
Spinach	D1	266	N N S R S L H F F L A A W P V V G I W F
Synechococcus	D1	266	N N S R S L H F F L G A W P V V G I W F
Rp. viridis	L	225	- G A L S I H R L G L F L A S N I F L T
			|.....E.....
Spinach	D1	286	T A L G I S T M A F N L N G F N F N - Q
Synechococcus	D1	286	T S M G I S T M A F N L N G F N F N - Q
Rp. viridis	L	244	G A F G T I A S G P F W T R G W P E W W
			.....| |.....e...

Fig. 1. Sequence alignment of the segments of the L subunit of the *Rp. viridis* reaction centre and the D1 protein of spinach and *Synechococcus* 7942 involved in herbicide and Q_B binding. The sequences are from ref. [24] (L subunit), [25] (spinach D1) and [26] (*Synechococcus* D1).

Table I. Conserved residues in D1 of PS II and the L subunit of the *Rp. viridis* reaction centre in the region of the herbicide/Q_B binding site.

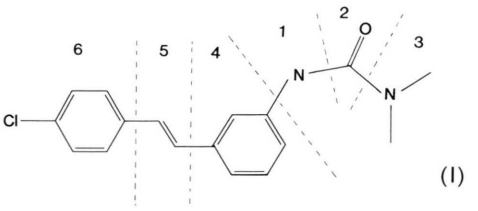
D1	L subunit	Comments
His 215	His 190	Fe ligand
Gly 216	Gly 191	
Leu 218	Leu 193	
Tyr 254	Tyr 215	
Phe 255	Phe 216	Interacts with terbutryne in *Rp. viridis* reaction centre. Mutation affects herbicide binding [27–29]
Ser 264	Ser 223	Hydrogen bonds to terbutryne in *Rp. viridis* reaction centre. Mutation affects herbicide binding [18, 19, 27, 30]
His 272	His 230	Fe ligand

Methods

When the D1 model used in this work was created, the atomic coordinates for the *Rp. viridis* reaction centre were not available on the Brookhaven Protein Structural Database. A representation of the atomic coordinates of the Q_B/herbicide-binding site of the L subunit was therefore obtained by digitization of the stereogram drawing of this site (from Fig. 5 of [5]) and the alpha-carbon structure in Fig. 2 of [4]. Atomic coordinates were then calculated from the digitized stereogram by a triangulation algorithm similar to that of Rossman (available from the Brookhaven Protein Database). We have since obtained the L subunit coordinates from the Brookhaven Database, and the calculated coordinates show good agreement with the crystallographic data. The polypeptide sequence for D1 from *Synechococcus* 7942 [26] was manipulated on to the calculated coordinates of the *Rp. viridis* L subunit using the molecular graphics programme MMS written by Steve Dempsey (University of California at San Diego). To establish a fit, two residues of D1 (phe 260 and gln 261) were added at the carboxyl-terminal end of the de helix, and one residue (asn 266) to the beginning of the E helix (see Fig. 1 for sequence alignments of D1 and the L subunit). In the extra-membraneous segment of D1 connecting the top end of helix D to the de helix, there are 14 residues with no equivalents in the L subunit. These have not been included in the D1 model.

The D1 model was viewed and manipulated using the Sybyl 5.22-C molecular-modelling software (Tripos Associates, Inc.) on an Evans and Sutherland PS 330 Graphics Terminal. Herbicide molecular structures were created using the Sybyl software, fitted with Gasteiger-Huckel charges and minimized.

