Stereospecific Inhibitor Probes of the PS II Herbicide Binding Site

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The influence of steric factors on the activity of 2-cyanoacrylic esters as inhibitors of the Hill reaction was examined. The spatial arrangement of the different groups in the inhibitor molecule was found to be an important factor in determining potency. The positioning of the phenyl ring in aralkylamino derivatives and the steric properties of the β-substituent are particularly significant in the interaction of molecules with the hydrophobic domain of the receptor site. The difference in activity observed with optically active α-methylbenzylamino derivatives confirmed the importance of the orientation of the phenyl ring and indicated an interaction with a specific hydrophobic region.

Introduction

2-Cyanoacrylates of general formula 1 are potent inhibitors of photosynthetic electron transport [1–6] and appear to act at a receptor site common to the amide-type of photosystem II (PS II) inhibitors. The Hill reaction in isolated pea chloroplast fragments has been used to establish the structural requirements for activity in this group of compounds. The potency of 2-cyanoacrylates in blocking photosynthetic electron flow was found to be extremely sensitive to minor structural variation so that these compounds have proved to be particularly suited to probing the topography of the herbicide binding site on the Qb protein in the PS II complex. The spatial arrangement of the different groups in the inhibitor molecule is crucial in determining activity suggesting a very precise orientation of the more potent inhibitors on the binding site. With some insight into the secondary and tertiary structure of the herbicide binding protein Qb beginning to emerge [7] and the advent of modern computer modelling techniques, it should be possible to use the knowledge of the stereochemical requirements for binding of the cyanoacrylate molecules now available to provide a three-dimensional picture of the binding domain of these, and perhaps other, inhibitors of electron transport.

A knowledge of the topography of the binding niche should enable the design of new, more effective inhibitors. In particular, the steric requirements of the site might be expected to play an important role in the high affinity binding of particular inhibitors. This aspect of inhibitor binding has proved difficult to define in previous studies of amide-type inhibitors. A recent survey of the quantitative structure-activity relationships (QSAR) of PS II inhibitors [8] concluded that the molecular requirements for inhibitors are an active polarizable sp2 nitrogen atom attached to a large lipophilic moiety, with only minor contributions from steric effects. Steric factors were found to be important in a later study [9], but the QSAR could not define these requirements adequately. In studies with cyanoacrylate inhibitors, it has become obvious that the steric properties of particular molecules play a key role in binding.

This paper will identify parts of the molecule where spatial orientation is important for high activity and examine the significance of these observations in deducing the topography of the binding domain.

Materials and Methods

The synthesis and physical properties of new compounds discussed herein will be reported elsewhere. All structures were confirmed by PMR spectra and gave satisfactory microanalyses.

All compounds synthesized included an ether oxygen function (R₂ = CH₂CH₂OCH₂CH₃ or CH₃CH₂OCH₃) since this structural features was found to enhance the binding of 2-cyanoacrylates to the receptor site [2].

Compounds were assayed for inhibition of the Hill reaction using chloroplast fragments isolated from the leaves of 21 day-old plants of Pisum sativum (c.v. Victory Freezer), the electron acceptor being the indicator dye 2,3',6-trichlorophenolindophenol. The
Experimental procedure was as described elsewhere [10], with the chlorophyll concentration routinely set at about 8 µg/ml. The activity was expressed in terms of \(p_{50}\), i.e., \(-\log_{10}I_{50}\), where \(I_{50}\) was the molar concentration required to decrease the rate of dye reduction under illumination of saturating intensity to 50% that obtained in the absence of the compound. The \(p_{50}\) values given are for reactions performed under coupled conditions and are the mean of at least three separate determinations, with a variation between experiments of less than ± 0.2 for each compound.

Results and Discussion

No discussion of the steric requirements of cyanoacrylate binding would be complete without reference to the stereochemistry of the molecules about the double bond. The steric arrangement of groups appears to be an important determinant of high activity, a cis configuration of the amino and ester functions appearing to most closely conform to the requirements of the receptor site [4]. Certainly, compounds with a β-alkyl substituent are unequivocally cis [4], while compounds unsubstituted in the β-position can exist as either geometric isomer. In the latter compounds, the low energy barrier to rotation presumably enables them to adopt the preferred cis configuration on the receptor site.

