Molecular Modelling of the Interactions between Optically Active Triazine Herbicides and Photosystem II

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The Q_B binding site of photosystem II in green plants displays stereoselectivity for the (*S*) stereoisomer of the α-methylbenzyl derivative of atrazine but not for derivatives with smaller substituents such as *sec*-butyl. We have shown that interactive models reflect the experimental data by determining the intermolecular energies between the D 1 protein binding region (residues Leu 210 to Val 280) and the triazine analogs. The intermolecular energy was calculated by van der Waals and electrostatic interactions after energy minimization of the combined structures to reduce inter and intramolecular strain. On the basis of these assumptions the role of stereoselectivity for optically active triazines was calculated and the region of the binding site responsible such stereoselectivity was identified.

Introduction

The reaction centres of *Rhodobacter sphaeroides* and *Rhodopseudomonas viridis* are mechanistically analogues of photosystem II (PS II) in higher plant chloroplasts [1]. In both PS II and the bacterial reaction centres, a photon of light causes the oxidation of a special reaction centre chlorophyll, with the resultant transfer of an electron to a tightly bound quinone (Q_A) which is then oxidized by a secondary quinone (Q_B) to form a stable semiquinone Q_B^-. A second photoreduction results in a second electron transfer to Q_B producing a fully reduced quinol which diffuses away from the reaction centre. The commercially available triazine herbicides such as atrazine and terbutryn are inhibitors of electron transport from Q_A to Q_B in both PS II and the bacterial reaction centres [2, 3]. They act by displacing the secondary quinone from its binding site. The binding site of the triazines is not absolutely identical to that of the quinone, but there is considerable spacial overlap [4].

Knowledge of the herbicide and Q_B binding site of the PS II reaction centre is important for the design of new herbicides and for the generation of herbicide resistant plants by molecular biologists. The binding site of terbutryn and its mode of binding to the reaction centre of *Rps. viridis* has been established by X-ray crystallography [5]. Terbutryn forms two hydrogen bonds with the protein matrix. One is between the N 3 triazine ring nitrogen and the backbone NH group of the Ile L 224 residue and the other between the side chain hydroxyl of Ser L 223 and the aminoethyl NH group of terbutryn. In addition, Phe L 216 is involved in stacking interactions with the triazine ring system and close contacts also exist between the herbicide and Glu L 212, Ile L 229 and Val L 220 [6, 7]. Different sensitivities of mutant bacteria to closely related triazines suggests different but partially overlapping binding sites for atrazine and terbutryn in purple bacteria [8, 9]. The differences in size and hydrophobicity of the substituents on the basic triazine ring system may cause different interactions with the protein. However, this assumption has to be proven by X-ray structure analysis of atrazine bound to the Q_B site. In the absence of high resolution crystal structures for PS II, molecular models have been constructed based upon functional and partial sequence homologies found between the L and M subunits of the photosynthetic bacteria and the PS II D 1 and D 2 proteins of higher plants [10–13]. In conjunction with hydropathy considerations, this has led to the proposal that the D 1 and D 2 proteins each consist of five hydrophobic helical spans through the photosynthetic membrane in a similar manner to the L and M subunits of the bacterial reaction centres [14, 15]. Studies by Sayre *et al.* [16] using antibiotics to map exposed segments add evidence to this proposed structure. Significantly, the Q_B and herbicide binding domains of the L and D 1 proteins

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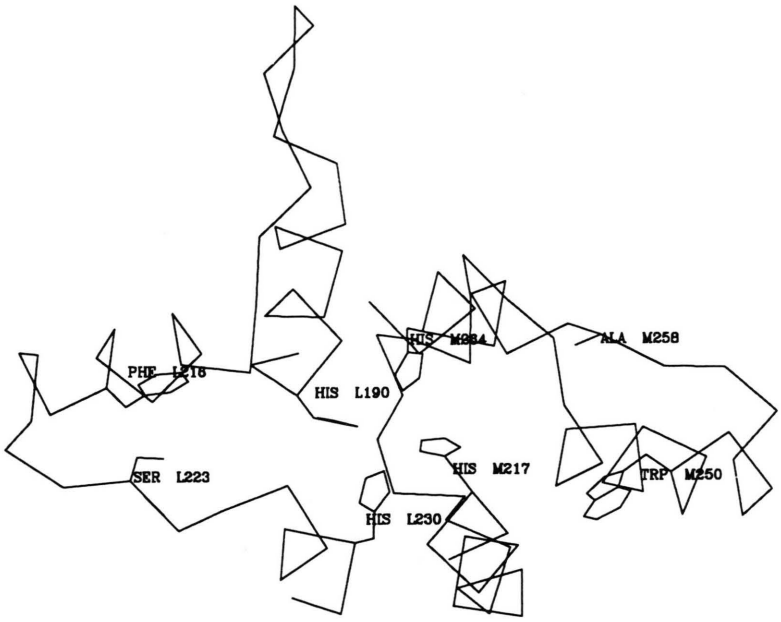


