Structure Activity Correlation of Herbicides Affecting Plastoquinone Reduction by Photosystem II: Electron Density Distribution in Inhibitors and Plastoquinone Species

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Molecular orbital calculations of the net charge and the π charge distribution in several inhibitors and herbicides of the functionally related group of the diuron and dinoseb type are reported. They confirm the model that urea, aminotriazinone and triazine herbicides all have in common a positive π-charge at a particular atom considered to be essential for binding. Phenol type inhibitors have different charge distribution and a model for their essential features is presented. The calculations support the finding that two different subunits with different binding characteristics are involved in inhibitor and plastoquinone function on the acceptor side of photosystem II.

Force-field model building and MO calculations of the charge distribution of a plastoquinone analogue with a butenyl side chain, of two of its semiquinone forms and of the hydroquinone, are reported, as well as their conformation with the lowest energy content and their likely anionic forms.

Introduction

The mode of action of two chemically different classes of photosystem II inhibitors (referred to as of the DCMU/triazine type and of the phenol type) consists in interfering with the reduction of plastoquinone at the acceptor side of photosystem II. The binding of these inhibitory compounds to the thylakoid membrane as well as the mutual displacement from the membrane of members of either class of inhibitors in spite of the different chemical elements led to the concept of individual though overlapping binding sites on a common binding area [1, 2]. A similar concept was invoked to explain the cross resistance in herbicide resistant plants [3]. The identification of the peptide subunits of the herbicide binding area by photoaffinity labelling, as reviewed in [2–5], indicated that two subunits are involved: a 32 kD subunit binding azidoatrazine [6] and a 47 kD subunit binding an azidodinoseb [7]. Both peptides had been known before their herbicide binding was recognized; the 32 kD peptide was known as a photogene rapidly turning over peptide [8] and the 47 kD peptide as a chlorophyll carrying peptide. Both are part of the architecture of the reaction center complex of photosystem II, which consists of at least three, but possibly four, integral peptides of 51, 47 and 34 and 32 kD, carrying antenna and the reaction center chlorophyll, the primary donor Z and two acceptor plastoquinones, called Q_A and Q_B. To it further peripheral peptides are attached for oxygen evolution. The arrangement of the integral peptides in the membrane is deduced from the hydropathy

Abbreviations: Atrazine, 2-chloro-4-(ethylamino)-6-(isopropylamino)-*s*-triazine; CNDO, complete neglect of differential overlap; DCMU (diuron), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; dinoseb, 2,4-dinitro-6-*sec*-butylphenol; EHT, extended Hückel theory; HMO, highest occupied molecular orbital; IEHT, intermediate extended Hückel theory; INDO, intermediate neglect of differential overlap; MO, molecular orbital; PPP, Pariser-Parr-Pople; Q_A, primary quinone electron acceptor of photosystem II; Q_B, secondary quinone electron acceptor.

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analysis of their aminoacid sequence (some not yet published), which indicates that all the peptides span the membrane and wind through the membrane several times. This is true also for the 32 kD peptide [9], originally assumed to be only a shielding peptide [10]. The interaction of the peptide spans through the membrane of the same or of an adjacent subunit yields a strong, though not necessarily rigid, structure of helical clusters. This interaction is indicated by the displacement from the membrane of an inhibitor by any member of the two inhibitor groups, already mentioned, but also by some quinones (these proceedings). Furthermore the change of functional properties in photosystem II particles [11, 12], where the 32 kD peptide is dislodged from the chlorophyll complexes as well as the influence of membrane phosphorylation [13], indicate the cooperation of several peptides in plastoquinone reduction and herbicide binding.

