Chapter 3

Insect Symbionts
A Promising Source of Detoxifying Enzymes

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Insects are the most common organisms on earth in terms of numbers of species. Many are exposed to a diversity of toxins, including plant polymers such as lignins. In feeding on natural hosts such as plants or fungi, insects must detoxify these chemicals to survive. Many insects contain symbiotic microorganisms that are known to provide nutrients for their host, but also may contribute to detoxification. Detailed work with the cigarette beetle and its symbiotic yeast indicated that activity towards a representative substrate (1-naphthyl acetate) is significantly reduced in symbiont-free insects. Larvae were also more susceptible to plant toxins when symbionts were absent. Cultures of the symbiont were able to utilize (and apparently detoxify) representative plant toxins, mycotoxins, and insecticides. Intact cells hydrolyzed parathion, and cell-free extracts dechlorinated 1-chloro, 2,4-dinitrobenzene. The 1-naphthyl acetate esterase produced in culture was relatively resistant to inhibition by paraoxon, was relatively stable in organic solvents, and could be induced by exposing the cells to toxins. The tremendous variety of symbionts in other insects suggests that these microorganisms are a promising source of detoxifying and other novel enzymes.

Insects are the most successful group of organisms on earth; an estimated 726,000 species have been identified (1), and an equal or greater uncategorized number are thought to exist in the tropics and other areas. Due to their feeding habits, insects may potentially encounter a diversity of toxins. Both plants (2) and microorganisms such as fungi (3) make a diversity of chemicals that act as agents to defend against insects and other predators, including plant polymers such
as lignins that interfere with digestion. Those insects that feed on plants or fungi must be able to detoxify these toxins, whether by excretion, sequestration, or metabolic conversion in order to successfully feed on their hosts. The diversity of defensive compounds includes classes such as alkaloids, cardiac glycosides, cyanogenetic compounds, flavonoids, phenolic compounds, and terpenoids in higher plants, and such mycotoxins as aflatoxin B₁, trichothecenes (e.g. T-2 toxin), and tremor-inducing compounds in fungi. Insects are known to detoxify enzymatically representatives of all major categories of plant toxins (4, 5). Detoxification of mycotoxins by insects can be inferred, based on apparent resistance of insects feeding on mycotoxin-contaminated materials, or documented by actual detoxification studies (6).

Exposure to natural toxins probably enables insects to adapt more readily to man-made materials such as insecticides (7). Insects have developed resistance to cyanide, chlorinated hydrocarbons, organophosphates, carbamates, synthetic pyrethroids, and other insecticides (8). This is not surprising when considering the same complex of detoxifying enzymes, mainly represented by hydrolytic, conjugative, and oxidative enzymes (9) is capable of detoxifying natural toxins as well as man-made materials. This ability is due to appropriate enzymes and/or isozymes that results in broad-substrate capabilities. For insects that feed on a wide variety of hosts (polyphagy), the spectrum of toxins that can be dealt with is truly remarkable.

Detoxification by Insect Symbionts

In an incisive discussion of insect-microbe relationships, Jones (10) postulated that insect-microbial associations, known to involve catabolic (e.g. cellulose-degrading) and anabolic (e.g. biosynthesis of vitamins, sterols, and amino acids) processes necessary to the survival of the host, could also include detoxification abilities. Most investigations in this area have been limited (11). Nevertheless, some studies indicate detoxification of terpenoids (12, 13), lignin (14), and insecticides (15) by apparent symbionts. The roles other microbial symbionts of insects play in detoxification are discussed below.