In order to study the binding of the phenylurea (**I**) to D1, residues extraneous to the quinone-binding site were deleted to simplify the model. Hydrogens and Kollman charges were loaded on



(I)

to all the remaining protein atoms. The phenylurea (**I**) was docked by eye into an extended cleft identified in the Q_B site, after making minor alterations to its conformation to optimize the fit. Some consideration was also given to potential intermolecular hydrogen bonds. The combined structures (D1 and **I**) were then relaxed using the Maximin 2 energy minimizer and electrostatics option within the Sybyl Optimization software. To compare the binding of the phenylurea (**I**) to the L subunit of *Rp. viridis*, residues were deleted from the L subunit structure taken from the Brookhaven Protein Database to produce a simplified structure similar to that created above. This structure was fitted to the minimized D1 using a partial least squares procedure by matching conserved residues (L-his 190 to D1-met 214; L-his 230 to D1-his 272; L-tyr 215 to D1-tyr 254; L-phe 216 to D1-phe 255). The "D1-relaxed urea (**I**)" was then extracted from D1 and transposed into the L subunit. Urea-protein interactions were compared by eye.

All stereograms are drawn such that if viewed in the following way they will produce the correct perspective. Hold a pen vertically, in front of and between the two images. Focus on the two stereo images, and move the pen until the two out-of-focus images of the pen are superimposed over the two stereo images. Focus on the pen; a third image, out-of-focus of the protein structure, appears behind the pen. Remove the pen without changing focus, and concentrate on the middle

image. Within a few seconds, it should appear as a stereo image.

Results and Discussion

Fig. 2 shows a stereogram "side-view" of the model of the herbicide-binding domain of D1. The purpose of this figure is to provide an overall picture rather than to examine specific molecular interactions. For clarity, the Q_B site is shown vacant. It shows helix D in front, the so-called parallel de helix, which is in fact tilted with respect to the membrane-plane, and helix E, behind helix D. Other studies with this D1 model [33] suggest that the isoprenoid side chain of the quinone is located approximately in the region of the arrow indicating the entrance to the herbicide-binding cleft. In this region it appears to be projecting into empty space; at least part of this space would normally be occupied by membrane lipid.

A second view (Fig. 3) is from above the reaction centre looking into the thylakoid membrane from the stromal side. It can be seen from the views in Fig. 2 and 3 that asn 266, which on mutation to thr results in resistance to the phenolic herbicide ioxynil [34], and val 249, which is photoaffinity-labelled by azidoioxynil [22], are on the periphery of the quinone-binding cleft. The model would therefore suggest a more peripheral binding site for the phenolic herbicides. Although highly

