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Research Paper

Molecular mechanisms of the microsomal mixed function oxidases and biological and pathological implications

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ABSTRACT

The cytochrome P450 mixed function oxidase enzymes play a major role in the metabolism of important endogenous substrates as well as in the biotransformation of xenobiotics. The liver P450 system is the most active in metabolism of exogenous substrates. This review briefly describes the liver P450 (CYP) mixed function oxidase system with respect to its enzymatic components and functions. Electron transfer by the NADPH-P450 oxidoreductase is required for reduction of the heme of P450, necessary for binding of molecular oxygen. Binding of substrates to P450 produce substrate binding spectra. The P450 catalytic cycle is complex and rate-limiting steps are not clear. Many types of chemical reactions can be catalyzed by P450 enzymes, making this family among the most diverse catalysts known. There are multiple forms of P450s arranged into families based on structural homology. The major drug metabolizing CYPs are discussed with respect to typical substrates, inducers and inhibitors and their polymorphic forms. The composition of CYPs in humans varies considerably among individuals because of sex and age differences, the influence of diet, liver disease, presence of potential inducers and/or inhibitors. Because of such factors and CYP polymorphisms, and overlapping drug specificity, there is a large variability in the content and composition of P450 enzymes among individuals. This can result in large variations in drug metabolism by humans and often can contribute to drug–drug interactions and adverse drug reactions. Because of many of the above factors, especially CYP polymorphisms, there has been much interest in personalized medicine especially with respect to which CYPs and which of their polymorphic forms are present in order to attempt to determine what drug therapy and what dosage would reflect the best therapeutic strategy in treating individual patients.

Introduction

The cytochrome P450 mixed function oxidase system plays a major role in the metabolism of important endogenous substrates such as in cholesterol biosynthesis and cholesterol conversion to bile acids, formation of steroid hormones, androgens and estrogens, metabolism of vitamin D_3 to the active 1,25-dihydroxyvitamin D_3, omega hydroxylation of fatty acids, as well as biotransformation of exogenous xenobiotics. The biological effectiveness and the potential toxicity of many drugs are strongly influenced by their metabolism, much of which is accomplished by P450-dependent monoxygenase systems. The wide array of chemical reactions performed by P450 makes this enzyme one of the most versatile catalysts known. The liver, lung and skin microsomal P450s in particular are important in converting lipophilic xenobiotics including drugs, insecticides, carcinogens, food additives, and environmental pollutants to more polar compounds which are easier to excrete. Intestinal CYPs, especially CYP3A4, may be very important in promoting first pass metabolism of many drugs. Since many of these compounds, lose their activity or potency after being metabolized to polar and excretable metabolites, P450 was considered to be important as a cellular detoxification system. However, with certain compounds although the parent xenobiotic is not toxic, metabolism by the P450 system can generate reactive intermediates which are highly toxic e.g. CCL_4, nitrosamines and acetaminophen.

The term P450 designates a broad family of heme-containing proteins found in bacteria, yeast, plants, invertebrates and vertebrates. About 150 forms of P450 have been identified and a nomenclature based on structural homology, largely deduced from the corresponding cDNAs is used to classify these multiple forms of P450 [1]. The nomenclature is based on evolutionary relationships between CYP450 enzymes and not on similarity in substrate profiles because of the overlapping substrate profiles of many CYP

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enzymes and the ability of multiple CYPs to modify a single substrate at the same or even at different sites [2]. Many in vitro studies using isolated microsomal fractions or intact hepatocytes or cell lines have provided critical and basic information on drug metabolism by CYPs despite the relative short term limitations of such systems including lack of conjugation and other cytosolic enzymes, loss of CYPs in culture, limited number of liver functions expressed, lower levels of CYPs compared to in vivo. Studies to improve such in vitro systems in order to be more reflective of the in vivo state are an important research front.

Several of the contributions to the common theme series in Redox Biology entitled "Role of CYP2E1 and Oxidative/Nitrosative stress in the Hepatotoxic Actions of Alcohol" discuss the P450 enzyme CYP2E1. The goal of this brief overview is to summarize the molecular mechanisms of the cytochrome P450 microsomal drug oxidation system and perhaps be helpful as an educational tool analogous to the Graphical Review by Dr. B. Kalyanaraman on "Oxidants, Antioxidants and Disease Mechanisms" published in Redox Biology [3].

