Frontiers in Biotransformation

Volume 2

Principles, Mechanisms and Biological Consequences of Induction

Edited by Klaus Ruckpaul and Horst Rein

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Introduction

H. Rein and K. Ruckpaul

Volume 2 of the series "Frontiers in Biotransformation" is focussed on the induction of enzymes involved in biotransformation. Induction as an effector mediated stimulation of the biosynthesis of distinct enzymes represents a regulation mechanism of enzymatic activity which is controlled on the cellular level. Thus the content of volume 2 continues and extends the scope of volume 1, which dealt with problems of regulation of cytochrome P-450 catalysed reactions on the molecular and membrane level of integration.

Biological and medical consequences of induction resulting in changed biotransformation activities are manifold and important for toxification and detoxification as well. In addition, inducing agents have become to be seen as promotors and may thus initiate malignant transformation. Therapeutic effects can be altered by inducing drugs and in this way, e.g., the protective effect of contraceptive steroids may vanish. Induction modifies the isozymic pattern, which originates from the genetically determined polymorphism. The changed isozymic pattern determines their individual capability of metabolizing endogenous and exogenous compounds alike.

A huge number of articles dealing with the induction of enzymes involved in biotransformation have generated a real need for reviews. Therefore the interest of the editors in selecting the contributions for this volume was not to look for outstanding casuistic articles but rather to put together such papers which would comprehend and condense problems more general in character. Volume 2 is introduced by an article by Parke, summarizing the general principles and biological consequences of induction. More detailed studies of molecular orbitals of inducers have revealed that the type of cytochrome P-450 induced is governed by the spatial dimensions and electronic structures of the inducing agents. Corresponding to the different types of inducers there exist different mechanisms of induction. An increase of the monooxygenase concentration but also of the NADPH dependent cytochrome P-450 reductase and further enzymes is observed at induction by the phenobarbital type inducers. Moreover, proliferation of the endoplasmic reticulum

and cellular hypertrophy of liver is observed. Obviously, phenobarbital increases both the transcriptional and translational activity.

The amino acid sequences of about 100 cytochromes P-450 led to a proposal for a unified nomenclature of the P-450 gene superfamily described by NEBERT and GONZALEZ in this volume. Most members of these families are inducible, with the assignment to a distinct family being mainly determined by the degree of homology but also by the specific inducer. The considerable increase in molecular biological studies on cytochrome P-450 in the last few years has led to a better understanding of constitutive and inducible gene expression of these key enzymes of biotransformation. The identification and characterization of *cis*-acting DNA regulatory elements, and the cloning of genes that encode *trans*-acting regulatory proteins interacting with these elements, are important steps in our knowledge of gene expression as well as of the developmental, sex and tissue specificities.

In the review of BRESNICK and HAUSER the polycyclic hydrocarbon specific cytochrome P-450 is described in detail. The gene of cytochrome P-450c was isolated and its DNA sequence determined. By genetic construction of specific gene segments their importance for interaction with the 4S polycyclic hydrocarbon-binding protein could be shown. The authors suggest that this protein could be the transcriptional factor which shuttles between the cytosolic compartment and the nucleus.

Already very early a sex-specific effect of inducers was observed, especially concerning the sex-related metabolism, such as hexobarbital hydroxylation and aminopyrine N-demethylation. The molecular basis of these differences is now understood in terms of a sex-specific induction of cytochrome P-450 isozymes in female and male animals. In the review of KATO and YAMAZOE not only this aspect of sex-dependent regulation of cytochrome P-450 expression is described but also a detailed analysis of sex-related cytochrome P-450 forms is given. The authors developed an immunoquantitative method for quantitation of cytochrome P-450-male and cytochrome P-450-female. These sex-specific forms clearly differ from each other in catalytic activity, molecular weight, physico-chemical properties and amino acid sequence too. Their specificity in drug oxidation was well documented by use of highly specific antibodies. Such inhibition studies offer an excellent method for classification of isozyme specific contributions in drug metabolism.