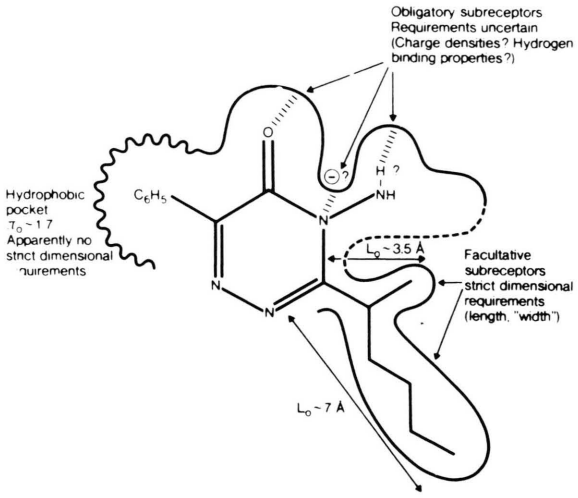


Fig. 1. QSAR-derived model for the binding site for a triazinone herbicide to its binding niche in the photosynthetic membrane.

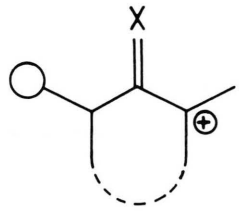


Fig. 2. Schematic representation of the essential atoms of a herbicide binding to the 32 kD peptide of photosystem II indicating the sp^2 hybrid with x = usually O, S or C = attached to a lipophilic group (O−) and the essential + charge.

Results and Discussion

The nature of the binding site(s) of the inhibitors and of plastoquinone on the peptides of photosystem II is naturally of great importance for the attempts to rationalize the design of new and better inhibitors and from there the development of new herbicides. The state of knowledge on the chemistry of the inhibitors, as it is related, important and essential for their high affinity binding to their receptor sites on the membrane, as prerequisite for an effctive inhibitor has been reviewed often. The relation of chemical structure to inhibitory potency of a herbicide indicated the essential atoms of a DCMU-type inhibitor: a sp^2 hybrid attached to a lipophilic moiety. A dependence of potency on electronic substitution parameters and lipophilicity was established. A fitting of an aminotriazinone herbicide into a niche of the acceptor site on the membrane is shown in Fig. 1 [14]. Furthermore, a particular charge sequence −++ on the essential atoms of the inhibitors was proposed by Draber (Fig. 2). Similar considerations, fittings and calculations have been reported [15–18]. Shipman [16, 18], in particular, concluded that the hydrophilic part of a herbicide binds electrostatically to the terminus of an α-helix at a highly charged amino-acid like arginine. The hydrophobic part of the inhibitors would extend into the hydrophobic part of the membrane. It is already tempting to fit this into the hydropathy prediction of its orientation in the membrane of the 32 kD peptide [9].

The DCMU/atrazine binding characteristics to the 32 kD and structure activity correlation show quite different pattern from that of the phenol herbicides binding to a 47 kD peptide. Electron and lipophilic substitution parameters were of little importance, but with certain steric parameters (those of Verloop) a close fit was obtained [1]. The different binding characteristics of the phenol-type, as against those of DCMU-type inhibitors, were supported recently by MO calculations [19]. This is documented again and extended to more compounds in the following examples.

Fig. 3 shows again an example of the distribution of net and π-charges of a nitrophenol compound. There are only very weak positive charges. From the

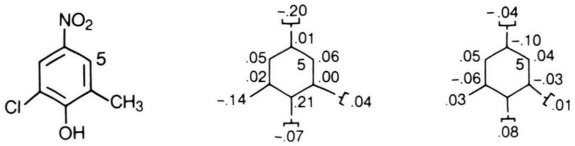


Fig. 3. The net and π-charge distribution of a phenolic type inhibitor obtained by CNDO. This type binds to a 47 kD peptide and does not carry the positive charge essential for those herbicides binding to the 32 kD peptide.

