Hydrogen Bonding of Cyanoacrylates with the D 1 Peptide

John N. Phillips and Wendy K. Banham

CSIRO Division of Plant Industry, Canberra, Australia

Z. Naturforsch. **48c,** 132–135 (1993); received November 16, 1992

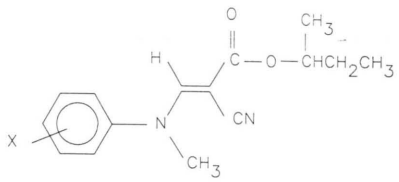
Brassica napus, Cyanoacrylates, D 1 Peptide, H-Bonding, Photodegradation

Representatives of two structural types of cyanoacrylate PS II inhibitors have been studied in respect to their pI_{50} values and qualitative rate of binding with wild type and S 264 G mutant thylakoids isolated from *Brassica napus*. Both are potent inhibitors of photosynthetic electron transport and both show a large discrimination between wild type and mutant thylakoids under equilibrium conditions. However one, an N-methylanilino cyanoacrylate, has an initial rapid reaction with both wild type and mutant thylakoids, but continues to react slowly with the wild type species until equilibrium is reached, while the other, a benzylamino cyanoacrylate, equilibrates rapidly with both species as does the classical PS II inhibitor, atrazine. These differences in kinetic behaviour have been interpreted in terms of different H-bond interactions with the serine-264 hydroxyl group. It is suggested that the slow binding reaction is due to the N-methylanilino compound interacting as an H-bond acceptor with the serine-264 hydroxyl hydrogen thus disrupting an intramolecular ser-264–his-252 H-bond within the D 1 peptide. Rapid equilibration on the other hand, has been attributed to the benzylamino derivative acting as an H-bond donor to the serine-264 hydroxyl oxygen and strengthening the 264–252 H-bond by conjugation. It is proposed that atrazine and other classical PS II inhibitors act in this way and that this may explain their ability to inhibit trypsin degradation of the D 1 peptide, if the 264–252 intramolecular H-bond plays an important role in stabilizing the peptide conformation. It is also speculated that photodegradation may be related to the ability of Q_B^- to act as an H-bond acceptor and disrupt the 264–252 H-bond.

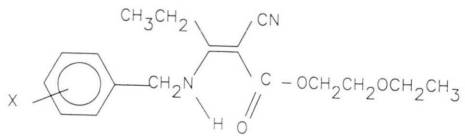
Introduction

3-N-Methylanilino (**A**) and 3-benzylamino (**B**) cyanoacrylates are active herbicides and potent inhibitors of photosynthetic electron transport at the PS II level. Although superficially similar from the structural point of view their requirements for activity are quite distinct. Thus inhibitory activity is associated with a *trans* configuration of the amino and ester functions in **A** but a *cis* configuration in **B;** a tertiary amino nitrogen in **A** but a secondary amino nitrogen in **B;** and a monosubstituted 3-methene carbon in **A** but a disubstituted 3-methene carbon in **B.** Furthermore, replacement of an ethyl by a 2-ethoxyethyl ester can reduce activity in **A** but promote it in **B,** while the order of activity of substituents in different positions of the aryl ring is 3 > 2 >>> 4 in **A** but 4 > 3 > 2 in **B** [1–5].

Such differences in the structural requirements for activity for these two classes of cyanoacrylate inhibitors imply differences in their molecular binding sites within the photosystem II reaction center (PS II RC). However, the observation that they each show a large discrimination between wild type and S 264 G mutant thylakoids isolated from *Brassica napus* [6, 7] suggests that in the wild type both classes interact with the serine-264 hydroxyl group. Differences in the nature of this in-



A



B

A: Secbutyl-2-cyano-3-N-methyl(subst)phenylamino acrylate (N-methylanilino cyanoacrylate type).
B: Ethoxyethyl-2-cyano-3-ethyl-3-(subst)benzylamino acrylate (benzylamino cyanoacrylate type).

Reprint requests to Dr. J. N. Phillips.

Verlag der Zeitschrift für Naturforschung,
D-W-7400 Tübingen
0939–5075/93/0300–0132 $ 01.30/0

teraction are reflected by **A** equilibrating slowly with the wild type and rapidly with the mutant and **B** equilibrating rapidly with both forms [7].

The purpose of this contribution is to interpret similarities and differences in the behaviour of these two classes of cyanoacrylates with wild type and mutant thylakoids in terms of their possible hydrogen (H) bonding interaction with the D1 peptide in the region of the PS II RC. It is also concerned with relating such H-bonding to the mode of binding of other classical type inhibitors such as atrazine and with speculating on the significance of such H-bonding to the conformational stability of the D1 peptide.

