# Interaction of Organophosphorus Insecticide Methylparathion with Calf Thymus DNA and a Synthetic DNA Duplex

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The interaction of an organophosphorus insecticide methylparathion (*O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate) with double-stranded DNA was characterized by UV and circular dichroism (CD) spectroscopy. Two kinds of DNA were employed: calf thymus DNA (CT DNA) and a synthetic two-stranded oligomer of sequence 5'-d(TTGGATCCGAATTCAAGCTT)-3'. Melting curves and CD spectra were taken for the DNAs in the presence of the insecticide at methylparathion/DNA base pair molar ratio of 0.5. The insecticide evoked a decrease of the melting temperature and a broadening of the transition range for CT DNA. Similar effects were observed for the synthetic oligomer but they were less pronounced than in the case of CT DNA. Methylparathion evoked a slight shift and an increase in the amplitude of the negative band in the CD spectra of both DNAs. Obtained results indicate that methylparathion may perturb the thermal stability and conformation of DNA, which is an evidence that the insecticide has an ability to interact directly with DNA.

## Introduction

Chemical pesticides are a group of agents used to control insects, weeds or other pests, but, although not showing any immediate adverse effect (at least at concentrations normally used in agriculture), some of them may pose a significant long-term hazard to man (Bianchi *et al.*, 1994; Hrelia *et al.*, 1994). Among the possible adverse health effects of pesticides, genotoxicity is of special significance because of the long latent period between the exposure and effects becoming apparent (Tungul *et* al., 1991). Although many pesticides have genotoxic properties, as demonstrated in various tests (Carbonell *et* al., 1993; Dolara *et al.*, 1994), there are, in general, very few studies on the molecular basis of this feature.

To assess interaction between the insecticides and genetic material an *in vitro* system which looks at the effect of insecticides on purified DNA directly can be used. This system has several advantages. First, it is organism-independent, that is, it does not rely on the biology of a particular organism for detecting any interaction with DNA. Second, the masking of DNA damage by the repair system need not to be considered.

In the present work the interaction between DNA *in vitro* and an organophosphorus insecticide methylparathion has been investigated using spectroscopic techniques. Since the early 70s organophosphorus insecticides have become the most extensively used pesticides due to their effectiveness in cholinesterase inhibition in insects and low persistence (Barnett and Rodgers, 1994). Methylparathion is a commonly used insecticide useful for insect control in agriculture and prevention medicine and there is some evidence for its genotoxic activity (Dolara *et al.*, 1994; Heath *et al.*, 1993; Mathew *et al.*, 1992).

## Materials and Methods

### *DNAs*

Calf thymus (CT) DNA was purchased from Sigma Chemicals (St. Louis, MO) and was then suspended in 10 mM phosphate buffer, pH 7.4, containing 100 mM NaCl and 0.1 mM EDTA.. Synthetic DNA oligomer of the sequence 5'-d(TTGGATCCGAATTCAAGCTT)-3' and

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oligomer with sequence complementary to it, purified by semipreparative anion-exchange HPLC were obtained from the Department of Bioorganic Chemistry, Polish Academy of Sciences (Lodz, Poland) as lyophilised single strands. Equal concentrations of the complementary single-stranded oligomers were combined in 10 mM pH 7.0 phosphate buffer and 0.1 M NaCl and heated at 70°C for five minutes. The strands were then annealed by cooling slowly for several hours at ambient temperature. Concentration of DNAs was determined spectrophotometrically.

*Spectroscopic measurements*

UV absorbance at 260 nm was monitored as a function of temperature from 5 to 95°C to examine helix-coil transition of DNAs. Experiments were performed using a Hewlett-Packard Diode Array Spectrophotometer Model 8452A. The absorbance versus temperature plots were normalized to an absorbance of 1.00 at 5°C (LeBlanc and Morden, 1991).

CD spectra were recorded with a Jasco J-700 spectropolarimeter interfaced to and controlled by an IBM compatible computer. Run parameters were the following: band width, 1 nm; time constant, 1–8 s; scan speed 5–20 nm/min. Sample temperature during the CD measurements was 20 ± 0.5°C. CD spectrum of the solvent blank was subtracted from each of DNA spectral scans.

*Methylparathion treatment*

Methylparathion (*O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate) of purity 99.9% was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany), its chemical structure is displayed in Fig. 1. The insecticide was derived from stock (50 mM) ethanolic solution to give final molar insecticide/DNA base pair ratio $r = 0.5$. The control received, instead of insecticide, ethanol, the final concentration of which was equal to 0.18%. The samples were equilibrated for 24 h at 37°C before the measurements.

