Genetic Analysis of Factors Controlling High-Level Expression of Cytochrome P450, CYP6D1, Cytochrome b5, P450 Reductase, and Monooxygenase Activities in LPR House Flies, *Musca domestica*

Nannan Liu and Jeffrey G. Scott

Received 21 July 1995—Final 29 Jan. 1996

To understand better the biochemical genetics of cytochrome P450 monooxygenase-mediated insecticide resistance, we examined the microsomal monooxygenases in insecticide-susceptible (aabys) and pyrethroid-resistant (LPR) house fly strains, as well as 15 house fly lines derived from crosses of LPR and aabys. In comparison to the aabys strain, LPR had higher levels of total cytochromes P450, cytochrome b5, P450 reductase, CYP6D1, and three P450 monooxygenase activities: 7-ethoxycoumarin O-deethylase (ECOD), methoxyresorufin O-demethylase (MROD), and aromatic hydrocarbon hydroxylase (AHH). The elevated levels of cytochrome b5 were linked to factors on autosomes 1 and 2. This is similar to previous reports on monooxygenase-mediated resistance and is consistent with the idea that elevated cytochrome b5 levels are involved in monooxygenase-mediated resistance in the LPR strain. Linkage of the elevated P450 reductase is different from that of monooxygenase-mediated resistance. Strains having high levels of CYP6D1 (i.e., like LPR) had high levels of P450 reductase, while strains having intermediate levels of CYP6D1 also had high levels of reductase. Therefore, there is no clear evidence that the elevated P450 reductase in the LPR strain is required for the increased monooxygenase activity. Overexpression of total cytochromes P450, CYP6D1 (mRNA and protein), and CYP6D1-mediated monooxygenase activities (MROD and AHH) in LPR microsomes was linked to a combination of factors on autosomes 1 and 2. This demonstrates that increased expression of CYP6D1 in the LPR strain is both cis regulated by a factor(s) on autosome 1 and trans regulated by a factor(s) on

1 Department of Entomology, Comstock Hall, Cornell University, Ithaca, New York 14853-0901.
2 To whom correspondence should be addressed.
The correlation between the overexpression of CYP6D1 mRNA and protein suggests that CYP6D1 expression is regulated transcriptionally. Monooxygenase-mediated resistance in LPR is controlled by factors on autosomes 1 and 2, which supports previous claims that CYP6D1 is responsible for monooxygenase-mediated resistance in the LPR strain.

KEY WORDS: pyrethroid resistance; Insecta; cytochrome P450 monooxygenases.

INTRODUCTION

The microsomal cytochrome P450 monooxygenases (P450 monooxygenases) are important in detoxification or activation of xenobiotics and are an important mechanism by which insects become resistant to insecticides (Wilkinson, 1983; Oppenoorth, 1985). The P450 monooxygenases have three major components: an unknown number of cytochrome P450 isoforms, which act as the substrate binding proteins (and terminal oxidases) (Golly et al., 1988); NADPH-cytochrome c (P450) reductase, which transfers electrons from NADPH to cytochrome P450; and cytochrome bs, which may donate the second electron to cytochrome P450 or may modulate P450 monooxygenase activity in an isoform-specific manner (Peterson and Prough, 1986; Zhang and Scott, 1994). Monooxygenase-mediated resistance is often associated with increased levels of total cytochromes P450 and P450 monooxygenase activities (Oppenoorth, 1985; Scott, 1991), cytochrome bs and/or P450 reductase (DeVries and Georghiou, 1981; Lee and Scott, 1989b; Scott and Georghiou, 1986b; Scott et al., 1990). Cytochrome bs has also been implicated in certain P450 monooxygenase activities in house flies (Zhang and Scott, 1994). However, the role of elevated levels of cytochrome bs and P450 reductase in monooxygenase-mediated resistance is not well understood.

In insects, only one P450 has been linked clearly to insecticide resistance. This P450, CYP6D1, is responsible for monooxygenase-mediated pyrethroid resistance in the LPR house fly strain (Scott and Georghiou, 1986a; Wheelock and Scott, 1992b). CYP6D1 has been sequenced (Tomita and Scott, 1995; Tomita et al., 1995) and mapped to autosome 1 (Liu et al., 1995). CYP6D1 protein and mRNA levels are nine-fold higher in the LPR strain compared to susceptible strains (Scott and Lee, 1993; Tomita et al., 1995). However, linkage analysis of the factors responsible for overexpression of CYP6D1 protein and mRNA has not yet been investigated.

Certain monooxygenase activities are characteristic of some cytochromes P450 (Burke and Mayer, 1974; Lubet et al., 1985). For example, ethoxyresorufin O-deethylase (EROD) activity was characteristic for P450 1A1 found in polycyclic hydrocarbon-induced rat liver (Burke and Mayer, 1974) and pentoxyresorufin O-depentylase (PROD) activity was specific for