Molecular Modelling of the Interaction between DCMU and the Q_B-Binding Site of Photosystem II

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The prefered binding orientations for the herbicide DCMU within the Q_B-binding site of the D 1 protein model from a photosystem II reaction centre have been determined. Calculation of the intermolecular energy between the herbicide and the binding site has been instrumental in obtaining optimum positions reinforced by experimental results from mutation studies and herbicide binding to analogous bacterial reaction centres. We have shown that two binding sites are possible, one involving a hydrogen bond to the His 215 residue of the Q_B-binding site and the other to the Ser 264 residue. In both cases, it appears to be the van der Waals forces which are more important for the stabilization of the interactions.

Introduction

In the absence of X-ray crystallographic data for the photosystem II (PS II) reaction centres of higher plants, molecular modelling provides us with a useful technique for investigating potential interactions between herbicides and the secondary quinone (Q_B) binding site of the protein. High resolution crystal structures of the reaction centres from the photosynthetic bacteria *Rhodopseudomonas viridis* [1, 2] and *Rhodobacter sphaeroides* [3–6] have been used as templates for the construction of three-dimensional models of a number of PS II reaction centre subunits based upon functional and partial sequence homologies between the two systems [7–10]. In particular, the Q_B- and herbicide-binding domains located on the D 1 protein (higher plants) and the L protein (bacteria) possess a number of conserved residues which are believed to participate in similar ways with respect to structure and function [11–14].

Previous attempts [13, 14] at modelling the interaction of herbicides with the Q_B site in the D 1 protein have been concerned with positioning the herbicide within the site to agree with the binding parameters determined from the bacterial data and from mutation studies of the Q_B-binding domain which affect the interaction. Modelling the interaction between phenylurea type herbicides such as DCMU with the Q_B site of PS II has lead to the suggestion that hydrogen bonding takes place between the phenylamino NH of the herbicide and residues present at the top of the Q_B-binding site such as Ser 264, Phe 265 or Ser 268 with hydrophobic interactions between the phenyl group and the side chains of Phe 265 and Phe 255 [13, 15, 16]. Reliance simply on visualization could lead to very unfavourable steric interactions which are not apparent without quantification. Such studies also rely completely on hydrogen-bonding interactions to position the herbicide in the site. This study suggests satisfaction of steric requirements is the more crucial. We have evaluated the non-bonded energy between the herbicide and the Q_B site to determine the optimum orientation(s) in the PS II D 1 protein based on the enthalpy of binding. The effects of this energy of reported site-directed mutagenesis in the Q_B-binding site of the D 1 protein have also been investigated. The intermolecular energies between the herbicide and individual amino acids have been calculated to identify those residues involved in binding. The D 1 model also includes a fitting of the intrathylakoid loop which has not been a part of previous similar studies.

Methods

The source of the parameters used to calculate non-bonded energies is the forcefield which is the empirical fit to the potential energy surface of the molecules involved. It defines the coordinates used, the mathematical form of the equations involving the coordinates and the parameters adjusted in the empirical fit of the potential energy sur-

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face [17, 18]. The forcefield employs a combination of internal coordinates (bond distances, angles and torsions) to describe the bonded part of the potential energy surface, and interatomic distances to describe the van der Waals and electrostatic interactions between atoms. For the purpose of calculating the intermolecular energies between the atoms of two molecules, we are interested in the expressions which determine the non-bonded interaction [19, 20].

