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8 Insect cytochrome P450s: Thinking beyond detoxification

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Abstract

The cytochrome P450-dependent monooxygenases are an extremely important metabolic system involved in the metabolism of xenobiotics and endogenous compounds. The number of P450s in a given insect species currently ranges from 48 in Apis mellifera to 164 in Aedes aegypti. For nearly forty years studies of insect P450s have focused primarily on metabolism of xenobiotics, including studies of insecticide resistance, insecticide metabolism and insect-plant interactions. This has led to the perception, held by at least some in the scientific community, that the majority of P450s in a given species are primarily for detoxification of xenobiotics. However, there are an

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ever increasing number of studies that indicate P450s have a much broader role in insects. Herein, it is postulated that metabolism of xenobiotics might be the role for only a minority of the P450s in a given insect species. Examples of P450 metabolism (known and predicted) of compounds that are not xenobiotics are presented.

1. Introduction

The cytochrome P450-dependent monooxygenases (monooxygenases) are an extremely important metabolic system because of their involvement in regulating the titers of endogenous compounds such as hormones, fatty acids and steroids, and in the catabolism and anabolism of xenobiotics such as drugs, pesticides and plant toxins. For example, P450s can be involved in intermediate steps of biosynthetic pathways, activation of prohormones to hormones and in the catabolism of hormones. Similarly, P450-mediated metabolism can result in detoxification of insecticides such as pyrethroids, or can be involved in both the bioactivation and detoxification of insecticides such as organophosphates.

Monooxygenases are found in virtually all aerobic organisms, including organisms as diverse as insects, plants, mammals, birds and bacteria [1]. Cytochrome P450 (P450) is a hemoprotein which acts as the terminal oxidase in monooxygenase systems. In eukaryotes, most P450s are found in the endoplasmic reticulum or mitochondria. P450s in the mitochondria use a different electron transport system (than the P450s in the endoplasmic reticulum) and are more closely related to the primitive prokaryote P450s than eukaryote P450s [2]. Monooxygenases are unusual in that they can oxidize widely diverse substrates and are capable of catalyzing a large array of reactions [3-5]. This is because each species contains numerous P450s and because of the broad substrate specificity of some isoforms.

One remarkable feature of the monooxygenases is the large variation in substrate specificity of different P450s. For example, human CYP1A1 can metabolize more than 20 substrates, while CYP7A1 has only one known substrate [4]. Certain P450s have overlapping substrate specificity (e.g. CYP2C subfamily in humans) [4] so that a single compound may be subject to metabolism by multiple P450s. In addition, some P450s produce only a single metabolite from a given substrate, while other P450s can produce multiple metabolites. These features are further complicated by the fact that the change of a single amino acid in a P450 (i.e. Cyp2a4) can alter its substrate specificity [6]. More information about known monooxygenase substrates can be found in several reviews [3, 4, 7-14].

2. Insect cytochrome P450s

The spectrophotometric detection of P450 (from mammalian liver) was first reported in 1965 [15], and two years later was found in insects [16].

Research began to suggest that there was not a single cytochrome P450, and many researchers found convincing evidence for multiple P450s in several insect species (refs in [7]). The majority of studies of insect P450s from the 1960s until the 1980s focused on insecticide metabolism and insecticide resistance. Starting in the 1970s several excellent studies were also carried out to examine the role of P450s in plant-insect interactions, although these were also aimed at understanding the P450s involved in toxin metabolism (primarily metabolism of toxic plant alleochemicals). Thus, for nearly four decades studies of insect P450s have focused primarily on toxin metabolism.

Insect monooxygenases can be detected in a wide range of tissues. Highest monooxygenase activities are usually associated with the midgut, fat bodies and Malpighian tubules [9], but again the expression of individual P450s can vary between these tissues [17]. For example, CYP6D1 is found in all tissues of adult house fly [18, 19], while CYP6L1 is expressed only in the testis of adult *Blattella germanica* [20].