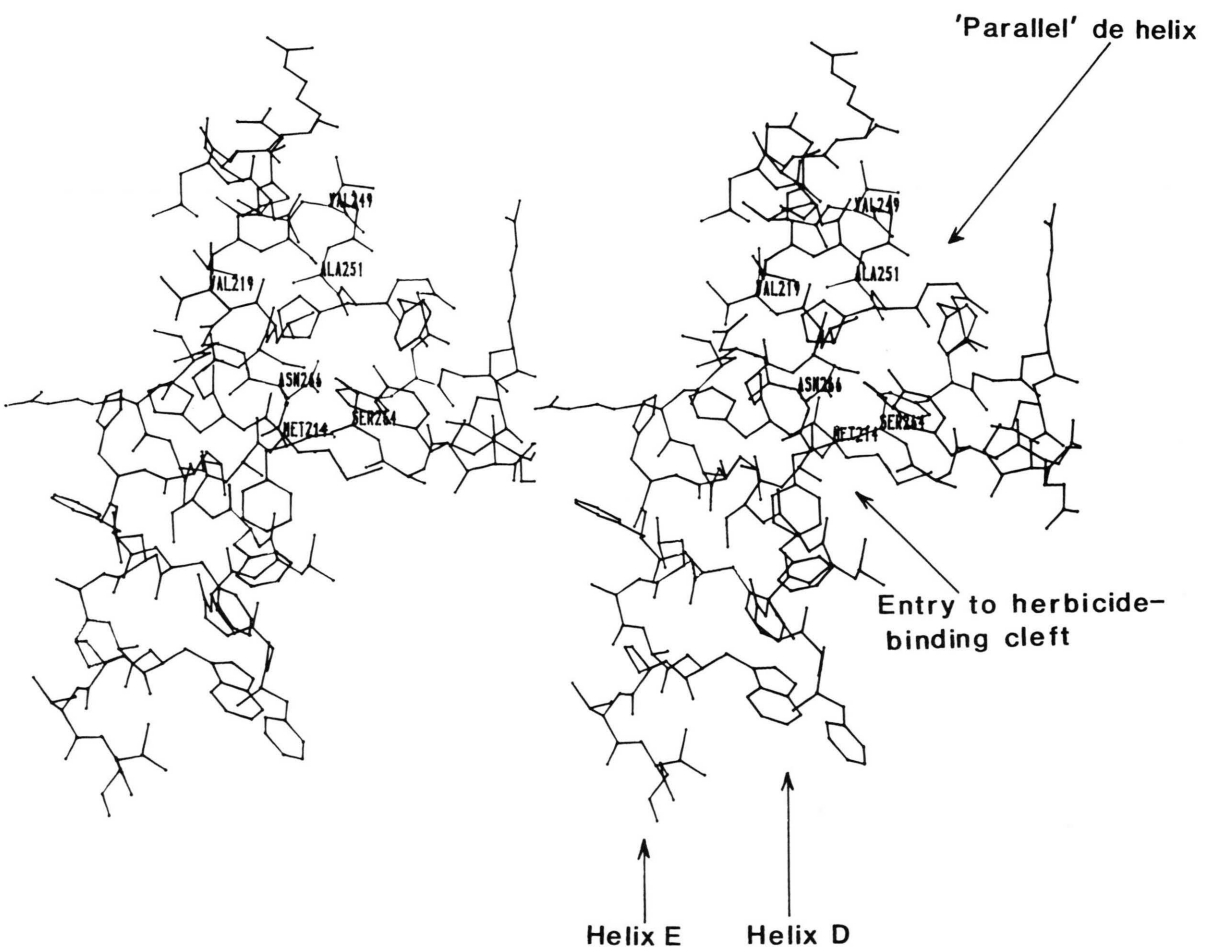


Fig. 2. Stereogram of a side view of the hypothetical model of the herbicide-binding region of D1 in the region of the membrane stromal phase interface. The Q_B site is not occupied. Hydrogen atoms are not shown.

