Cyanoacrylate Inhibitors of Photosynthetic Electron Transport in Atrazine Susceptible and Atrazine Resistant *Brassica* Chloroplasts

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Comparison of the pI_{50} values for a series of cyanoacrylate derivatives in chloroplasts isolated from atrazine susceptible (wild type) and atrazine resistant (mutant) *Brassica napus* biotypes reveal that the degree and direction of discrimination can vary from being 200- fold more active against the wild type to 10-fold more active against the mutant. There appears to be a direct correlation between the level of inhibitory activity in thylakoids isolated from "susceptible" chloroplasts and the level of discrimination between "susceptible" and "resistant" chloroplasts – a correlation which can be improved by allowing for variations in molecular hydrophobicity. Studies with optically active ethoxyethyl-3-alkyl-2-cyano-3-α-methylbenzylamino acrylates suggest that there are specific receptor sites present in both "susceptible" and "resistant" chloroplasts for both the α-methylbenzyl chiral centre and the 3-alkyl moiety. There is a direct relationship between photosynthetic electron transport inhibitory activity and herbicidal activity of optical isomers.

Introduction

Many photosynthetic inhibitor herbicides are known to act by blocking photosynthetic electron transport (PET) close to photosystem II reaction centres (PS II RC's) in the thylakoid membranes of plant chloroplasts [1]. Competitive displacement studies have indicated that a variety of these PS II PET inhibitors, including the ureas, triazines and cyanoacrylates, interact with the same receptor region and this region has been associated with the D_1 peptide of the PS II RC in a number of plant species [2, 3]. Such inhibitors are believed to exert their effect by displacing the secondary plastoquinone electron acceptor, Q_B, from its binding niche near the reaction centre, thus interfering with the electron transport process.

The serine $_{264}$ residue in the D_1 peptide of wild type chloroplasts has been found to be altered to glycine in the analogous peptide isolated from chloroplasts of triazine resistant weeds [4]. Moreover, different PET inhibitors show different levels of discrimination between wild type and mutant thylakoids even though they competitively displace each other from the binding domain. Pfister and Arntzen [5] explained this differential sensitivity but competitive interaction in terms of a model where part of each inhibitor molecule interacted with a common binding domain and the remainder of the molecule interacted specifically elsewhere. Trebst and Draber [6] on the other hand suggested that for each class of PS II herbicide there were multiple binding sites to account for the differential sensitivity and an overlap of some of these sites between the different PS II herbicide types to account for their competitive interaction. The latter concept is consistent with a recent QSAR analysis of structure-activity relationships of amide and triazine type PS II herbicides [7].

Previous comparative studies of the effects of PET inhibitors on wild type and mutant plant and algal species have been concerned with compounds of widely different chemical structure. This study has been focussed on a closely related series of PET inhibitors of the cyanoacrylate class with the aim of exploring differences in detail between wild type and triazine resistant *Brassica* biotypes.

Results

Table I records pI_{50} data for a series of 3-alkyl, 3-aralkyl and 3-aryl derivatives of methoxyethyl-2-cyano-3-*p*-chlorobenzylamino acrylates as inhibitors of photosynthetic electron transport in both wild type and mutant *Brassica* thylakoids and in pea thylakoids [9] under coupled (basal) conditions. There is reasonable agreement between pI_{50} values in pea and wild type *Brassica* thylakoids but values determined with mutant *Brassica* thylakoids are generally lower except for the 3-phenyl and 3-benzyl derivatives.