Fig. 1. Quinone binding sites in the L and M subunits from the photosynthetic reaction centre of *Rhodopseudomonas viridis*. The residue numbers of the L subunit are prefixed by the letter L and by the letter M for the M subunit.

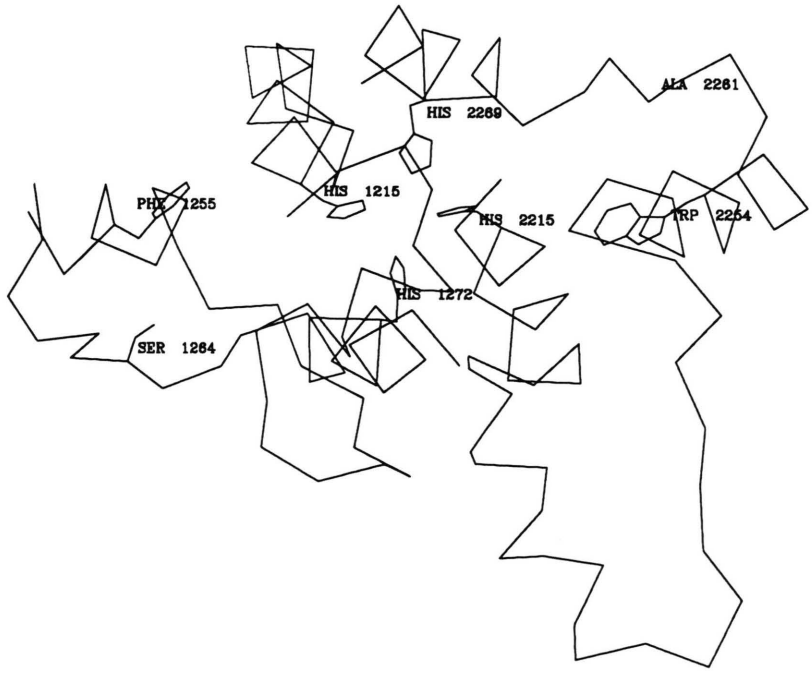
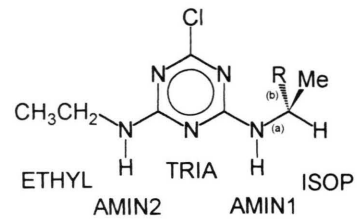


Fig. 2. Quinone binding sites in the D1 and D2 proteins from PS II of *Pisum sativum* showing the intrathylakoid loops. The residue numbers of the D1 protein are prefixed by the number 1 and by the number 2 for the D2 protein.

located on and between the forth and fifth transmembrane helices, possess a number of conserved residues and molecular models of this region are structurally similar [10–13]. However, because the interhelical sequence of the D1 protein is longer than the bacterial system, to construct a model of equivalent structure around the Q_B site which incorporates the amino acid residues involved in Q_B and herbicide binding necessitates the inclusion of an intrathylakoid loop consisting of the extra amino acids [10, 11, 13] (Fig. 1 and 2).

Previous attempts [17, 18] at modelling the interaction of herbicides with the Q_B site in the D1 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 [19–23]. 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 nonbonded energy between the herbicide and the Q_B site to determine the optimum orientation in the PS II D1 protein based on the enthalpy of binding. The intermolecular energies between the herbicide and individual amino acids have been calculated to identify those residues involved in binding.