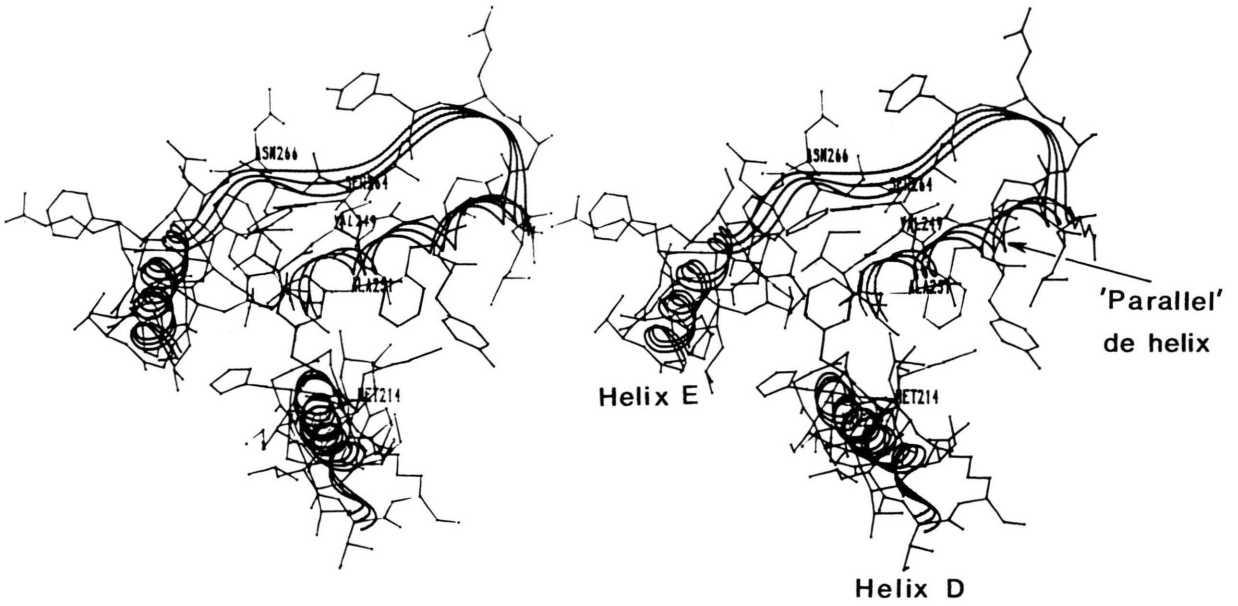


Fig. 3. Stereogram of the herbicide-binding region of D 1, viewed from the stromal phase looking into the membrane (into the plane of the paper). The Q_B site is not occupied. Hydrogen atoms are not shown.

speculative, this would be consistent with other studies on phenolic herbicide binding to D 1 which indicate that they bind in a different fashion to the urea/triazine family [18, 35–38]. The triazine herbicide terbutryne may be bound into a site on D 1 very similar to that in the L subunit of *Rp. viridis*. Hydrogen bonds analogous to those found in *Rp. viridis* are possible between N 5 of the triazine ring as acceptor and the peptide NH of phe 265 as donor, and between the side chain OH of ser 264 as acceptor and the 4-ethylamino side chain nitrogen as donor (not shown).

In order to study the binding of a phenylurea to D 1, we chose a large inhibitory phenylurea ((N,N-dimethylcarbamoyl)4-amino-4′-chloro-*trans*-stilbene) (**I**), which binds with high affinity to PS II in thylakoids (J. Whitelegge, P. Jewess, P. Camilleri, and J. R. Bowyer, unpublished data), and, by virtue of its size, has greater limitations on its possible binding domain than the "standard" phenylurea, diuron (DCMU; 3-(3,4-dichlorophenyl)-1,1-dimethylurea).

The phenylurea is located in a cleft approximately located between helices D and E, and the membrane-facing surface of the loop connecting helices D and E (Fig. 4). Using the structure shown in Fig. 2 to define directions, the dimethylurea group of the phenylurea (**I**) is directed upwards, and the hydrophobic stilbene moiety is directed downwards into the membrane. Fig. 5 shows a view of the phenylurea bound to D 1, designed to illustrate more clearly some of the phenylurea/protein interactions. Some of the many interactions between D 1 and **I** are listed in Table II. Hydrogen bonds are possible between the phenylamino NH and both the side chain hydroxy group of serine 264 and the peptide carbonyl oxygen of ala 251. In a slightly different binding position, the side chain hydroxy group of ser 268 can also form a hydrogen bond to the phenylamino NH group. These alternative or additional hydrogen bonds could explain the relatively small effect of the serine 264 to glycine mutation on DCMU binding [39–41]. The proximal styrene moiety corresponding to the dichlorophenyl group of DCMU is sandwiched between phe 265 and phe 255. Comparison of the binding of the phenylurea (**I**) and plastoquinone indicate that both molecules approach the cleft from the membrane phase in essentially the same manner (see also [42]); the stil-

