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CYTOCHROME P450 POLYMORPHISM – MOLECULAR, METABOLIC AND PHARMACOGENETIC ASPECTS. I. MECHANISMS OF ACTIVITY OF CYTOCHROME P450 MONOOXYGENASES

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Abstract: Cytochrome P450, initially perceived as a type of cell pigment, was soon identified as a hemoprotein with an enzymatic activity characteristic for monooxygenases with an affinity for differentiated endo- or exogenous substrates, including drugs. So far in the human organism 58 CYP isoenzymes belonging to 18 families have been described. Most from the CYP monooxygenases superfamily turned out to be integral elements of hepatocytic reticular monooxygenase complexes which also contain NADPH-dependent cytochrome P450 reductase (CPR). Later investigations indicated the possibility of the participation in electron transport for reticular CYP isoenzymes, alternative NADH-dependent reticular system composed of cytochrome b_5 reductase (CBR) and cytochrome b_5. The demonstration of the activity of some CYP superfamily isoenzymes not only in hepatocytes but also in many other cells of the human organism, numerous plant and animal tissues and even in cells of fungi, protists and prokaryotes has contributed to the significantly increased understanding of the role of CYP in biological systems. In addition, some CYP isoenzymes were found to be characteristic for the inner mitochondrial membrane monooxygenase complexes which contain NADPH-dependent adrenodoxin reductase (AR) and adrenodoxin (Ad), which is identical with ferredoxin-1 (Fd-1) and hepatoredoxin (Hd).

Keywords: cytochrome P450, monooxygenase activity, membrane-bound electron transport complexes

Abbreviations: **Ad** – **a**drenod**o**xin; **AR** – **a**drenodoxin **r**eductase (EC 1.18.1.2); **CBR** – **c**ytochrome $\mathbf{b_5}$ **r**eductase (EC 1.6.2.2); **CPR** – **c**ytochrome **P**450 **r**eductase (EC 1.6.2.4); **CYP** – **c**y**t**ochrome **P**450; **cyt** – cytochrome; **ER** – **e**ndoplasmic **r**eticulum; **FAD** – **f**lavin-**a**denine **d**inucleotide; **Fd** – **f**erre**d**oxin; **FMN** – **f**lavin **m**ononucleotide; fp – **f**lavo**p**rotein; **Hd** – **h**epatore**d**oxin; **MSR** – **m**ethionine **s**ynthase **r**eductase (EC 1.16.1.8), **NAD**$^+$ – **n**icotinamide **a**denine **d**inucleotide, oxidized form; **NADH+H**$^+$ – **n**icotinamide **a**denine **d**inucleotide, reduced form; **NADP**$^+$ – **n**icotinamide **a**denine **d**inucleotide **p**hosphate, oxidized form; **NADPH+H**$^+$ – **n**icotinamide-**a**denine **d**inucleotide **p**hosphate, reduced form; **NOS** – **n**itrogen **o**xide **s**ynthase (EC 1.14.13.39); $\mathbf{PGH_2}$ – **p**rosta**g**landin $\mathbf{H_2}$.

Research on cytochrome P450 polymorphism has made an important contribution to the development of modern pharmacokinetics, pharmacology and pharmacogenetics, creating and extending the possibility of developing optimal, individualized pharmacotherapeutic strategies, using the knowledge of the individual differences in reactions of the organism to a drug and the occurring interactions between the administered drugs, their metabolites and the food components and environmental toxins, and all this under the conditions of natural variation of the functional efficiency in differentiated physiological and pathological states. These investigations affect the strategy of new drug developement and serve to ensure safe and effective pharmacotherapy, adapted to individual physiological properties and health requirements of the patient.

Development of knowledge about cytochrome P450 (CYP) enzymatic activity

The name *cytochrome P450* was introduced into the scientific literature in 1962 to describe a pigment detected in the microsomal hepatocyte fraction, which in a reduced form after binding carbon monoxide showed the ability to absorb electromagnetic radiation in the visible range (1). The maximum of absorption at wavelength $\lambda = 450$ nm was the source of the numerical symbol of the new cytochrome. The appearance of a wavelength of 450 nm in the absorption spectrum of the microso-

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mal hepatocyte fraction after reduction and saturation with CO had already been observed in 1958, though the cause of this effect was unknown at the time (2, 3).