The non-bonded van der Waals interaction are represented by the first two terms in Eqn. (1) where A_{ij} and B_{ij} are parameters with units of kcal mol^{-1} $angstrom^{-12}$ and kcal mol^{-1} $angstrom^{-6}$ respectively and R_{ij} is the distance between the atoms i and j in angstroms. The second component of the non-bonded intermolecular energy is the electrostatic energy, which is represented by the third expression in Eqn. (1) where q_i and q_j are the charges on atoms i and j and D is the dielectric constant. The intermolecular energy is computed by summing the energy contributions between atoms of the two molecules. The contribution between atoms interacting with atoms in the same molecule is ignored.

$$E_{\text{interaction}} = \sum_i \sum_j \left[\frac{A_{ij}}{R^{12}_{ij}} - \frac{B_{ij}}{R^{6}_{ij}} + \frac{q_i q_j}{D R_{ij}} \right] \quad (1)$$

Enclosure analysis focuses on a smaller region of a molecular system in order to generate intermolecular energies between a ligand and individual amino acid residues within its binding site. In doing so, Eqn. (1) is used to calculate the interaction energy between the atoms of the ligand and the atoms of the residues which fall into a defined sphere of a given radius around that ligand. This allows the identification of the main residue-ligand non-bonded interactions which make up the intermolecular energy between the two molecules.

The model used was the D1 protein from the PS II photosynthetic reaction centre of *Pisum sativum* [21]. The Q_B-binding domain was represented by residues Leu 210 to Val 280 and all other residues were deleted from the model for simplification. All hydrogen atoms, polar and non-polar were included. The models of DCMU in both the *cis*- and *trans*-amide conformations were constructed using the *Insight II* molecular graphics modelling program [22] and minimized accordingly. Atom partial charges and potentials for both protein and herbicide models were assigned according to the parameters defined within the Consistent Valence Force Field (CVFF) used by the *Discover* molecular simulation program [23].

The herbicide was orientated within the Q_B-binding site employing the three-dimensional stereo viewing facility of the *Insight II* modelling program until a minimum intermolecular energy was achieved. Energy minimization of the combined structures involved constraining the herbicide heavy atoms and the backbone atoms of the peptide whilst allowing the amino acid side chains to relax to relieve further unfavourable interactions between the protein and the herbicide. This was performed using steepest descents and conjugate gradients algorithms successively until the average first derivative was less than 0.005 kcal mol^{-1} $angstrom^{-1}$. The cancellation of the non-bonded interactions between atoms after a specified cut-off distance was not carried out during minimization in order to achieve a more accurate final structure. A dielectric constant of one was employed throughout the study. The same minimization procedure was adopted after replacing amino acid residues in line with reported mutations. A sphere of 8 angstrom radius around each functional group of the DCMU was used to calculate the non-bonded interaction energy between the herbicide and individual amino acid residues of the binding site.

Results and Discussion

The model for the Q_B-binding domain of the L protein in Fig. 1 shows the end and beginning of the transmembrane α helices D and E and the α helix in the connecting segment. Because the interhelical sequence of the D1 protein is longer than the bacterial system [24], to construct a model of equivalent structure around the Q_B which incorporates those residues involved in plastoquinone and herbicide-binding necessitates the inclusion of an intrathylakoid loop consisting of the extra amino acids [7, 10, 11] (Fig. 2).

Previous modelling discussions have focussed upon the interaction between DCMU and the top of the Q_B-binding domain of PS II [13, 15, 16]. However, the absence of any change in the binding interaction when Val 219 is replaced by an Ile residue [13], a mutation which induces resistance to DCMU [25], suggests that other binding orienta-

tions may be possible. This mutation may alter DCMU binding directly by being near to the His 215 residue at the bottom of the Q_B-binding pocket [26], which has been proposed to interact with the herbicide [27]. Studies investigating the effect of herbicides on the EPR spectra of Fe(III) in PS II [28–30] suggest that the urea may have one binding site similar to that of *o*-phenanthrolene which may interact with His 215 of the D1 protein in an analogous manner to it forming a hydrogen bond with His L190 in the bacterial centre [31]. In a separate development, the characterization of the triazine-resistant mutants of *Rps. viridis* revealed that one of the mutants, referred to as T4 (Tyr L222 to Phe) was sensitive to the urea type inhibitors [32] in common with the PS II reaction centre. In addition, the semiquinone-iron EPR signal of Q_B^- in T4 resembled that reported for PS II [33]. X-ray crystallographic analysis of the reaction centre from T4 with bound DCMU [34] provide us with potential ligand-residue binding interactions with the D1 protein. In light of these observations, we propose that one binding orientation for DCMU in the Q_B-binding pocket is stabilized by a predominantly electrostatic interaction between the carbonyl group (CARB) of the herbicide and the imidazoyl side chain of His 215 (Fig. 3), equivalent to His L 190 of the T4 reaction centre (Table I).