The lipophilic moiety, \(R_1\)

Earlier studies [1, 2] suggested that long-chain alkylamino-2-cyanoacrylates (1, \(R_1 = C_nH_{2n+1}\)) behaved as though the alkyl group was interacting with a large, unconstrained lipophilic area in the thylakoid membrane. The variation in \(p_{50}\) with carbon chain length of the alkyl group suggested that inhibitors of this type partition into the membrane as they would into octanol. This observation was consistent with results obtained with other amide-type PS II inhibitors [8]. However, studies with aryl and aralkyl-amino derivatives presented a somewhat different picture [5], with the lipophilic interactions being modified by steric effects. The phenylamino derivatives (2, \(n = 0\)) are weak Hill inhibitors, but inclusion of a methylene group between the phenyl and amino functions (2, \(n = 1\)) produces a large increase in activity (Fig. 1). Further lengthening of the carbon chain produces a step-wise increase in potency as the chain length increased. Fig. 1 illustrates this effect for three series of compounds, in which the carbon chain length is extended from 0 to 4. This step-wise effect, common to each series, is reminiscent of crystal packing phenomena, and may be explained in terms of a preferred orientation of the aryl group for interaction with a specific region within the hydrophobic matrix. The greater binding affinity observed when the phenyl ring is in the “correct” orientation (2, \(n = 1\) and 3) may arise from interaction with the lipid groups associated with the membrane-spanning helices of the 32 kDa herbicide binding protein [5].

Substitution in the phenyl ring can profoundly affect Hill inhibition. In the benzyl series (2, \(n = 1\))

Fig. 1. Schematic representation of the \(p_{50}\) values as a function of the number \((n)\) of carbon atoms between the phenyl and amino functions in general structure 2. Each series has a different β-substituent: × \(R_2 = CH_3\); ■ \(R_2 = C_2H_5\); ▲ \(R_2 = C_3H_7\).
particularly, the presence of a 4-chloro substituent raises activity by 100-fold, from $p_{I/50}$ of 6.20 to 8.20 [5]. This compound is one of the most active inhibitors of photosynthetic electron transport yet reported, being greater than an order of magnitude more active than DCMU in the assay system used.

The $\beta$-substituent, $R_2$

In the alkyl-, aryl- and aralkyl-amino series studied [4, 5], activity was increased by alkyl substitution at the $\beta$-carbon, reaching a maximum when a $\beta$-ethyl substituent was present in the molecule and declining as the chain length of the group was further increased. This effect is illustrated schematically in Fig. 2 for benzylamino-, 4-chlorobenzylamino- and $n$-octylamino-2-cyanoacrylates ($I$, $R_1 =$ benzyl, 4-chlorobenzyl, $n$-octyl, respectively, $R_3 =$ ethoxyethyl). Fig. 2 emphasizes the parabolic nature of the $\beta$-alkyl effect, which is qualitatively similar in all series containing straight-chain $\beta$-alkyl substituents so far studied. However, Fig. 2 also shows that, quantitatively, the effect can be quite different. The greater than 100-fold increase in the transition from compounds unsubstituted in the $\beta$-alkyl position to the corresponding $\beta$-ethyl derivatives in the benzyl- and 4-chlorobenzyl-amino series contrasts strongly with the 6-fold increase in $p_{I/50}$ observed for the same change in the octylamino series. The same large activity enhancement (of about 100-fold) has been observed in all aryl- and aralkyl-amino series so far examined [5], irrespective of the activity of the $\beta$-unsubstituted molecule. It would appear, then, that the steric constraints of the space surrounding the $\beta$-position of the cyanoacrylate molecule must play a key role in the alignment of the molecule on the site. The much larger $\beta$-alkyl effect in series containing an aryl ring indicates that a well-fit $\beta$-alkyl group is particularly important in positioning the phenyl ring to maximize interaction with the specific binding region within the hydrophobic domain referred to above.