Cytochrome P450, initially perceived as a type of cell pigment, was soon identified as a hemoprotein with an enzymatic activity characteristic for monooxygenases (mixed function oxidases) using molecular oxygen and reductive equivalents supplied by NADPH + H^+ (3). Initially, in addition to typical cytochrome P450, with an affinity for differentiated small molecule substrates of endo- or exogenous character, cytochrome P488 with a maximum absorption at slightly shorter wavelengths (λ = 448 nm) and an affinity for larger compounds subject to monooxygenation was also isolated from the liver microsome fraction. Experiments indicated that the former of the two then isolated cytochromes – P450 – was induced by phenobarbital, and the latter – P448 – by polycyclic aromatic hydrocarbons (4).

The initially isolated cytochromes P450 and P448 were the first known isoenzymatic variants of a very large hemoprotein superfamily now known under the unified joint name ***cy****tochrome **P****450*** and designated by the abbreviation CYP (prev. CytP-450, Cyt P450, $cytP_{450}$) (5, 6). Most eukaryotic hemoproteins from the CYP superfamily turned out to be integral elements of reticular monooxygenase complexes which also contain flavoprotein 1 (fp_1) with a **c**ytochrome **P**450 **r**eductase activity (CPR; EC 1.6.2.4; syn.: NADPH – CytP-450 reductase, NADPH : cyt P450 oxidoreductase, P450R). It contains the diflavin system – FAD and FMN, as a pair of prosthetic groups allowing the transport of electrons from NADPH + H^+ to the CYP, interacting with the appropriate substrate. Further detailed investigations indicated the role of the phospholipid milieu of the endoplasmic reticulum (ER) membranes in the activity of CYP family monooxygenases with special emphasis on the role of phosphatidylcholine in the stabilization of interactions between CPR and CYP (7).

Later investigations in eukaryotes indicated the possibility of the participation in electron transport for reticular CYP isoenzymes, alternative to CPR, using NADH+H^+, a reticular system composed of flavoprotein 2 (fp_2) with a **c**ytochrome $\mathbf{b_5}$ **r**eductase activity (CBR; EC 1.6.2.2; syn.: NADH- $Cytb_5$ reductase, NADH : cyt b_5 oxidoreductase, B5R) and cytochrome b_5 (6, 8).

In addition to numerous CYP variants localized in the ER in hepatocytes some isoenzymes from this superfamily were found to be characteristic for the inner mitochondrial membrane e.g. CYP24A1, CYP27A1 (6). The presence of CYP molecular species identical to isoenzymes present in the ER in the external membrane of the nucleus was demonstrated, which confirms the developmental relationship of these biological membranes. According to some authors unfavorable relationships are possible between some active metabolites (e.g. epoxides) formed with the participation of these CYP family monooxygenases and mRNA transported from the nucleus to the cytoplasm (9, 10).

The demonstration of the activity of some CYP superfamily isoenzymes not only in hepatocytes but also in many other cells of the human organism, numerous plant and animal tissues and even in cells of fungi, protists and prokaryotes has contributed to the significantly increased understanding of the role of CYP in biological systems (11). So far several hundred CYP isoenzymes have been described. Many of them occur in several or even several dozen isoforms, generally as the result of genetic polymorphisms, i.e. the occurrence of differentiated alleles of *CYP* genes due to point mutations of precursor genes. In some cases the CYP molecular species are the result of differentiated transcription initiation mechanisms of one gene common for these forms (6, 12-14).

X-ray diffraction crystallography has permitted the analysis of the molecular structure of numerous CYP isoenzymes, from prokaryotic and mitochondrial forms to the fundamentally important eukaryotic reticular enzymes, whose analysis became possible after elaboration of a method of separating of the N-terminal domain anchoring CYP in reticular membranes (10).

Molecular mechanisms of the enzymatic activity of CYP superfamily monooxygenases

The basic requirement for CYP superfamily monooxygenase activity, in addition to the availability of molecular oxygen (O_2), is the coupling of the cytochrome with an enzymatic system capable of effectively providing electrons indispensable for the heme system interacting with the oxidized substrate. For the CYP5A1 isoenzyme, showing tromboxane A synthase 1 (EC 5.3.99.5) activity and CYP8A1, showing prostacyclin synthase (EC 5.3.99.4) activity, the availability of molecular oxygen is not required, as the structure of substrates for these enzymes, i.e. PGH_2, contains two partially reduced oxygen molecules of the endoepoxide system (11, 15).