Table I. The main interactions between amino acid residues of the D1 protein with DCMU when hydrogen bonded to His 215.

Residue	Intermolecular energy [kcal mol^{-1}] PHE	AMIN	CARB	DMA
His 215	−0.1	−0.8	−3.2	−2.5
Leu 218	−0.8	−0.9	−1.3	−1.3
Val 219	−0.0	−0.2	−0.6	+1.6
Ala 251	−1.6	+1.5	+0.2	−1.1
Phe 255	−0.2	−0.5	−0.1	−0.2
Ser 264	+3.7	−0.1	−0.0	−0.1
Asn 266	−3.2	+0.5	−0.1	+0.2
Asn 267	−1.5	+0.1	+0.6	−0.3
Ser 268	−0.5	−0.1	+0.0	+1.7

The urea adopts the more stable *trans*-amide conformation [35] and consequently reduces potential van der Waals repulsive forces between the dimethylamino moiety (DMA) and amino acids in the vicinity, with the exception of Val 219. Replacement of Val 219 by Ile in line with the mutation inducing DCMU resistance [25] increases the intermolecular energy between the more bulky Ile side chain and the DMA group (Table II) and makes binding unfavourable. Non-bonded stabilization also occurs between the phenyl moiety (PHE) of the DCMU and Ala 251 and Asn 266 but ring stacking between this group and the side chain of Phe 255 in a similar manner to the interaction with Phe L216 of the bacterial reaction centre [34] was not apparent. However, replacement of Phe 255 by Tyr does not significantly alter the overall binding energy between DCMU and the protein (Tables II and III) in agreement with its reported failure to induce resistance to the herbicide [36].

In our model, replacement of Ala 251 by Val, another mutation reported to induce resistance to DCMU [37], produces a high intermolecular energy between the phenylamino group (AMIN) of the herbicide and the protein as a consequence of the steric interaction with the more bulky side chain of the mutated residue (Table II). The Ser to Thr [38] and Ser to Ala [39–41] mutations induce resistance to DCMU whereas Ser to Gly [41, 42] does not. Replacement of Ser 264 with Gly in our model results in a slightly improved intermolecular en-

Table II. Intermolecular energies between DCMU and the mutated D1 protein when hydrogen bonded to His 215.

DCMU	Intermol. energy with protein [kcal mol^{-1}] Val 219 to Ile	Ala 251 to Val	Phe 255 to Tyr	Ser 264 to Gly
PHE	−15.2	−12.7	−11.1	−19.8
AMIN	+4.7	+189.0	+4.9	+4.8
CARB	−5.0	+0.7	−4.9	−5.0
DMA	+289.8	−3.9	−4.4	−4.4
Total	+274.3	+173.1	−15.5	−24.4

