Minireview

CHOLESTEROL-METABOLIZING CYTOCHROMES P450

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ABSTRACT:

By catalyzing the first steps in different pathways of cholesterol degradation, cytochromes P450 (P450s) 7A1, 27A1, 11A1, and 46A1 play key roles in cholesterol homeostasis. CYP7A1 is a microsomal liver-specific enzyme that converts cholesterol to 7α-hydroxycholesterol. CYP27A1 is a ubiquitous expressed mitochondrial P450 that metabolizes cholesterol to 27-hydroxycholesterol. CYP11A1 also resides in mitochondria but is expressed mainly in steroidogenic tissues, where it catalyzes the conversion of cholesterol to pregnenolone. Finally, CYP46A1 is a brain-selective microsomal monoxygenase producing 24S-hydroxycholesterol from cholesterol. Catalytic efficiencies of cholesterol-metabolizing P450s vary significantly and probably reflect physiological requirements of different organs for the rate of cholesterol turnover. P450s 7A1, 27A1, 11A1, and 46A1 represent a unique system for elucidation of how different enzymes have adapted to fit their specific roles in cholesterol elimination. Studies of cholesterol-metabolizing P450s suggest that their activities could be modulated post-translationally and that they should also be considered as targets for regulation of cholesterol homeostasis.

In mammals, excess cholesterol (5-cholestene-3β-ol) is removed mainly through conversion to bile acids, and only a small portion is used for production of steroid hormones (Turley and Dietschy, 1982). Enzymes called cytochromes P450 (P450s) initiate all quantitatively significant pathways of cholesterol degradation. The P450 proteins contain a single heme group and have a characteristic absorption at 450 nm when reduced and form a complex with CO (for recent reviews on P450s, see references: Nebert and Russell, 2002; Meunier et al., 2004; Aguiar et al., 2005; Coon, 2005; Denisov et al., 2005; Guengerich, 2005; Johnson and Stout, 2005). P450s from different families share low sequence identity (<40%); nevertheless, they have a similar overall structural fold and generally carry out monooxygenation reactions (Graham and Peterson, 1999). This review describes only those P450s that act directly on cholesterol (Fig. 1); information about P450s that hydroxylate cholesterol derivatives can be found in other reviews (Russell, 2003; Payne and Hales, 2004). Furthermore, the major focus will be on human enzymes because some interspecies differences exist both in maintenance of cholesterol homeostasis and in the regulation of the activity of cholesterol-metabolizing P450s.

Physiological and Medical Significance of Cholesterol-Metabolizing P450s

CYP7A1. There are several metabolic routes that lead to the formation of bile acids and only one pathway to produce steroid hormones. In the liver, the main organ for cholesterol degradation, bile acid biosynthesis, is initiated and controlled by a liver-specific enzyme, CYP7A1, that converts cholesterol to 7α-hydroxycholesterol (Myant and Mitropoulos, 1977). This reaction represents the first and rate-limiting step in the classical or neutral bile acid biosynthetic pathway, which dominates under normal physiological conditions. Approximately 400 to 600 mg of cholesterol are eliminated through this pathway on a daily basis by the human liver (Sabine, 1977; Turley and Dietschy, 1982). Humans lacking cholesterol 7α-hydroxylase activity as a result of the mutation in the CYP7A1 gene have significant elevation of total and LDL cholesterol levels, substantial accumulation of cholesterol in the liver, and a markedly decreased rate of bile acid excretion (Pullinger et al., 2002). Increased plasma LDL cholesterol levels are also observed in some subjects carrying nucleotide substitutions in the CYP7A1 promoter region and, presumably, having reduced cholesterol 7α-hydroxylase activity (Wang et al., 1998). In two population studies, a frequent A-204C polymorphism (∼38–46% of the population carries the -204C allele) was found to have significant or modest effect on LDL cholesterol levels (Wang et al., 1998; Couture et al., 1999), whereas in three studies, the effect was inconsistent or nonsignificant (Hegele et al., 2001; Kovar et al., 2004; Abrahamsson et al., 2005). Thus, different results in the five studies are explained by differences in diet and in genetic background of the population. In addition to a medical significance, it is possible that the A-204C genotype has a pharmacological relevance because it was shown to be associated with a poor response to a cholesterol-lowering drug, atorvastatin, and to influence the response of plasma lipids to increased intake of dietary cholesterol and cafestol (Hofman et al., 2004; Kajinami et al., 2005).

