# Plant metabolism of xenobiotics

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Metabolism of foreign chemicals (xenobiotics) by plants generally proceeds in three phases: transformation, conjugation and compartmentation. The participating enzymes have numerous similarities not only to the enzymes of normal secondary plant metabolism, but also to those of xenobiotic metabolism in mammalian liver. Plants may therefore be considered as a 'green liver', acting as an important global sink for environmental chemicals.

**BOTH PLANTS AND ANIMALS** are exposed to numerous potentially toxic foreign chemicals (xenobiotics). In animals, a main site of xenobiotic metabolism is the liver where the normally nonpolar and thus lipophilic xenobiotics are metabolized to more soluble forms that are then typically excreted in urine. Xenobiotic metabolism in the liver can be divided into three phases. Phase I enzymes oxidize, reduce or hydrolyse the substrate, introducing a reactive group that can serve as a 'handle' for subsequent conjugation to moieties such as glutathione or glucuronate catalysed by phase II enzymes. In phase III, the hydrophilic conjugates are excreted in urine or faeces.

Plant xenobiotic metabolism can also be divided into three phases, although plants have no effective excretion pathways. Instead, transformation (phase I) and conjugation (phase II) reactions are coupled to internal compartmentation and storage processes (phase III). Cellular storage sites are the vacuole (for soluble conjugates) and the cell wall (for 'insoluble conjugates'). These basic processes have been reviewed in Ref. 1.

Plants further resemble the animal liver by being able to metabolize a great number of xenobiotics, including polychlorinated and polycyclic hydrocarbons. It was recognized some 15 years ago that plants contain enzymes that resemble, by a number of criteria, two major enzyme systems of the liver, cytochrome P450 monooxygenases and glutathione transferases[2]. Plants may therefore function as 'green livers'[2] and act as a global sink for environmental pollutants through the metabolism of such chemicals. This proposal is analogous to the concept of plants as the 'green lungs' of the earth due to their production of oxygen through photosynthesis. It is well worth emphasizing that the use of 'green liver' is more of an ecological analogy than a specific anatomical description. The data summarized in this review considerably support and extend the green liver concept.

## Enzymes for xenobiotics

As a main site of xenobiotic metabolism in mammals, the liver contains several families of enzymes including the cytochrome P450 monooxygenases[3], glutathione transferases[4] and UDP-glucuronosyltransferases[5]. Similar enzymes have been detected in plants and were active against pesticides or other xenobiotics (see Table I). Plant *O*-glucosyl and *O*-malonyl transferases as well as *N*-glucosyl and *N*-malonyl transferases with specificity for chlorinated xenobiotics and a carboxylesterase with specificity for a plasticizer chemical have also been characterized (Table I). The specific substrates listed in Table I for plant cytochromes P450, glutathione transferases and carboxylesterases are also utilized by liver enzymes. The *O*- and *N*-glucosyl and *O*- and *N*-malonyl transferases have no mammalian counterpart. However, the negatively charged *O*-malonyl-(*O*-glucosyl)-double conjugates may correspond to the likewise negatively charged mammalian *O*-glucuronosyl conjugates. No microsomal UDP-glucuronosyltransferases involved in xenobiotic metabolism have been reported for plants. However, plant microsomal UDP-glucuronosyltransferases for cell wall biosynthesis are well known. Only those enzymes that have been highly purified are listed in Table I. Additional herbicides and xenobiotics have been successfully metabolized by what appear to be further plant enzyme species (see Ref. 6 for review). The enzyme species listed in Table I for natural substrates play defined roles in normal plant secondary metabolism. In contrast, the physiological role of the distinct purified enzyme species for xenobiotic substrates is presently obscure.

The purified xenobiotic enzymes from plants are of similar molecular masses to those of the liver, i.e. cytochromes P450 (~55 kDa), NADPH: cytochrome P450 reductase (~80 kDa), glutathione transferases (two subunits, ~25 kDa each). There is also great similarity in molecular mass (~50 kDa) and isoelectric points (pI 4.5–6.5) between the various plant *O*- and *N*-glucosyl- and *O*- and *N*-malonyltransferases for both xenobiotic and natural substrates (see references in Table I). These similarities are consistent with the existence of plant enzyme families. In a few cases cDNA sequences have been determined. The cDNA sequence of a plant *O*-glucosyltransferase (for natural flavonol substrates) has been determined[19], but no comparison to other sequences in the protein sequence databases has been reported. The cDNA sequence of an avocado cytochrome P450 species of unknown function had characteristics of a typical P450: an amino-terminal hydrophobic membrane anchor, a conserved binding domain in the carboxy-terminal region and 'patches of similarity' to various P450 family members[20]. However, the avocado P450 shared less than 40% positional identity with any characterized members of the cytochrome P450 families and was therefore placed in a separate family[20]. Maize glutathione transferase enzymes have significant protein sequence homology to the rat and human enzymes[10], and to a glutathione transferase from *E. coli*[21]. The maize glutathione transferase enzyme III for the herbicide alachlor contained a region of 44 amino acids that shares 66% positional identity with

