CYTOCHROME P450: Structure-Function

1. General P450 Characteristics and Taxonomy
2. Human P450s – Substrate and Inhibitor Selectivities
3. Structure-Function Aspects of Ligand Binding, P450 Reduction and Oxygen Activation

References

P450 Homepage -http://drnelson.utmem.edu/CytochromeP450.html
Drug elimination is dominated by metabolic processes, which in turn are dominated by **cytochrome P450-mediated oxidative (Phase I) metabolism.**

**Phase 0 - Uptake**
**Phase I - Functionalization**
**Phase II - Conjugation**
**Phase III - Efflux**

Hydroxylations and Dealkylations are the most common P450 reactions. They serve to decrease the lipophilicity of parent drug and enhance excretion.

**Aliphatic hydroxylation**

\[ \text{Aliphatic hydroxylation} \]

**Epoxidation**

\[ \text{Epoxidation} \]

**Aromatic hydroxylation**

\[ \text{Aromatic hydroxylation} \]

**Dealkylation**

\[ \text{Dealkylation} \]

**Heteroatom oxygenation**

\[ \text{Heteroatom oxygenation} \]

**Alcohol and Aldehyde oxidations**

\[ \text{Alcohol and Aldehyde oxidations} \]

**Dehydrogenation**

\[ \text{Dehydrogenation} \]

**Deformylation**

\[ \text{Deformylation} \]

**Dehydration**

\[ \text{Dehydration} \]

**Reductive dehalogenation**

\[ \text{Reductive dehalogenation} \]

**Isomerization**

\[ \text{Isomerization} \]
Cytochrome P450s - Basic characteristics

- Named for wavelength of maximal absorption of the Fe^{2+}-CO complex
- Occurs at 450 ± 4 nm; ε ~ 100 mM cm^{-1}
- Superfamily of heme-containing oxygenases with MWt of 55 ± 5 kDa
- ~ 500 amino acids

Fe^{2+}-CO vs Fe^{2+} difference spectrum of P450

Purified P450s from Rat
(Levin et al., 1980)

Difference spectrum can be used to quantitate P450 by applying ε = 91 mM^{-1} cm^{-1} according to the relationship:

\[
[P450] (\mu M) = \frac{\Delta A\ (450-490) \times 1000}{91 \times 1} = 0.007 \times 11 = 0.08 \mu M
\]

- P450s are ubiquitous in nature, >8000 named genes as of 2/2008
- There are >200 P450 genes in rice, and in plants P450s often represent up to ~1% of the total genome.
- Humans have 115 P450 genes, but only ~50% of these are full-length.
- The 57 functional human P450s are arranged into 18 families that are conserved in all mammals.
- Only 3 P450 families are important to hepatic drug clearance.
### Substrate Selectivity of the 57 Human P450 Enzymes

<table>
<thead>
<tr>
<th>XENOBIOTICS</th>
<th>STEROIDOGENIC</th>
<th>FATTY ACIDS/EICOSANOIDS</th>
<th>UNKNOWN</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>CYP11A1</td>
<td>CYP4A11</td>
<td>CYP2A7</td>
</tr>
<tr>
<td>CYP1A2</td>
<td>CYP11B1</td>
<td>CYP4B1</td>
<td>CYP2S1</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>CYP11B2</td>
<td>CYP4F2</td>
<td>CYP2U1</td>
</tr>
<tr>
<td>CYP2A6</td>
<td>CYP17A1</td>
<td>CYP4F8</td>
<td>CYP2W1</td>
</tr>
<tr>
<td>CYP2A13</td>
<td>CYP19A1</td>
<td>CYP4F12</td>
<td>CYP3A43</td>
</tr>
<tr>
<td>CYP2B6</td>
<td>CYP21A2</td>
<td>CYP5A1</td>
<td>CYP4A22</td>
</tr>
<tr>
<td>CYP2C8</td>
<td>CYP8A1</td>
<td></td>
<td>CYP4F11</td>
</tr>
<tr>
<td>CYP2C9</td>
<td>BILE ACID</td>
<td></td>
<td>CYP4F22</td>
</tr>
<tr>
<td>CYP2C18</td>
<td>CYP7A1</td>
<td>VITAMIN D</td>
<td>CYP4V2</td>
</tr>
<tr>
<td>CYP2C19</td>
<td>CYP7B1</td>
<td>CYP24A1</td>
<td>CYP4X1</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>CYP8B1</td>
<td>CYP26C1</td>
<td>CYP4Z1</td>
</tr>
<tr>
<td>CYP2E1</td>
<td>CYP27A1</td>
<td>CYP27B1</td>
<td>CYP20A1</td>
</tr>
<tr>
<td>CYP2F1</td>
<td>CYP39A1</td>
<td>CYP2R1</td>
<td>CYP27C1</td>
</tr>
<tr>
<td>CYP2J2</td>
<td>CYP46A1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A4</td>
<td>CYP51A1</td>
<td>RETINOIC ACID</td>
<td></td>
</tr>
<tr>
<td>CYP3A5</td>
<td>CYP26A1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP3A7</td>
<td>CYP26B1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP4F3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Andrew Parkinson, 2009
P450 TAXONOMY - Basic Nomenclature Rules:

