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ACTION OF METHOMYL ANALOGUES ON MAIZE MITOCHONDRIA

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Key Word Index—*Zea mays*, Gramineae, maize, male sterility, mitochondria, swelling, *Helminthosporium maydis* race T toxin, Methomyl

Abstract—Methomyl analogues were assayed on mitochondria isolated from male sterile (T cytoplasm) and male fertile maize None of them was more selective than Methomyl The analogues which were more efficient than Methomyl displayed no selectivity The results suggest stringent steric requirements for Methomyl action and stress the importance of the electron conjugated system in the Methomyl molecule for its efficiency

INTRODUCTION

Maize (*Zea mays* L) with a mitochondrial gene for male sterility (Texas male sterile cytoplasm, or T) is highly susceptible to *Helminthosporium maydis* race T (or HMT) A host-specific pathotoxin produced by HMT was purified and showed the same specificity as HMT [1] A growing body of evidence suggests that mitochondria are a site of toxin action [2] The insecticide Methomyl, or *S*-methyl-*N*-(methylcarbamyl) oxy-thioacetimidate, was found to mimic the action of HMT toxin, both in plants [3] and on isolated mitochondria [4] The selectivity of the insecticide is less than that of HMT toxin though high enough to allow biochemical studies

In order to get more information about the interaction between Methomyl and its target in the mitochondrial membrane, we compared the action of several Methomyl analogues A first part of the study dealt with their action on root elongation in male sterile and male fertile maize [5] In the present paper, we report about the action of these compounds on mitochondria isolated from male sterile (F_7T) and male fertile (F_7N) maize coleoptiles

RESULTS AND DISCUSSION

Figure 1 shows the action of Methomyl on the absorbance of mitochondrial suspensions in the case of F_7T and F_7N mitochondria Methomyl at a concentration of 5 mM had no, or a very slight effect on F_7N mitochondria the slight absorbance decrease observed was probably mostly due to the addition of 1% solvent in the reaction medium Results were subsequently corrected to take into account a 1% dilution Conversely, 5 mM Methomyl brought about an 11% decrease in the absorbance of F_7T mitochondrial suspensions Such an action was first demonstrated by Gegenbach *et al* [6] using HMT toxin and was interpreted as an energy-independent swelling Electron micrographs indicated that the swelling was caused by a specific disruption and vesiculation of the inner mitochondrial membrane This could account for the absorbance decrease in two ways (1) Alteration of the optical properties of the mitochondrial membrane (2) Increased permeability of the membrane to the osmoticum (in our case, mannitol) resulting in its entry into the organelle and in swelling Increased permeability to low MW compounds was suggested by swelling studies [6] and demonstrated for NAD^+ [7] As mannitol is a smaller molecule than NAD^+, permeability to mannitol of the inner mitochondrial membrane is very probably increased

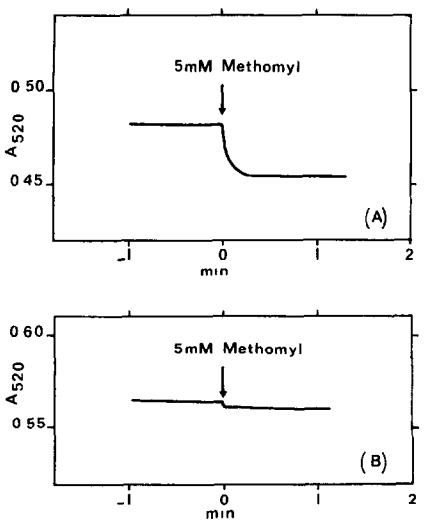

Fig 1 Typical experiment showing the action of 5 mM Methomyl on (A) a suspension of F_7T mitochondria, (B) a suspension of F_7N mitochondria Initial $A_{520\,nm}$ were 0 512 (A) and 0 565 (B) Other experimental details are in the text

To assess the activity of Methomyl analogues, we used swelling tests instead of respiratory tests for several reasons (1) Respiratory tests give different results according to the substrates used inhibition of O_2 consumption with malate–glumate, stimulation with NADH [2] (2) Results with respiratory tests change quickly with aging of the mitochondria [8] On the contrary, responses to swelling tests were more stable In addition they could be performed three times more quickly than respiratory tests, which limited eventual effects of mitochondrial

aging (3) Effect of Methomyl on swelling occurred within a few seconds (Fig 1) Effect on oxygen consumption could take more than 1 min to be fully exhibited (data not shown)