CYP27A1. Analysis of the CYP7A1 gene knockout mice and then
subsequent characterization of the human subjects carrying the CYP7A1 null mutation revealed that when the classical bile acid biosynthetic pathway is suppressed, an alternative or acidic pathway of bile acid biosynthesis is up-regulated (Ishibashi et al., 1996; Schwarz et al., 1996; Pullinger et al., 2002). Under normal conditions, this pathway is initiated in extrahepatic tissues and accounts for a daily elimination of 18 to 20 mg of cholesterol (Duane and Javitt, 1999). The alternative pathway complements the HDL-mediated reverse cholesterol transport to the liver and is initiated by a ubiquitously expressed CYP27A1 that converts cholesterol to 27-hydroxycholesterol (Anderson et al., 1972; Lund et al., 1996). CYP27A1 is a polyfunctional enzyme and, in addition to hydroxylation of cholesterol in extrahepatic tissues, it also oxygenates bile acid intermediates in the liver and vitamin D₃ in the kidney (Wikvall, 1984; Masumoto et al., 1988; Okuda et al., 1988). The CYP27A1 products 27-hydroxycholesterol and 7α-hydroxycholesterol are the ligands for the nuclear receptors LXRα and LXRβ that activate the transcription of several genes involved in lipid metabolism (Song and Liao, 2000; Fu et al., 2001). However, the significance of CYP27A1 in transcriptional regulation of cholesterol homeostasis via generation of biologically active oxysterols is currently unclear (Fu et al., 2001; Hall et al., 2001; Bjorkhem, 2002; Javitt, 2002; Meir et al., 2002). Deficiency of CYP27A1 activity as a result of genetic mutations causes a disease called cerebrotendinous xanthomatosis (CTX), which is characterized by abnormal deposition of cholesterol and cholestanol (5α-saturated analog of cholesterol) in multiple tissues (Cali et al., 1991; Bjorkhem et al., 1995). Phenotypic manifestations and clinical progression of CTX are variable and may include bilateral cataracts, premature atherosclerosis, tendon xanthomas, neurological and neuropsychiatric abnormalities, and osteoporosis (Bjorkhem et al., 1995). CTX is believed to be a rare disease. It has been reported in more than 200 people worldwide (Moghadasian, 2004). Most recent studies suggest that CTX is underdiagnosed, and the prevalence of CTX is approximately 3 to 5 per 100,000 people (Lorincz et al., 2005). Over 40 different mutations in CYP27A1 have been described (Verrills et al., 2000; Lee et al., 2001). Of them, about half are deletion/insertion, splice site, and nonsense mutations (resulting in premature stop codon) that probably abolish the enzyme expression and, consequently, the activity. The other half constitute missense mutations that result in amino acid substitution. No genotype-phenotype correlations have been established thus far, possibly because CYP27A1 is involved in several metabolic pathways, the contribution of each of which to the phenotype is poorly understood at present (Verrills et al., 2000). Studies in this laboratory (presented in the next section) may provide some insights into mechanisms underlying phenotypic heterogeneity associated with CTX. CTX is a treatable disease. Oral supplementation with chenodeoxycholic acid halts CTX progression and leads to significant neurological recovery (Beringer et al., 1984).

CYP11A1. In steroidogenic tissues (adrenal glands, ovaries, testis, placenta, and brain), cholesterol is converted to pregnenolone. This reaction represents the first step in the overall steroid hormone biosynthesis and is catalyzed by CYP11A1 (Stone and Hechter, 1954). Approximately 40 to 50 mg of cholesterol is used every day to produce steroid hormones (Sabine, 1977; Turley and Dietschy, 1982). The conversion of cholesterol to pregnenolone was long thought to be the rate-limiting step in overall steroidogenesis. However, it is now clear that the critical step is cholesterol delivery to the inner mitochondrial membrane where CYP11A1 resides (Stocco, 2000). Intracellular cholesterol transport is mediated by NPC1L1 and SR-B1, and it plays a role in the regulation of cholesterol metabolism.
mitochondrial cholesterol transport regulates cholesterol availability to CYP11A1 and, consequently, the enzyme activity and is mediated by the steroidogenic acute regulatory protein (Stocco, 2001). Steroidogenic acute regulatory protein is expressed in many steroidogenic tissues but not all: it is not found in the placenta. Therefore, in this organ, the rate-limiting step in steroid synthesis is controlled by CYP11A1 (Tuckey, 2005). Mutations that completely abolish the CYP11A1 activity are believed to be incompatible with human term gestation because this enzyme is needed for placental biosynthesis of progesterone, an essential hormone required to maintain pregnancy (Miller, 1998). Recently, a patient homozygous for a nucleotide deletion leading to a premature stop codon was described, indicating that in rare cases, fetuses with a complete CYP11A1 deficiency may be viable (Hiort et al., 2005). The child was born prematurely, and had a complete sex reversal and congenital lipoid adrenal hyperplasia, a severe disorder of steroidogenesis in which cholesterol accumulates within the steroidogenic tissues and the synthesis of all adrenal and gonadal steroids is impaired. The adrenal crisis was treated with the hormone replacement therapy; the child has survived, and was 2 years old at the time of the paper writing. Different partially inactivating CYP11A1 mutations are also described in two patients who were born after normal pregnancies (Tajima et al., 2001; Katsumata et al., 2002).