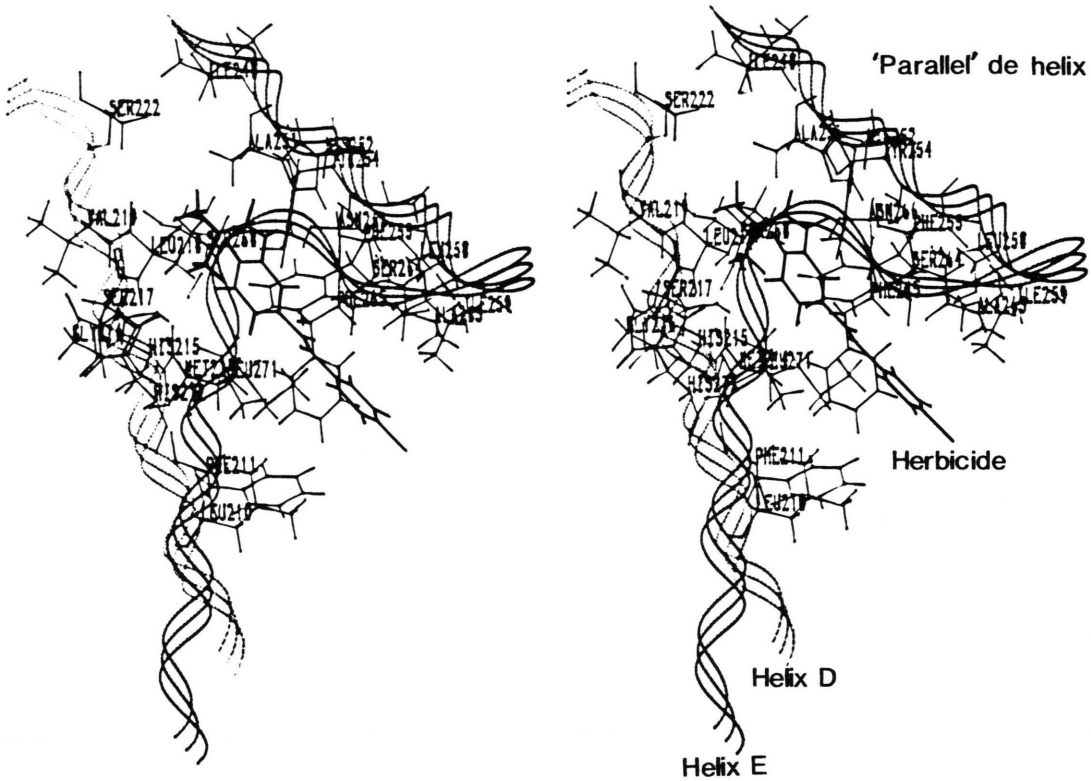


Fig. 4. Stereogram of phenylurea (**I**) bound to the herbicide-binding region of D1, showing a "side" view in the region of the membrane-stromal phase interface, similar to that shown in Fig. 2. To aid clarity, some residues have been deleted, and a ribbon is superimposed over the polypeptide backbone.

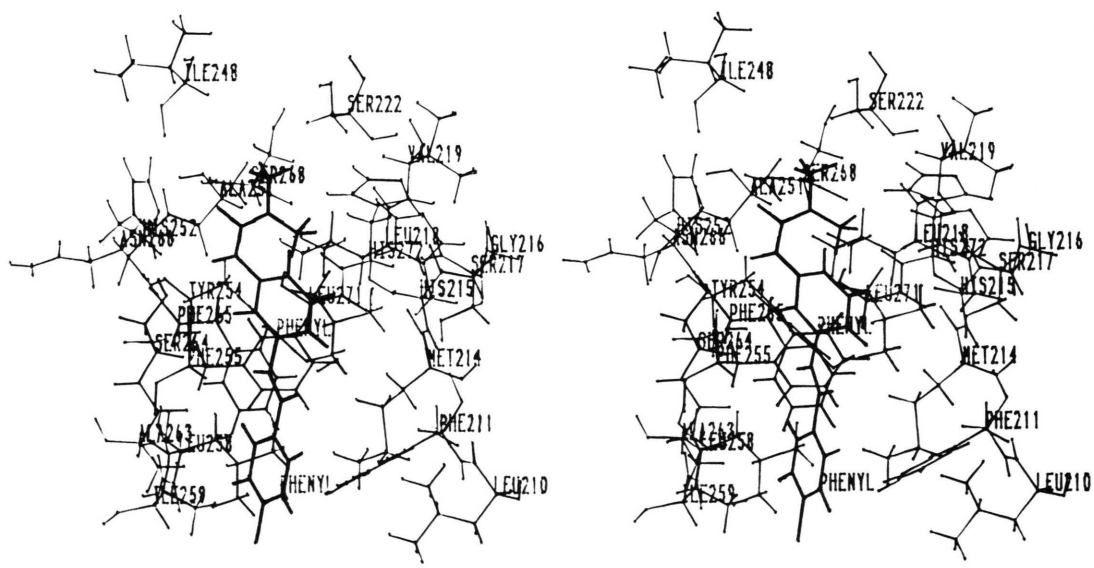


Fig. 5. Stereogram of phenylurea (**I**) binding to D1. The view is from within the membrane (above the plane of the paper) looking towards the stromal side (below the plane of the paper). Helix D is on the right, the "parallel" de helix (leu 248 to ileu 259) is located behind the phenylurea, and the polypeptide connecting the parallel de helix to the top of helix E (phe 260 (not shown) to leu 271) is located in front of the phenylurea. To aid clarity, some residues have been deleted.