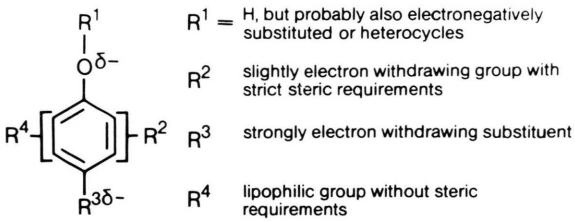
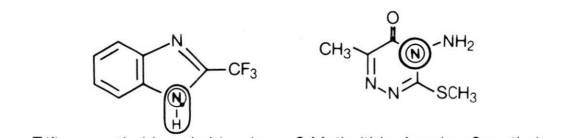


Fig. 4. An attempt to generalize the essential feature of a phenol-type herbicide.



Trifluoromethyl-benzimidazole — 3-Methylthio-4-amino-6-methyl-triazin-5-one

	Inhibitory activity	
pI_{50}	3.82	4.2
	Charge s by CNDO/2	
(net) planar	+.028	-.036
(π) planar	+.369	+.385

Fig. 5. Net and π-charge density of the encircled atoms in two herbicides obtained by CNDO. The positive π-charge is characteristic of a herbicide binding to the 32 kD peptide.

structure activity correlation [1] and the charge distribution pattern [6] we would like, with some caution, to propose the general model of Fig. 4 for this type of inhibitors.

The distribution of charges on essential elements in the phenol herbicides is different from the atoms essential in Fig. 2 for the DCMU/triazine family. As reported already [19], CNDO calculations did support Fig. 2 for an aminotriazinone herbicide. Fig. 5 compares the π-charge on the essential nitrogen atoms of an aminotriazinone with that of a derivative of another potent group of photosystem II inhibitors, trifluorobenzimidazoles (the pI_{50} value given in Fig. 5 is that of the basic compound that can be calculated, but does not carry the substituents needed to arrive at a very potent derivative). Clearly there is a positive π-charge on the encircled atoms in the planar state (Fig. 5). Fig. 6 shows the net and π-charge distribution for diuron. The positive π-charge (in a planar form) at the essential atom according to the model of Fig. 2 is shown for diuron and atrazine in Fig. 7. For the calculations the energy of all molecules had been minimized by a force-field based model builder prior to carrying out the CNDO calculations. With the three heterocyclic molecules in Figs. 5 and 6 we did not encounter any difficulties to calculate the charge density, which we consider significant. However, we had to force DCMU into a planar conformation, which was not obtained by the model builder, to be able to calculate the π-charge density. We believe that indeed the planar form is a prerequisite for a herbicide to bind to the membrane.

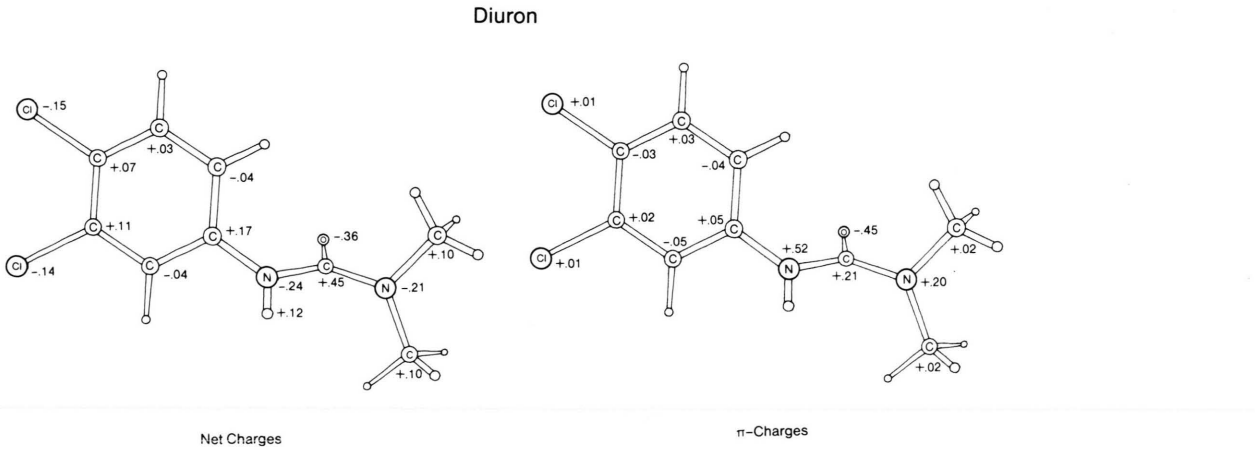


Fig. 6. Net and π-charge density distribution in diuron (DCMU).

