ADRENODOXIN REDUCTASE OF MITOCHONDRIAL CYTOCHROME P450 SYSTEMS: STRUCTURE AND REGULATION OF EXPRESSION

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1. The role of adrenodoxin reductase in mitochondrial cytochrome P450 systems

Cytochrome P450 type enzymes generally function in the hydroxylation of small hydrophobic molecules in diverse metabolic and biosynthetic pathways (7). The mitochondrial P450s catalyze critical steps in the biosynthesis of steroid hormones, bile acids and vitamin D, all of which share the steroid structure (Table 1). The reactions catalyzed by these P450s are either a single hydroxylation (monooxygenation) at a specific position on the substrate, or a series of consecutive monooxygenations which result in C-C bond cleavage. Each P450 catalyzed hydroxylation has the following stoichiometry:

$$\text{Substrate-H} + \text{NADPH} + \text{H}^+ + \text{O}_2 \longrightarrow \text{Substrate-OH} + \text{NADP}^+ + \text{H}_2\text{O}$$

thus consumes two electrons, a H^+, and O_2. The two electrons from NADPH are transferred to P450 by specific electron carrier proteins. In the mitochondrial P450 systems this function is performed by two proteins: adrenodoxin reductase (ferredoxin-$NADP^+$ reductase, which is a flavoenzyme with FAD) and adrenodoxin (which is a ferredoxin type iron sulfur protein) (8, 9, 20). The microsomal P450 systems are dependent on P450 reductase, (which is a flavoenzyme with FAD and FMN) wherein the cofactor FMN fulfils a role similar to that of adrenodoxin in the mitochondrial P450 systems as a single electron acceptor (Fig. 1). The molar ratios of these proteins (10, 37) and kinetic studies of enzyme-enzyme interactions (8, 9, 20, 29) indicate that the P450 system enzymes do not form static complexes on the membrane and are independently mobile.

2. Gene number and organization

The first cloned cDNA for adrenodoxin reductase was isolated by screening bovine adrenal cortex cDNA expression libraries using specific antibodies (11, 24, 32). The bovine cDNA was then used to isolate the human cDNA for the same enzyme (35). In both bovine and human genome there is only one gene that hybridizes with the adrenodoxin reductase cDNA (11, 35). The human gene is located on chromosome 17 (35), is approximately 12 kb long and consists of 12 exons (21).

The finding of a single gene for adrenodoxin reductase established that the two different mitochondrial cytochromes P450 in the adrenal cortex and P450scc in the ovary and testis must be dependent on the same reductase for electron transfer (11). Until this determination of gene number, the possibility that there could be distinct isozymes specific for each P450 could not be eliminated based on protein chemistry alone. Two dimensional gel electrophoresis indicated the presence of two forms of adrenodoxin reductase

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Table 1. Mitochondrial Cytochrome P450 Systems

Enzyme	Substrate	Reaction	Tissue	Concentration (pmol/mg protein)[a]	Reference
P450scc	Cholesterol	Side chain cleavage	Adrenal cortex	400	10
"			Corpus luteum	80-400	10, 37
"			Ovary	<5	10, 37
"			Placenta	<50	34
"			Testis Leydig cells	-	23
"			Brain	<10	39
P450c11	Steroids	C-11 hydroxylation	Adrenal cortex	400	10
P450c1	Vitamin D	C-1 "	Kidney	<20[b]	3, 6, 19, 27
"			Placenta	-	42
P450c24	Vitamin D	C-24 "	Kidney	-	3, 6
P450c25	Vitamin D	C-25 "	Liver	-	4
P450c26	Bile acids	C-26 "	Liver and most	-	1, 4, 26, 41
"	Cholesterol	C-26 "	tissues		26, 41
"	Vitamin D	C-25 "			4

P450c25 is listed as a separate enzyme, but since P450c26 is also capable of catalyzing the same reaction these two activities may reflect the function of one enzyme (4). In addition to the P450s listed here, two xenobiotic inducible mitochondrial P450s have been observed in rat liver (31). These P450s also show catalytic activities similar to P450c26 (31).

[a] The concentrations for placenta, brain and kidney were calculated assuming that the mitochondrial fraction represents 20% of the total cellular protein.
[b] This value represents the concentrations of all mitochondrial P450s in the kidney.

