Degradation of \(^{14}\)C-malathion \textit{in vitro} by \textit{Culex pipiens quinquefasciatus} strains resistant and susceptible to malathion

Tadashi Miyata,* Tetsuo Saito* and Kazuo Yasutomi**

*Laboratory of Applied Entomology and Nematology, Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464, Japan
**Department of Medical Entomology, National Institute of Health, 2-10-35, Kamiosaki, Shinagawa, Tokyo 141, Japan

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\textbf{Abstract:} \textit{In vitro} degradation of \(^{14}\)C-malathion by \textit{Culex pipiens quinquefasciatus} was studied. The optimum temperature of \(^{14}\)C-malathion degradation \textit{in vitro} by \textit{C. p. quinquefasciatus} 900 g supernatant fraction was 25°C. Most of \(^{14}\)C-malathion degradation activity was found in the 105,000 g supernatant fraction. \(^{14}\)C-Malathion degradation activity of the 105,000 g supernatant fraction was 20–35-fold higher in the resistant strain than in the susceptible one. \(^{14}\)C-Malathion degradation activity was higher in adults than in larvae.

\textbf{Introduction}

Georghiou and Pasture (1978) and Yasutomi (1983) reported that the malathion resistant strains of \textit{Culex pipiens quinquefasciatus} showed higher \(\beta\)-naphthyl acetate hydrolyzing enzyme activity than the susceptible ones. Yasutomi (1983) reported also that synergists such as TPP (triphenyl phosphate) and \(K_2\) (2-phenoxy-4H-1,3,3-benzodioxaphosphoric 2-oxide) showed higher synergistic activity with malathion in the resistant strain, but not in the susceptible strain. From these results, the enhanced malathion degradation in the resistant strain was thought to be the most important mechanism of the malathion resistance in \textit{C. p. quinquefasciatus} as reported in other insects (Perry and Agosin, 1974; Oppenorth and Welling, 1976; Miyata, 1983), however nothing has been reported about the properties of enzymes degrading malathion in \textit{C. p. quinquefasciatus}. In this paper, certain properties of malathion degrading enzymes of \textit{C. p. quinquefasciatus} will be discussed.

\textbf{Materials and Methods}

\textit{Insect:} The insects used were the Okinawa (R) strain and the Ogasawara (S) strain. The Okinawa strain was collected from the open sewer in Koza, Okinawa in 1977 and has been selected in the laboratory. The Ogasawara strain was collected from the open sewer in Chichijima, Ogasawara in 1968 and has been reared in the laboratory without exposure to any insecticides. The larvae were reared at a temperature of 25±1°C and 16 hr illumination per day, being fed on dry beer yeast. The 4-th
instar larvae and 1–2 day adults were used for the experiment.

*Insecticide:* 14C-Malathion (methyl carbon 14 labelled) used here has been described previously (Miyata et al., 1976). The specific activity was 1,500 dpm/µg determined by an Aloka LSC-700 liquid scintillation spectrometer.

*Enzyme preparation:* Freshly prepared larvae or adults were homogenized in an ice cold buffer solution by means of a Potter-Elvehjem glass homogenizer. The standard buffer was 0.05 M tris-HCl buffer, pH 7.4 containing 0.25 M sucrose. The homogenate was first centrifuged at 900 x g for 10 min at 4°C, and the resultant supernatant was used as an enzyme source (*i.e.*, 900 g supernatant).

Various subcellular fractions were separated by differential centrifugation of the above 900 g supernatant at 4°C in a Hitachi 55P-2 ultracentrifuge. The mitochondrial fraction was separated by centrifuging 900 g supernatant at 10,000 x g for 10 min and the sediment was washed with the standard buffer solution twice. Microsomal and soluble fractions were obtained by further centrifugation at 105,000 x g for 60 min. The mitochondrial and the microsomal fractions were resuspended in the standard buffer.

*In vitro degradation of 14C-malathion:* The reaction mixture used in the 14C-malathion degradation study contained 0.5 ml each of 900 g supernatant and the standard buffer. A 10 µl aliquot of 14C-malathion (10^-5 M) in absolute ethyl alcohol was added to the reaction mixture. The system was kept at 25°C for 30 min with shaking. To investigate the effect of incubation temperature on 14C-malathion degradation, the incubation temperature from 20 to 40°C were utilized. To investigate the effect of enzyme concentration on 14C-malathion degradation, different enzyme concentrations were tested. For the time course experiment, various incubation intervals were tested. For the 14C-malathion degradation study by subcellular fractions, the enzyme concentrations used were 8 and 2% for the Ogasawara and the Okinawa strains, respectively.

The reaction was terminated by adding 1.0 ml of chloroform. The chloroform extraction was repeated three times. Radioactivity in the aqueous fraction (0.7 ml) was determined for its degradation products with 10 ml of Bray's scintillator (Bray, 1960) by an Aloka LSC-700 liquid scintillation spectrometer.