Table II summarizes pI_{50} data for a series of optically active ethoxyethyl-3-alkyl-2-cyano-3-α-methyl-

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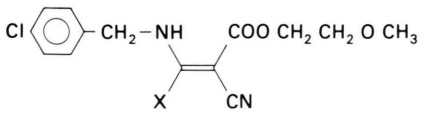
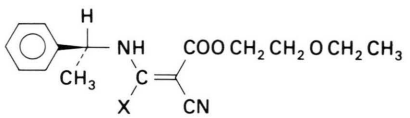
Table I. The effect on PET in wild type and mutant *Brassica* thylakoids of a series of 3-substituted cyanoacrylates of general formula:



Compound[a] X =	pI_{50} (*Brassica*)[b] pI_{50}^{BW} (wild type)	pI_{50}^{BM} (mutant)	ΔpI_{50}^{BW-BM}	pI_{50}^{P} (peas)[c]
H	4.80	3.75	1.05	
Me	6.80	5.10	1.70	6.25
Et	7.30	5.50	1.80	7.20
Pr	6.75	5.30	1.45	6.50
Pr_i	8.40	6.75	1.65	8.20
Bu	5.50	4.65	0.85	5.00
Bu_s	7.90	6.55	1.35	7.95
Bu_i	5.80	4.90	0.90	5.35
Bu_t	4.75	4.15	0.60	4.30
2Pentyl	7.20	5.75	1.45	6.95
3Pentyl	8.10	6.60	1.50	
2Hexyl	6.55	5.65	0.90	
Phenyl	4.70	5.20	−0.50	4.10
Benzyl	4.10	5.20	−1.10	3.65

[a] Synthesis and physical properties of new compounds will be reported elsewhere. All structures were confirmed by PMR spectra and gave satisfactory microanalyses.
[b] Compounds were assayed as inhibitors of the Hill reaction using thylakoids isolated from the leaves of 21 day old *Brassica napus* seedlings with results expressed as pI_{50} values in accord with the experimental procedure described earlier [8]. Atrazine susceptible (wild type) and atrazine resistant (mutant) *B. napus* seeds were kindly supplied by Dr. John Kirk of the CSIRO Division of Plant Industry, Canberra.
[c] Data from reference [9].

Table II. The effect on PET in wild type and mutant *Brassica* thylakoids of the *R* and *S* isomers of general formula:



Compound[a] Isomer	×	pI_{50} (*Brassica*)[b] pI_{50}^{BW} (wild type)	pI_{50}^{BM} (mutant)	ΔpI_{50}^{BW-BM}	ΔpI_{50}^{B} ($S-R$ isomer) wild type	mutant	pI_{50}^{P} (peas)	ΔpI_{50}^{P} ($S-R$ isomer)
S	Me	6.90	5.15	1.75	2.30	1.45	6.2	2.0
R	Me	4.60	3.70	0.90			4.2	
S	Et	7.75	5.85	1.90	2.35	1.80	7.1	2.2
R	Et	5.40	4.05	1.35			4.9	
S	Pr	6.45	4.85	1.60	1.90	0.70	6.15	1.8
R	Pr	4.55	4.15	0.40			4.35	
S	Bu	5.05	4.50	0.55	1.05	0.10	4.85	1.05
R	Bu	4.00	4.40	−0.4			3.80	

[a] Compounds described in reference [10].
[b] See footnote [b], Table I.
[c] See reference [10].

benzylamino acrylates as inhibitors of photosynthetic electron transport in both wild type and mutant *Brassica* thylakoids and in pea thylakoids [10] under coupled conditions. The *S* isomers are more active than the *R* isomers as PET inhibitors in all three thylakoid systems. Moreover, all the *S* isomers and all except one of the *R* isomers are more active against the wild type than the mutant *Brassica* thylakoids.

Discussion

In view of the highly conserved nature of the herbicide binding site in plant chloroplasts, it is not surprising that cyanoacrylates inhibit photosynthetic electron transport at similar levels in thylakoids isolated from *Pisum sativum* (pI_{50}^{P}) and *Brassica napus* (pI_{50}^{BW}) species (see Tables I and II). The slightly higher pI_{50} values usually observed with the brassica species could indicate that thylakoids isolated from that species are less highly coupled than those isolated from peas since, under otherwise comparable conditions, pI_{50} values tend to be greater in uncoupled than in coupled systems [11].