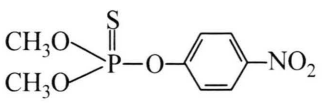


Fig. 1. Chemical structure of methylparathion, *O,O*-dimethyl *O*-4-nitrophenyl phosphorothioate.

## Results and Discussion

As mentioned above, very few studies were done on the interaction of organophosphorus insecticides with DNA *in vitro*. Because it is very hard to relate a concentration of an insecticide in a man environment to the actual concentration of this insecticide in the DNA environment (mostly cell nucleus), a insecticide/DNA base pair molar ratio 0.5 has been chosen to be consistent in order with the work done in other laboratories (Griffin and Hill, 1978; Richardson and Imamura, 1985; Bartoli *et al.*, 1991).

UV absorption spectra of methylparathion, CT DNA alone and in the presence of the insecticide are displayed in Fig. 2A. Fig. 2B displays UV absorption spectra of methylparathion, oligomer alone and in the presence of the insecticide. It can be seen from the figures that addition of methylparathion does not change the nature of the UV absorption spectrum of either DNA – the result-

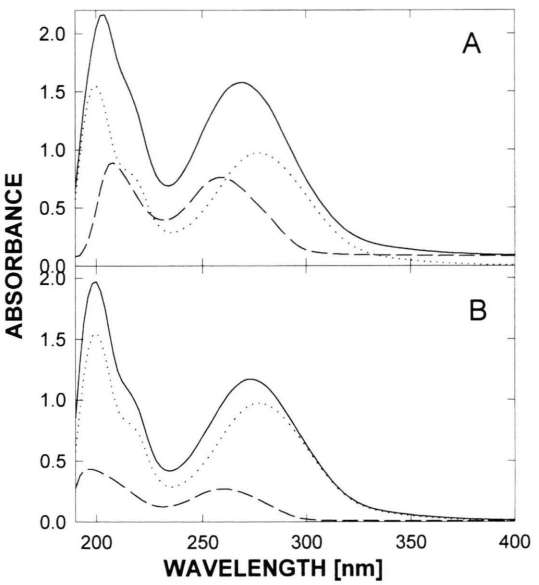


Fig. 2. UV absorption spectra of DNA (·····), methylparathion (----) and DNA in the presence of methylparathion at insecticide/DNA base pair molar ratio 0.5 after 24 h incubation at 37°C (——) for calf thymus DNA (A) and two-stranded oligomer of sequence 5'-d(TTGGATCCGAATTCAAGCTT)-3' (B).

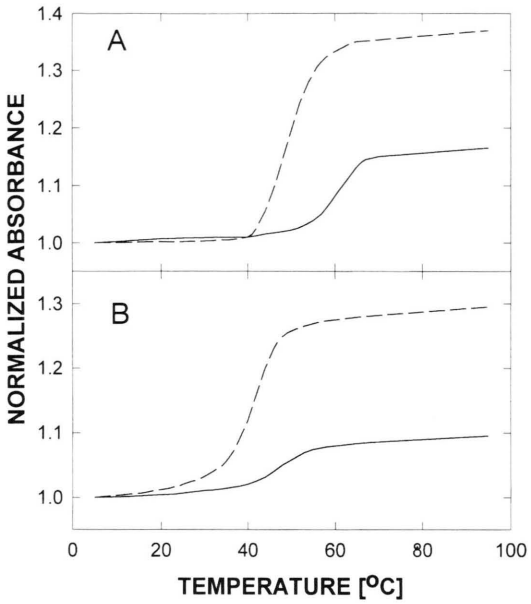


Fig. 3. Melting curves for calf thymus DNA (A) and two-stranded oligomer of sequence 5'-d(TTGGATCCGAATTCAAGCTT)-3' (B) in the presence of methylparathion (——) after 24 h incubation at 37°C as compared with control DNA (– – –). Insecticide/DNA base pair molar ratio was 0.5. The absorbances were normalized to absorbance of 1.00 at 5°C.

ing spectra are a sum of the spectrum of either DNA alone and the spectrum of the insecticide.

The lack of changes in the UV absorption spectra indicates that the possible interaction between the insecticide and DNA does not change substantially the secondary structure of the DNA in the degree which can be resulted in the changes in its UV absorption spectrum and that the amount of molecules of the insecticide bound to DNA may be quite low.