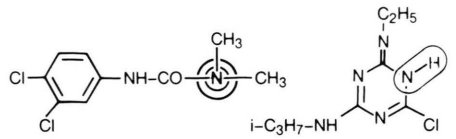
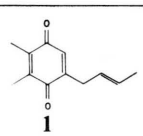
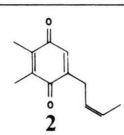
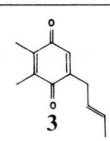
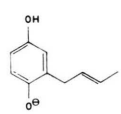
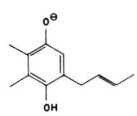
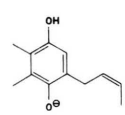
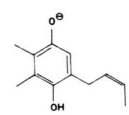
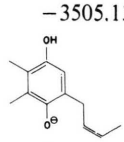
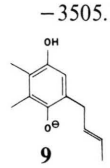
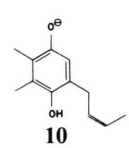
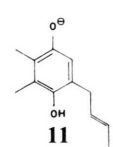
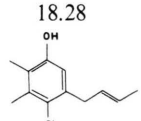
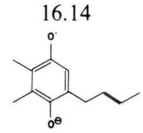


Fig. 7. Net and π-charge density of the encircled atoms in two herbicides obtained by CNDO. The positive π-charge is characteristic of a herbicide binding to the 32 kD peptide.

The atrazine molecule is written in an unusual way. Only that way it yields a positive charge on the ring-N-1. If it is written in the normal *s*-triazine form, however, the C-atoms in the ring carry a slightly positive π-charge, whereas the N's are corresponding negative. This may look somewhat forced, though, we believe it is not. A. Pullman [20] had carried out nearly 15 years ago a comparison of the then available MO calculation methods (EHT, IEHT, CNDO, PPP), into which she had included the unsubstituted uracil. The π-charges given by her correspond very well with ours, *i.e.* with a positive charge on the nitrogens. We have obtained similar results by HMO and later by CNDO/2 calculations of the following classes of molecules, which are known to include active photosystem II inhibitors: 1,2,4-triazol-5-ones, 1,3,4-oxadiazol-5-ones, 1,2-pyrazol-5-ones, 4-chloro-1,2-diazin-3-ones, 3-isoxazolo-(4,5-d)pyrimidin-6-ones, and 1,3,5-triazol-2,4-diones.

As discussed above, it has been suggested that the DCMU-herbicide binding site on the 32 kD is identical with the plastoquinone binding site. As we

Table I. Energies for the different conformational or charged forms of plastoquinone analogue with a 2-butenyl side chain, hydroquinone anions and semiquinone anion. Minimized strain energies were obtained by force-field model building and the total energy content by either CNDO/2 or INDO.

Quinone **1** without, **2** and **3** with intramolecular hydrogen bond	 **1**	 **2**	 **3**	
Minimized strain energy (kJ/mol)	26.99	31.50	35.33	
E_{tot} (eV) by CNDO/2	−3609.23	−3608.72		
Hydroquinone anion **4**–**7** without intramolecular hydrogen bond	 **4**	 **5**	 **6**	 **7**
Minimized strain energy (kJ/mol)	9.09	9.59	10.15	9.83
E_{tot} [eV] by CNDO/2 INDO	−3634.83 −3505.13	−3505.11		
Hydroquinone anion **8**–**11** with intramolecular hydrogen bond	 **8**	 **9**	 **10**	 **11**
Minimized strain energy (kJ/mol)	18.28	16.14	15.84	10.04
Semiquinone (**12**) and *Semiquinone anion* (**13**)	 **12**	 **13**		
E_{tot} [eV] by INDO	−3505.28	−3482.94		