The interaction between PS II and the triazine herbicides has been studied extensively in terms of structure-activity relationships [24–26], however it is only recently that the role of stereoselectivity has been investigated [27–30]. This is probably due to most commercially important PS II inhibitors not having a chiral centre. The incorporation of an optically active α-methylbenzyl or 2-butyl (Fig. 3) produces stereoisomers with different activities [27, 28, 30]. When tested for their ability to displace specifically bound [^{14}C]atrazine from thylakoid membranes of *Pisum sativum*, there were differences in the affinities for the binding site between some of the stereoisomers [28]. If the chiral carbon possessed a large substituent such as a phenyl group, the (*S*) isomer had a higher binding affinity than the (*R*) isomer. If a smaller substituent was present, such as an ethyl group, both (*S*) and (*R*) isomers had similar binding affinities indicating that the protein matrix was only stereoselective when larger groups were involved.



Compound	*R* group	Isomer
1	Me	(−)
2	Et	(*S*)
3	Et	(*R*)
4	Ph	(*S*)
5	Ph	(*R*)

Fig. 3. Functional group nomenclature of stereoisomeric atrazine derivatives.

We have modelled the interaction of atrazine with the Q_B binding domain of a D1 protein model from *P. sativum* [13] based on the parameters which govern the interaction of the triazines with bacterial reaction centres. We then introduced a chiral centre into the atrazine and calculated the intermolecular energies between the protein and the stereoisomers to determine whether they reflect the trends displayed in reported experimental data.

Methods

The source of the parameters used to calculate nonbonded 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 surface [31, 32]. 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 inter-

actions 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 nonbonded interaction [33, 34].

The nonbonded van der Waals interaction is represented by the equation:

$$E_{ij} = \frac{A_{ij}}{R^{12}_{ij}} - \frac{B_{ij}}{R^{6}_{ij}} \quad (1)$$

where A_{ij} and B_{ij} are parameters with units of kcal mol^{-1} $Å^{-12}$ and kcal mol^{-1} $Å^6$ respectively and R_{ij} is the distance between the atoms i and j in Å.

The second component of the nonbonded intermolecular energy is the electrostatic energy, expressed as

$$E_{ij} = \sum_i \sum_j \frac{q_i q_j}{D R_{ij}} \quad (2)$$

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 other atoms in the same molecule is ignored. Thus:

$$E_{\text{interaction}} = \sum_i \sum_j \left[\frac{A_{ij}}{R^{12}_{ij}} - \frac{B_{ij}}{R^{6}_{ij}} + \frac{q_i q_j}{D R_{ij}} \right]. \quad (3)$$

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. (3) is used to calculate the interactive 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 nonbonded 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* (coordinates supplied by J. H. A. Nugent, University College London). 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 the triazines in the minimum energy *cis* conformation with respect to both NH functions [35] were constructed using the *Insight II* molecular graphics modelling program (Version 2.0.0, Biosym Technologies, Inc., San Diego, CA 92121) 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 (Version 2.7.0, Biosym Technologies, Inc., San Diego, CA 92121).

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 and to relieve any intramolecular strain. This was performed using steepest descents and conjugate gradients algorithms successively until the average first derivative was less than 0.005 kcal mol^{-1} $Å^{-1}$. The cancellation of the nonbonded interactions between atoms after a specified cutoff 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. Stereoisomers **2–5** (Fig. 3) were constructed using the *Insight II* modelling program and based on the coordinates of atrazine in its optimum minimized position within the protein. Both bonds (a) and (b) (Fig. 3) were rotated to achieve a minimum intermolecular energy between the herbicide and the protein. The heavy atoms of the protein and the herbicide were constrained with the exception of those in the *R* group and the combined structures minimized as before. A sphere of 8 Å radius around each functional group of the triazine (Fig. 3) was used to calculate the nonbonded interactive energy (van der Waals and electrostatic) between the herbicide and individual amino acid residues of the binding site.