- When describing a P450 gene, CYP1A2 for example, CYP is italicized and designates the gene as a segment coding for cytochrome P450. The first arabic numeral designates the P450 family. This is followed by a capital letter designating the subfamily, and another arabic numeral to distinguish members within a subfamily.

- When describing the gene product, either CYP or P450 can be used in front of the family designation; for example, CYP1A2.

- P450 isoforms are assigned to specific families on the basis of amino acid sequence homology. The P450 protein sequences within a given family are >40% identical (some exceptions exist).

- P450 sequences within the same sub-family are > 55% identical. The degree of homology for distinct gene products from the same sub-family varies between 55 and 98% (e.g. rat CYP2B1 and CYP2B2 differ only at 13 positions out of a total of 491 amino acids).

- When considering genetic variants of a P450 gene, an asterisk is placed after the arabic numeral for sub-family designation, and each allelic form is assigned an arabic number, e.g. CYP2C9*2 represents the first allelic form of this gene discovered (relative to the reference sequence which usually has the *1 designation).

- Homologous P450s are related genes that can be identified on the basis of sequence similarity alone, e.g. human CYP2C9, rat CYP2C11 and monkey CYP2C43. They likely evolved from a common ancestor before species divergence.

- Orthologous P450s are related gene products that maintain functional similarities. Examples of P450 orthologs are the CYP2E1 enzymes found in the rat, rabbit, monkey, and human, - all of which have very similar catalytic properties. In contrast, it is difficult to identify species orthologs to the human CYP3A and CYP2C isoforms.
ALL the Human P450 genes in Families CYP1-CYP3

CYP1A1, CYP1A2, CYP1B1

CYP2A6, CYP2A7, CYP2A13
CYP2B6
CYP2C8, CYP2C9, CYP2C18, CYP2C19
CYP2D6
CYP2E1
CYP2F1
CYP2J2
CYP2R1
CYP2S1
CYP2U1
CYP2W1

“Orphan P450s” identified through large-scale sequencing projects including the Human Genome Project

CYP3A4, CYP3A5, CYP3A7, CYP3A43

HUMAN LIVER P450 CHEATSHEET
(see also http://www.fda.gov/cder/drug/drugInteractions/tableSubstrates.htm)

• The average amount of P450 in human liver microsomes is ~500 pmols/ mg microsomal protein. CYP3A and CYP2C proteins make up more than 75% of total liver P450.

