Effect of a Safener Towards Thiocarbamates on Plant Lipid Metabolism*

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It has been found that various thiocarbamate herbicides, known to alter surface wax and cutin synthesis, inhibit the elongation of fatty acids. We have proposed this as a mode of action of such compounds. Because it is believed that the sulphoxide metabolites of thiocarbamates are the active intermediates, we have examined the action of 1-aminobenzotriazole (an inhibitor of sulphoxide formation) on the inhibition of very long-chain fatty acid biosynthesis. In all tissues tested, aminobenzotriazole was able to block the specific inhibitory effect of thiocarbamates on fatty acid elongation. These results add further support to our proposal that fatty acid elongation is a sensitive target site for thiocarbamate herbicides in plants.

Introduction

The selectivity of a number of modern herbicides can be improved by the use of safeners [1]. In particular, the latter are utilized where there are problem weeds, in order to allow the use of effective but non-selective herbicides, when high herbicide doses are usually needed and where weed species closely related to the crop are present. Safeners can act in a number of ways [2]. They may interfere with herbicide uptake, increase metabolic inactivation of the herbicide or modify the target site thus reducing sensitivity. Other safeners can act by stimulating overproduction of the target enzyme.

We have carried out previous work with various thiocarbamate herbicides which are known to affect the surface layers of sensitive plants. Very long-chain fatty acids are precursors of many of the components of wax, cutin and suberin [3, 4] and the formation of these acids is sensitive to thiocarbamates. We have, therefore, suggested that fatty acid elongation may be a primary mode of action of such herbicides [5].

It is thought that thiocarbamates are activated by oxidation reactions to form their sulphoxide derivatives, which are the active form of the herbicide [6]. Further metabolism *via* glutathione conjugation and other reactions then leads to their inactivation [2]. Safening compounds could reduce herbicidal activity by lowering the initial oxidation reaction or, alternatively, as in the case of naphthalic anhydride, by stimulating glutathione S-transferase [7]. Our previous results suggested that thiocarbamate sulphoxides rather than the herbicides themselves were responsible for the selective action on fatty acid elongation [8]. Therefore, we wished to use a safener which could reduce the formation of thiocarbamate sulphoxides in order to test this possibility and also to confirm that thiocarbamate action on fatty acid elongation was of significance in their herbicidal activity. Such a safener is 1-aminobenzotriazole which is known to inhibit cytochrome P_{450}-dependent mixed-function oxidases [9] which is one mechanism for the oxidation of sulphide xenobiotics [10]. Because thiocarbamate safeners are used only against monocotyledonous crops [2] we used various cereal tissues, which we had shown previously [11] to have high rates of fatty acid elongation that was sensitive to the thiocarbamate herbicide diallate [11].

Materials and Methods

Growth of cereal tissues, incubation of samples and analysis of radiolabelled fatty acyl groups was carried out as described previously [11]. In general, pre-incubations of 4 h were carried out with the herbicide and/or aminobenzotriazole followed by 4 h radiolabelling period with [1-^{14}C]acetate. After extraction of lipids, the radiolabelled fatty acids were analyzed as their methyl esters by radio-gas liquid chromatography [11].

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Results and Discussion

1-Aminobenzotriazole was tested for its own effect on fatty acid synthesis from [1-^{14}C]acetate. In barley *(Hordeum vulgare)* leaf blades increasing concentrations of aminobenzotriazole up to about 20 mg/l caused a reduction of total fatty acid labelling. Above 20 mg/l there was little further increase in inhibition up to about 100 mg/l. The general inhibition produced by 1-aminobenzotriazole applied to the labelling of all fatty acids and was due to the compound's action on the precursor's uptake. Since 50 mg/l concentrations of aminobenzotriazole should have been antagonistic towards the usual concentration of diallate (S-(2,3-dichlorallyl)diisopropylthiocarbamate) used (10^{-5} M), we used this concentration in further experiments.

The action of aminobenzotriazole on labelling of leaf blades of spring barley (*Hordeum vulgare* L. *cv.* Doublet) is shown in Table I. It will be seen clearly that aminobenzotriazole reduced the radiolabelling of all fatty acids and to approximately the same extent. As discussed above this was due to a reduction in the availability of precursor [1-^{14}C]acetate within the tissue. By contrast, diallate caused a selective increase in the labelling of very long-chain fatty acids and had no significant effect on *de novo* synthesis as seen by the labelling of palmitate and stearate (Table I). The inhibition of elongation by 10^{-5} M diallate was comparable to that seen in previous experiments with barley [11]. When aminobenzotriazole was used at the same time as diallate, it completely reversed the selective inhibition of elongation caused by the herbicide alone. These results are fully in keeping with our suggestion that it is the sulphoxide derivative of diallate, rather than the herbicide itself, which selectively inhibits fatty acid elongation [8].