Results and Discussion

Based on the homology between the L subunit of the purple bacteria and the D1 protein of higher plants, Shigematsu *et al.* [36] have suggested that

the triazine binding site is formed by Ser 264 (equivalent to the Ser L 223 residue in the bacterial reaction centre), Phe 265 and the hydrophobic region around Phe 255 (equivalent to Phe L 216). Bowyer *et al.* [17] have proposed that hydrogen bonds analogous to those found in the bacterial reaction centres are possible between the triazine ring and the peptide NH of Phe 265 and between the side chain hydroxyl of Ser 264 and the ethylamino NH of terbutryn. On the same basis, Tietjen *et al.* [18] have suggested similar hydrogen bonding between atrazine and the Q_B binding domain.

The D 1 protein is specifically labelled by the *s*-triazine photoaffinity analogue azido [^{14}C]atrazine which competitively inhibits binding of atrazine and *vica versa* [37]. The evidence suggests that the chloro substituent of the triazine ring, which is replaced by the azido group in the labelling analogue, is in proximity to Met 214 [38, 39]. Our model of the interaction of atrazine with the Q_B binding domain of PS II from *P. sativum* (Fig. 4) shows this interaction having a repulsive energy of 1.0 kcal mol^{-1} (Table I). Terbutryn, which binds in a similar position [40] has a greater repulsive force with this residue (2.6 kcal mol^{-1}) as would be expected due to the large methylthio substituent (data not shown). A favourable interaction energy between the ethylamino group of atrazine and the

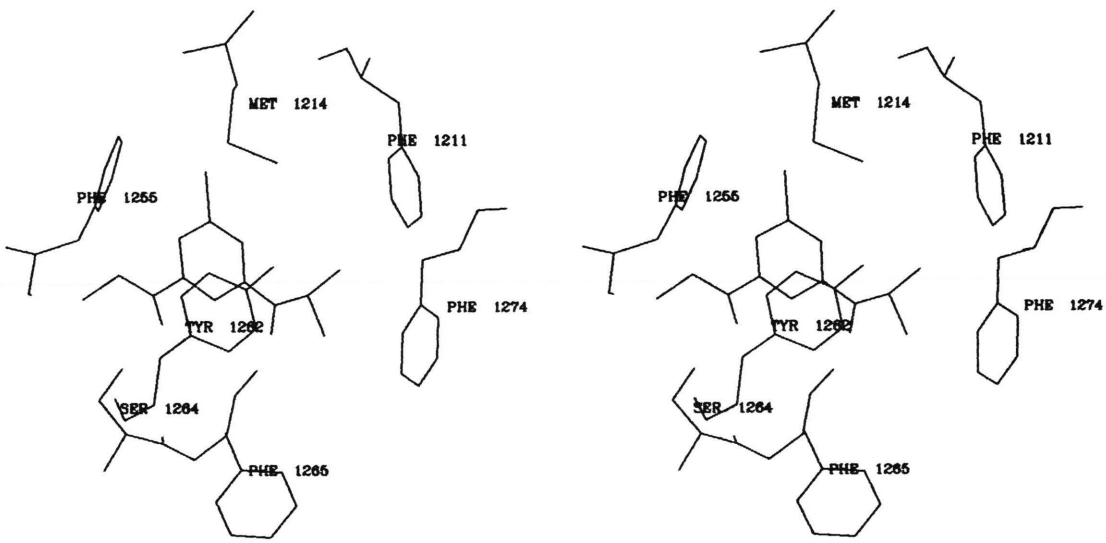


Fig. 4. Atrazine binding to the D 1 protein of *Pisum sativum*. The residue numbers of the D 1 protein are prefixed by the number 1.

Table I. The main interaction energies between atrazine and amino acid residues in the Q_B binding domain of PS II.

Residue of D 1 Protein	Interaction energy with functional group [kcal mol^{-1}] TRIA	CHLO	AMIN 1	ISOP	AMIN 2	ETHYL*
Met 214		+1.0				
Ala 251						−2.0
Phe 255	−2.5				−3.0	+3.8
Tyr 262	−4.2					−1.1
Ser 264					−4.0	+3.2
Phe 265			−1.1	−1.2		
Asn 266	−2.9					−1.5
Asn 267	−1.6					
Leu 271	−1.4					
Phe 274				−3.1		

* For functional group identification, see Fig. 3.