Detoxification by Symbionts of the Cigarette Beetle

The cigarette beetle feeds on a wide variety of plant material (16), indicating a broad-spectrum ability to deal with plant toxins, including mycotoxin ochratoxin A (17). When the symbionts, Symbiotaphrina kochii Jurzitza ex. W. Gams and v. Arx c, were removed from the insect by hatching surface-sterilized eggs (the means of generation to generation transfer of the yeast) or by treatment with fungicides, the resulting symbiont-free insects became more susceptible to representative plant toxins. Qualitative screening procedures were used to test toxins as sole carbon sources for growth of the cigarette beetle symbionts. By this method, we found the symbionts could utilize (and apparently detoxify) a diversity of plant flavonoids and phenolics including rutin, quercetin, caffeic acid, tannic acid, and gallic acid (11),
as well as insecticides such as parathion, diazinon, and malathion; herbicides such as dinoseb, glyphosate, and 2,4-D; mycotoxins such as sterigmatocystin, (an aflatoxin precursor), ochratoxin A (a carcinogen), deoxynivalenol (a trichothecene), mycophenolic acid and citrinin; and meal toxins such as amygdalin, phytic acid and stachyose (18, Shen and Dowd, unpublished data). Presently we are working on demonstrating actual detoxification of representative toxins and on identifying and characterizing enzymes of interest. For example, ca. 4 pmole of \textsuperscript{14}C parathion can be metabolized (to a \textsuperscript{4}-nitrophenol hydrolysis product) in one hour by a suspension of \textsuperscript{10} cells (Shen and Dowd unpublished data). Dechlorination of 1-chloro, 2,4-dinitrobenzene, by a cell-free extract (\textsuperscript{1000} g supernatant) occurred at a rate of 20 nmole/min/mg protein (Shen and Dowd unpublished data). This dechlorination reaction appears to be performed by a glutathione transferase-like enzyme, since the reaction is glutathione dependent. Both \(\alpha\)-glucosidase and \(\beta\)-glucosidase activity have also been detected (Shen and Dowd unpublished data).

Most of our work on enzyme properties has concentrated on 1-naphthyl acetate esterase. The enzyme responsible for most of the activity has a specific activity of ca. 1 \(\mu\) mole/min/mg protein and a molecular weight of ca. 38,000 when partially purified by gel filtration chromatography (Shen and Dowd unpublished data). Isoelectric focusing indicated a pI of 4.6-4.8 for the major source of enzyme activity (Shen and Dowd unpublished data). This enzyme is interesting in that it appears to have relatively few external charges, because it moves relatively slowly by conventional polyacrylamide gel electrophoresis (Rf of ca. 0.33 in a 7.5% gel, with bromophenol blue as a tracking dye), in spite of its relatively low molecular weight (Shen and Dowd unpublished data). Although it is not particularly thermostable (inactivated at ca. 40\(^{\circ}\)C.), it is stable when refrigerated or frozen (at least 1 week) (Shen and Dowd unpublished data). Another interesting property of the enzyme is that the activity is fairly stable in organic solvents; the rate of hydrolysis in a 50:50 acetone:buffer solution (after 1 hour preincubation) is still ca. 30% of control (Shen and Dowd unpublished data). The activity is somewhat resistant to inhibition by organophosphorous pesticide derivatives such as the extremely active paraaxon (ca 50% inhibition at \(10^{-4}\) M), but is more susceptible to inhibition by heavy metal ions, such as Hg\(^{2+}\) (ca. 80% inhibition at \(10^{-4}\) M) (Shen and Dowd unpublished data). Thus, the 1-naphthyl acetate activity from the cigarette beetle yeast does have properties, especially the solvent stability, that are likely to promote use for decontamination of lipophilic toxins. In addition, this activity (and in some cases additional molecular forms detected by gel electrophoresis) can be stimulated (based on higher activity relative to solvent controls) by malathion, \(\beta\)-pinene, griseofulvin, and flavone (1.8 fold); a relatively high level for a hydrolytic enzyme (19).

Potential for use of Insect Symbionts in Detoxification

The cigarette beetle symbiont has many desirable properties for use in decontamination. As indicated earlier, it has a broad-spectrum
ability to detoxify, although we do not yet know if these reactions will occur with mixed substrates. The evidence of stimulation of 1-naphthyl acetate hydrolysis, however, suggests appropriate enzyme activity will be present with substrate mixtures as well. Since it is an obligate symbiont, it is not likely to persist in the environment when used apart from its host. Histochemical work indicates the symbiont appears to secrete or, at least, have externally bound hydrolytic enzymes, which overcomes some potential problems when detoxifying enzymes are located internally, and toxins are not taken up. However, the organism also appears to take up phenolic acids, (20). It is not known to produce any toxins and, in fact, it is known to produce B-vitamins, sterols and amino acids for its insect host (21). In many situations, however, its relatively slow growth compared to other microorganisms is likely to be a disadvantage since it can be overgrown by other organisms. In collaboration with Dr. Nancy J. Alexander at our lab, we are attempting to obtain faster growing strains or recombinant organisms that retain detoxifying capabilities.

Literature Cited

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