What Are the Principal Enzymes Oxidizing the Xenobiotics in Plants: Cytochromes P-450 or Peroxidases? (A Hypothesis)

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Abstract. Effectively no information is available at present concerning the enzymes which directly participate in the *in vivo* oxidation of xenobiotics in plants. Based on the known data a hypothesis is presented suggesting that only a minor part of the oxidation of xenobiotics in plants may be catalyzed by cytochrome P-450, the majority of xenobiotics being oxidized by plant peroxidases.

Key words: Oxidation of xenobiotics in plants — Enzyme — Cytochrome P-450 — Peroxidase

Introduction

While the biotransformation reactions of xenobiotics in animals are well known, information on the plant systems is scarce. Several differences in the biotransformation of xenobiotics between animals and plants have been suggested (Baldwin 1977).

During phase I of xenobiotics biotransformation in an organism the oxidative reactions lead to the formation of polar compounds, which can be further conjugated during phase II of biotransformation. Another possibility is the formation of toxic, mutagenic or carcinogenic metabolites, which are not conjugated and, due to their reactivity, bind covalently to biological macromolecules. Hence, the oxidative reactions are crucial for the further activity of xenobiotics and for the understanding of their fate in the organism.

Evaluation of the possible role of cytochrome P-450 in the metabolism of xenobiotics in plants

The oxidative reactions in animals are supposed to be catalyzed mainly by mixed function oxidases with cytochrome P-450 as the terminal oxidase. However, it is questionable whether the same enzymes are also the major enzymes participating in oxidative reactions of xenobiotics in plants.

Cytochrome P-450 has been found in many plants (Higashi 1985; O'Keefe et al. 1987; Riviere and Cabanne 1987) and its physiological roles have been investigated.

In general, plant cytochromes P-450 have not a broad substrate specificity typical for cytochrome P-450 of the animal livers (Riviere and Cabanne 1987; Higashi 1988). The endogenous substrates have only been studied from the point of view of the substrate specificity of isolated plant cytochromes P-450, since the isolated plant cytochromes P-450 do not convert exogenous substrates *in vitro* (Reichhart et al. 1979; Higashi 1985; Riviere and Cabanne 1987). Moreover, the metabolism of only xenobiotics catalyzed by plant microsomes has been studied, including herbicides such as monuron (Frear et al. 1969), 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid (Hamilton et al. 1971; Makeev et al. 1977; Adele et al. 1981) and other xenobiotics, e.g. N,N-dimethylanilide (Gordeziani et al. 1987), dimethylnitrosoamine (Gichner and Veleminský 1984; 1986; Gichner et al. 1985), benzo(a)pyrene (Higashi et al. 1981, 1982; Higashi 1988), aminopyrine (Fonne-Pfister et al. 1988), etc. (see Higashi 1988). The suggestion that a plant cytochrome P-450 is involved in these reactions has been based solely on indirect evidence (requirement of oxygen molecules, of reducing equivalents NADPH or NADH, and inhibition by carbon monooxide) (Higashi 1988).

The amounts of cytochrome P-450 in plants (0.015—0.2 nmol . mg^{-1} protein) are much lower than those present in animal tissues (0.8—1.1 nmol . mg^{-1} protein) (Riviere and Cabanne 1987; Higashi 1988). Furthermore, plant cells do not contain as much protein per unit mass as do mammalian cells.

Moreover, oxidation of several exogenous compounds in plants is mediated by other mechanisms rather than by cytochrome P-450-dependent reactions (Lamoureux and Frear 1979; Dohn and Krieger 1981; Sandermann 1988).

The question thus arises, which other enzymic systems are important for the oxidation of exogenous compounds in plants.

Comparison of benzo(a)pyrene metabolism by plant microsomal enzymes and by peroxidase

Benzo(a)pyrene is a foreign compound, the metabolism of which in plants has been thoroughly studied. This exogenous compound has been supposed to be metabolized by plant microsomal cytochrome P-450 (v.d. Trenck and Sandermann 1980, 1981; Higashi et al. 1981). We suggest that this may not be the fact.