One of the patients developed symptoms of adrenal insufficiency at the age of 7 months and the other at the age of 4 years. In a genetic male, attenuation of CYP11A1 activity caused pseudohermaphroditism and in a genetic female, lack of secondary sexual characteristics. A substitution with unknown effect on enzyme activity, V179I, was found in a hyperandrogenic hirsute woman (Calvo et al., 2001). Because this was a conservative replacement, the authors do not believe that it plays a major role in the pathogenesis of hirsutism and hyperandrogenism.

**CYP46A1.** Cholesterol elimination from the human brain begins with 24S-hydroxylation catalyzed by CYP46A1 (Lund et al., 1999). In contrast to cholesterol, 24S-hydroxycholesterol can cross the blood-brain barrier, enter the circulation, and then be delivered to the liver for further degradation. The brain does not appear to contribute significantly to the whole-body cholesterol balance: only 6 to 7 mg of cholesterol are 24S-hydroxylated every day in the brain (Heverin et al., 2004). The significance of CYP46A1 may not be limited to involvement in cholesterol degradation. 24S-Hydroxycholesterol is a potent activator of the LXR receptors (Janowska et al., 1999); therefore, CYP46A1 may play a regulatory role by producing a biologically active product. It is possible that CYP46A1 may also be involved in subsequent metabolism of 24S-hydroxycholesterol because, in vitro, it was found to convert 24S-hydroxycholesterol to 24,25- and 24,27-dihydroxycholesterols with 24S-hydroxycholesterol being a much better substrate for CYP46A1 than cholesterol (Mast et al., 2003). Furthermore, in vitro studies indicate that CYP46A1 has a broad substrate specificity and metabolizes a number of structurally diverse compounds including different cholesterol derivatives and drugs (Mast et al., 2003). Accordingly, CYP46A1 may participate in metabolism of neurosteroids and drugs that are targeted to the central nervous system. Accumulating evidence suggests that cholesterol is a risk factor for Alzheimer’s disease (Burns and Duff, 2002; Puglielli et al., 2003; Raffai and Weisgraber, 2003); therefore, CYP46A1 deficiency was proposed to play a role in the pathogenesis of this neurological disorder. Several single nucleotide polymorphisms have been identified in the introns of the CYP46A1 gene, and two of them were studied. The results are controversial, and as of the end of 2005, six published papers suggest that there is a link between the polymorphisms and Alzheimer’s disease (Kolsch et al., 2002; Raffai and Weisgraber, 2003; Russell, 2003). Some drugs (fibrates), hormones (thyroid, steroid, pituitary, and insulin), and nutritional factors (glucose and high fat diet) as well as diurnal rhythm and such physiological
conditions as obesity and age also have effects on CYP7A1 activity (Chiang, 1998). An important mechanism of regulation of CYP7A1 activity is believed to take place at the level of gene transcription, because changes in enzyme activity were found to parallel those in mRNA levels. A number of reports, however, indicate that control of the CYP7A1 activity could also include post-transcriptional events such as cholesterol availability, cytosolic factors, disulfide bonds in the enzyme structure, and phosphorylation (Chiang, 1998). The mRNA changes do not always correlate with changes in enzyme activity (Pandak et al., 1994; Cheema et al., 1997; Li et al., 2004), also suggesting post-transcriptional regulation. Finally, it is not clear at present why CYP7A1 activity varies over a 5- to 10-fold range among healthy individuals (Nicolaou et al., 1974; Oda et al., 1990; Reinhart et al., 1990). The mechanism of regulation of CYP7A1 activity varies over a 5- to 10-fold range among healthy individuals (Nicolaou et al., 1974; Oda et al., 1990; Reinhart et al., 1990). The mechanism of regulation of CYP7A1 activity is just beginning to be elucidated, and virtually nothing is known about the regulation of CYP46A1. Human CYP27A1 mRNA and enzyme activity is down-regulated by bile acids; however, the responses are less prominent than those of CYP7A1 (Chen and Chiang, 2003; Ellis et al., 2003). Two reports in the literature suggest post-transcriptional regulation of CYP27A1 activity, showing a large discrepancy in the magnitude of increase in the CYP27A1 mRNA and protein levels versus enzyme activity (Hall et al., 2001), and the other, significantly reduced enzyme activity when mRNA levels remained normal (Matsuzaki et al., 2002). With CYP11A1, there is acute and chronic regulation of the enzyme activity. Acute regulation occurs at the level of cholesterol availability, whereas chronic regulation takes place at the transcriptional level and involves pituitary trophic hormones acting via the cAMP-dependent pathway (Sewer and Waterman, 2003).