Doubtless the ontogenetic development of biotransformation activity is regulated and, or moreover, determined by the induction since it is known that the administration of phenobarbital to neonates has been shown to "imprint" or "programme" the microsomal enzymes of the adult. Distinct forms of cytochrome P-450 are induced in the foetal liver after administration of selected inducers to pregnant rats and treatment of neonates with inducers or their uptake via the maternal milk increases distinct cytochrome P-450 dependent enzyme activities. These facts are considered in the review of

KLINGER describing the biotransformation during the ontogenetic development. Although the total cytochrome P-450 concentration is a function of age, evidently, this enzyme concentration is responsible neither for the age dependence of drug oxidation, nor for the age dependent induction by the inducers of the phenobarbital and 3-methylcholanthrene group. Several reasons determine this behaviour. Different developmental patterns are observed for the multiple forms of cytochrome P-450. For the activity of a relevant isozyme, however, developmental changes in the microenvironment of the enzyme are of importance, i.e. phospholipids and their fatty acid composition which change with age, thereby changing structural and functional properties of the membrane and the cytochrome P-450 reducibility likewise. This knowledge is of importance to understand the prolonged half-life times in blood for most drugs in human neonates and to avoid therapeutic accidents as observed after chloramphenicol administration in newborns.

From the toxicological point of view, postoxidation reactions of xenobiotics are of importance because by these reactions, not in all cases highly water soluble metabolite of decreased pharmacological activity but also more toxic products are formed. Examples of such activation pathways are given in the chapter of LANGNER, BORCHERT and PFEIFER who describe the sulfo-, methyl-, and acetyltransferases in detail, including a lot of substrates which are conjugated by these enzymes. The fact that postoxidation enzymes are also inducible by different inducers is of interest in medicinal practice because enhanced bilirubin blood levels can be normalized by induction of glucuronosyltransferase which conjugates bilirubin. Instead of phenobarbital with many undesired side effects, the development of specific inducers for glucuronosyltransferase is necessary which requires deep insight into its structure and regulation. Definite data currently available for rat cDNAc are the basis of the review of MACKENZIE dealing with the analysis of structural features, substrate preferences and regulation of glucuronosyltransferases predominantly of this species.

One of the main conjugation pathways is catalysed by glutathione transferases. These multifunctional enzymes are associated with the detoxification of drugs and carcinogens, the reduction of organic hydroperoxides and nitrate, the binding and intracellular transport of lipophiles and the biosynthesis of the local hormone leucotriene C. In the review of KETTERER and TAYLOR the reader will not only find a broad account of reactions with GSH which are catalysed by GSH transferases but the current situation regarding the distribution of these enzymes in the animated world, their structure and, finally, what is known at present of their genetic regulation.

In a last chapter THOMAS and OESCH deal with the most recent findings about the enzyme epoxide hydrolase which catalyses the hydration of epoxides. Normally this reaction is considered a step of detoxification. However, in the special case when dihydrodiol is formed from benzo(a)pyrene 7,8-oxide, a

further activation by a monooxygenase takes place resulting in the ultimate carcinogen, i.e. benzo(a)pyrene 7,8-diol-9,10-oxide. Biotransformation of foreign compounds is a complex process including steps of both toxification and detoxification. Clearly, the balance between the toxification and detoxification pathways depends on several factors; most importantly the genetically determined concentration of individual enzymes involved in biotransformation steps and their inducibility, too.

Contents

Chapter 1

Induction of Cytochromes P-450 – General Principles and Biological Consequences

D. V. PARKE

1. Introduction

The cytochromes P-450, a ubiquitous family of enzymes which have been detected in all living systems examined, function as mixed-function oxygenases involved in the biogenesis of sterols, steroid hormones and prostanoids, the oxidation of fatty acids and steroids, and the metabolic oxygenation of a multitude of diverse exogenous xenobiotic chemicals. These chemicals range from the polycyclic aromatic hydrocarbons from combustion of fossil fuels to the products of human ingenuity for creating chemicals of high social value, such as the polychlorinated biphenyls (electrical insulators) or the benzodiazepine drugs (hypnotics). The cytochromes P-450 were among the first mammalian enzymes discovered to exhibit substrate-induced genomal regulation of their enzyme activity (enzyme induction), which until 30 years ago had been considered to be a phenomenon confined to microorganisms. In 1956 CONNEY et al. showed that treatment of animals with the polycyclic aromatic hydrocarbon, 3-methylcholanthrene, increased their ability to metabolize methylated azo dyes, and REMMER and ALSLEBEN (1958) found that tolerance to the barbiturate drugs was the result of these drugs enhancing their own metabolism, by induction of the cytochromes P-450.