Benzo(a)pyrene-quinones have been isolated as *in vivo* metabolites from cultured plant cells (Harms et al. 1977; v.d. Trenck and Sandermann 1981) as well as soyabean leaves (Sakamoto et al. 1984) or primary leaves of pea (v.d. Trenck and Sandermann 1980). More than 90 % of radioactivity of labeled

benzo(a)pyrene appears in the quinones. The *in vitro* metabolism of [^{14}C] benzo(a)pyrene by microsomes from tulip bulbs and Jerusalem artichoke tubers leads mainly to the formation of benzo(a)pyrene-quinones, and a small amount of benzo(a)pyrene-phenols is also formed. No benzo(a)pyrene-diol-derivatives are formed, which are typical products of the metabolism of benzo(a)pyrene by rat liver microsomes containing cytochrome P-450 (Karasaki and Higashi 1982; Higashi 1988). Unfortunately, the conversion of benzo(a)pyrene by isolated plant cytochromes P-450 has not been studied as yet.

Based on the results obtained in several laboratories it has recently been suggested by Higashi (1988) that there might be differences in the metabolism of benzo(a)pyrene between plant tissues and rat liver. The question, however, arises whether these differences are due to differences in the reactions catalyzed by plant and rat liver cytochromes P-450, or whether enzyme(s) other than cytochrome P-450 are responsible for the metabolism of benzo(a)pyrene in plants.

A detailed study of *in vitro* benzo(a)pyrene conversion by horseradish peroxidase and by prostaglandin H synthase was recently published by Cavalieri et al. (1988). These authors observed the formation of benzo(a)pyrene-quinones (1,6-dione, 3,6-dione, 6,12-dione) from the horseradish peroxidase- or prostaglandin H synthase-mediated reactions. These results may help in estimating the principal enzyme system which is responsible for the oxidation of benzo(a)pyrene in plants.

It follows from all the three types of studies of benzo(a)pyrene metabolism: in plants *in vivo* (Harms et al. 1977; v.d. Trenck and Sandermann 1980; Sakamoto et al. 1984); *in vitro* by plant microsomes (Karasaki and Higashi 1982; Higashi et al. 1982); *in vitro* by plant peroxidases (Cavalieri et al. 1988) that essentially the same products are formed (benzo(a)pyrene quinones, see above).

Unfortunately, the products of the benzo(a)pyrene metabolism by isolated plant cytochrome P-450 are unknown as no study of this type has been performed as yet. Moreover, previously it has been reported that plant cytochromes P-450 have an extremely low stability when present in preparations of microsomes isolated from plant tissues (Higashi 1988). It is, thus, questionable whether there actually are active cytochromes P-450 in isolated plant microsomes. On the other hand, several peroxidases were found in microsomal fractions obtained from plant tissues. Peroxidases are known to be present in almost all plant cell organelles, including the microsomes (Scandalios 1974; van Huystee 1987; van Huystee and Chibbar 1987).

Formulation of the hypothesis

Based on all the above data a suggestion can be drawn that products found both in *in vivo* experiments and in *in vitro* plant microsomal studies are formed mainly by the peroxidase-type mechanisms (with only little, if any, participation of the cytochrome P-450-oxygenase-mechanism), and that in plants *in vivo* the peroxidases could be the principal enzymes converting benzo(a)pyrene.

This and further facts (see below) allow the formulation of the hypothesis that in plants peroxidative reactions catalyzed by several types of peroxidases may play a significant role not only in the metabolism of benzo(a)pyrene, but also in the metabolism of other xenobiotics.

Experimental evidence in support of the hypothesis on the effective function of peroxidases in the oxidation of xenobiotics in plants

As a matter of fact, *in vitro* peroxidases in the presence of H_2O_2 and/or O_2 oxidize and/or oxygenate various organic compounds such as phenols, amines, diamines (Lamoureux and Frear 1979; Saunders et al. 1964; Boyd and Eling 1984), polycyclic aromatic hydrocarbons (Ortiz de Montellano and Grab 1987; Ortiz de Montellano et al. 1987; Cavalieri et al. 1988), azo dyes (Stiborová et al. 1988), nitroalkanes (Lamoureux and Frear 1979; Meunier 1987), aminopyrine (Griffin and Ting 1978), etc. (see Saunders et al. 1964). The substrate specificity of plant peroxidases is thus very wide, including substrates which enter in the plants as exogenous chemicals (e.g. pesticides or other agricultural chemicals) (Lamoureux and Frear 1979; Higashi 1988; Sandermann 1988).