Thus, activity of one of four cholesterol-metabolizing P450s is established to be regulated post-translationally, and there is a possibility, based on the literature data, that there may be post-translational regulation of activity of the other two enzymes (CYP7A1 and 27A1). Keeping this information in mind, as well as questions outlined in the beginning of this section, the following research is being undertaken.

**Structure/Function Studies of Cholesterol-Metabolizing P450s**

Characterization of cholesterol-metabolizing P450s is mainly carried out using purified recombinant enzymes that are produced by *Escherichia coli* when cells are transformed with the expression plasmid containing the P450 cDNA (Wada et al., 1991; Karam and Chiang, 1994; Pikuleva et al., 1997; Mast et al., 2003).

**Active Site.** The active site of CYP7A1 and 27A1 was investigated using substrate analogs in combination with homology modeling and site-directed mutagenesis (Mast et al., 2005; N. Mast, D. Murtazina, H. Liu, S. Graham, I. Bjorkhem, J. R. Halpert, J. Peterson, and I. A. Pikuleva, manuscript submitted for publication). In the case of CYP7A1, 41 mutants, encompassing 26 amino acid residues, were generated and characterized. Based on the homology model and phenotype of the mutant P450s, seven active site residues were identified (V280, A284, N288, A358, L360, and L485) and suggested to determine the enzyme specificity for cholesterol (Fig. 2). Studies of CYP7A1 indicate that there is a tight complementarity fit between cholesterol and the enzyme active site, and a “goodness of fit” seems to be the feature that contributes in part to strict substrate specificity and high catalytic efficiency of this P450 (Mast et al., 2005).

CYP27A1 has a broader substrate specificity than CYP7A1, accommodating substrates with significantly different three-dimensional structures. For example, cholesterol, the enzyme substrate in the alternative bile acid biosynthetic pathway, is a flat molecule and has only one hydroxyl group in the steroid nucleus, the 3β-hydroxyl. In contrast, 5β-cholestan-3α,7α,12α-triol, the CYP27A1 substrate in the classical bile acid biosynthetic pathway, has a bend at the A/B ring junction, the 3-hydroxyl in the α-position instead of the β-position.
identified, and mutations were introduced in this region (Pikuleva et al., 2001). Wild-type and mutant P450s were compared with respect to catalytic activity, product pattern, substrate binding, formation of hydrogen peroxide, and interaction with reduct partner. Results indicate that the mutated residues are important for delivery of the correctly oriented substrate to the P450 active site. The I211K and F215K mutations, for example, affected the regioselectivity of CYP27A1-dependent hydroxylation reactions and conferred the P450 capacity to cleave one of the terminal methyl groups of 5β-cholestan-3α,7α,12α-triol during the catalytic cycle. Studies of CYP11A1 indicate that F202 has functions similar to those of its counterpart in P450 27A1 (F215). Thus, CYP27A1 and 11A1 seem to have the substrate access channel that provides an additional mechanism to control regioselectivity of hydroxylation in mitochondrial P450s (Pikuleva et al., 2001).

