Extraction of Microsomal Fraction from Midguts of Western Spruce Budworm *Choristoneura occidentalis* F., and Rat Liver, and their Ability to Epoxidize Aldrin

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Key words: Microsomal fractions; western spruce budworm; *Choristoneura occidentalis* F.; rat liver; epoxidation; aldrin.

**ABSTRACT**

Microsomal fractions were isolated from midguts of western spruce budworm larvae and rat liver. The microsomal preparations were then assayed for cytochrome P-450 content and aldrin epoxidase activity. Both microsomal preparations showed distinct spectrum of cytochrome P-450. Relative to protein content, the cytochrome P-450 content of midguts of western spruce budworm was lower by 7–9 times compared to that of the rat liver. Epoxidase activity is present in the two microsomal preparations as indicated by their ability to convert aldrin to dieldrin. The activity of both microsomal preparations was inhibited by piperonyl butoxide.

**INTRODUCTION**

The oxidative enzymes known as microsomal oxidases play a central role in the metabolism of organic insecticides in animal body. The enzyme has an extremely broad spectrum of substrates and catalyze a wide variety of transformation, with cytochrome P-450 acting as the oxygen-activating enzyme component to carry out the reaction. Cytochrome P-450 and its implication as the terminal oxidase component has been discovered in mammalian and insect microsomes (Williamson and Schechter 1970).

Microsomal fractions of insect tissues and rat liver constitute a ready source of mixed-function oxidases (Wilkinson 1979; Wong and Terriere 1965). These microsomal oxidases of the microsomal fractions in several lepidopterous insects and in rat livers are known to parallel
that of epoxidase activity (Brattsten and Wilkinson 1973; Chan et al., 1967; Wong and Terriere 1965). Therefore, aldrin, a model substrate to measure the total enzyme activity (by epoxidation) of mixed-function oxidases, is often used for comparison.

This paper reports on the isolation of microsomal fractions from midguts of western spruce budworm, an important lepidopterous insect pest, and testing of the activity of the enzyme that is involved in insecticide metabolism. The presence of the cytochrome P-450 spectrum and aldrin epoxidation were used to demonstrate the activity of the isolated enzyme fractions. The activity of the enzyme fractions from rat liver was also studied for comparison.

MATERIALS AND METHODS

Enzyme Preparation

The microsomal fraction was prepared from two sources; the midguts of last-instar larvae of western spruce budworm and the liver of six-month-old male Wistar rats.

The larval midguts were isolated, sectioned longitudinally and rinsed in cold buffer solution (0.1 M phosphate, pH 7.6) to remove their contents, and then cleaned thoroughly in the fresh ice-cold buffer medium. The cleaned midguts (20 midguts/5 ml cold buffer) were homogenized for 15 min using a Potter-Elvehjem homogenizer fitted with a motor-driven teflon pestle. The homogenized samples were later combined for centrifugation at 0° to 4°C for 15 min at 10,000 g. The supernatant was filtered through glass wool and then recentrifuged at 0° to 4°C for 60 min at 105,000 g in a Sorval OTD 75B ultracentrifuge with a Ti60 fixed-angle rotor according to Yu and Terriere (1979). The supernatant was decanted and the microsomal pellets were resuspended in cold buffer solution, at the equivalent of 20 midguts/ml, by gentle homogenization.

The livers of rats, killed by decapitation, were perfused with the cold buffer solution by inserting a hypodermic needle into the portal vein. The perfused liver was then removed from the thoracic cavity, washed thoroughly, and homogenized for 30 sec in cold buffer solution at 1 g/4 ml, using a VirTis blender. The homogenate was centrifuged for 30 min at 10,000 g and 0° and 4°C. Microsomes were prepared from the supernatant as described above, and finally buffer was added at the equivalent of 1 g wet liver weight/ml.

Protein concentration was estimated for each preparation by the technique of Lowry et al., (1951). The microsomal suspension was either used immediately or stored frozen at −12°C, and used within a week without loss of the activity.

Cytochrome P-450 Determination

Cytochrome P-450 was measured as its carbon monoxide-binding complex according to Omura and Sato (1964). A 3-ml microsomal preparation was reduced with about 100 mg of sodium dithionite (Na₂S₂O₄). Carbon monoxide (CO) was then slowly bubbled through the reduced sample for about 1 min, and CO difference spectra were immediately determined with a Cary-14 recording spectrophotometer by scanning from 390−520 nm. The concentration was determined by the peak height of the spectrum between 450 and 490 nm (ΔOD450−490).

Epoxidation of Aldrin

The epoxidation of aldrin was done using a typical reaction mixture (Wong and Terriere 1965). Each incubation mixture contained 0.5 ml microsomal preparation, 1 ml of NADPH-generating solution (containing 2 μmoles of NADPH, 20 μmoles of glucose-6-phosphate dehydrogenase, and 25 μmoles of magnesium chloride, in phosphate buffer), and 5.5 ml of buffer. It was shaken in a Dubnoff metabolic shaker at 37°C for 10 min, before 10 μg of aldrin in 50 ml methyl cellulose was added as substrate compound. The incubation times were 0, 15, 30 and 60 min. To one of the incubation media, 10 −4M piperonyl butoxide was added, and incubated for 60 min. The reaction was...
MICROSOMAL FRACTION EXTRACTION AND EXPOXIDATION OF ALDRIN

TABLE 1
Protein and Cytochrome P-450 contents of microsomal preparations from midguts of spruce budworm larvae and rat livers used in aldrin epoxidation