Table II. Groups interacting with phenylurea (**I**) on D 1 and the L subunit of *Rp. viridis* at the Q_B site. The numbers in brackets refer to particular parts of phenylurea (**I**) (see diagram of structure **I**).

Residue in D 1	Nature of interaction with phenylurea	Analogous residue in L subunit and interaction
Ser 264	Hydroxy OH as ligand to phenylamino NH [1]	Ser 223 forms a similar interaction
Ala 251	Peptide C=O as ligand to phenylamino NH [1]	Glu 212 α-C=O forms a similar interaction
Ser 268	α-CH and β-CH_2 close to dimethyl [3]	Ala 226 – similar interaction possible
	Hydroxy OH could act as alternative ligand to phenylamino NH [1] in different binding position	Ala 226. No interaction available
His 252	Imidazol ring near C=O [2]	Not available
Ala 251	β-Methyl has unfavourable interaction with C=O [2] but interacts with methyl [3]	Glu 212 β-CH_2 and δ-CO_2^- occupy space where C=O [2] and methyl [3] are located. Very unfavourable
Leu 218	δ-Methyl groups have favourable interaction with methyl [3]	Leu 193 δ-methyl groups have favourable interaction with methyl [3]
Phe 255	Phenyl ring contacts phenylalkene [4]	Phe 216 forms similar interaction
Leu 271	Side chain isopropyl contacts proximal phenylalkene [4]	Ileu 229 side chain also forms contact
Phe 265	Phenyl ring contacts alkene [4]	Ileu 224 side chain forms contact
Phe 211	Phenyl ring contacts distal phenyl [5]	Ala 186 some distance away
Ileu 259	Side chain methyl contacts phenyl ring [5]	Val 220 forms a similar contact
Met 214	Side chain contacts phenyl ring [5]	Leu 189 side chain has less favourable contact

bene axis has the same orientation and overlaps partially with the binding region of the isoprenoid side chain of the quinone.

Our model for the binding of **I** to D 1 would not predict that a 3-azido group on the proximal phenyl ring of **I** would react with tyr 254 as has been observed using 3-azidomonuron [21], since phe 255 is located between these two groups. However, azidomonuron would presumably have more freedom within the site than **I** owing to its reduced size, and this must enable it to bind between tyr 254 and phe 255. Mutation of ala 251 to val results in a 5-fold increase in the I_{50} for DCMU [43]. Our model for the interaction between a phenylurea and D 1 suggests that this might result from steric interference with the binding of the N,N-dimethylcarbamoyl moiety. It is not immediately apparent from the model why mutations in leu 275 or val 219 should affect DCMU binding [28, 44]. The model predicts that phenylureas do not directly interact with the histidine ligands to the Fe atom (his 215 and his 272), consistent with the relatively minor effects of DCMU on the EPR spectrum of the oxidized Fe moiety [45].

When the binding of **I** to the L subunit of *Rp. viridis* is analyzed (assuming a similar binding location to that on D 1), many of the interactions available on D 1 are also present in the L subunit (Table II and Fig. 6). Unfavourable or less favourable interactions which may be responsible for preventing DCMU binding to the L subunit [32] include the lack of binding interactions provided by the side chain hydroxyl of ser 268, the imidazol ring of his 252, the phenyl ring of phe 211 and the side chain of met 214. The side chain β-CH_2 and δ-CO_2^- groups of glu 212 occupy the space where part of the dimethylurea moiety was located in D 1, and this would be expected to disfavour phenylurea binding. The model of D 1 used here does not include the extra-membraneous loop containing the so-called PEST sequence at which D 1 is cleaved during light-dependent turnover [46]. Since DCMU not only inhibits cleavage at this site by the endogenous protease responsible for D 1 turnover [47], but also tryptic cleavage at the nearby arg 238 [48], it seems likely that this extra-membraneous loop which is absent in *Rp. viridis* plays an important role in creating the phenylurea binding domain.