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the analogous region in a glutathione transferase from *Drosophila*[22]. Like the cytochrome P450 species, the plant glutathione transferase enzymes seem to belong to an enzyme superfamily, and possess evolutionary relationships to bacteria as well as mammals[21,22]. The kind of selection pressure that has led to the evolution of these enzyme families is unknown, since widespread environmental pollution has begun only in this century and no obvious 'natural pollutant analogues' are known.

**Table I. Plant enzyme classes**

| Enzyme class | Xenobiotic substrates | Refs | Natural substrates | Refs |
|---|---|---|---|---|
| Cytochromes P450 | 4-Chloro-*N*-methyl-aniline | 7 | Cinnamic acid, pterocarpans | 8 |
| Glutathione transferases | Fluorodifen,<br>alachlor, atrazine | 9<br>10,11 | Cinnamic acid | 9 |
| Carboxylesterases | Diethylhexylphthalate | 12 | Lipids, acetylcholine | 12 |
| *O*-Glucosyltransferases | Chlorinated phenols | 13 | Flavonoids, coniferyl alcohol | 13,14 |
| *O*-Malonyltransferases | β-D-Glucosides of pentachlorophenol- and of 4-hydroxy-2,5-dichlorophenoxyacetic acid | 13,15 | β-D-Glucosides of flavonoids and of isoflavonoids | 14,15 |
| *N*-Glucosyltransferases | Chlorinated anilines, metribuzin | 13,16 | Nicotinic acid | 17 |
| *N*-Malonyltransferases | Chlorinated anilines | 13,18 | 1-Aminocyclopropyl carboxylic acid,<br>D-amino acids,<br>anthranilic acid | 18 |

## Inducibility

Many of the plant xenobiotic enzymes are constitutive, since extensive metabolism of numerous xenobiotics has been observed in plant cell cultures without any prior induction treatment. In contrast, certain subsets of liver detoxifying enzymes are induced above their constitutive levels by foreign agents of which phenobarbital is best known[3–5]. Phenobarbital and other inducers can also induce plant cytochromes P450 (Ref. 23), but these induction processes have not been thoroughly characterized. The best example of induction of detoxifying enzymes in plants is the induction of glutathione transferases (e.g. the enzyme for alachlor) by herbicide antidotes, such as naphthalic anhydride[10,11,24]. Interestingly, transcription of the gene encoding a glutathione transferase was strongly induced upon infection of wheat with the powdery mildew fungus *Erysiphe graminis*[25].

## Processing pathways

Natural and xenobiotics *O*-β-D-glucosides are often further processed in plants by addition of an *O*-malonyl substituent[13–15]. The chemically labile *O*-malonyl substituent is, however, easily lost during conventional isolation procedures. Facile loss of the malonyl substituent has, for example, been demonstrated for the (*O*-malonyl)-*O*-glycosides of the apiin complex and the (*O*-malonyl)-*O*-glucoside of pentachlorophenol[26]. The malonyl substituent may act as a signal for transport into the vacuole[15,27], or into the extracellular space[26,28].

Early studies indicated that plants further process the glutathione conjugate of the herbicide atrazine and that the processing products differed from the mercapturic acids that are the final processing products in mammalian systems. The present list of plant processing products from the primary glutathione conjugates includes *N*-malonyl-cysteinyl, thiolactyl, thiol, methylthio[11] and additional unknown soluble and insoluble derivatives. Processing reactions are thus another feature that plant and mammalian systems of xenobiotic metabolism share.

## Compartmentation processes

As mentioned above, phase III of plant xenobiotic metabolism involves compartmentation and storage, rather than excretion as in mammalian xenobiotic systems. Three major pathways have so far been detected for phase III in plants, but the underlying mechanisms are still unknown: (1) export into cell vacuoles (e.g. Ref. 27); (2) export into the extracellular space (e.g. Refs 26,28); (3) deposition into lignin or other cell wall components (reviewed in Ref. 29). These processes explain much of the so-called 'bound' residue fraction in plants.