Dramatic variation in monooxygenase activities and P450 levels occur during the development of most insects. In general, total P450 levels are undetectable in eggs, rise and fall in each larval instar, are undetectable in pupae and are expressed at high levels in adults [7]. The patterns of expression of individual P450s can vary within and/or between life stages [17, 21-24].

During the 1970s and 1980s a large number of P450s were purified and characterized (primarily from mammals) and the number of P450s present in a given organism became a topic of considerable debate. As increasing numbers of P450s were isolated and named by different groups, there became more and more confusion in the literature regarding the relatedness of these isoforms. In 1987 a new nomenclature was proposed [25] and, although subject to revisions [26-28], it remains the preferred system [29, 30]. Sequences are named *CYP* (for <u>CY</u>tochrome <u>P</u>450), followed by a number, a letter and a number indicating the family, subfamily and isoform, respectively [29]. Alleles are designated *v1*, *v2*, etc. Since this nomenclature is based on overall sequence similarity, no information regarding the function of a P450 should be assumed by its classification within this system [31]. Overall, there is limited similarity between the P450s (in terms of overall amino acid similarity) between species, unless they are very closely related (such as insects within the same genus [32]), making it clear that the P450 gene superfamily is rapidly evolving. However there are a few exceptions, especially among the P450s involved in biosynthetic pathway of 20-hydroxyecdysone, which are highly similar between insects [24].

The first insights into the number of P450s in eukaryotes came with the completion of the human genome, where 57 P450s were found [33, 34]. The completion of the first insect genome (*Drosophila melanogaster*) revealed 87 P450s in this species [35]. A summary of the ever growing number of insect P450s is shown in Table 1. Currently the number of P450s (not counting pseudogenes)

Table 1. Total number of P450s (excluding pseudogenes) determined from the genomes of six insect species, relative to four mammals.

Species	P450s
Aedes aegypti	164
Tribolium castaneum	134
Anopheles gambiae	105
Drosophila melanogaster	84
Drosophila pseudoobscura	79
Apis mellifera	48
Mus musculus	102
Ratus norvegicus	89
Homo sapiens	57
Canis familiaris	54

in different insect species ranges from 48-164, but this information changes frequently. Thankfully, Dr. David Nelson maintains a very useful web site (http://drnelson.utmem.edu/CytochromeP450.html) where current information about the P450s in different species is available.

3. Why are there so many P450s in some insect species?

Over the last 40 years the study of insect P450s has evolved from "Do insects have cytochrome P450?" to "How many P450s do insects have?" to "Why do insects have a large number P450s?" It is this latter question that will be the focus of the remainder of this paper.

There are two general schools of thought about why insects have so many P450s. The first group considers xenobiotic detoxification to be the primary purpose of insect P450s. In this school of thought, detoxification of the numerous environmental and dietary toxins constitutes the primary selective force for maintenance of P450 diversity (between species) as well as the presence of numerous P450s in each species. The root of this thinking is logically traced back to the original studies on P450s involved in metabolism of insecticides and plant toxins (see above). However, there is an alternative school of thought. This group believes the majority of P450s in a given insect are not involved in xenobiotic detoxification, but rather in the metabolism of other compounds.

Given the importance of human P450s to health, there has been a very large amount of research to identify the specific P450(s) involved in xenobiotic (especially drug) metabolism. Of the 57 P450s found in humans, it has been estimated that nine of the P450s are responsible for 75% of the phase I metabolism in humans [36]. What is the role of the other 48 P450s? Certainly there will be other human P450s involved in xenobiotic detoxification, but how many? Is it reasonable that another 25-35 P450s are needed to account for the remaining 25% of the xenobiotic metabolism? Would 10-15 be more likely?

If we are to extrapolate the approximate number of insect P450s that are involved in xenobiotic metabolism (based on the data from humans) we could estimate that of the 164 P450s in *Aedes aegypti,* perhaps 100 or more may be involved in metabolism of compounds other than xenobiotics. What are these other groups of substrates and how many P450s might be involved with each group? A highly speculative approximation is provided in Table 2. Examples of P450 metabolism of substrates other than xenobiotics are provided below. It should be pointed out that some P450s may be able to metabolize both xenobiotics and endogenous compounds, making their classification more difficult.