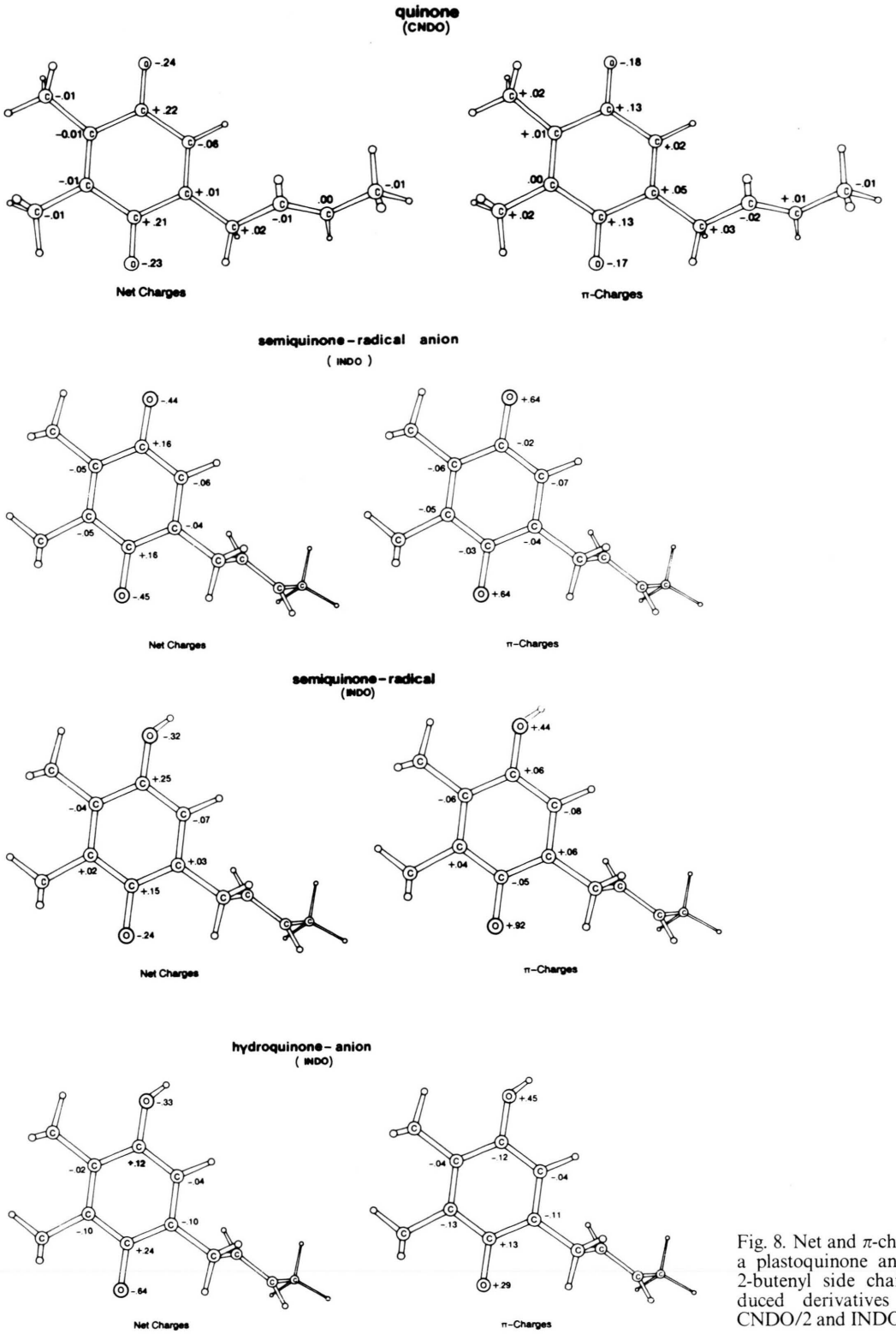


Fig. 8. Net and π-charge density of a plastoquinone analogue with a 2-butenyl side chain and its reduced derivatives obtained by CNDO/2 and INDO.