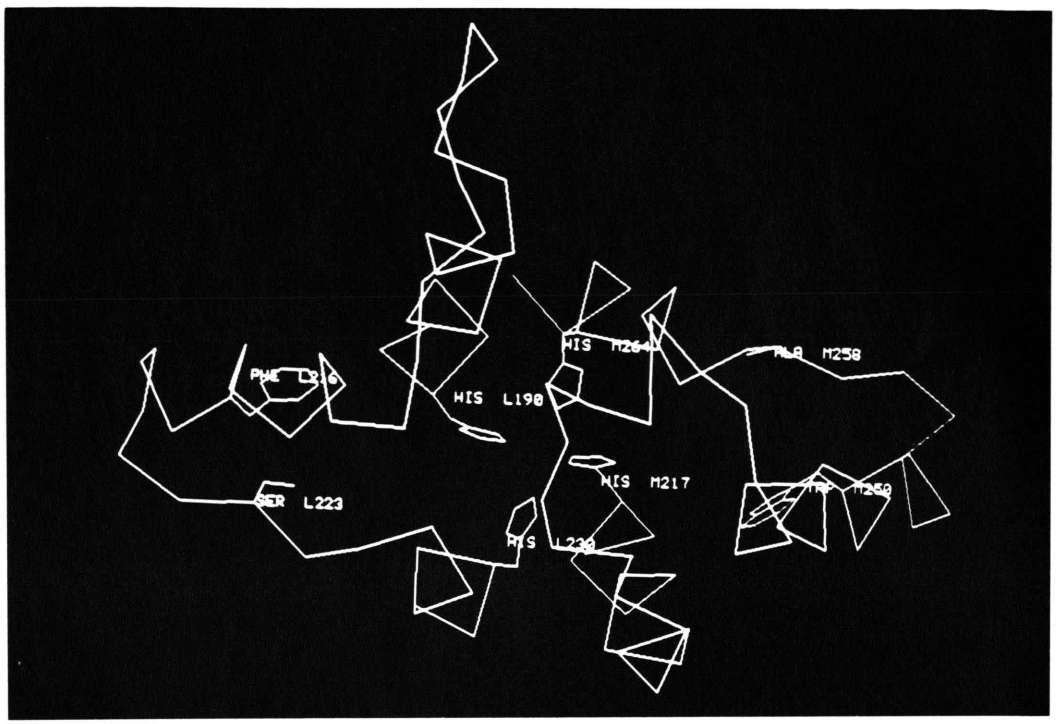

Fig. 1. Quinone-binding sites in the l and m subunits from the photosynthetic reaction centre of *Rps. viridis*.