Biosynthesis of hormones is one of the processes in which the role of insect P450s has been well documented. For example, the work of Larry Gilbert and his co-workers have already identified five P450s that are involved in the biosynthetic pathway from cholesterol to 20-OH ecdysone [24]. There are likely to be many more P450s involved in cholesterol metabolism, because at least 14 P450s have been found to be involved in the processing of cholesterol and its metabolites in mammals [37, 38]. In insects, P450s are also involved in juvenile hormone biosynthesis [39] and potentially in aggregation pheromone biosynthesis [40].

The first sex-specific insect P450 was found in the male reproductive system of *Blattella germanica* [20], and subsequently male-specific P450s were found in *D. melanogaster* [41] and *Ips paraconfusus* [40]. The role of the male specific P450s in *B. germanica* and *D. melanogaster* are not known. In *I. paraconfusus* the proposed role is in aggregation pheromone biosynthesis [40]. Using microarray analysis of *D. melanogaster* females, it was shown that 28 P450s were regulated (i.e. expression changed more than 2-fold) by mating, including 11 that were regulated exclusively by sperm and two that were regulated exclusively by accessory gland proteins transferred from the male [42]. The functions of these P450s in the reproductive process, as well as the

Table 2. A highly speculative estimation of the relative percentages of P450s involved in various biological processes of a generalized insect.

% of P450s involved	Process
65	Biosynthetic processes
	Hormone and pheromone biosynthesis (35-50%)
	Reproduction (5-15%)
	Pigmentation (5-10%)
	Other (10%)
30	Xenobiotic metabolism
5	Sensory physiology

identification of other sex specific (and reproduction related) P450s deserves further attention.

Several recent studies have shown that insect P450s are found in sensory organs associated with ”smell” and “taste” [43-47]. Evidence is accumulating that these P450s are responsible for degrading the molecules that are detected by the sensory neurons. The rapid turn-over of these stimuli by P450s is likely necessary for the normal functioning of the sensory organs. These P450s may be unique, because they do not appear to be located in the endoplasmic reticulum or mitochondria (W. Leal, personal communication).

It is well known that plants utilize P450s in the biosynthetic pathways of pigments [48]. For example, dramatic variation in Petunia colors arise from the up or down regulation of P450 genes [48]. Insects that synthesize their own pigments do so using some of the same starting materials as plants [49], providing reasonable conjecture that insects also have P450s involved in pigment biosynthesis. However, no P450s with this putative function have yet been identified in insects.

There may well be other functions of insect P450s that we have not yet discovered. For example, what is the role of the brain specific *Cyp4g15* [50] in *D. melanogaster*? Given that P450 expression is temporally regulated in termites [51], do they have a role in caste differentiation? Identification of unique P450s, as well as the expression patterns of P450s will be an important first step toward discovering new roles for P450s in insects.

4. The future of insect P450 studies

Identification of the P450 responsible for a specific phenotype has always been a difficult scientific challenge, and with as many as 164 P450 genes in an insect species (*Ae. aegypti*) the task is indeed daunting. However the discovery of P450s involved in metabolism of endogenous compounds can be greatly facilitated by use of microarrays and proteomics. For example, Kevin White and his colleagues conducted a microarray analysis of gene transcription throughout the development of *D. melanogaster* [52]. This data set readily identifies the developmental changes in expression and it includes many P450s. In the case of *An gambiae*, an array was developed which included all of the P450 genes [53]. This array was named the “detox chip”, but in reality it can be far more powerful and significant by using it to identify transcripts that are sex, tissue, age, or condition (such as blood fed) specific. Using this array as a “physiology chip” is likely to have far greater implications in understanding *An. gambiae*, and in potentially defining essential P450s that might be used in the development of novel control methods.

The future for studies of insect P450s is very promising. While studies on the xenobiotic metabolizing P450s will continue to be important and significant, it is likely that much of the new (and perhaps revolutionary) information will come from studies of the P450s that metabolize compounds that are not xenobiotics.

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