In general, cyanoacrylates behave like triazines in being less active PET inhibitors in thylakoids isolated from atrazine resistant (mutant) than from atrazine susceptible (wild type) *Brassica* species. However, as the data in Tables I and II indicate, the degree and direction of discrimination varies from being 200-fold more active against the wild type ($\Delta pI_{50}^{BW-BM} = +2.3$) to 10-fold more active against the mutant ($\Delta pI_{50}^{BW-BM} = -1.1$).

There appears to be a general relationship between the level of PET inhibitory activity with wild type thylakoids (pI_{50}^{BW}) and the level of discrimination between the wild type and mutant thylakoids (ΔpI_{50}^{BW-BM}) – the more active the inhibitor with the wild type species the higher the discrimination in favour of the wild type. This is shown in Eqn. (1) which represents the relationship between pI_{50}^{BW} and ΔpI_{50}^{BW-BM} for all data in Tables I and II.

$$pI_{50} = 1.38\ (\pm 0.23)\ \Delta pI_{50} + 4.70\ (\pm 0.29) \quad (1)$$
Correlation coefficient, $r = 0.80$.

The general trend evident in Eqn. (1) can be broadly accounted for in terms of the multiple site binding concept [6]. If there are a number of possible interacting sites within the herbicide binding domain and different molecules can interact with different combinations of them then the same molecule could also interact with different combinations of sites, *i.e.* it could interact with the binding domain in different ways. These interactions could range from being highly specific and involving many sites on the receptor peptide down to non-specific where no peptide site interactions were involved. In all cases, however, the binding affinity of a molecule would be influenced by its tendency to escape from the aqueous medium to the non-aqueous membrane environment, *i.e.*, by its hydrophobicity. For molecules of comparable hydrophobicity it is likely that changing environments, *e.g.*, from the wild type to the mutant, will have a greater effect on binding affinity and hence on ΔpI_{50} values the more highly specific the interaction, *i.e.*, the higher the activity. This is in accord with the trend of the relationship in Eqn. (1). Moreover, when allowance is made for hydrophobicity, the correlation coefficient (r) of the pI_{50} *vs* ΔpI_{50} relationship is further improved. This is evident from a comparison of Eqn. (2) based on pI_{50} values for the alkyl series excluding the phenyl and benzyl derivatives in Table I with Eqn. (3) based on pI_{50} values for the same series corrected for hydrophobicity by subtracting the Hansch π value for the alkyl moiety [12].

$$pI_{50} = 2.5\ (\pm 0.6)\ \Delta pI_{50} + 3.5\ (\pm .8) \qquad r = 0.78 \quad (2)$$
$$pI_{50}(\text{corr}) = 3.1\ (\pm 0.4)\ \Delta pI_{50} + 0.8\ (\pm .5) \quad r = 0.93 \quad (3)$$

A similar improvement in correlation coefficient is evident between Eqn. (4) based on values for the *R* and *S* optical isomers of ethoxyethyl-3-alkyl-3-α-methylbenzylamino-2-cyanoacrylate derivatives in Table II and Eqn. (5) based on the π corrected pI_{50} values for the same series:

$$pI_{50} = 1.5\ (\pm 0.3)\ \Delta pI_{50} + 4.1\ (\pm .4) \qquad r = 0.90 \quad (4)$$
$$pI_{50}(\text{corr}) = 2.0\ (\pm 0.3)\ \Delta pI_{50} + 2.2\ (\pm .3) \quad r = 0.95 \quad (5)$$

It would appear from the relative sensitivity of electron transport in chloroplasts isolated from *Brassica* biotypes that, in general, cyanoacrylates interact more specifically with wild type than mutant thylakoids. However, the converse is true for the 3-phenyl and 3-benzyl derivatives recorded in Table I which are more active PET inhibitors with mutant *Brassica* thylakoids than with wild type *Brassica* or pea thylakoids. This suggests that there is a receptor site in the mutant thylakoid which is not present in the wild type and which is capable of interacting specifically with an aryl or ara alkyl group in the 3 position of the cyanoacrylate molecule.