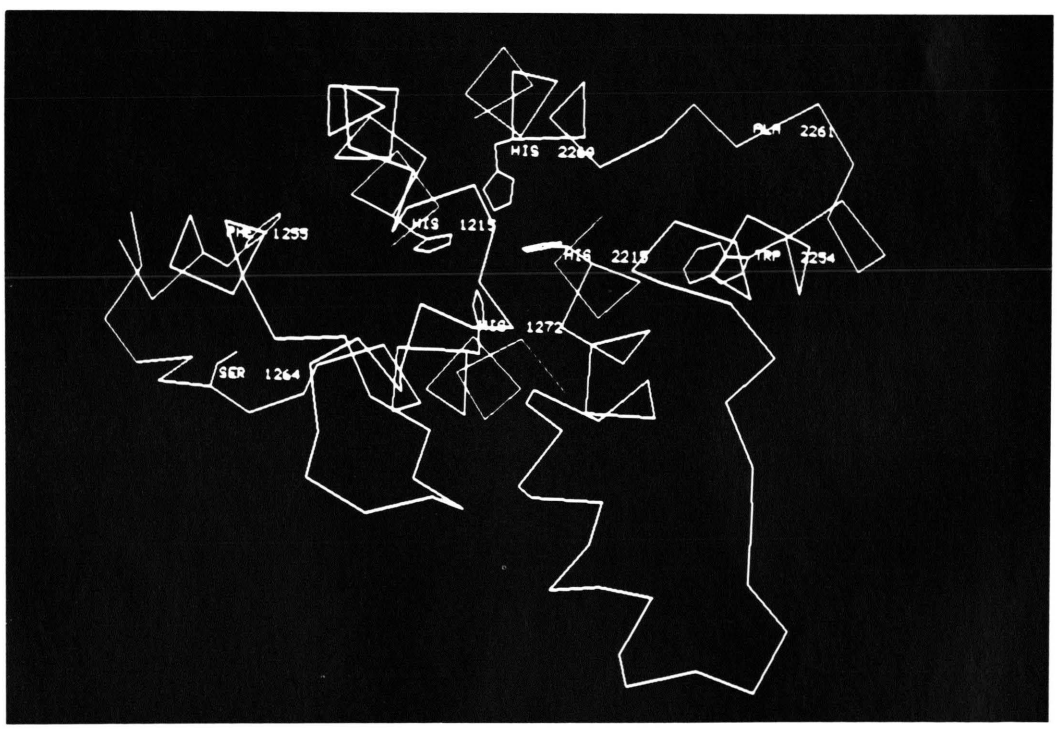

Fig. 2. Quinone-binding sites in the D 1 and D 2 proteins from PS II of *P. sativum* showing the intrathylakoid loops. The residue numbers for the D 1 protein are prefixed by the number 1 and by the number 2 for the D 2 protein.

Table III. The intermolecular interactions of different binding orientations of DCMU within the Q_B-binding site of the D1 protein model from *P. sativum*.

DCMU		Intermolecular energy [kcal mol^{-1}] PHE	AMIN	CARB	DMA	Total
(1)	vdW	−10.0	−1.0	−5.5	−5.0	−21.5
	elect	−5.3	+6.0	+0.5	+0.6	+1.8
	total	−15.3	+5.0	−5.0	−4.4	−19.7
(2)	vdW	−10.2	−3.1	−3.0	+1.5	−14.8
	elect	−5.3	+3.7	−2.8	+2.2	−2.2
	total	−15.5	+0.6	−5.8	+3.7	−17.0

(1) DCMU forming a hydrogen bond between its carbonyl group and His 215.
(2) DCMU forming a hydrogen bond between its phenylamino NH group and Ser 264.

ergy (Tables II and III) between the herbicide and the binding site due to the removal of the repulsive effect between the hydroxymethyl side chain of Ser 264 and the phenyl ring. In the absence of hydrogen bonding between Ser 264 and DCMU [43] as we predict, little change in binding and therefore activity would be expected. Mutations of Ser 264 to Ala or Thr may produce conformational changes in the Q_B-binding pocket [39, 44] which lower the relative affinity of DCMU with respect to the natural quinone for the protein. Shigematsu *et al.* [44] predicted a conformational change to a β sheet in the region of residues 262 to 264 which was absent in the Ser to Gly mutant. Without such a conformational change in our model, the Ser to Ala or Thr mutations have little effect on binding energies (data not shown). It is difficult to predict the structural effect of such a conformational change on the rest of the protein without recourse to crystallographic data and consequently, we have not attempted to model such an interaction.

Table IV. The main interactions between amino acid residues of the D1 protein with DCMU when hydrogen bonded to Ser 264.

Residue	Intermolecular energy [kcal mol^{-1}] PHE	AMIN	CARB	DMA
Leu 218	−0.6	−0.5	+0.0	+2.8
His 252	−0.6	−0.1	−2.8	−1.3
Phe 255	−2.2	−1.3	−0.1	−0.9
Tyr 262	+1.2	−0.2	+0.0	+0.1
Ser 264	−0.9	−1.4	+0.2	+0.2
Asn 267	−0.6	−0.3	−0.5	+2.7
Leu 271	−0.4	−0.6	−0.1	−1.1

