Methylation of Guanine *in vivo* by the Organophosphorus Insecticide Methamidophos*

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Dedicated to Professor Erich Hecker on the occasion of his 60th birthday

DNA, RNA, Alkylation, Insecticides, Methamidophos

The methylating capability of methamidophos, assayed by the formation of [7-^{14}C]methylguanine in mouse liver, was investigated using a ^{14}C-insecticide labelled at the O–CH_3 group. Following i.p. administration of the toxicant, [7-^{14}C]methylguanine could be isolated from liver nucleic acids of treated mice. The amount of ^{14}C-label reached its maximum 6 h following administration of the insecticide. At maximum ^{14}C-labelling, the amount of 7-methylguanine calculated as fraction of applied dose, was $20-22 \times 10^{-4}$ and $98-104 \times {}^{-4}$, for DNA and RNA, respectively. The results obtained indicate also, that an appreciable amount of ^{14}C-activity is incorporated *via* the C-1 pool.

Introduction

Methamidophos (O,S-dimethyl phosphoramidothioate (**I**) is an important broad spectrum insecticide with pronounced systemic properties [1] and is sold under the brand names monitor (Chevron Chemical Co. USA) and Tamaron (Bayer Chemical Co. Germany). It possesses high mammalian toxicity [2] and is a potent inhibitor for plasma and RBC-cholinesterase [3–5]. It was recently reported [6] that methamidophos induces different types of chromosome aberrations in root tips of *Vicia faba* plant.

A number of organophosphorus insecticides were classified as alkylating agents [7] and the methylating capability of some of the 0,0-dimethylester type was reported recently [8–10]. In the present study, the *in vivo* alkylating capability of methamidophos towards N-7 of guanine of mouse liver nucleic acids was investigated. For this study an O$^{14}CH_3$-labelled preparation was used.

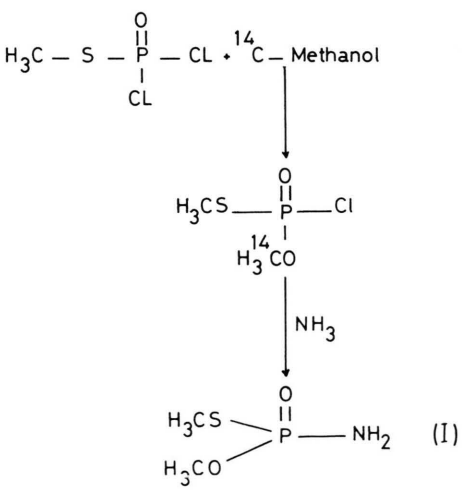
Materials and Methods

Chemicals

All chemicals were of analytical grade. [^{14}C]Methamidophos had a specific activity of 4.16 mCi/g and a radiometric purity over 98%. Methylated bases (Sigma) were used as markers.

[OCH_3-^{14}C]Methamidophos

Methamidophos was prepared from S-methyl phosphorodichloridothioate mainly according to procedures described [11] (Scheme 1). The dichloridothioate was allowed to react with one equivalent of [^{14}C]methanol in chloroform at 0–2 °C. After stirring for 30 min, the reaction mixture was saturated with dry ammonia at temperatures below 10 °C. The isolated product was purified by partitioning between chloroform and water.



Scheme 1. Synthesis of [O-methyl-^{14}C]methamidophos.

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Animals

Males of randomly bred Swiss mice, 20–30 g of weight and 3–4 months of age were given standardized food and water ab libitum. A solution of [^{14}C]methamidophos was injected intraperitoneally into the mouse. The doses used were 1.6, 4.4 and 5.3 mg/kg body weight. Doses were chosen in such a way to avoid signs of severe intoxications. At different time intervals, mice were killed with chloroform and organs were removed and stored at −20 °C till analysis.

Isolation of liver macromolecules

The radioactivity in mouse liver was determined at 3, 6, 24 and 48 h following treatment of mice. Total cell RNA and DNA were isolated from the liver homogenate in 0.03 M phosphate buffer, pH 6.8 using a phenol-extraction method based on techniques described earlier [12–15]. Nucleic acids were precipitated from the combined aqueous phases by addition of 2 volumes of ethanol and keeping overnight at −20 °C. Protein was isolated from phenolic phases by addition of methanol followed by centrifugation at 1000 × *g* for 10 min [15]. The separated protein was washed in succession with water, 5% TCA, ethanol:chloroform (1:1), acetone, ethanol:ether (1:1) and finally with ether.