Cytochrome P450 General characteristics

The presence of a carbon monoxide-binding pigment in rat liver microsomes was initially reported in 1958 [4,5]. The oxidized and reduced spectrum of one member of the cytochrome P450 family of enzymes, CYP2E1, is shown in Fig. 1. The pigment, when reduced, displayed a maximal absorbance at a wavelength of 450 nm when binding carbon monoxide and was called P (pigment)-450. Spectral evidence revealed that P450 was a heme-containing protein [6] and P450 plus cytochrome b_5 accounted for most of the hemoproteins found in liver microsomes. The content of cytochrome P450 (nmol/mg microsomal protein) can be calculated from the ferrous carbon monoxide P450 versus ferrous P450 difference spectrum as described in detail in [7]. Cytochrome P450 was subsequently shown to function as the oxygen-activating oxidase associated with microsomal oxygenation reactions such as steroid C-21 hydroxylation, xenobiotic hydroxylation and oxidative dealkylations [8,9]. Elevated activity of the microsomal mixed function oxidase system after in vivo administration of certain drugs was shown to be related to cytochrome P450 and its inducibility [10]. CYPs are b-type cytochromes, containing protoporphyrin IX as the prosthetic group.

Cytochrome P450 is present in various vertebrates, invertebrates and plants. In mammals, P450, while present at highest levels in microsomes from liver (where it plays a major role in detoxification reactions) is also present in microsomes from kidney, small intestine, lungs, adrenal cortex, skin, brain, testis, placenta and other tissues [9]. Mitochondria, especially from liver and endocrine tissue, contain P450. The nuclear envelope and plasma membranes contain low amounts of P450 [11,12]. In plants, P450s are involved in the synthesis of lignins and alkaloids.

Most P450s are made up of about 400–500 amino acids with molecular weights of about 50,000 Da. About half of the amino acids are non-polar. While earlier studies proposed P450 to be buried in the membrane, it is now recognized that much of the

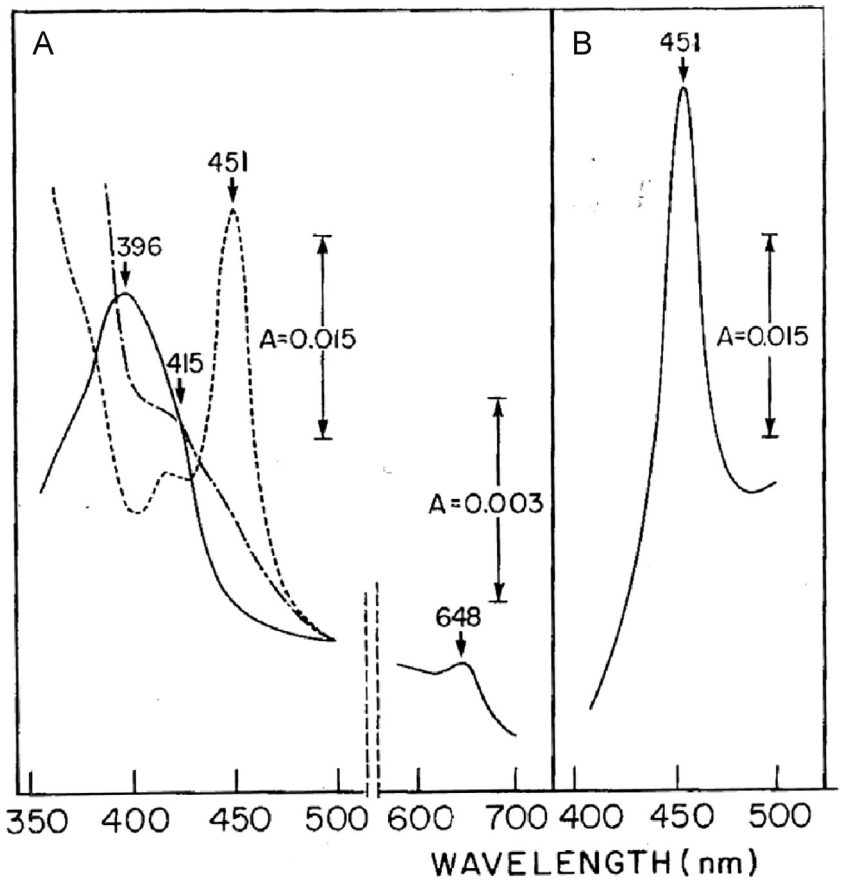

Fig. 1. Spectral characteristics of cytochrome P450. A: Absolute spectra of CYP2E1 purified from pyrazole-treated rats [101]. The sample cuvette contained 0.39 nmol of CYP2E1 in 0.1 M KPi, pH 7.4 buffer, 20% glycerol, 0.1 mM EDTA, 0.2% Emulgen 911 and 0.5% sodium cholate in a final volume of 1 ml. The reference cuvette was identical except for the omission of the CYP2E1. The scanned spectra were: oxidized CYP2E1 (solid line); dithionite reduced CYP2E1 (dot/dashed lines -.-.-.-.-.); carbon monoxide-bound reduced CYP2E1 (dashed line - - - -). B: The CO-CYP2E1 difference spectrum. Dithionite-reduced CYP2E1 (0.39 nmol/ml) was present in the sample and reference cuvettes. The sample cuvette was saturated with CO and spectra recorded over the indicated wavelengths.