**RESULTS AND DISCUSSION**

Both enzyme preparations showed the highest 14C-malathion degradation activity at 25°C among the temperature tested (from 20 to 40°C). At 35°C, 14C-malathion degradation activity was highly inhibited (Fig. 1). Therefore, for further studies, the incubation temperature was fixed at 25°C. The similar phenomenon was reported by Shrivastava et al. (1971) with C. p. quinquefasciatus. They showed that as the incubation temperature was decreased from 40 to 25°C, the enzyme activity metabolizing propoxur increased. In contrast of C. p. quinquefasciatus, maximum enzyme activity for various enzyme reactions was obtained at 30°C in the housefly (Tsukamoto and Casida, 1967).

Matsumura and Brown (1963) reported the carboxylesterase from Culex tarsalis was sensitive to destruction of its carboxylesterase activity by temperature increasing from 30°C. The resistant material showed a greater

![Fig. 1 Effect of the incubation temperature on 14C-malathion degradation by 900 g supernatant of C. p. quinquefasciatus.](image-url)
sensitivity to destruction than the susceptible one. Houk and Hardy (1981) reported that the optimal temperature of nonspecific esterase of *C. tarsalis* was 30°C, with a range of approximately 1°C depending on mosquito strain.

Effect of incubation concentration of the enzyme solution and effect of incubation time on 14C-malathion degradation were shown in Figs. 2–5. From these results, the resistant strain showed higher malathion degrading activity than the susceptible strain. Between the larval and the adult homogenates, the adult homogenate showed higher 14C-malathion degrading activity than the larval one.

The highest degradation activity of 14C-malathion was observed in a 105,000 *g* supernatant fraction with a higher activity in the R strain than the S strain. *In vitro* degradation activity of 14C-malathion by 105,000 *g* supernatant fraction from larvae and adults of the R strain was 35- and 20-fold higher than that from larvae and adults of the S strain, respectively (Table 1). Addition of GSH and NADPH to the microsome and the 105,000 *g* supernatant fractions,
Fig. 5 Effect of the incubation time on \(^{14}\)C-malathion degradation by 900 \(g\) supernatant of \(C.\) \(p.\) \(quinquefasciatus\) (adult).

Table 1 \(In\) \(vitro\) degradation of \(^{14}\)C-malathion by subcellular fraction of \(C.\) \(p.\) \(quinquefasciatus\) strains resistant and susceptible to malathion

<table>
<thead>
<tr>
<th>Enzyme sources</th>
<th>Degradation activity ((\mu g/g/30) min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Larva</td>
</tr>
<tr>
<td></td>
<td>S</td>
</tr>
<tr>
<td>Nuclei and cell debris</td>
<td>1.0</td>
</tr>
<tr>
<td>Mitochondria</td>
<td>6.7</td>
</tr>
<tr>
<td>Microsome</td>
<td>1.0</td>
</tr>
<tr>
<td>105,000 (g) Supernatant</td>
<td>3.7</td>
</tr>
<tr>
<td>(Original homogenate)</td>
<td>4.3</td>
</tr>
</tbody>
</table>

respectively, showed no effect on the degradation on the \(^{14}\)C-malathion degradation (Miyata \(et\) \(al.,\) unpublished observation). Therefore, enhanced malathion degradataon activity was thought to be a main cause of malathion resistance in \(C.\) \(p.\) \(quinquefasciatus\) as reported in other insect pests (Perry and Agosin, 1974; Oppenooth and Welling, 1976; Miyata, 1983). However, in \(Anopheles\) \(albimanus\) resistant to organophosphates and carbamates, the resistance mechanism based on reduced sensitivity of acetylcholinesterase has been reported (Ayad and Georgiou, 1975).

Carboxylesterases are predominantly (60–80\%) in the microsomal fraction with 10–25\% appearing in the cytoplasm (Chow and Ecobichon, 1973). While malathion degrading carboxylesterase is present in the soluble phase of animal cells (Ahmad and Forgash, 1976). \(^{14}\)C-Malathion degrading activity of \(C.\) \(p.\) \(quinquefasciatus\) was found in the 105,000 \(g\) supernatant fraction as reported in \(Nilaparvata\) \(lugens\) (Miyata \(et\) \(al.,\) 1983). However, Matsumura and Brown (1961) reported that the mitochondrial fraction contained the highest malathion degrading carboxylesterase activity and Niwa \(et\) \(al.,\) (1977) reported that malathion degrading carboxylesterase activity was observed both in the microsome and the 105,000 \(g\) supernatant fractions in \(Musca\) \(domestica.\)

High synergistic action of TPP and \(K_{2}\) in malathion resistant \(C.\) \(p.\) \(quinquefasciatus\) (Yasutomi, 1983) was probably due to the inhibition of malathion degrading carboxylesterase (Plapp and Eddy, 1961; Ohkawa \(et\) \(al.,\) 1968).

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**摘 要**

マラチオン抵抗性および感受性ネットイエカによる14C-マラチオンの分解

マラチオン抵抗性および感受性ネットイエカによるin vitroでの14C-マラチオンの分解をしらべた。ネットイエカのマラチオン分解の最適温度は25℃であった。細胞分画した酵素液による分解をしらべたところ、14C-マラチオンの分解作用はおもに105,000 g上清分画で認められた。また幼虫と成虫では、成虫で分解作用が高かった。