In agreement with earlier studies [13, 15, 16], we have also been able to demonstrate a second potential binding site for DCMU within the Q_B-binding domain which has a binding energy similar to that of the first orientation (Table III). The DCMU hydrogen bonds *via* its phenylamino NH to the side chain hydroxyl oxygen of the Ser 264 residue at the top of the Q_B-binding pocket (Fig. 4). A more favourable interaction is achieved when the the DCMU adopts the *cis*-amide conformation. Whilst the *trans*-amide isomer is considered to be the more stable conformation [35], *in vivo* adoption of the *cis*-amide form cannot be discounted. By assuming this form, a negative ring stacking interaction energy can be achieved between the phenyl ring of the urea and the phenyl side chain of the Phe 255 residue (Table IV). The intermolecular energy between the phenylamino NH group and Ser 264 is predominantly electrostatic, as is the interaction between the urea carbonyl group and the imidazoyl ring of His 252. The dimethylamino group is responsible for mainly van der Waals repulsive interactions between Leu 218 and Asn 267 although it interacts favourably with His 252 and Leu 271 (Table IV). Mutations affecting the Phe 255 [36] and Ala 251 [37] residues give the expected changes in binding energy (Table V), however a discrepancy between experimental and theoretical results occurred for the Val 219 to Ile mutation. We can report no significant change in the binding interaction between DCMU and the mutated protein, a change at the bottom of the pocket, as demonstrated by Bowyer *et al.* [13]. Little change to the binding energy occurs when Ser 264 is replaced by Gly even though the electrostatic interaction with the phenylamino NH is lost.

One important observation from the overall intermolecular energies between the DCMU in both orientations in the protein is that the major contribution is from the van der Waals non-bonded interactions (Table III). The electrostatic attractive forces, which are more appreciable over larger interatomic distances than van der Waals forces, may act as an initial guide and anchor for the herbicide to a residue in the binding site, for example His 215 or Ser 264. Once within the site, the herbicide may then orientate to achieve a maximum van

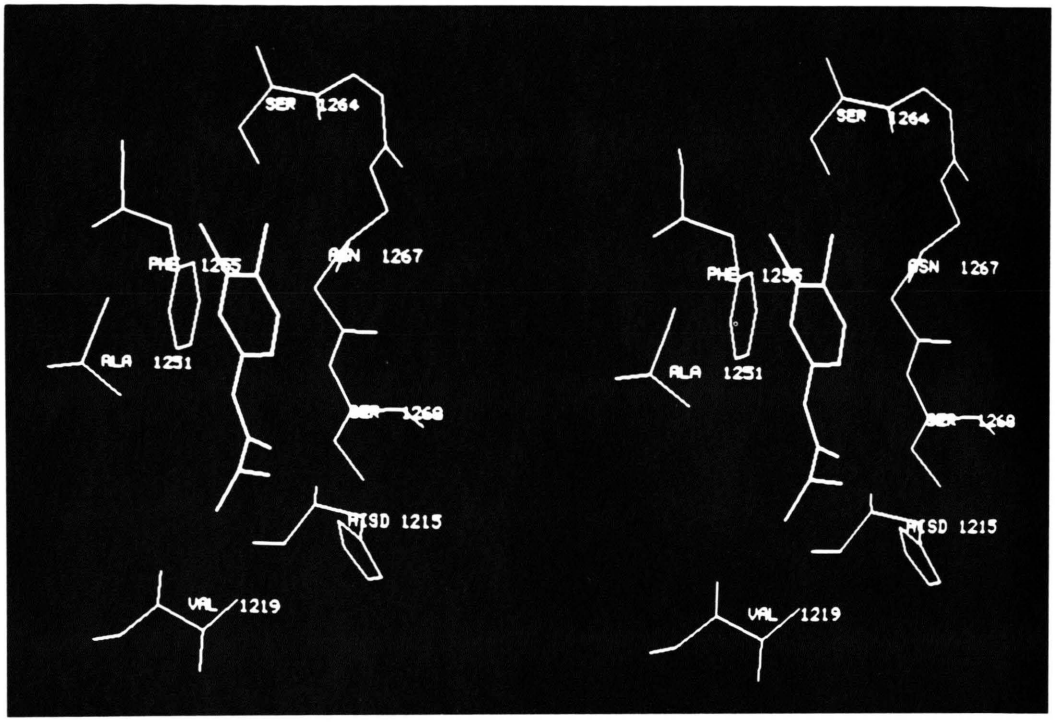

Fig. 3. DCMU binding in the *trans*-amide conformation to the Q_B-binding site of *P. sativum* and forming a hydrogen bond with the His 215 residue. The residue numbers for the D 1 protein are prefixed by the number 1.