CYP3A4 (50-350 pmol/mg)
• The major constitutive isoform in human liver and intestine, responsible for the metabolism of up to 50% of all drugs, cleared by oxidative processes.
• Highly inducible form of P450 (e.g. by rifampin, phenytoin, phenobarbital).
• Key drug substrates include lovastatin, alfentanil, nifedipine, midazolam, R-warfarin, lidocaine, quinidine, carbamazepine, ethynyl estradiol, erythromycin.
• Marker reactions: midazolam 1’-hydroxylation, testosterone 6β-hydroxylation,
• Inhibitors: ketoconazole, itraconazole, azamulin
• Activator: α-naphthoflavone

CYP3A5 (0-200 pmol/mg)
• Present at significant levels in humans in only ~15% of the adult Caucasian population due to genetic polymorphism.
• Similar, albeit slightly distinct substrate specificity to CYP3A4
• Marker reaction: midazolam 1’-hydroxylation
• Inhibitor: ketoconazole (all weaker inhibitors than for CYP3A4).
CYP2E1 (~50 pmol/mg)
- An important constitutive isoform in both human and animal liver.
- Inducible by ethanol
- Key substrates: ethanol, acetaminophen, volatile anesthetics (enflurane and sevoflurane), and a myriad of organic solvents.
- **Marker reaction**: chlorzoxazone 6-hydroxylation
- **Inhibitor**: diethyl dithiocarbamate (disulfiram metabolite)

CYP2D6 (0-15 pmol/mg)
- Relatively uninducible form that prefers to metabolize basic drugs.
- Highly polymorphic, > 60 alleles known.
- Key substrate classes, β-blockers, many CNS drugs.
- **Marker reaction**: dextromethorphan O-demethylation
- **Inhibitor**: quinidine

CYP2C9 (40-80 pmol/mg)
- Major form, prefers to metabolize mildly acidic drugs
- Key substrates: phenytoin, tolbutamide, S-warfarin.
- **Marker reaction**: S-warfarin 7-hydroxylation, diclofenac 4’-hydroxylation
- **Inhibitor**: sulfaphenazole, benzbromarone
- **Activator**: dapsone

CYP2C19 (0-30 pmol/mg)
- Important polymorphic isoform, prefers basic or neutral substrates
- Key substrates: omeprazole, citalopram, proguanil.
- **Marker reaction**: (S)-mephenytoin 4’-hydroxylation
- **Inhibitor**: (S)-benzyl nirvanol

CYP2C8 (10-25 pmol/mg)
- Key substrates: taxol, carbamazepine
- **Marker reaction**: paclitaxel 6α-hydroxylation, amodiaquine de-ethylation
- **Inhibitor**: montelukast
CYP2B6 (0-50 pmol/mg)
• Highly inducible
• Key substrates: bupropion, efavirenz
• **Marker reaction**: bupropion hydroxylation
• **Inhibitor**: thiotepa, clopidogrel, (sibutramine)

CYP2A6 (0-20 pmol/mg)
• Key substrates: nicotine and several tobacco smoke carcinogens. Some overlap with CYP2E1 substrates
• **Marker reaction**: coumarin 7-hydroxylation
• **Inhibitor**: methoxypsoralen, tranylcypromine,

CYP1A2 (10-50 pmol/mg)
• Inducible by cigarette smoke and polycyclic aromatic hydrocarbons.
• Key substrates: caffeine, theophylline, phenacetin, several some pro-mutagens (2-acetylamino-fluorine and by-products of charcoal broiled meats).
• **Marker reaction**: phenacetin O-deethylation, caffeine N-3 demethylation
• **Inhibitor**: furafylline

**Summary – The Seven Samauri:**
**Key Drug Metabolizing and Bioactivating Human P450s**
- Diagnostic Substrates and Inhibitors

<table>
<thead>
<tr>
<th>Isoform</th>
<th>Typical substrate</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A2</td>
<td>Caffeine</td>
<td>Furafylline&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>2C8</td>
<td>Amodiaquine</td>
<td>Montelukast&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>2C9</td>
<td>Flurbiprofen, (S)-Warfarin</td>
<td>Sulfaphenazole&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>2C19</td>
<td>(S)-Mephenytoin</td>
<td>(S)-Benzyl-nirvanol&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>2D6</td>
<td>Dextromethorphan</td>
<td>Quinidine&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>2E1</td>
<td>Chlorzoxazone</td>
<td>Disulfiram&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>3A4</td>
<td>Midazolam</td>
<td>Ketoconazole&lt;sup&gt;1&lt;/sup&gt;, TAO&lt;sup&gt;2&lt;/sup&gt; CYP3cide&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>nM Ki  <sup>2</sup>Mechanism-based