Table 1 shows the results obtained with some of the Methomyl analogues tested (for their chemical formulae see the Experimental) Only compounds 2 and 3 retained the selectivity exhibited by Methomyl but none was as efficient From the remaining ones, only compound 6 showed a small activity but had no selectivity Compounds 4 and 5 were insoluble at 5 mM When tested at lower concentrations, they displayed no activity Compounds 8–10 had a peculiar effect which prevented us to integrate them in Table 1 Their addition to the mitochondrial suspensions resulted in an instantaneous increase in absorbance followed by a decrease taking place in *ca* 1 min (Fig 2) They were as effective on N mitochondria as on T mitochondria (data not shown) They showed no significant absorbance at 520 nm thus the instantaneous absorbance increase they triggered reflected optical changes in mitochondria In our experimental conditions, it is difficult to explain why mitochondria would shrink, since an increase in membrane permeability would lead to the opposite result, namely swelling One must notice that the absorbance increases we observed were far greater and far quicker than those obtained through true shrinkage [9] We hypothesize that due to their *n*-octyl moiety, compounds 8–10 can be inserted between the fatty acid chains of membrane phospholipids The result could be a modification of several physical properties of the mitochondrial membrane, for instance its optical properties (leading to an absorbance increase) and an increase in permeability (leading to the subsequent swelling we observed)

Table 1 Effect of some Methomyl analogues on mitochondria isolated from male sterile (F_7T) and male fertile (F_7N) maize

		F_7N		F_7T	
1 (Methomyl)	(+)	−0 4	(0 3)[a]	−11 00	(0 8)[b]
2	(+)	−0 7	(0 4)[a]	−4 7	(0 8)[b]
3	(+)	−0 3	(0 4)[a]	−2 9	(0 2)[b]
4	(−)	IS		IS	
5	(−)	IS		IS	
6	(−)	−0 7	(0 1)[b]	−0 9	(0 4)[b]
7	(−)	−0 2	(0 2)[a]	−1 8	(1 2)[a]
11	(−)	−0 2	(0 4)[a]	0 0	(0 1)[a]
12	(−)	−0 5	(0 3)[a]	−1 2	(0 8)[a]
13	(−)	0 0	(0 2)[a]	0 0	(0 1)[a]

All compounds were tested at 5 mM Results are expressed as percent variation of $A_{520\,nm}$ 1 min after addition of the compound under test Figures in parentheses are s d 's [a] Not significantly different from 0, [b], significantly different from 0, (+), compound whose effect on T mitochondria is significantly greater than that on N mitochondria, (−), compound displaying no selectivity between N and T mitochondria, IS, insoluble at 5 mM For all statistical tests $P = 0\,05$

The rationale behind some of the alterations we made in the Methomyl molecule was to increase its lipophilicity in order to increase its efficiency At first, we interpreted the decrease in absorbance observed after addition of Methomyl to a mitochondrial suspension as a detergent-like effect (selective for T mitochondria) In such conditions, the lipidic part of the mitochondrial membrane would have been the target of Methomyl and we expected an increase in efficiency from an increase in lipophilicity The results were opposite as shown by the comparisons Methomyl vs compounds 2–5 and 12 The requirements for efficiency and selectivity were shown to be very stringent complete loss through R_1 substitution (compound 12), sharp decrease through R_2 substitution (compounds 2 and 4), even sharper decrease through R_3 substitution (compound 3), complete loss following the replacement at the X position of the oxygen atom by a sulfur one This suggests very strong steric requirements for Methomyl action Instead of acting through dissolution into the lipidic part of the membrane, Methomyl seems to have a specific target, probably a protein It is known that isolated T and N mitochondria synthesize different polypeptides [10] Methomyl target could be one of the polypeptides, which could explain the selectivity between N and T mitochondria

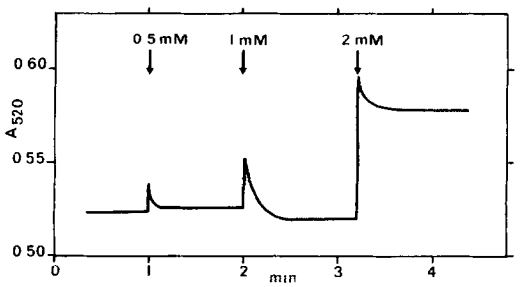

Fig 2 Typical experiment showing the effect of addition of increasing amounts of compound 9 to a F_7N mitochondrial suspension