The above experiment was repeated with other monocotyledonous crop species in order to check that the safening action of aminobenzotriazole towards diallate was seen in a number of plants. Table II shows data for a winter barley variety and Table III shows those from experiments with two varieties of oats *(Avena sativa)*. In all cases amino-

Table I. Effect of diallate and 1-aminobenzotriazole (ABT) on labelling of fatty acids from [1-^{14}C]acetate in leaf blades of *Hordeum vulgare cv.* Doublet. Diallate was used at 10^{-5} M and ABT at 50 mg/l. Results are expressed as means ± S.D. (n = 3). The barley *cv.* Doublet used was a spring variety and was used 9 days after germination and growth in the dark at 20 °C. n.d. = none detected; 18C = 18 carbon fatty acids which were not separated from each other on the SE-30 column used for radio-GC.

Additions	Labelling of fatty acids [dpm × 10^{-2}]				
	16:0	18C	20:0	22:0	22:0
None (control)	147 ± 6	194 ± 7	51 ± 6	101 ± 13	91 ± 9
Diallate	146 ± 5	178 ± 8	15 ± 8	34 ± 4	n.d.
ABT	128 ± 4	155 ± 5	37 ± 3	56 ± 4	60 ± 2
Diallate + ABT	128 ± 6	142 ± 7	39 ± 8	57 ± 7	53 ± 8

Table II. Effect of diallate and 1-aminobenzotriazole (ABT) on the proportion of radioactivity in the fatty acids labelled from [1-^{14}C]acetate in leaf blades of winter barley. *Hordeum vulgare cv.* Halcyon was used. Other details as in the legend to Table I.

Additions	Total labelling [dpm × 10^{-2}]	Distribution of radioactivity [% [^{14}C]fatty acids]				
		16:0	18C	20:0	22:0	24:0
None (control)	477 ± 48	27 ± 3	40 ± 4	7 ± 2	20 ± 3	7 ± 1
Diallate	338 ± 59	36 ± 2	50 ± 2	5 ± 1	8 ± 2	n.d.
ABT	380 ± 67	31 ± 2	43 ± 1	8 ± 1	12 ± 1	6 ± 1
Diallate + ABT	295 ± 61	31 ± 1	44 ± 2	8 ± 1	12 ± 2	6 ± 2

Table III. Effect of diallate and 1-aminobenzotriazole (ABT) on fatty acid labelling from [1-^{14}C]acetate in leaf blades from oats. Oats were grown for 9 days in the dark. Other details as in Table I. tr. = trace (<0.5).

Additions	Labelling [dpm × 10^{-2}]	Distribution of radioactivity [% [^{14}C]fatty acids] 16:0	18C	20:0	22:0	24:0
cv. Peniarth						
None (control)	298 ± 56	33 ± 1	47 ± 1	7 ± 1	8 ± 1	5 ± 1
Diallate	246 ± 51	39 ± 2	52 ± 1	4 ± tr.	5 ± 1	n.d.
ABT	257 ± 37	30 ± 1	47 ± 1	8 ± 1	10 ± 1	5 ± tr.
Diallate + ABT	230 ± 43	32 ± 1	48 ± 1	7 ± 1	8 ± 1	5 ± 1
cv. Pennal						
None (control)	327 ± 40	35 ± 1	50 ± 1	4 ± 1	7 ± 1	4 ± tr.
Diallate	281 ± 44	37 ± 1	59 ± 1	1 ± tr.	3 ± 1	n.d.
ABT	279 ± 32	32 ± tr.	55 ± 3	5 ± 2	8 ± 1	2 ± 1
Diallate + ABT	268 ± 40	34 ± tr.	53 ± 1	5 ± 1	8 ± 1	1 ± tr.

benzotriazole caused some decrease in total labelling but the effect was the same for all fatty acids because it was due to reduction in the available precursor [1-^{14}C]acetate. Diallate, on the other hand, always caused a selective effect on the labelling of very long-chain fatty acids and had no significant inhibition on *de novo* synthesis. When diallate and aminobenzotriazole were used together, the P_{450}-oxygenase inhibitor prevented the selective action of diallate.

Conclusion

The results described here reinforce the conclusions previously made in our laboratory that thiocarbamates have a primary mode of action on fatty acid elongation [5] and that the active compound is the sulphoxide derivative [8]. Thus, any compound which can reduce the metabolism of the thiocarbamate herbicide to its sulphoxide or which can increase the rate of removal of the sulphoxide is likely to safen the crop because fatty acid elongation (and, hence, surface layer formation) is preserved.

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