Ser 264 residue of -4.0 kcal mol^{-1} which is predominantly electrostatic in nature is also evident. A hydrogen bond is formed between these two functions in a similar manner to the triazines hydrogen bonding to the Ser L 223 residue in bacterial reaction centres [5, 6], and in agreement with other models for PS II [17, 18, 36]. However, we propose that a second hydrogen bond is formed between the peptide carbonyl group of the adjacent Phe 265 residue and the isopropylamino NH group of atrazine as opposed to involving the triazine ring nitrogen. The triazine ring system formed a favourable interaction energy with both the aromatic Phe 255 (equivalent to Phe L 216 in bacterial reaction centres) and Tyr 262 residues. The narrowness of the binding region [30, 41] around the ethylamino function is evident from the repulsive energy between the ethyl group and the Phe 255 and Ser 264 residues which is counterbalanced by its favourable interaction with Ala 251, Tyr 262 and Asn 266, and between the amino group and Phe 255 and Ser 264.

Replacement of a methyl group in the isopropyl moiety of atrazine with an ethyl group (**2** and **3**) produces little change in the intermolecular energies in either the (*S*) or (*R*) conformation (Table II). Similarily, the experimentally determined binding affinities of these two compounds do not differ significantly [27, 28]. However, replacement with a larger group such as a phenyl ring system results in major differences in both experimentally and theoretically determined binding affinities between stereoisomers, the (*S*) isomer having a significantly more favourable intermolecular energy with the Q_B binding site than the corresponding (*R*) isomer. In Table III, we have shown the contributions to the overall intermolecular energy with the protein made by the different substituents at the chiral centre in both stereochemical conformations. The larger phenyl group has a repulsive energy of 26.1 kcal mol^{-1} with the protein when in the (*R*) conformation (**5**). Significantly, there is a noticable improvement in the van der Waals contribution from the phenyl moiety when in the (*S*) conformation (**4**). This is due to its interaction with the aromatic side chains of the Phe 211, Tyr 262, Phe 265 and Phe 274 residues (Table IV). Its planar nature suggests some favourable ring stacking interactions are present which appears to involve the Phe 274 residue (Fig. 5).

Superimposition of the (*S*) isomer of the phenyl analogue (**4**) onto the secondary plastoquinone within the Q_B binding domain revealed that the large hydrophobic group occupies the same region in the protein as the isoprenoid side chain (Fig. 6) as previously suggested [42]. When in the (*R*) conformation, the same group appears to interact unfavourably with the Tyr 262 and Phe 265 residues. In conclusion, we have shown that the intermolecular energies between optically active isomers of atrazine derivatives and the Q_B binding domain of PS II reflect the different binding affinities for

Table II. Comparison of the total intermolecular energies between the triazine derivatives and the Q_B binding site.

Compound (see Fig. 3)	Intermolecular energy [kcal mol^{-1}] van der Waals	Electrostatic	Total
1	−27.0	−4.5	−31.5
2	−28.7	−4.3	−33.0
3	−25.4	−4.9	−30.4
4	−37.9	−4.5	−42.4
5	+1.7	−4.7	−3.0

Calculated according to Eqn. (1) and (2).

Table III. Interaction energies between *R* substituent of the chiral carbon and the Q_B binding site.

Compound	Interaction energy of *R* group [kcal mol^{-1}] van der Waals	Electrostatic	Total
1	−3.7	+0.4	−3.3
2	−5.8	+0.4	−5.3
3	−0.9	−0.3	−1.2
4	−14.3	+0.3	−13.9
5	+26.1	−0.0	+26.1

Calculated according to Eqn. (3).

Table IV. Interaction energies between the *R* substituent and amino acid residues of the D 1 protein in the stereoselective region of the Q_B binding domain.

Compound (see Fig. 3)	Interaction energy with amino acid [kcal mol^{-1}] Phe 211	Tyr 262	Phe 265	Phe 274
1	−0.3	−0.8	−0.7	−1.2
2	−0.7	−1.3	−0.8	−1.9
3	−0.5	+1.4	−1.4	+0.6
4	−2.2	−3.4	−3.1	−4.0
5	−1.2	+21.0	+11.1	−2.4

Calculated according to Eqn. (3).