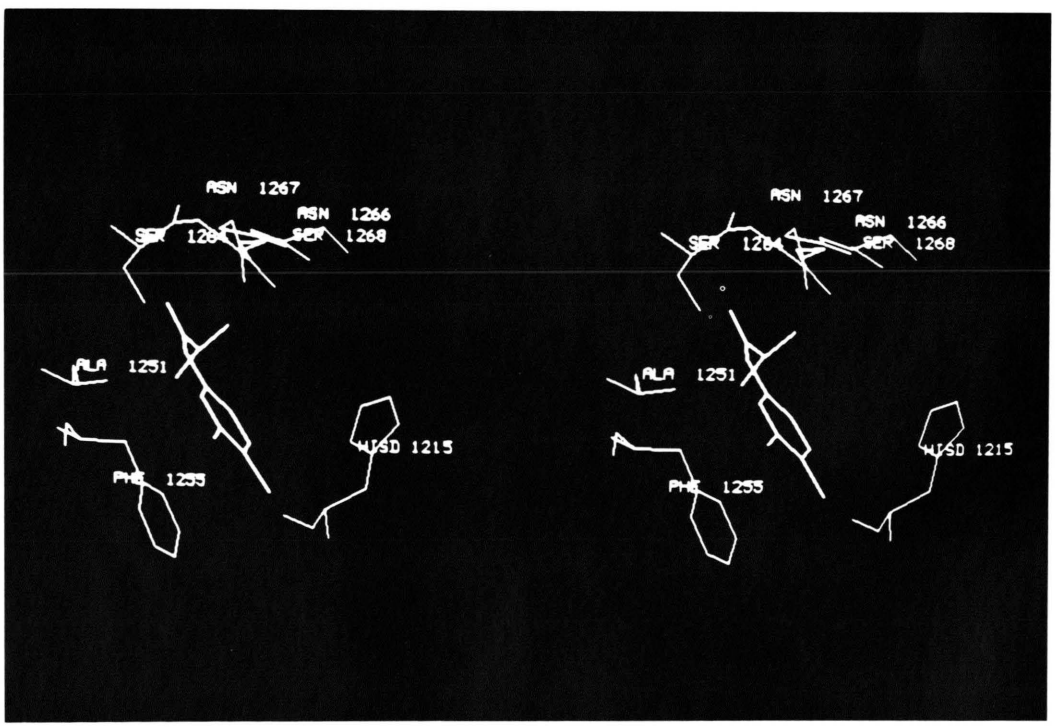

Fig. 4. DCMU binding in the *cis*-amide conformation to the Q_B-binding site of *P. sativum* and forming a hydrogen bond with the Ser 264 residue. The residue numbers for the D 1 protein are prefixed by the number 1.

Table V. Intermolecular energies between DCMU and the mutated D1 protein when hydrogen bonded to Ser 264.

DCMU	Intermol. energy with protein [kcal mol^{-1}] Val 219 to Ile	Ala 251 to Val	Phe 255 to Tyr	Ser 264 to Gly
PHE	−15.9	−16.4	−11.2	−14.9
AMIN	+0.7	+1.0	+0.9	+2.1
CARB	−5.7	−6.5	−5.8	−5.8
DMA	+2.5	+6021.6	+3.1	+2.1
Total	−18.4	+5999.7	−13.0	−16.5

der Waals dispersion interaction with the binding pocket residues.

Until a high resolution structure for the PS II reaction centre complexed with DCMU is available, models of the interaction remain speculative. The idea for more than one binding site is not unique, and we feel that in view of the mutation studies we have performed in addition to the results from the work with bacterial T4 reaction centres, a binding site involving the His 215 residue is a strong possibility.

Acknowledgements

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