These two distinctly different examples of induction of the cytochromes P-450 were associated with two different forms of this enzyme, which at that time were termed 'cytochrome P-450' (induced by phenobarbitone) and 'cytochrome P-448' (induced by carcinogenic polycyclic hydrocarbons), the names being derived from the wavelength (450 and 448 nm) of their reduced CO-ligand absorption spectra. Although initially it was considered that cytochrome P-448 was merely a derivative of cytochrome P-450 in which the polycyclic hydrocarbon inducing agent had become irreversibly bound to the enzyme molecule, later studies in which the liver microsomal enzyme was solubilized and rigorously purified revealed that these were two distinctly different enzyme proteins, under separate genetic control. Today, the cytochromes P-450 are considered to comprise a superfamily of enzymes, many of which have been shown to exhibit induction following exposure of the intact animal to a typical substrate or certain chemical agents. Studies of the amino acid structures and gene regulation of the many forms of cytochrome P-450 have revealed valuable information concerning the structural and functional relationships of the members of this superfamily of enzymes, but unfortunately have also resulted in a confused variety of different nomenclatures. This has led to some obfuscation of the functional differences of these isozymes and of the differences in their regulation. Recent endeavours to clarify the problem has resulted in the publication of new recommendations for cytochrome P-450 nomenclature, based on the evolution of these enzymes (NEBERT and GONZALEZ, 1987; NEBERT et al., 1987). This new nomenclature

will be used in the present chapter, in addition to the more distinctive, familiar, original terminology (see Table 1).

Evidence is now available for the existence of eight mammalian P-450 gene families (I, II, III, IV and the steroid hydroxylases XI, XVII, XIX and XXI), and of at least five sub-families within the P-450 II family (NEBERT and GONZALEZ, 1987; NEBERT et al., 1987). The P-450 I gene family of polycyclic aromatic hydrocarbon-inducible cytochromes comprises only one sub-family with only two genes, A1 and A2. The P-450 II gene family comprises P-450 II A, inducible by 3-methylcholanthrene (MC) and possibly also by phenobarbital (PB), a number of PB-inducible forms (P-450 II B and C – some non-inducible), the ethanol-inducible form, II E, and form II D. The P-450 III family comprises one sub-family (A1 and A2) of steroid-inducible genes which are also induced by phenobarbital and by macrolide antibiotic

Table 1. Cytochromes P-450 and their induction

Isozyme		Typical inducing agent	Specific enzymic activity induced
New nomenclature	Original name		
P-450 I A1	P-448 (rat P-450c, rabbit LM6, mouse P_1-450)	3-methyl-cholanthrene	7-ethoxyresorufin-O-deethylase
P-450 I A2	P-448 (rat P-450d, rabbit LM4, mouse P_3-450)	3-methyl-cholanthrene, isosafrole	7-ethoxyresorufin-O-deethylase, phenacetin O-deethylase
P-450 II A1	P-450 (rat P-450a)	3-methyl-cholanthrene	testosterone-7-hydroxylase
P-450 II B1	P-450 (rat P-450b)	phenobarbital	7-pentoxyresorufin-O-deethylase
P-450 II B2	P-450 (rat P-450e)	phenobarbital	7-pentoxyresorufin-O-deethylase
P-450 II C1–10	(rat P-450, PB1 and P-450f)	phenobarbital	—
P-450 II D1 & 2	P-450	—	debrisoquine-4-hydroxylase
P-450 II E	(rat P-450j)	ethanol, isoniazid	aniline hydroxylase

Table 1. (continued)

Isozyme		Typical inducing agent	Specific enzymic activity induced
New nomenclature	**Original name**		
P-450 III A1	P-450 (rat pcn 1)	pregnenolone-16α-carbonitrile	aminopyrine-N-demethylase, ethylmorphine-N-demethylase
P-450 III A2	P-450 (rat pcn 2)	pregnenolone-16α-carbonitrile	aminopyrine-N-demethylase, ethylmorphine-N-demethylase
P-450 IV	P-452	clofibrate	lauric acid ω and ω−1 hydroxylase
P-450 XI A1 P-450 XI B1	bovine and human scc bovine and human 11β		
P-450 XVII A1	bovine and human 17α		
P-450 XIX A1	human aromatase		
P-450 XXI A1 P-450 XXI A2	bovine, murine and human steroid 21-hydroxylase		
P-450 LI A1	yeast 1 an		
P-450 CI A1	*Pseudomonas putida* cam		