Discussion

Table I sets out equilibrium pI_{50} values and qualitative rates of binding for the interaction of a typical 3-N-methylanilino- (**1, A,** X = H) and 3-benzylamino- (**11, B,** X = 4C1) cyanoacrylate with thylakoids isolated from wild type and S264G mutants of *Brassica napus*. The classical PS II inhibitor, atrazine (**111,** 2,6-diethylamino-4-chloro-1,3,5-triazine) has been included for purposes of comparison. In the case of compounds **11** and **111** equilibrium is established rapidly *i.e.* within a mixing time of seconds in both the wild type and mutant thylakoids and the same is true for compound **1** with the mutant thylakoids. However in the case of compound **1** with the wild type species there is an initial fast reaction followed by a much slower binding phase.

The three compounds **1, 11** and **111** are comparably active against the wild type thylakoids and all show a large discrimination ($\Delta pI_{50} > 2.0$ units) between the wild type and the S264G mutant in favour of the former. This suggests that all three interact with the serine-264 hydroxyl group in the wild type species. Such an interaction is most likely to involve H-bonding with the serine hydroxyl acting either as an H-bond donor *via* the hydroxyl hydrogen or as an H-bond acceptor *via* the hydroxyl oxygen. Compound **1,** because it lacks any readily dissociable hydrogen atom, can only act as an H-bond acceptor *via* the ester carbonyl (C=O) function. However compounds **11** and **111** can act as either H-bond donors *via* the amino (NH) group in **11** and **111** or as H-bond acceptors *via* the ester carbonyl (C=O) in **11** or the azene (−N=) group in **111.** Most known PS II inhibitors are capable of acting as H-bond donors or acceptors *i.e.* like compounds **11** and **111.** It is the N-methylanilino cyanoacrylate class, represented here by compound **1,** that tends to be somewhat different.

Large differences in the rate of binding of the N-methylanilino cyanoacrylate **1** with the wild type and mutant species implicate the serine-264 hydroxyl group in the kinetics of the binding process. It is suggested that an intramolecular H-bond between the serine-264 hydroxyl, acting as an H-bond donor, and some nearby amino acid residue, acting as an H-bond acceptor, must first be broken or at least weakened before interaction can take place. Modelling considerations have suggested that histidine-252 forms such an intramolecular

Table I. Equilibrium pI_{50} values and qualitative rates of equilibrium for the interaction of a 3-N-methylanilino cyanoacrylate (**1**) (**A,** X = H), a 3-benzylamino cyanoacrylate (**11**) (**B,** X = 4C1) and atrazine (**111**) with wild type and S264G mutant thylakoids isolated from *B. napus*.

	Wild type[a]		Mutant[b]		
Compound[e]	pI_{50}[c]	Equil. rate[d]	pI_{50}[c]	Equil. rate[d]	ΔpI_{50}
1	6.9	slow	4.7	fast	2.2
11	7.2[f]	fast	4.9[f]	fast	2.3
111	6.5[f]	fast	3.6[f]	fast	2.9

[a] Wild type *B. napus* thylakoids.
[b] S264G mutant *B napus* thylakoids.
[c] pI_{50} values at equilibrium.
[d] Equilibration >15 min = slow; equilibration <30 sec = fast.
[e] Compound **1** = structure **A;** X = H; compound **11** = structure **B;** X = 4C1; compound **111** = atrazine.
[f] Reference [7].

H-bond with serine-264 in the wild type D 1 peptide [8].

Although **1, 11** and **111** all appear to interact with the serine-264 hydroxyl group in the wild type species, **1,** which can only accept H-bonds, equilibrates slowly, while **11** and **111,** which can either accept or donate H-bonds, equilibrate rapidly. Such differences in equilibration rates may reflect differences in their H-bonding interactions and since **1** must act as an H-bond acceptor then **11** and **111** may act as H-bond donors to the oxygen of the serine-264 hydroxyl group. This is in accord with X-ray crystallographic studies that have shown terbutryne, a triazine analogue of atrazine **111,** to bind in this way to the equivalent serine-223 in the reaction center of *Rhodopseudomonas viridis* [9]. It is also in agreement with the speculation, based on modelling considerations, that atrazine acts as an H-bond donor to the serine-264 hydroxyl in the D 1 peptide of *Chlamydomonas reinhardtii* [8]. However it is not in agreement with a further modelling speculation that a 3-benzylamino cyanoacrylate interacts with the serine-264 hydroxyl *via* the ester carbonyl function *i.e.* as an H-bond acceptor [8].