Base analyses

The nucleic acids were hydrolyzed with 1 N HCl at 100 °C for 1 h and the hydrolysate was fortified with guanine, adenine and 7-methylguanine (200 µg each). Released bases were then fractionated on an ionexchanger column (10 × 1 Cm) of Dowex 50WX-12(H^+), 100–200 mesh using a gradient elution with 1–4 N HCl. The peak of 7-methylguanine was isolated and rechromatographed using the same system. The eluted fractions (5 ml each) were then measured for their UV absorption at 260 nm on a CE-595 Double Beam Spectrophotometer. Fractions were then dried over P_2O_5 in a desiccator. Residues were taken in 2 ml methanol and diluted with 10 ml scintillator for counting of the radioactivity.

Radiomeasurements

Solutions of radioactive samples were analyzed for ^{14}C-activity in a Nuclear Enterprises "Model 8310" liquid scintillation spectrometer. Tissues and protein samples were combusted in a Packard-Oxidizer system 306 and then assayed for radioactivity. The scintillation cocktail consisted of permablend (5.5 g) and naphthalene (120 g) in one liter dioxan.

Results and Discussion

The ^{14}C-activity in mouse liver following i.p. injection of different doses of the radioactive insecticide into male mice is shown in Fig. 1. The highest

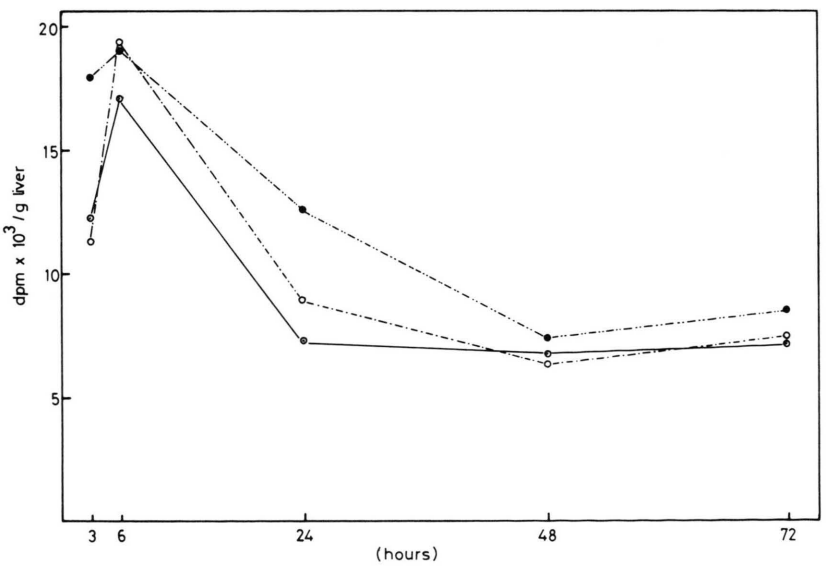

Fig. 1. ^{14}C-activity in mouse liver following i.p. administration of different dose of [methyl-^{14}C]-methamidophos to male mice. Each point a mean of 5 mice. ⊙———⊙ 1.6 mg/kg body weight; ○—·—·—○ = 4.4 mg/kg body weight; ●—··—··—● = 5.3 mg/kg body weight.

radioactivity in liver was reached to 6 h following administration of the labelled insecticide.

The isolated nucleic acids and protein contained ^{14}C-activity. Table I shows the time course of ^{14}C-labelling in relation to time and dosage. In general, the specific activity of the isolated macromolecules increased with increase of applied dose and reached its maximum 6 h following administration of methamidophos. The data obtained indicate a tendency of the insecticide to react with RNA than with DNA.

Base analyses were carried out so as to differentiate between ^{14}C-incorporation and alkylation. Fig. 2 shows the elution curve and radioactivity profile obtained from chromatography of the HCl-hydrolysate of DNA on Dowex 50WX-12.

Analysis of RNA-hydrolysate using the same system as in DNA gave a comparable picture. In both cases, ^{14}C-activity was found to be present in the adenine, guanine and 7-methylguanine fractions of the column effluent. Rechromatography of the 7-methylguanine fractions on Dowex 50WX-12 showed that about 76–80% of the chromatographed radiodose was associated with 7-methylguanine. The amount of 7-methylguanine formed contributed to about 9% and 13% of the radioactivity in DNA and RNA, respectively. At maximum ^{14}C-labelling, the amount of 7-methylguanine formed, calculated as fraction of total applied dose, was $20-22\times10^{-4}$ and $98-104\times10^{-4}$, for DNA and RNA, respectively