In human cells, similarly as in all eukaryotes, the main electron transport system for CYP superfamily monooxygenases is the previously men-

tioned cytochrome P450 reductase – CPR. It is one of the four diflavin proteins occurring in humans and other mammals, i.e. a protein containing FAD and FMN as prosthetic groups linked to a single polypeptide chain. The other three are: nitrogen oxide synthase – NOS (EC 1.14.13.39), methionine synthase reductase – MSR (EC 1.16.1.8), and the NR1 protein. The pre-CPR with a molecular weight of 76 kDa and containing 677 amino acids is encoded by the nuclear *POR* gene (locus 7q11.2; 15 exons). The C-terminal region of CPR with closely located FAD and FMN binding domains (flavoxin-like domain) has the catalytic activity. Tryptophan, the penultimate – 676th amino acid (Trp-676) of the CPR polypeptide chain is crucial for FAD binding. The N-terminal CPR sequence binds CYP, thus is the C-terminal domain of each of the reticular complexes of CYP superfamily monooxygenases. The molecular forms of CYP anchored through their N-terminal domains in the reticular membrane on the cytoplasmic side are typical for the ER. Reduction equivalents derived from NADPH + H$^+$ are transported by CPR, with the successive use of FAD and FMN, so that finally the electrons are directed to the CYP, interacting with the proper substrate (7, 10).

An electron transport system alternative to CPR for reticular monooxygenases in man and most other eukaryotic organisms is the NADH+H$^+$-dependent reticular system of **c**ytochrome **b**$_5$ **r**eductase (CBR) and **cyt**ochrome **b**$_5$ (cyt b$_5$) (11, 16). The pre-CBR has a molecular weight of 34 kDa and contains 301 amino acid residues. It is encoded by the nuclear *CYB5R3* gene (locus 22q13.2; 9 exons) (6, 17). This protein has a C-terminal catalytic domain which interacts with NADH+H$^+$, whose reductive equivalents are transferred to the FAD, bound in the vicinity. Then the electrons are made available to the heme system of cyt b$_5$, bound to CBR through two histidines. Cyt b$_5$ occurs in two isoforms: a basic one – CYB5A.1, which binds to ER membranes, and a soluble one – CYB5A.2, which occurs in erythrocyte cytoplasm (11, 16). Isoforms are the result of differential splicing of the nuclear *CYB5A* gene (locus 18q23; 6 exons). Single electrons may be transferred through cyt b$_5$ to CYP (12, 17).

In the case of mitochondrial CYP isoenzymes electron transport is performed by **a**drenodoxin **r**eductase (AR; EC 1.18.1.2; syn.: NADPH : adrenodoxin oxidoreductase, ferredoxin reductase, ferredoxin-NADP$^+$ reductase, AdR, FR, FdR), present in the matrix (16). The pre-AR with a molecular weight of 58 kDa contains 491 amino acids and is encoded by the nuclear *FDXR* gene (locus 17q24-q25; 12 exons; syn. *ADXR*) (6). This protein, which is a flavoprotein, allows transfer of reductive equivalents from NADPH+H$^+$, and also NADH+H$^+$, to FAD which is the AR prosthetic group. Electrons from FAD are then transferred to **a**drenodoxin (Ad), which is identical with ferredoxin-1 (Fd-1) and hepatoredoxin (Hd), a soluble iron sulfur proteins (Fe_2S_2) of the mitochondrial matrix (11, 16). Pre-Ad

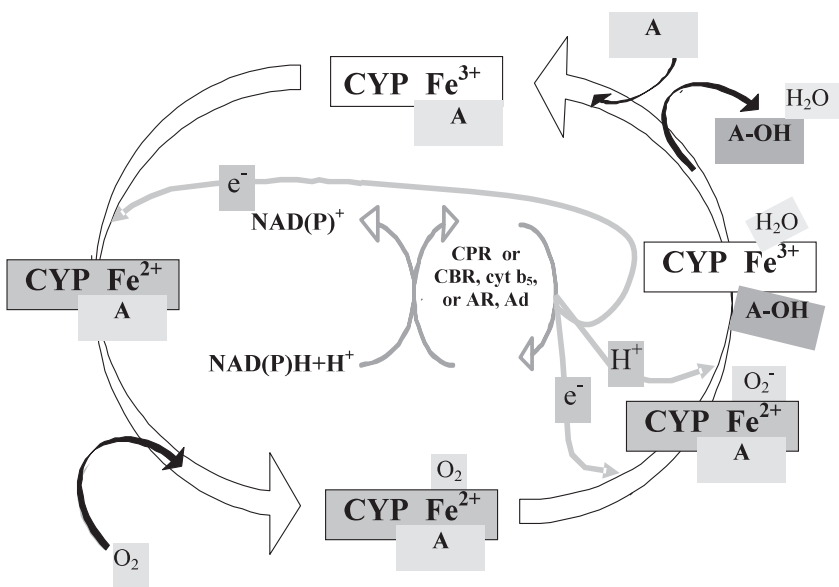

Figure 1. The enzymatic activity cycle of CYP superfamily monooxygenases. A – substrate, A-OH – product (oxidized substrate) (7, 8).