<table>
<thead>
<tr>
<th>Source of microsomal preparation</th>
<th>Preparation no.</th>
<th>Protein (mg ml⁻¹)</th>
<th>Cytochrome P-450 (nmoles ml⁻¹/protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Midguts of spruce budworm larvae</td>
<td>1</td>
<td>2.13</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.03</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2.05</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.90</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>1.95</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>X ± S.D.</td>
<td>2.01 ± 0.09</td>
<td>0.25 ± 0.02</td>
</tr>
<tr>
<td>Rat livers</td>
<td>1</td>
<td>10.00</td>
<td>9.56</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>8.00</td>
<td>7.14</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>12.50</td>
<td>9.78</td>
</tr>
<tr>
<td></td>
<td>X ± S.D.</td>
<td>10.17 ± 2.25</td>
<td>8.83 ± 1.46</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

The classical method for preparing microsomal fractions is to centrifuge the mitochondria-free supernatant at about 105,000 g for 1 hour. Using this method, the microsomes could be isolated from homogenates of midguts of western spruce budworm larvae and of rat livers to make up the microsomal preparations. The typical spectral difference curves of the cytochrome P-450-CO complex of the microsomes from the two species are shown in Figures 1a and 1b. They clearly show that absorption maxima to be at 450 nm in both cases, thus indicating an almost complete absence of the degradation product cytochrome P-420, suggesting a fully active sample of the cytochrome. The protein and cytochrome P-450 contents were determined as index of "activity". Results are shown in Table 1. Since the main purpose was to test if the microsomal fractions activated insecticide in vitro, no attempt was therefore made to compare the specific contents of the protein and the cytochrome P-450 in the preparations from the two sources. It was enough to note the consistency of the preparations within each source, as each source of the preparations will represent a replicate in the epoxidation work done later in the study.
Several workers have reported the isolation of microsomal fractions from the midguts of some other lepidopterous larvae. It was interesting to note that the content of cytochrome P-450 based on µmoles/mg protein obtained from the western spruce budworm larvae in the present preparations was about the same as that obtained from the southern armyworm by Brattsten and Gunderson (1981), but was two or three times lower than those obtained from alfalfa looper and cabbage looper larvae, respectively (Farnsworth et al., 1981), and about two times higher than that obtained from the variegated cutworm (Yu et al., 1979).

Incubation of the microsomal preparations with the NADPH-generating system to test for epoxidation of aldrin in vitro gave significant results (Table 2). Incubation of up to 60 min did not result in reduced recovery. At least 88% of the added substrate compound was recovered, either epoxidized to dieldrin or as unchanged parent compound. The conversion occurred very rapidly: at least 71% of the total recovery after 15 min of incubation was dieldrin. At 15 min and 60 min after the incubation, the conversion levels were, 73.23% and 82.96% respectively of the total recoveries from rat liver MFO, and 71.14% and 79.36% for those incubated with MFO of midguts of spruce budworm larvae. There was no evidence that a product other than an epoxide (dieldrin) was produced. The chromatogram obtained only showed two distinct...

**TABLE 2**

*In-vitro* aldrin epoxidation by microsomal preparations at 37°C

<table>
<thead>
<tr>
<th>Incubation time (min)</th>
<th>Aldrin added (µg)</th>
<th>Aldrin (µg)</th>
<th>Dieldrin (µg)</th>
<th>%</th>
<th>Aldrin (µg)</th>
<th>Dieldrin (µg)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>9.63</td>
<td>0</td>
<td>0</td>
<td>9.19</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>10</td>
<td>2.69</td>
<td>6.63</td>
<td>71.14</td>
<td>2.65</td>
<td>7.25</td>
<td>73.23</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>1.99</td>
<td>7.50</td>
<td>79.03</td>
<td>2.09</td>
<td>7.50</td>
<td>78.21</td>
</tr>
<tr>
<td>60</td>
<td>10</td>
<td>1.99</td>
<td>7.65</td>
<td>79.36</td>
<td>1.69</td>
<td>8.23</td>
<td>82.96</td>
</tr>
<tr>
<td>60³</td>
<td>10</td>
<td>8.80</td>
<td>0</td>
<td>0</td>
<td>8.50</td>
<td>1.99</td>
<td>18.97</td>
</tr>
</tbody>
</table>

¹N = 2.

²Dieldrin. % of aldrin and dieldrin.

³Incubation with $1 \times 10^{-4}$M piperonyl butoxide.
The results obtained in the present study were comparable with those reported by several other workers. Williamson and Schecter (1970) showed that the rate of aldrin epoxidation by MFO obtained from whole larvae of tobacco budworm was rapid for 20 min and reached a maximum after 40 min. Wong and Terriere (1965) obtained similar results with rat liver microsomes. Using diazinon as the substrate, Shishido et al., (1972) found that the rate of the oxidative metabolism was higher with rat liver MFO than with that of American cockroach fat body.

**CONCLUSION**

Using the conventional method of preparation, microsomal fractions were able to be isolated from the midguts of western spruce budworm larvae. The microsomal preparations and those obtained from rat liver have in common that they show the presence of cytochrome P-450 (with spectral difference peak at 450 nm), they epoxidize aldrin with the requirements of NADPH-generating system, and are inhibited by piperonyl butoxide. Since liver microsomes have been shown to perform a variety of biological oxidations, it seems likely that microsomal enzymes in the midguts of western spruce budworm larvae would also catalyze a wide variety of transformations in metabolism of insecticides.

**REFERENCES**


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