Further investigation of the $\beta$-alkyl effect has shown that the parabolic variation of activity with alkyl chain length, indicating a specific size constraint in this region of the binding site, was an oversimplification. The $p_{I/50}$ data for a series of straight- and branched-chain alkyl substituents, a $\beta$-phenyl and $\beta$-benzyl substituent are shown in Fig. 3.* The

* In this series, lipophilic interaction was maximized by inclusion of a 4-chlorobenzyl residue. However, to compensate a methoxyethyl ester residue was used. This has the effect of reducing activity by 10-fold compared with the corresponding ethoxyethyl esters, thus ensuring that the activity of all compounds remained within the activity range (about 5 $p_{I/50}$ units) imposed by the assay system [5].
most active compounds is the $\beta$-iso-propyl derivative, which has a $pK_{50}$ value 1.7 units higher than the $n$-propyl isomer. The difference between the isomeric $\beta$-butyl compounds is also pronounced, the sec-butyl isomer being an exceptionally potent inhibitor, whereas the isomeric iso-, tert- and $n$-butyl derivatives are only weakly active. The sec-butyl function, therefore, conforms well to the steric requirements of this region of the binding site. Compounds carrying the other butyl groups in the $\beta$-position, on the other hand, are not well-fit and are only capable of low affinity binding. The bulky phenyl and benzyl derivatives are also poor inhibitors.

While it does appear that a branch at the $\alpha$-carbon of a $\beta$-alkyl substituent is a particularly favourable structural feature, the exact nature of the steric requirements of this region of the binding domain are not altogether obvious. The picture which emerges from Fig. 3 of a highly constrained pocket which can accommodate a methyl or ethyl group well may be a simplistic one, since the $\beta$-alkyl substituents are interacting hydrophobically while at the same time subject to steric constraint. It is difficult to reconcile these two binding parameters. Nevertheless, it is hard to discount the view that the position occupied by a methyl group at the $\alpha$-carbon (in iso-propyl, sec-butyl, etc.) is critical for high affinity binding. The increase of almost 100-fold in the activity of $\alpha$-methylbutyl over the $n$-butyl derivative would seem to support this conclusion.

Chirality in the lipophilic moiety, $R_I$

The importance of the steric requirements of the inhibitor binding site is highlighted by a comparison of enantiomers with a chiral centre in the group ($R_I$) interacting with the lipophilic region of the site. The $S(\,\text{+}\,)$ isomer of compound 3 is 200-fold more active than the corresponding $R(\,-\,)$ isomer. Compounds in the urea [11] and triazine [12] class of PS II inhibitors containing the same chiral centre also show differential activity in the Hill reaction. However, the level of discrimination was much less than observed with the optical isomers of compound 3. That the level of discrimination was different for the different classes of inhibitors having the same chiral centre may indicate that the cyanoacrylates interact with a different region of the hydrophobic matrix than do the ureas and triazines.

A detailed study of enantiomers related to compound 3 indicated that the activity of the $S$-isomer and the relative inactivity of the $R$-isomer must involve only the orientation of the phenyl ring and its capacity to interact with the specific binding region within the hydrophobic matrix [6]. The preferred orientation of the phenyl ring above the plane of the cyanoacrylate system was established by the inspection of space-filling models [6]. It is possible to imagine the cyanoacrylate molecule fitting the binding niche in the recently proposed model of the 32 kDa polypeptide [7], with the cyanoacrylate moiety binding close to the lipid-water interface and the lipophilic residues dipping down into the hydrophobic matrix of the membrane. The phenyl ring of the aralkylamino derivatives would then be favourably placed to interact with the hydrophobic amino acid residues within the membrane, particularly when the phenyl ring was correctly oriented.

The studies outlined above have established that high affinity binding is critically dependent on the steric arrangement of the groups attached to the cyanoacrylate molecule. As the correct orientation for precise fit in different regions of the site becomes better defined, a sharper picture of the topography of the binding domain will ultimately emerge to complement and improve current models of the architecture of the binding niche on the 32 kDa protein.