Melting curve of CT DNA incubated with methylparathion is compared with that of the control in Fig. 3A. The analysis of the melting curves brought parameters of the helix-to-coil transition for methylparathion-treated and control DNA given in Table I. It can be seen from the Table that methylparathion evoked a broadening of the transition range and a shift of the transition temperature toward lower temperature. These effects are also apparent from Fig. 3A where the melting curve of DNA in the presence of methylparathion differs from the melting curve of the control DNA.

Table I. Melting temperature ($T_m$), low ($T_1$) and high ($T_2$) temperatures of the transition and width of the transition range ($\Delta T$) of methylparathion-treated calf thymus (CT) DNA and two-stranded oligomer (one strand sequence 5'-d(TTGGATCCGAATTCAAGCTT)-3') ($r = 0.5$) as compared with appropriate control DNAs.

| Sample | $T_m$ [°C] | $T_1$ to $T_2$ [°C] | $\Delta T$ [°C] |
|---|---|---|---|
| control CT DNA | 49.5 | 39.4 to 59.6 | 20.2 |
| methylparathion-treated CT DNA | 47.6 | 36.7 to 60.8 | 24.1 |
| control oligomer | 44.0 | 31.0 to 50.0 | 19.0 |
| methylparathion-treated oligomer | 42.9 | 23.0 to 50.0 | 27.0 |

The effect of methylparathion on melting properties of synthetic oligomer is illustrated in Fig. 3B and the corresponding parameters are summarized in Table I. The effects are qualitatively the same as in the case of calf thymus DNA – the insecticide evoked a shift of the transition temperature toward a lower temperature and a broadening of the transition range.

Fig. 4 shows the CD spectra of the CT DNA and the oligomer in the 200–350 nm region. Both the

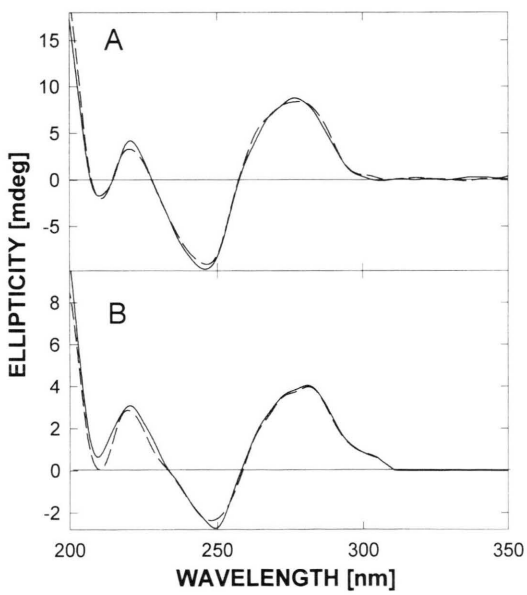


Fig. 4. Circular dichroism spectra of calf thymus DNA (A) and two-stranded oligomer of sequence 5'-d(TTGGATCCGAATTCAAGCTT)-3' (B) in the presence of methylparathion (——) after 24 h incubation at 37°C as compared with control DNA (– – –). Insecticide/DNA base pair molar ratio was 0.5.

insecticide-treated and control DNAs showed no CD signal in the 330–400 nm region. The positive band centered at around 280 nm as well as isodichroic points remained unchanged in the presence of methylparathion. However, the spectra of the control and treated samples show slightly different features in the wavelength region below 260 nm. In particular, the negative band observed near 250 nm of the insecticide-treated sample was red-shifted in the case of calf-thymus DNA and blue-shifted in the case of synthetic oligomer as compared with the control samples. The amplitude of the band was slightly increased in both cases.

Inspection of the CD spectra in Fig. 4 suggests that methylparathion does not significantly alters the global conformation of treated DNA relative to the corresponding untreated DNA. In other words, after the insecticide treatment, DNA retains the CD signature characteristic of a right-handed B-form duplex DNA (Plum and Breslauer, 1994; Krishnamurthy *et al.*, 1994).

Obtained results indicate that there was a direct methylparathion – DNA interaction. The interaction affected thermal behavior of both calf thymus and a synthetic duplex DNA and caused minor conformational changes in both kinds of DNA. The exact character of the changes cannot be deduced from this study, but it is important that experiments indicate an ability of methylparathion, a commonly used xenobiotic, to interact directly with DNA *in vitro*. This does not mean that the insecticide is mutagenic or carcinomic, but does, however, suggest that it has a potential to cause a damage to DNA *in vivo* (Griffin and Hill, 1978). That is why the results obtained warrant further investigation.

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