and the clofibrate-inducible P-450 IV comprises yet another family, with probably two or three genes (NEBERT et al., 1987). The P-450 XI, XVII, XIX and XXI familes are the proteins involved in steroidogenesis, and the P-450 LI and CI families comprise the yeast and *Pseudomonas* cytochromes respectively. Several other unique P-450 gene families, including those com-

prising the microsomal cholesterol 7α-hydroxylase and the renal mitochondrial 25-hydroxyvitamin D_3-1α-hydroxylase, remain to be cloned and characterized (NEBERT and GONZALEZ, 1987). From considerations of the structures of the P-450 I and P-450 II gene families, the amino acid residue numbers at each exon-intron junction, the location in the coding triplets in which the exons are split, and homologies among introns and exons, it has been deduced that these two gene families probably diverged from a common ancestor more than 200 million years ago and that P-450 I A1 and A2 genes split from each other about 65 million years ago (GONZALEZ et al., 1985).

The cytochrome P-450-mediated mixed-function oxidation of xenobiotics (drugs, pesticides, industrial chemicals, etc.) involves primarily the P-450 I – IV families, and is concerned with detoxication, namely, the oxidative metabolism of xenobiotics to more-polar, biologically-inactive, readily-excretable metabolites. However, the same microsomal enzymes can also catalyse the oxidative formation of reactive intermediates and ultimate carcinogens, leading to covalent binding, DNA damage, mutations and malignancy, and other pathological processes. This catalysis of the opposing pathways of detoxication and activation of chemical carcinogens by the same group of enzymes has been described by GELBOIN (1983) as the paradox of chemical carcinogenesis and cancer. However, chemical carcinogenesis has long been associated with the specific induction of the cytochromes P-448 (P-450 I) (CREAVEN and PARKE, 1966), and more recently it has been shown that the P-448 family of enzymes is specifically concerned with the activation of chemicals and carcinogens to reactive intermediates and the formation of mutagens and carcinogens (IOANNIDES et al., 1984).

The present review will be limited to considerations of the induction of the P-450 I – IV families of microsomal cytochromes P-450, and will not be concerned with induction of the mitochondrial cytochromes P-450 of steroidogenesis (XI, XVII, XIX and XXI), or the induction of the cytochromes P-450 of yeast (LI) or of *Pseudomonas putida* (CI).

2. Induction of specific cytochromes P-450

2.1. Cytochromes P-448 (P-450 I)

The cytochromes P-448 are induced by planar molecules, which are their preferred substrates, such as the polycyclic aromatic hydrocarbons, e.g. benzo(a)pyrene and 3-methylcholanthrene (MC) (THOMAS et al., 1983; IOANNIDES et al., 1984), and by planar polyhalogenated biphenyls (PARKINSON et al., 1983) such as 3,3′,4,4′,5-pentachlorobiphenyl (OZAWA et al., 1979) and 2,3,3′,4,4′,5-hexabromobiphenyl (ROBERTSON et al., 1981), by 3,3′,4,4′-tetrachloroazobenzene (HSIA and KREAMER, 1979), aminoazobenzenes (DEGAWA

et al., 1985), the anticancer drug, ellipticine (CRESTEIL et al., 1982) and the muscle relaxant, dantrolene (JAYYOSI et al., 1987), and to a lesser extent by aromatic amines (IOANNIDES et al., 1984) and amides (ASTRÖM and DE PIERRE, 1985; IWASAKI et al., 1986).

The most potent inducer of cytochrome P-448 is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) a contaminant of the herbicide 2,4,5-trichlorophenoxyacetic acid which, because of its resistance to biodegradation, is a persistent environmental contaminant (POLAND and GLOVER, 1974). TCDD is 10^4 times more potent than MC in the induction of P-448. Of the analogues of TCDD, 2,3,7,8-tetrabromodibenzo-p-dioxin has 50% of the potency of TCDD, 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin has 10%, and all other analogues had markedly lower activities ranging from 10% to 10^{-7} that of TCDD (BRADLOW et al., 1980). TCDD induces P-448 in human lymphocytes and in a number of different cell lines derived from a variety of animals (NIWA et al., 1975). Induction of P-448 by TCDD is tissue specific, and in rabbit liver it induces forms LM6 (P-450 I A1) and LM4 (P-450 I A2), but in lung and kidney only LM6 is induced (LIEM, 1980). TCDD markedly induces both isozymes of P-448 (P-450c and d) in rat liver, but only P-450c (P-450 I A1) in extrahepatic tissues; induction of P-450c is greatest in liver > kidney > lung > intestine > spleen > testes (GOLDSTEIN and LINKO, 1984).

