The cytochrome P450 IV family: constitutive and inducible haemoproteins

G. GORDON GIBSON
Molecular Toxicology Group, Biochemistry Department, University of Surrey, Guildford, Surrey GU2 5XH, U.K.

Mammalian cytochrome P-450 exists as a multigene family expressing a spectrum of structurally and functionally distinct isoenzymes involved in the biotransformation of both exogenous and endogenous substrates [1, 2]. Although a generally accepted isoenzyme nomenclature based on divergent evolution and nucleotide/amino acid homologies is now widely used [1], it is still useful, under certain circumstances, to consider the cytochromes P-450 under the more broad subdivisions of constitutive and inducible enzymes. However, the problem with the use of this broader nomenclature lies in the difficulty in defining what a constitutive enzyme actually is. According to a recent definition [3], constitutive enzymes are 'enzymes which, in contrast to the inducible enzymes, are constitutively produced by the cell, irrespective of the growth conditions'. This definition infers that constitutive cytochromes P-450 are regulated by endogenous or dietary inducers in vivo or by the existence of a control mechanism governing constitutive expression, and that the constitutive cytochromes P-450 are probably associated with relatively ancient genes.

Based on the amino acid sequences of the cytochromes P-450, an evolutionary tree has been constructed [4]. There are two major divisions in this phylogenetic tree, the first occurring at the separation of the eukaryotic and prokaryotic cytochromes P-450 (approximately 1400 million years ago), and the second separating the enzymes that catalyse the metabolism of endogenous versus exogenous substrates (approximately 100 million years ago). The first gene duplication event occurred approximately 1360 million years ago and gave rise to the cytochromes P-450 in two different cellular organelles, namely the mitochondrion and the endoplasmic reticulum. According to this evolutionary analysis, the early history of mammalian cytochrome P-450 appears to be related to the evolution of cholesterol, as the earliest eukaryotic cytochrome P-450 sequences reported are the cholesterol side-chain cleavage enzymes of the mitochondrion.

Accordingly, the above description of constitutive and inducible cytochromes P-450 serves as a useful operational definition to classify the factors that control and regulate the expression of cytochrome P-450 isoenzymes. In this context, the cytochrome P450 IV family of genes is an excellent example of the expression of both constitutive and inducible isoenzymes in that a constitutive high-level enzyme expression is attained [5], and this regulation of a 'constitutive' enzyme is additionally regulated by xenobiotics such as the oxysterobutyrate hypolipidaemic drugs, such as clofibrate, in rat liver and kidney, and has been recently rationalized by the significant induction (approximately 5-fold) of catalytically active cytochrome P450 IVA1 protein [5, 6]. Recent evidence, using cDNA probes for cytochrome P450 IVA1, has demonstrated that clofibrate pretreatment results in an accumulation of cytochrome P450 IVA1 mRNA [9, 10], due to transcriptional activation of the cytochrome P450 IVA1 gene, and not due to increased transcription or repression of the gene [10], and hence additionally contributes to the overall control of cytochrome P450 IVA1 gene expression.

Interestingly, induction of cytochrome P450 IVA1 in rat liver is not solely confined to oxysterobutyrates, such as clofibrate and its analogues, but also includes structurally diverse inducers such as 3T4,14,6,4,3 arsphenin, and the phthalate ester plasticizers including di-(2-ethylhexyl)-phthalate [6, 7]. This, and other observations based on the lack of inducer binding to subcellular liver homogenates [18], would tend to cast some doubt on the existence of a 'classical' nuclear/cytoplasmic receptor for the above inducers of cytochrome P450 IVA1, as has been described for the 2,3,7,8-tetrachlorodibenzo-p-dioxin which regulates cytochrome P450 I receptor [19]. In addition, based on cytohexamide-inhibition experiments, it would appear that clofibrate induction of the cytochrome P450 IVA1 gene does not involve the intermediation of a clofibrate-inducible protein factor, as has been postulated for the cyclic AMP-mediated production of labile proteins in the regulation of steroid hydroxylase cytochrome P450 gene expression in the adrenal cortex [20]. How then is the cytochrome P450 IVA1 gene regulated by clofibrate? Does the induction mechanism involve the same control and regulatory elements that are responsible for maintenance of the constitutive level of...
expression or are other molecular mechanisms operative? Unfortunately, we do not have precise answers to these questions at present owing to lack of information on the 5' upstream regulatory elements of genomic cytochrome P450 IVA1.

It is interesting to draw attention to the mechanism proposed by Elcombe and his colleagues, whereby lipid bio-transformation is again involved [15], similar to the possible regulation of constitutive cytochrome P450 IVA1 gene expression described above. In this particular mechanism, the inducer is taken up by the hepatocyte and initially directly inhibits the mitochondrial β-oxidation enzyme specific for medium-chain fatty acids, and/or the carnitine acyl transferase responsible for the transport of medium-chain acylcarnitines across the mitochondrial membrane. Alternatively, it has been postulated that the CoA derivatives of oxysubertic acid inducers per se or CoA sequestration by fatty acids are responsible for the initial inhibition of fatty acid oxidation. Accordingly, the cellular levels of medium-chain fatty acids accumulate (either per se or as their CoA esters), and it is postulated [15] that these lipids induce microsomal cytochrome P450 IVA1 to maintain lipid homeostasis, as this haemoprotein will efficiently oxidize these fatty acids [12, 21]; this is, therefore, another possible example of the well-documented phenomenon of substrate regulation of gene expression. This substrate overload perturbation of lipid metabolism may then be the common factor that readily explains the diversity of chemical structures and physiological conditions that have been reported to alter cytochrome P450 IVA1 gene expression.

The research described herein was supported in part by project grants from the M.R.C. and the Wellcome Trust.


Received 11 August 1989

Cytochrome P-450 and oxidative metabolism in invertebrates

DAVID R. LIVINGSTONE
N.E.R.C. Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth PL1 3DH, U.K.

Introduction

The cytochrome P-450-dependent mono-oxygenase or mixed-function oxidase (MFO) system is an apparently universally distributed enzyme system, involved in the metabolism of a variety of endogenous and exogenous compounds, including steroids, prostaglandins, fatty acids, natural and pollutant xenobiotics, and chemical carcinogens [1–2]. The ancestral gene for cytochrome P-450, the terminal component of the system, is thought to be more than 2 billion years old, and subsequent divergent evolution has produced many different gene forms and gene products [1, 3]. To date, the cytochrome P-450 gene superfamily is known to contain more than 70 members, divided into 14 families, most of the information available being for mammals [4].

Over a million described species of greatly diverse physiology, biochemistry and ecological lifestyle make up the animal kingdom. Five percent of these constitute the vertebrate phylum, with the remainder comprising some 25 or more invertebrate phyla [5]. Cytochrome P-450 has been studied to any degree in only four of the invertebrate phyla, by far the most being known for insects (phylum: Arthropoda) [6–12], with considerably less information available for crustaceans (Arthropoda) [13], the Mollusca [14–16], Annelida and Echinodermata [17] (also see [18–20]). Cytochrome P-450 activity has also been demonstrated or indicated in the Cnidaria [21], Platyhelmintes [21] and Nematoda [22]. Comparative aspects of cytochrome P-450 form and function in invertebrates are presented below and in the following Tables.

Synopsis of information

1. Cytochrome P-450 and the MFO system can have a wide tissue distribution, but are generally found in highest contents/activities in tissues associated with the processing of food. In addition to the major sites described in Table 1, other examples of tissues include the gills in bivalves, green gland in crabs, Malpighian tubules in insects, and haemal plaxes in holothurians. The microsomes are a major sub-