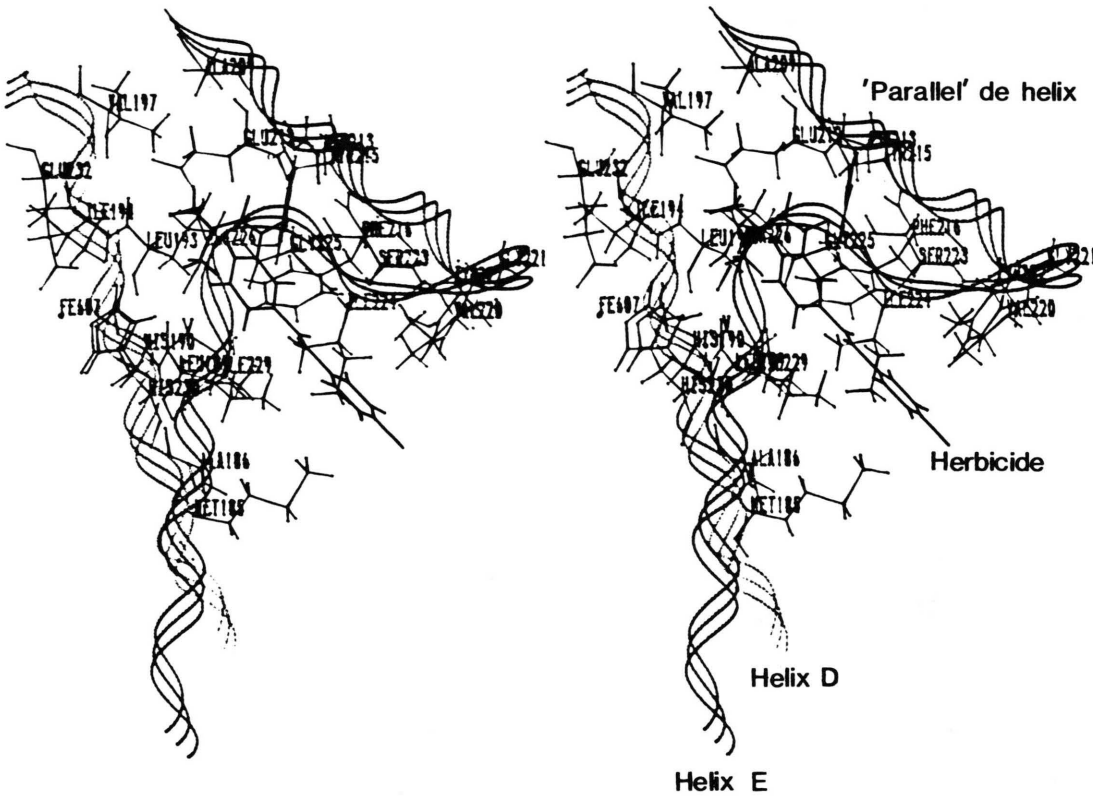


Fig. 6. Stereogram of phenylurea (**I**) binding to the L subunit of *Rp. viridis* in a very similar orientation to that in Fig. 4. To aid clarity, some residues have been deleted.

This study should obviously be considered as a rather preliminary and speculative appraisal of phenylurea binding to PS II. Nevertheless, it provides the basis for a number of site-directed mutagenesis experiments which might verify some of the interactions. The availability of a DCMU-sensitive mutant of *Rp. viridis* (L-tyr 222 to phe) [27, 32] may enable the binding of DCMU to the *Rp. viridis* reaction centre to be studied by X-ray crystallography. This should provide further valuable insights into the possible mode(s) of binding of phenylurea herbicides to PS II.

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