INSECT CYTOCHROME P450

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INTRODUCTION

Cytochrome P450 (P450) dependent monooxygenase systems occur in most if not all organisms, from bacteria to mammals and higher plants. In insects, the biochemical functions ascribed to this enzyme system include the metabolism of a wide variety of endogenous substrates such as hormones and the oxidation of xenobiotics such as pesticides and secondary plant metabolites. Insect monooxygenase systems appear to be involved in many different physiological processes with roles in growth and development, insecticide resistance and in the interaction of phytophagous insects with multiple host plants. A number of reviews have appeared on these topics in recent years (1-5). Due to its importance in the study of the biochemistry and genetics of insecticide resistance the house fly (Musca domestica) has been a useful model species for the study of insect monooxygenases. The purification and characterization of multiple cytochrome P450 isozymes from this species, as well as their role in insecticide resistance is discussed. Recently with the cloning and sequencing of a P450 isozyme from the housefly (6,7) a beginning has been made in the study of the molecular biology of insect cytochrome P450. Heliothis spp are also useful model species since their polyphagy has led to the study of the role of P450 in insect-plant interactions.

DISTRIBUTION AND GENERAL PROPERTIES OF INSECT MONOOXYGENASES

P450 was first demonstrated in insects by Ray (8,9) and is now known to be distributed in many tissues and species, the highest concentrations being generally found in the midgut, fat body and Malpighian tubules. At the sub-cellular level, insect monooxygenases are found in both the endoplasmic reticulum (10,11), (the microsomal monooxygenases), and in mitochondria (12). As in mammals, insect microsomal monooxygenase systems consist of two components, the flavoprotein NADPH-cytochrome P450 reductase and the heme protein, cytochrome P450, the latter existing as multiple
isozymes displaying overlapping substrate specificities. The mechanisms of action appear to be similar to those seen in other animals. Cytochrome b5 is present in insect microsomes and, as in mammals and fish (13,14), appears to be involved synergistically in the oxidation of a number of substrates eg. benzo(a)pyrene and lauric acid (15,16) possibly supplying one electron from NADH via cytochrome b5 reductase. Spectral interactions between insect P450 and monooxygenase substrates and inhibitors are much the same as those seen in mammalian systems. A characteristic type I binding spectrum is often difficult to demonstrate but, when present, has an absorption maximum at 385-390 nm and a minimum at 420 nm. It is seen with the oxidized form of the cytochrome and substrates while type II binding spectra, with a maximum at 420-425 nm and a minimum at 390-410 nm, are seen with many nitrogen containing inhibitors. The reduced cytochrome shows the well known carbon monoxide difference spectrum and a type III spectrum with ethyl isocyanide and methylenedioxyphenyl compounds consisting of two pH dependant peaks at 430 nm and 455 nm. Spectral studies with insect microsomes have been considered in detail in recent reviews (3,17).

METABOLIC REACTIONS

Metabolism of Hormones and Hormone Analogues

P450-dependent monooxygenases play an important role in the synthesis and degradation of insect hormones. The terpenoid juvenile hormones (JHs), which suppress molting, and the steroid ecdysones, which promote it, have both been studied. The final step of JH-III (methyl,10,11-epoxy-3,7,11-trimethyl-trans-2,6-dodecadienoate), synthesis, an epoxidation, occurs in the corpora allata of several insects. This reaction has been shown to be due to a P450 dependent monooxygenase in microsomes from the corpora allata of both Blaberus giganteus (18) and Locusta migratoria (19). The anti-JHs, precocenes I and II (7-methoxy-2,2-dimethylchromene and 6,7-dimethoxy-2,2-dimethylchromene) which cause precocious molts and destroy the corpora allata are thought to act as "suicide substrates" for a form of P450 (20) found in this endocrine organ. Oxidative degradation of JH I (E,E,cis-methyl-10,11-epoxy-7-ethyl-3,7-dimethyl-2,6-tridecadienoate) has been demonstrated in housefly and blowfly microsomes (21) and JH I acts as a competitive inhibitor of several P450 dependant oxidations in housefly microsomes (22). Moreover, JH analogues of the alkyl 3,7,11-trimethyl-2,4-dodecadienoate type are easily oxidized by fly microsomes (23,24). However, despite this facile oxidation of JHs in vitro, much of the metabolism in vivo appears to be due to epoxide hydrolase and esterase (25,26). Oxidative metabolism of JH III could not be demonstrated in housefly microsomes (16) and only the JH dihydrodiol, JH acid and JH acid dihydrodiol, characteristic of epoxide hydrolase and esterase attack, were recovered.

Ecdysteroids that play a major role in insect development and metamorphosis are a group of 8 or 9 closely related C27 steroids whose synthesis and degradation appears to be carried out via a number of mitochondrial and microsomal monooxygenases present in different tissues (27). The best studied of these reactions is ec dysone 20-hydroxylase which