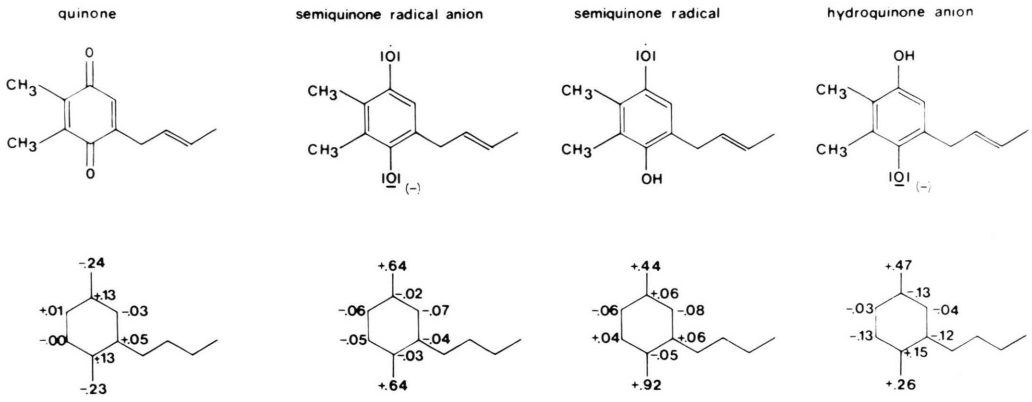


Fig. 9. Comparison of the π-charge density distribution in the redox states of a plastoquinone analogue, as obtained from the data in Fig. 8 and Table I, except for CNDO for the hydroquinone.

do not subscribe to this and rather assume an allosteric interference, we have calculated the electron densities for four species of three redox states of an analogue of plastoquinone with a short (butenyl) side chain (Figs. 8 and 9). There are different conformations of the side chain possible including a hydrogen bond to the oxygen group, as well as two forms of anions of the semiquinone and hydroquinone. The forms calculated in Fig. 9 have the lowest strain energy with 26.99 kJ/mol for the quinone analogue as well as for the semiquinone (see Table I), *i.e.* the intermolecular hydrogen bond is actually not favored. The energies given in kJ/mol (here and in Table I) are results from force-field calculations [21], which cannot be taken absolutely. However, they do give some indication of the relative stabilities of the respective conformations. MO calculations support this notion, as the values for the total energy content of the molecules indicated in Table I. The calculations were carried out by CNDO/2 or by INDO, which is more suitable for radicals [22]. There is also agreement between force-field model building and INDO calculations (see Table I), as to which hydroxyl is dissociated by the influence of the charge in the hydroquinone anion favoring the form shown in Fig. 9. The same is true for the semiquinone radical anion, as shown in Fig. 9. Again the preferred form has been determined from the results of the program and not by giving it into the input that means that no assumption has been made about the specific position of the single electron. The results show that the two oxygen atoms contribute equally to the electron density. Both contribute equally to the radical form in the semiquinone radical anion, whereas in the semiquinone the two oxygens are definitely different. The π-charge distribution in neither of the four species in Fig. 9 suggests strongly a similarity to the π-charge distribution of DCMU of Fig. 2, as considered essential for the herbicides. Thus we feel that MO calculations can be very helpful in understanding the mechanism of photosystem II inhibitor action as well as photosynthetic function.

Acknowledgement

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