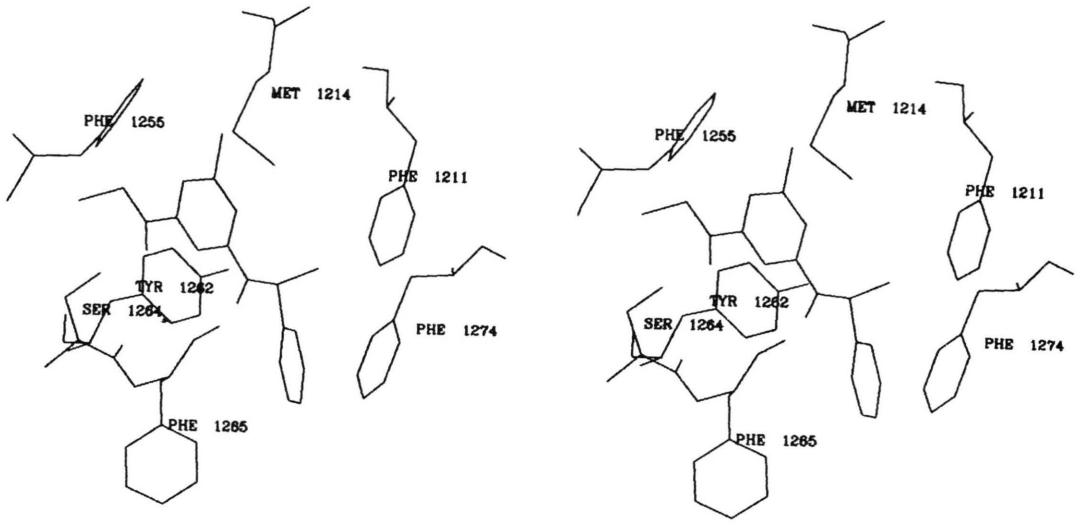


Fig. 5. Binding of (*S*)-α-methylbenzylatrazine **4** to the D 1 protein of *Pisum sativum*. The residue numbers of the D 1 protein are prefixed by the number 1.

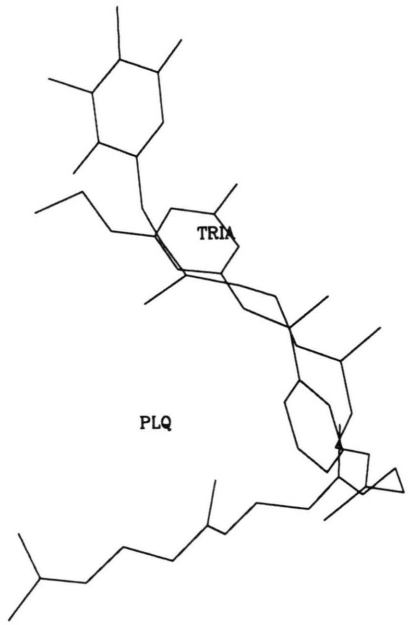


Fig. 6. Superimposed structures of the plastoquinone (PLQ) and the (*S*) isomer of the α-methylbenzyl derivative of atrazine (TRIA) in their respective binding orientations within the D 1 protein.

stereoisomeric pairs as determined experimentally. In addition, we have identified the amino acid residues responsible for stereoselectivity within the binding site. These results not only reinforce our proposal for the binding orientation of atrazine within the protein, but can also be viewed as further confirmation of the structural model of the Q_B binding site. However, the limitations of theoretical methods must be kept in perspective. Much progress has been made in the development of approaches to represent as accurately as possible the energy of complex molecules. Quantum mechanical calculations are not yet feasible for calculations involving biomolecules because of the computational expense for dealing with such large numbers of particles. The approximations of the potential energy function and mechanics employed in modelling determines the reliability of the results. The form of the potential energy reflects a balance of the requirements of computational efficiency and meaningful accuracy.

The minimized energies calculated for the inhibitor-receptor complexes have been used to estimate relative binding enthalpies. However, an entropy term has been neglected in such calculations. Direct calculation of entropy is a computationally intensive process and the extent of the errors introduced in omitting entropic considerations is difficult to estimate. When studying the relative binding

of two substrates to the binding site, entropic effects would be more important if dealing with one flexible and one rigid molecule. When comparing enantiomers, steric repulsion by residues in the binding site resulting as a consequence of stereoisomerism would negate entropic effects.

Acknowledgements

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