## Ecological plant biochemistry

In the past 15 years, a branch of plant biochemistry has developed known as 'ecological biochemistry'[30]. Plants produce a vast array of metabolites that are at first sight not essential for their own physiological function. The recognition that some of these secondary metabolites serve as natural pesticides protecting the host plant from predators has prompted their exploitation as a potential source of drugs, insecticides and herbicides for use by man. The exposure of plants to stressors, either natural or those introduced by man, usually induces specific biochemical responses in plants. The secondary metabolites produced may affect other organisms in the same ecosphere. For example, induction of phytoalexins can influence plant–fungus interactions and induction of certain terpenes can influence plant–insect interactions. Figure 1 illustrates the general framework of ecological biochemistry. It will be an important research task to integrate the above results on plant xenobiotic metabolism into this general picture of ecological biochemistry. Exposure of plants to xenobiotics will lead to the biochemical responses described in this review. Plants can thereby act as an important global sink for environmental chemicals, in many cases, detoxifying xenobiotics. However, in certain cases mutagenic activation of xenobiotics[31] and

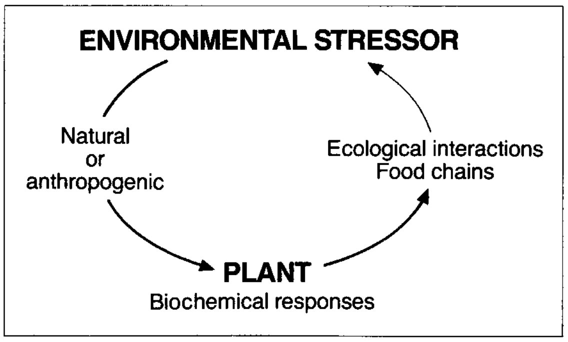

**Figure 1**
General scope of ecological biochemistry.

physiological effects on plant secondary metabolism[32] can occur. Furthermore, since xenobiotics are usually not completely degraded by plants, but stored in the form of soluble and insoluble conjugates, additional substances that may be xenobiotic for other organisms may be passed along food and decomposer chains. The existence in plants of xenobiotic metabolizing enzymes and their corresponding genes is also of considerable biotechnological interest. Possible applications are in biological decontamination or in pesticide development.

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# BOOKS in BRIEF

## Biophysical and Biochemical Aspects of Fluorescence Spectroscopy

*edited by* ***T. G. Dewey****, Plenum Press, 1991. $83.40 (xvii + 294 pages) ISBN 0 306 43627 2*

Fluorescence is such a versatile technique that ingenious researchers are constantly finding new applications in the biological sciences. The field is now so large that it would be impossible to cover all aspects in a single volume. Here the editor has opted for an eclectic mix of topics. The scope of the book is somewhat wider than the title suggests since some of the chapters strongly impinge on areas of cell biology. Thus Chapter 6 is concerned with analysis of ligand binding and crosslinking of cell surface receptors using immunoglobulin E as a model receptor. In Chapter 8, developing techniques for fluorescence imaging in conjunction with resonance energy transfer and fluorescence recovery after photobleaching (FRAP) are discussed. Applications of FRAP, including diffusion in and through cell membranes, are further considered in Chapter 9. Chapter 5 describes fluorescence methods for investigating receptor-mediated processes with particular emphasis on receptor–G protein coupling.

The techniques of fluorescence quenching, time-resolved fluorescence and resonance energy transfer each get an individual chapter. Although these topics are frequently covered in books on fluorescence, these chapters provide a solid introduction to some of the most widely used fluorescence methods. The remaining two chapters deal with studies of cation binding sites using fluorescent lanthanides and the development of fluorescent DNA sequencing techniques. Overall, this is a useful book which highlights a number of areas where fluorescence methodologies are currently making a significant impact.

**R. J. C.**

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## Conformations and Forces in Protein Folding

*edited by* ***Barry T. Nall*** *and* ***Ken A. Dill****, American Association for the Advancement of Science, 1991. $34.95 (x + 222 pages) ISBN 0 87168 394 6*

Of the increasing number of books on protein folding, this multi-authored collection places emphasis on the importance of charge effects and their calculation, and on the relation of amino acid sequences to folding, as well as an up-to-date review of the role of the molten globule in protein folding. Strangely, the deuterium protection technique of monitoring the early growth of H-bonded structures is not featured. An excellent review of the field, ideally to be read in conjunction with other complementary reviews and monographs of the subject, notably its AAAS predecessor edited by King.

**R. P.**