The differential activity between *S* and *R* optical isomers of ethoxyethyl-3-alkyl-2-cyano-3-α-methyl-

benzylamino acrylates with both wild type and mutant *Brassica* chloroplasts and with pea chloroplasts (Table II) implies that a specific receptor site capable of distinguishing between the *S* and *R* isomers in favour of the *S* isomer is present in both normal and mutant biotypes. Moreover, as Fig. 1 shows for both the *S* and *R* isomers with wild type *Brassica* thylakoids and for the *S* isomer with mutant *Brassica* thylakoids, there is a parabolic relationship between pI_{50} and the chain length of the 3-alkyl substituent with optimum activity associated with the ethyl derivative in each case. Similar parabolic relationships for the 3-straight-chain alkyl series have also been observed for other cyanoacrylate derivatives with pea thylakoids [11]. A specific receptor site for the 3-alkyl moiety is therefore likely to be present in both normal and mutant thylakoids. This leads to the conclusion that the ability of cyanoacrylates to differentiate between the wild type and mutant *Brassica* thylakoids must be due to regions of the molecule other than the α-methylbenzyl chiral centre or the 3 alkyl moiety.

The linear rather than parabolic relationship between pI_{50} and alkyl chain length observed for the *R* isomer with the mutant thylakoids (Fig. 1) suggests that the disadvantageous *R* conformation coupled with the weaker binding associated with the mutant thylakoids result in a largely non-specific interaction primarily influenced by hydrophobicity factors.

Optical isomers also show differential herbicidal activity. Thus the *S* isomer of ethoxyethyl-2-cyano-3-ethyl-3-α-methylbenzylamino acrylate kills atrazine susceptible *Brassica napus* seedlings in the glasshouse when applied post-emergence at 0.25 kg/ha, whereas the *R* isomer shows only minor contact phytotoxicity at 8 kg/ha. Such differentiation is consistent with that observed between the same isomers as PET inhibitors in isolated chloroplast systems (see Table II). Neither the *S* or *R* isomers, however, have any effect at 8 kg/ha on the atrazine resistant *Brassica* biotypes.

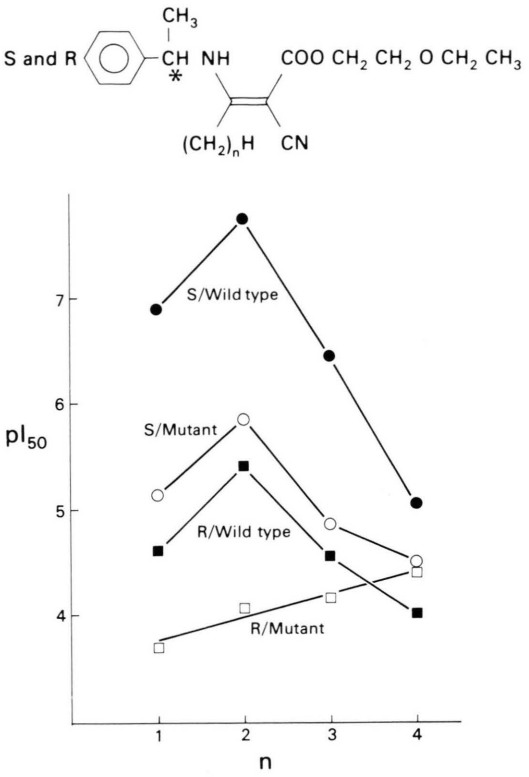


Fig. 1. pI_{50} values for *S* and *R* isomers of ethoxyethyl-3-alkyl-2-cyano-3-α-methylbenzylamino acrylates *vs* alkyl chain length (*n*) for both wild type and mutant *Brassica* thylakoids.

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