The observation that peroxidases have high affinities towards exogenous substrates K_m values between 10^{-6}—10^{-4} mol/l) (Saunders et al. 1964; Ortiz de Montellano and Grab 1987; Ortiz de Montellano et al. 1987; Meunier 1987) suggests that xenobiotics are very easily oxidized by peroxidase.

Furthermore, peroxidase is able to catalyze a variety of chemical reactions (oxidation, oxygenation, peroxidation, dehydrogenation, halogenation, dehalogenation, N-dealkylation, etc.) (Saunders et al. 1964; Lamoureux and Frear 1979; Ortiz de Montellano et al. 1987; Meunier 1987) resulting in the formation of a variety of products.

The same products were obtained *in vivo* from some xenobiotics in plants and in vitro from peroxidase-mediated reactions. For example, recent metabolic *in vivo* studies with the pesticide pentachlorophenol in wheat plants yielded the conversion of the compound to tetrachlorocatechol. Also, this herbicide was observed to be converted *in vitro* to tetrachlorocatechol by horseradish peroxidase (Sandermann 1988). Furthermore, *in vivo* N-dealkylation products of atrazine formed in plants are not the products of a plant microsomal system

(cytochrome P-450) (Frear et al. 1969; Higashi 1988), and the enzyme which metabolizes this herbicide by oxidative reactions remained unknown. Recently, we found that *in vitro* this compound is N-dealkylated by horseradish peroxidase (Anzenbacher and Stiborová 1989).

Further facts in support of the role of peroxidases in the metabolism of xenobiotics in plants are findings on the location of these enzymes and of the xenobiotics in plants. Exogenous compound get into almost all parts of the plant body. They are present in the extracellular space, they pass the cell walls and membranes, and enter the cytoplasm and cellular compartments (organelles), where they may be also fixed (deposited, accumulated) (Lichtner 1986; Hsu et al. 1988). Their location correlates with that of the peroxidases which apparently are located in any part and organelle of plant cells (Scandalios 1974; van Huystee 1987; van Huystee and Chibbar 1987). Moreover, peroxidases are also transported to different sites, where their activity is needed (Gaspar et al. 1985).

Another support to our hypothesis may be derived from the fact that peroxidases are relatively very stable and abundant in most plants. They may form up to one-sixth of the total proteins in plant cells (e.g. basic isoperoxidases of cultured peanut cells) (van Huystee and Chibbar 1987). Whether peroxidases are the inducible enzymes similarly as cytochrome P-450, and whether the concentrations of the enzyme can be raised by the *de-novo* synthesis initiated by substrates, remains unknown. Peroxidase activity has been reported to be enhanced by treating plants with various chemicals (Lamoureux and Frear 1979; Castillo 1986). The question however is whether the increased peroxidase levels are due to the induction of *de-novo* synthesis of the enzyme, or to the release of the bound enzyme forms.

In conclusion, the experimental evidence in support of the hypothesis on the effective functions of peroxidases in the oxidative reactions of xenobiotics in plants may be summarized as follows:

- large amounts of peroxidases in plants
- small ammounts of cytochrome P-450 in plant tissues
- a low substrate specificity of plant peroxidases as compared to the high specificity of the plant cytochromes P-450
- a wide range of action of plant peroxidases
- the similarity of *in vivo* metabolites of several xenobiotics in plants to those formed *in vitro* by peroxidases rather than to those resulting from cytochrome P-450-dependent *in vitro* reactions
- high affinities of peroxidases to exogenous substrates
- the location of peroxidases in plants; while peroxidases are located in all parts of plant cells, the plant cytochrome P-450 are located in the microsomal fraction only. The peroxidases are thus more available for interactions with xenobiotics than cytochrome P-450.

Further studies are needed to elucidate the exact roles of cytochrome P-450 and peroxidases in the metabolism of endogenous and exogenous substrates by plants. The following questions should be investigated:

1) the exact substrate specificity of isolated active plant cytochromes P-450 and isolated plant peroxidases;
2) comparison of *in vivo* metabolism of several types of xenobiotics by plants with the *in vitro* conversion of these compounds by isolated cytochromes P-450 and peroxidases.

These studies will extend our understanding of the participation of the enzymes mentioned in the metabolism of xenobiotics in plants, and strengthen or exclude the hypothesis presented herein.

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