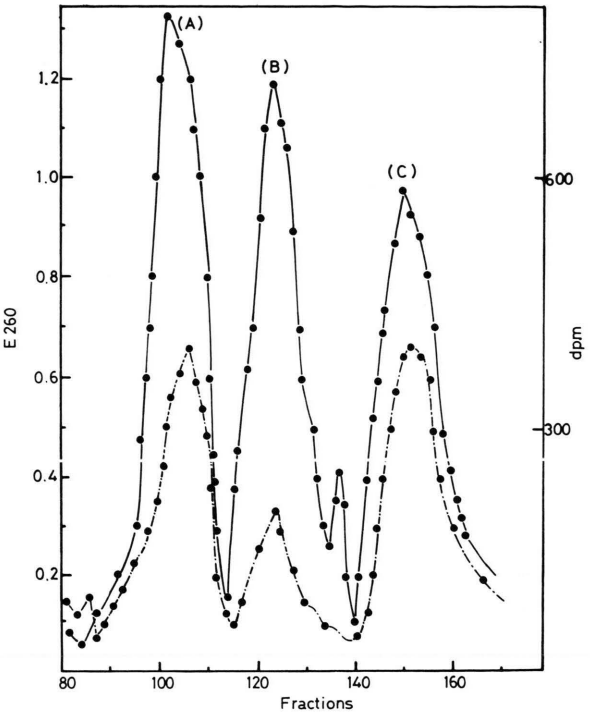

Fig. 2. Chromatographic and radioactive profile of acid hydrolysis of mouse liver DNA, *via* ion exchange chromatography on Dowex 50WX-12(H$^+$) eluted with 1–4 N HCl. Guanine (A), 7-methylguanine (B) and adenine (C) were added as tracers before analysis. ●———● = E 260; ●—·—● = ^{14}C-activity. Data are mean of 2 experiments.

Table I. Amount of ^{14}C as dpm and μg methamidophos/mg nucleic acid or protein in liver of male mice following i.p. administration of different doses of [OCH_3-^{14}C]methamidophos.

Time [hrs]	dpm/mg material* [^{14}C as μg methamidophos/mg material]					
	D_1[a]			D_2[b]		
	RNA	DNA	Protein	RNA	DNA	Protein
3	540 (0.1)	700 (0.13)	70 (0.012)	3200 (0.58)	1250 (0.23)	340 (0.06)
6	3560 (0.64)	4100 (0.74)	240 (0.042)	7070 (1.28)	4400 (0.78)	420 (0.075)
24	300 (0.054)	390 (0.069)	400 (0.072)	1200 (0.22)	3170 (0.57)	630 (0.114)
48	40 (0.008)	17 (0.003)	135 (0.024)	440 (0.078)	370 (0.07)	320 (0.057)

* Data are mean of three replicates.
[a] D_1 = 1.6 mg/kg body weight.
[b] D_2 = 5.3 mg/kg body weight.

(Table II). The extent of ^{14}C-labelling in RNA was higher than in DNA.

The alkylating properties of organophosphorus insecticides are well recognized and were already reviewed since 1967 [16]. A number of them were reported as potential alkylating agents [7], where the N-7 of guanine was considered to be the preferable site of alkylation [8, 9, 17–19]. The formation of labelled 7-methylguanine may be taken as indication for chemical alkylation of guanine moieties. On the other hand, the isolation of labelled guanine and adenine suggests incorporation of radioactivity, *via* C-1 pool in the biosynthesis of these nucleotides.

The decrease in ^{14}C-label in DNA with time *in vivo* finds analogy with results previously reported for genotoxic alkylating agents [20–22] and some organophosphorus insecticides [8, 10, 12]. This may be attributed to fresh synthesis of nucleic acids and/or to repair mechanisms. The latter, however, is known to be a slow process.

The results obtained indicate a fair methylating capability of methamidophos, as compared with other organophosphorus insecticides. Methamidophos was reported to be readily dealkylated *in vivo* to give S-methyl phosphoramidothioic acid [23]. Alkylation of N-7 of guanine with small groups, such as methyl or ethyl groups, was reported not to correlate with carcinogenic or mutagenic effects [24, 25]. In a test battery, however, alkylation of nucleic acids may be of significance in evaluation of the genotoxic potential of a chemical, since it provides an indication that the toxicant could reach and react with the target with the formation of an alkylated product.

Table II. Extent of ^{14}C-labelling in macromolecules of mouse liver 6 h following i.p. administration of [OCH_3-^{14}C]methamidophos.

Material	Applied dose [μg/mouse]	Extent of ^{14}C-labelling [as fraction of total dose $\times 10^{-4}$] in macromolecules	in 7 MeGu. moieties
DNA[a]	133	225	20
	160	243	22
RNA[a]	133	751	97.7
	160	800	104
Protein[a]	48	612	
	133	480	
	160	328	

[a] Data are mean of 3 experiments.

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