β-Naphthoflavone, a potent non-carcinogenic inducer of cytochrome P-448, increases this enzyme in mouse liver by a mechanism similar to that evoked by MC (BOOBIS et al., 1977). Cigarette smoke is also a potent inducer of P-448 in lung and kidney, but not liver, of rats, hamsters and guinea pigs (BILIMORIA and ECOBICHON, 1980), and in human placenta (WELCH et al., 1968); placental P-448 has also been induced in humans by exposure to polychlorinated biphenyls (WONG et al., 1986). 1,1-Dichloroethylene, a plastics copolymer, also resulted in a tissue specific induction of P-448, increasing the levels in mouse kidney, especially in the male, but not in mouse liver (KRIJGSHELD and GRAM, 1984). A novel form of P-448, induced in hamster liver by MC, and distinct from the MC-induced rat liver P-448, has been purified and shown to have high specificity for aflatoxin B_1, which it activates to a mutagen 50 times more effectively than does rat liver P-448 (MIZOKAMI et al., 1986).

The cytochromes P-448 may be quantified specifically by the 7-ethoxyresorufin (EROD) assay (PHILLIPSON et al., 1984), and are induced in liver, kidney and lungs of rats, hamsters, guinea pigs and mice by a variety of carcinogens and non-carcinogenic inducing agents (IWASAKI et al., 1986). The MC-induced pulmonary cytochrome P-448 isolated from rats is structurally identical to the rat liver enzyme (ROBINSON et al., 1986). The mixed-function oxidases of the intestines are induced in rat by β-naphthoflavone (LINDESKOG et al., 1986), and by various chemicals of the diet, for example, indole-3-carbinol and ascorbigen, metabolites of glucobrassicin present in cabbage and other vegetables, which induce EROD of rat intestinal mucosa and liver

(McDanell et al., 1987). TCDD and β-naphthoflavone also induce EROD activity in the smooth muscle of rabbit aorta, which may be associated with the role of carcinogens and mutations in atherosclerosis (Serabjit-Singh et al., 1985; Paigen et al., 1986).

Carcinogenic primary aromatic amines (2-aminofluorene, aminobiphenyl), secondary aromatic amines (N-methyl-4-aminoazobenzene) (Kimura et al., 1985), and the arylamide, 2-acetylaminofluorene (2-AAF) (Lotlikar et al., 1984), are metabolically activated to mutagens and proximate carcinogens only by the high spin form of cytochrome P-448 (P-448 H, P-450d, or P-450 I A2). This isozyme of P-448 is selectively induced by the azo dyes, such as 3-methoxy-4-aminoazobenzene and several other methyl and methoxyl derivatives of 4-aminoazobenzene (Degawa et al., 1986), and by the 'mixed' P-448 and P-450 inducer, hexachlorobenzene (Linko et al., 1986). Isosafrole is also a selective inducer of P-448 H, but as the induced cytochrome forms a complex with isosafrole metabolites, the induced enzyme is largely inactivated.

Table 2. Selective induction of rat liver cytochromes P-450

Inducing agent	Type of inducer	Cytochromes induced (ratio of induced/control)			
		P-450 I A1 (P-450c)	P-450 I A2 (P-450d)	P-450 II A1 (P-450a)	P-450 II B1 & B2 (P-450b & e)
Phenobarbital (PB)	PB	1	1	2	40
3-Methylcholanthrene (MC)	MC	72	11	4	1
Isosafrole	MC?	19	22	2	13
2,2′,4,4′,5,5′-Hexachlorobiphenyl	PB	1	1	2	73
3,3′,4,4′,5,5′-Hexachlorobiphenyl	MC	43	40	8	1
2,3,3′,4,4′,5-Hexachlorobiphenyl	mixed	43	10	6	47
Aroclor 1254	mixed	50	20	4	45

Immature rats were pretreated with the different inducing agents and the specific cytochromes P-450 were quantified by radical immunodiffusion using monospecific antibodies (data from Conney, 1986).