has a molecular weight of 19.4 kDa, contains 184 amino acids and is encoded by the nuclear *FDX1* gene (locus 11q22.3-q23.1; 4 exons; syn. *ADX*) (6, 13). Ad mediates electron transport to mitochondrial CYP isoenzymes anchored through their N-terminal domain in the matrix side of the mitochondrial inner membrane. An analogous system to the eukaryotic mitochondrial AR/Ad/CYP system has also been observed for CYP monooxygenase complexes in bacteria, where the role of Ad is played by: putidaredoxin, terpredoxin or rodocoxin (11, 16).

An electron transferred through one of the three above-mentioned routes to CYP(Fe^{3+}), interacting with the proper substrate, reduces Fe^{3+} in the heme system to Fe^{2+}, allowing a consequent interaction of the CYP(Fe^{2+})-substrate complex with O_2 (Figure 1). The second transferred electron allows for transformation of the O_2-CYP(Fe^{2+})-substrate complex into a form with an anion potentialized reactive oxygen species $^{\cdot}O_2$-CYP(Fe^{2+})-substrate, permitting destabilization of the bond between the atoms of the oxygen molecule to form a bond of one of the oxygen atoms with the substrate. The second oxygen atom is reduced to H_2O by the above-mentioned reductive equivalent transport systems (CPR, CBR/cyt b_5, AR/Ad) (4, 8,16).

The progress of knowledge about molecular mechanisms of the enzymatic activity of CYP superfamily monooxygenases and their role in drug biotransformation, is the basis of modern pharmacokinetics, used for the elaboration of optimal pharmacotherapeutic profiles and shaping of new drug development strategies. In the next part in this series we will analyze the participation of CYP molecular species in drug and endogenous substance metabolism and the influence of cyp genetic polymorphism on differentiation of drug metabolism phenotype.

REFERENCES

1. Omura T., Sato R.: J. Biol. Chem. 237, 1375 (1962).
2. McKinnon R.: Aust. J. Hosp. Pharm. 30, 54 (2000).
3. Estabrook R.W.: Drug Metab. Dispos. 31, 1461 (2006).
4. Beyer K-H.: Biotransformation der Arzneimittel. pp. 4-28, Springer-Verlag Berlin, Heidelberg 1990, .
5. IUBMB Enzyme Nomenclature: www.iubmb.org.
6. UniProt – The Universal Protein Knowledgebase: www.expasy.org/uniprot.
7. Gutierrez A., Grunau A., Paine M., Munro A.W., Wolf C.R., Roberts G.C.K., Scrutton N.S.: Biochem. Soc. Trans. 31, 497 (2003).
8. Murray R.K., Granner D.K., Mayes P.A., Rodwell V.W.: Harper's Biochemistry (Polish translation), Wydawnictwo Lekarskie PZWL, Warszawa 2006.
9. Ortiz de Montellano P.R. (ed.): Cytochrome P450. Structure, Mechanism, and Biochemistry. 3rd ed., Kluwer Academic/ Plenum Publishers, New York 2005.
10. Williams P.A., Cosme J., Sridhar V., Johnson E.F., McRee D.E.: Mol. Cell 5, 121 (2000).
11. McLean K.J., Sabri M., Marshall K.R., Lawson R.J., et al.: Biochem. Soc. Trans. 33, 796 (2005).
12. Source. Stanford University, Genetics Department: www. stnford.edu/cgi-bin/source/surcesearch.
13. Human Gene Lynx: www.genelynx.org.
14. Genatlas Gene Database: www. genatlas.org.
15. Entrez Protein. NCBI Sequence Viever v. 2.0. National Center for Biotechnology Information: www.ncbi.nlm.nih.gov/ entrez/query.fcgi?cmddb.
16. Hanukoglu I.: Adv. Mol. Cell. Biol. 14, 29 (1996).
17. Ensembl Human GeneViev: www.ensembl.org.

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