The slow equilibration associated with **1** may be explained by the need to disrupt an existing intramolecular H-bond before the final interaction can take place whilst the fast binding associated with **11** and **111** is consistent with little or no disruption of existing H-bonds.

Fig. 1 shows the proposed H-bonding scheme involving the serine-264 hydroxyl, histidine-252 and inhibitors **1, 11** and **111.** As the figure shows, compound **1,** acting as an H-bond acceptor and competing with the histidine, will tend to disrupt and weaken the 264–252 H-bond relative to the native peptide while compounds **11** and **111,** acting as H-bond donors, will tend to strengthen it by conjugating an additional H-bond to the system. If, as has been suggested [8], the 264–252 H-bond acts as a stabilizing influence on the conformation of the D 1 peptide then strengthening or weakening that bond may influence the susceptibility of the peptide to degradation. It is likely from the scheme outlined in Fig. 1 that H-bond donor type inhibitors such as **11** and **111** would stabilize the

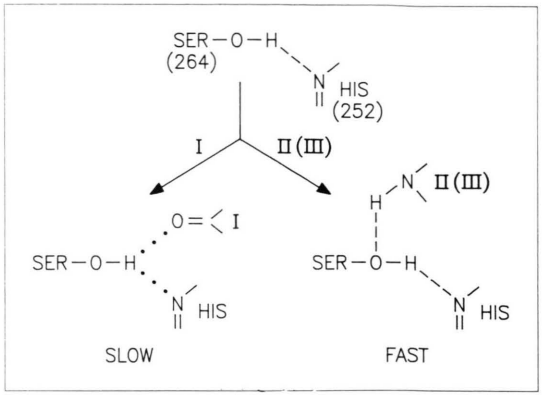


Fig. 1. Proposed H-bonding interactions between the PS II inhibitors **1** (**A,** X = H), **11** (**B,** X = 4C 1) and **111** (atrazine) and the serine-264 and histidine-252 residues of the D 1 peptide.

peptide and this is consistent with findings that atrazine and other classical type PS II inhibitors act as inhibitors of trypsin degradation [10]. On the other hand it could be predicted that H-bond acceptor type inhibitors, like **1** would tend to weaken the conformational stability of the D 1 peptide and hence facilitate breakdown, but this remains to be tested.

It is of interest to note that oxidized plastoquinone (Q_B) and its semiquinone (Q_B^-) might behave like the N-methylanilino cyanoacrylate (**1**) since they also can only act as H-bond acceptors. In particular (Q_B^-), because of its negative charge, would be a strong H-bond acceptor – possibly strong enough, if suitably oriented, to disrupt the 264–252 H-bond and weaken the conformational stability of the D 1 peptide. Such disruption by Q_B^- may explain the susceptibility of the D 1 peptide to photodegradation.

The observation that there can be discrimination between wild type and mutant species in relation to kinetic aspects of the binding process has added a new experimental parameter for exploring PS II inhibitor binding at the molecular level. It has also highlighted the importance of determining equilibrium pI_{50} values if accurate levels of resistance or supersensitivity are to be assessed.

[1] J. L. Huppatz, J. N. Phillips, and B. M. Rattigan, Agric. Biol. Chem. **45** (12), 2769–2773 (1981).
[2] J. N. Phillips and J. L. Huppatz, Z. Naturforsch. **39c,** 335–337 (1984).
[3] J. L. Huppatz and J. N. Phillips, Z. Naturforsch. **39c,** 617–622 (1984).
[4] J. L. Huppatz, H. G. McFadden, and L. F. McCaffery, Z. Naturforsch. **45c,** 336–342 (1990).
[5] W. K. Banham, J. L. Huppatz, and J. N. Phillips, Z. Naturforsch. **48c,** 136–139 (1993).
[6] J. N. Phillips and J. L. Huppatz, Z. Naturforsch. **42c,** 670–673 (1987).
[7] J. Phillips, Curr. Res. Photosynth., Proc. Int. Conf. Photosynth., 8th **1,** 587–590 (1989).
[8] K. J. Tietjen, J. F. Kluth, R. Andree, M. Haug, M. Lindig, K. H. Muller, H. J. Wroblowsky, and A. Trebst, Pestic. Sci. **31,** 65–72 (1991).
[9] J. Deisenhofer and H. Michel, EMBO J. **8,** 2149–2170 (1989).
[10] A. Trebst, B. Depka, B. Kraft, and U. Johanningmeier, Photosynth. Res. **18,** 163–177 (1988).