**Association with the Membrane.** Studies of the microsomal enzyme CYP2C5 suggest that both mitochondrial (e.g., 27A1) and microsomal (e.g., 7A1) P450s have a similar, monofacial mode of association with the membrane (von Wachenfeldt and Johnson, 1995; Williams et al., 2000). In microsomal P450s, a large hydrophilic domain is believed to be anchored to the lipid bilayer through the N-terminal segment and the adjacent surface of the protein formed by noncontiguous portions of the polypeptide chain. Mitochondrial P450s do not contain the N-terminal membrane anchor, and yet their membrane-binding motif is likely to be similar to that of microsomal P450s and includes one or more hydrophobic regions that are partially inserted in the membrane (Williams et al., 2000). A loop between the putative helices F and G and adjacent segments of these helices were proposed to be one of the regions involved in membrane binding in eukaryotic P450s (Graham-Lorence et al., 1995; Graham and Peterson, 1999). Computer modeling and quantification of subcellular distribution of the F-G loop mutants in *E. coli* were used to assess membrane topology of CYP7A1, 27A1, and 11A1 (Nakayama et al., 2001; Muratzena et al., 2002; Pikuleva, 2004). The validity of *E. coli* as a model system was justified by the fact that, despite differences in the phospholipid (PL) content of bacterial and eukaryotic membranes, cholesterol-metabolizing P450s stay associated with the membrane fraction when expressed in *E. coli* and are catalytically active when *E. coli* membranes containing these enzymes are used for reconstitution of the enzyme activity. Replacements of the amino acid residues in the putative F-G loop caused alteration of subcellular distribution of the three cholesterol-metabolizing P450s, strongly suggesting that the F-G loop is indeed a membrane-interacting area in CYP7A1, 27A1, and 11A1. Membrane binding of CYP11A1 was also studied by a different approach. Cysteine mutagenesis within the F-G loop was followed by fluorescent labeling of the mutated residues and then measurements of the changes in their fluorescence upon association of the mutant P450 with PL vesicles. The data obtained also support the notion that association of CYP11A1 with the membrane is mediated, at least in part, by the F-G loop (Headlam et al., 2003). The effect of the altered membrane-protein interactions on kinetic parameters for cholesterol hydroxylation of the F-G loop mutants was investigated in a reconstituted system containing exogenous NADPH, cholesterol, reduct partner(s), and the P450-containing *E. coli* membranes (Nakayama et al., 2001; Muratzena et al., 2002). In CYP7A1, several substitutions increased *K*_μ*_, but not *K_*μ, up to 12-fold, whereas in CYP27A1, there was no significant change of *K*_μ_. The *k*_cat values varied significantly in both P450s and ranged from undetectable activity to a 2- to 3-fold increased activity. An increase in *k*_cat in the CYP7A1 mutants was not enough to compensate for the increased *K*_μ, therefore, an overall effect was a decrease in catalytic efficiency of cholesterol hydroxylation (the *k*_cat/*K*_μ* ratio). In the CYP27A1
mutants, \( k_{\text{cat}} \), values were not significantly affected, and mutants with increased \( k_{\text{cat}} \) values had increased catalytic efficiencies. The K226R mutation that underlines CTX was reproduced during the course of this work (Murtazina et al., 2002). According to the computer model, K226 is located on the surface of the molecule in the putative F-G loop (Fig. 4). The K226R replacement weakened the CYP27A1 interaction with the membrane, insignificantly increased the \( k_{\text{mic}} \), and resulted in a 5-fold reduction of the \( k_{\text{cat}} \). Properties of the K226R mutant indicate that a complete disruption of the CYP27A1 activity is not required for CTX to develop; even a 5-fold decrease of catalytic efficiency of cholesterol hydroxylation is sufficient to cause the disease. Overall, kinetic studies clearly demonstrated that the way in which CYP7A1 and 27A1 interact with the membrane influences catalytic efficiency of cholesterol hydroxylation, and the effect is not always negative since, in some of the mutants, cholesterol hydroxylation became more efficient. Establishing that membrane-protein interactions contribute to efficiency of cholesterol hydroxylation raises a possibility that activity of cholesterol-metabolizing P450s provide insight into the factors that control the rates of cholesterol and 5\(^\text{a}\)-cholestane-3\(^\text{a}\),7\(^\text{a}\),12\(^\text{a}\)-triol hydroxylation, and, therefore, they have the potential to regulate the amount of cholesterol metabolized through the classical and alternative pathways of bile acid biosynthesis (Murtazina et al., 2004). Maintenance of cholesterol homeostasis is complex, involves many enzymes and proteins, and is controlled at several levels. A comprehensive investigation is required to assess how varying ratios between different fatty acids, especially between the \( n\)-6/\( n\)-3 PUFAs, simultaneously affect plasma lipid profile and activity and expression of the key enzymes and proteins in the pathways of cholesterol synthesis and degradation. It is very likely that through these studies, an optimal composition of the dietary fat will be found which maximally stimulates cholesterol degradation and has a strong overall hypocholesterolemic effect. If established that cholesterol balance could be regulated via post-translational modulation of activity of cholesterol-metabolizing P450s, a strong impetus will be given to studies of other classes of fatty acids. Conjugated linoleic acid could be one of such fatty acids because it has been reported to have numerous beneficial effects on human health, including cardiovascular disease (Belury, 2002).

**Conclusions**

There is a clear need to identify additional therapeutic strategies to lower plasma cholesterol levels. Structure and function studies of cholesterol-metabolizing P450s provide insight into the factors that control catalytic efficiency of these important metabolic enzymes and suggest that their activity could be modulated post-translationally. Further research is required to assess the potential of cholesterol-metabolizing P450s as targets for cholesterol lowering.

**References**


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