The complete loss of efficiency and selectivity (almost complete in the case of compound 3) when sulfur is omitted from R_3 (compounds 6 and 7) indicates that another requirement for Methomyl activity is the extension of the conjugated system N–CO–O–N=C–S The reasoning does not hold for compounds 8–10 for their *n*-octyl moiety brings about an effect of its own (see above) Compound 11, in which C=S replaces C=O, had no activity The greater polarizability of sulfur as compared to oxygen induces a greater polarization of the thione moiety as compared to the corresponding ketone [11] It results in a distortion in the electronic delocalization as compared to Methomyl Thus, the partial structural relation Me–NH–CO– is necessary for specific toxicity

In a previous work [5] it was shown that as far as selectivity towards F_7T and F_7N maize seedlings is concerned, structural requirements are far less stringent For instance, compound 3 was as efficient and selective as Methomyl So were compounds 11 and 13, in which the carbonyl oxygen is replaced by a sulfur atom This could well indicate that Methomyl and some of its analogues have other targets than mitochondria in F_7T maize cells Candidates could be enzymic activities located on plasmalemma [12]

Most of the Methomyl analogues can have two isomeric conformations, commonly referred as *Z* and *E* We have not attempted so far to determine whether they are equally effective Our current work deals with this topic in the case of Methomyl

In conclusion, Methomyl is the most selective compound in the series studied here A few compounds were

more efficient than Methomyl but without any selectivity The structural requirements for selectivity of action (both steric and electronic) were far more stringent in the case of mitochondria than in the case of seedlings

EXPERIMENTAL

Preparation of mitochondria Mitochondria were isolated from epicotyls of F_7T (male sterile) and F_7N (male fertile) maize (*Zea mays* L) lines The plants were grown on eight layers of wet germination paper for 5 days at 25° in the dark The epicotyls (*ca* 15 g fr wt) were ground with a pestle and mortar in a medium consisting of 0 4 M mannitol, 0 1 M morpholinopropanesulfonate buffer (MOPS), 1 mM EGTA and 2 mg/ml bovine serum albumin, pH 7 6 After straining through four layers of cheese cloth, the homogenate was centrifuged at 2000 *g* for 10 min, and the resulting supernatant fraction at 10000 *g* for 10 min The pellet from the second centrifugation was resuspended in grinding medium, then centrifuged at 10000 *g* for 10 min The resulting pellet contained 50–80 mg protein/ml and was kept on ice All the above procedures were performed at 0–4°

Assay of the Methomyl analogues Mitochondria (*ca* 0 5 mg protein) were suspended in a medium containing 0 3 M mannitol and 10 mM MOPS buffer (pH 7 2) $A_{520\,nm}$ was recorded with an Acta III (Beckman) spectrophotometer 1 min after suspension of the mitochondria, when drift was no longer observed, the compound under study was added and the subsequent variation in $A_{520\,nm}$ calculated after 1 min All expts presented in this paper were repeated at least × 3

Table 2 Formulae of the Methomyl analogues synthesized

	R_1	R_2	R_3	X
1 (Methomyl)	H	Me	SMe	0
2	H	C_2H_5	SMe	0
3	H	Me	C_2H_5	0
4	H	C_6H_5	SMe	0
5	H	C_6H_5	C_2H_5	0
6	H	Me	i-C_3H_7	0
7	H	Me	n-C_3H_7	0
8	H	Me	n-C_8H_{17}	0
9	Me	Me	n-C_8H_{17}	0
10	H	C_2H_5	n-C_8H_{17}	0
11	H	Me	SMe	S
12	Me	Me	SMe	0
13	H	Me	C_2H_5	S

General formulae (R_1R_2)-N-CX-O-N=C(H_3)-R_3

Synthesis of Methomyl analogues These were obtained by reaction of methyl-, ethyl- and thiomethyl isocyanates and *N*-dimethylcarbamyl chloride with the corresponding oximes The oximes were obtained from the suitable ketones [13] For more details about the Methomyl analogues synthesized, see ref [5] All compounds synthesized have spectroscopic data (IR ^1H and ^{13}C NMR) in agreement with the structure

The formula of each compound is explicated in Table 2

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