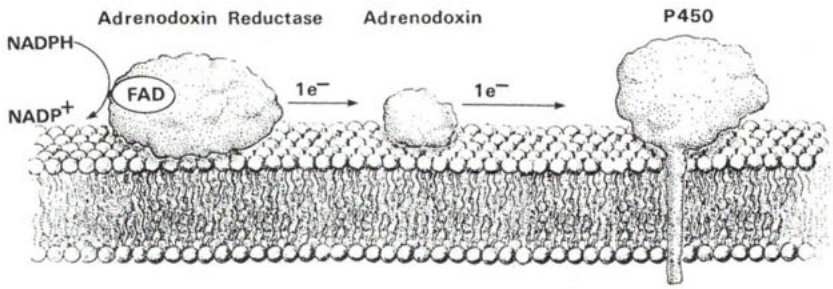

Figure 1. Model for the membrane association of the mitochondrial P450 system enzymes. The enzymes are located on the inner mitochondrial membrane and face the matrix (inner) side of the membrane (14). The membrane spanning region of the mitochondrial P450s is not known and is depicted in analogy to the microsomal P450s.

which were shown to differ in glycosylation (36). The finding of a single gene for adrenodoxin reductase further indicates that these two forms do not represent different gene products. The sequencing of the adrenodoxin reductase cDNAs revealed a minor species of a cDNA that encodes a protein with six extra residues (32, 35). The mRNA with these six residues is the product of alternative splicing, and represents only <10% of the total adrenodoxin reductase mRNAs (21, 35). It is not known whether this minor form of adrenodoxin reductase is translated to encode a functional enzyme.

3. Tissue specificity of expression

As mitochondrial P450s are found in most tissues (Table 1), adrenodoxin reductase would be expected to be present also in most tissues. The highest levels of expression of the adrenodoxin reductase gene are observed solely in steroidogenic tissues (11). A homologous mRNA could not be detected in the liver and kidney (11). If there is a homologous mRNA in these two tissues, its levels were estimated to be at least 40 fold lower than those in the adrenal (11). Similarly, the concentrations of the enzyme in the liver and kidney were estimated to be 25-100 fold lower than that in the adrenal cortex (cf. 10 and 25). Since there is only one gene for adrenodoxin reductase, the functional homologue of this enzyme in the liver, kidney, and other tissues can not be a closely related isozyme; it is either the product of the same gene or a gene that is so significantly different from adrenodoxin reductase that it does not show hybridization with its cDNA (11). Biochemical and immunochemical analyses have revealed no significant difference between ferredoxin reductases from the liver and adrenal cortex (2, 25, 28). However, there is at least one report indicating differences between liver and adrenal ferredoxins (38).

4. Sequence and structure

The comparison of the sequence of adrenodoxin reductase with the protein sequence databases revealed no significant similarity to any other sequence (12). Despite the lack of any overall sequence similarity to other proteins, the FAD and NADP binding domains of adrenodoxin reductase were identified by consensus sequences characteristic of Rossman fold type AMP binding sites in other oxidoreductases (12). The FAD binding domain of adrenodoxin reductase is on the amino terminus and a putative NADP binding dinucleotide fold is located close to the middle of the molecule (Fig. 2). The locations of the FAD and NADP binding sites are similar to those observed in some other flavoenzymes, including glutathione reductase (12). Thus, adrenodoxin reductase may be evolutionarily related to this group of flavoenzymes. Yet, the evolution of these enzymes and the domains remains an amazing enigma because of the lack of sequence similarity outside of these very short domains. One explanation for this may be found in the observation that the FAD and NADP binding domains are encoded by separate small exons (21).

The analyses that were used to identify the NADP binding site of adrenodoxin reductase led to the discovery of a consensus sequence that can identify the NADP binding site of many NADP binding enzymes (12). This consensus sequence showed a single highly conserved amino acid difference from that of NAD binding sites: an alanine instead of a glycine (12). Thus, it was suggested that this residue difference may play a role in determining NAD vs. NADP specificity of the binding site (12). This has been demonstrated to be true for glutathione reductase (33).

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A. reductase (Human)      1 MASRCWRWWGWSAWPRTRLPPAGSTPSFCHHF  29
             (Bovine)     1 --P------P--S-T------SR-IQN-GQ--  29

FAD Site:   * *  *   *      *
STQEKTPQICVVGSGPAGFYTAQHLLKHP QAHVDIYEKQPVPFGLVRFGVAPDHPEVKN  59
----Q----------------------HSR---------L------------------  60

VINTFTQTAHSGRCAFWGNVEVGRDVTVPELQEAYHAVVLSYGAEDHRALEIPGEELPGV 119
---------R-D----Y----------Q---D-------------Q--D--------- 120

                NADP site:     * *  *   *      *
CSARAFVGWYNGLPENQELEPDLSCDTAVILGQGNVALDVARILLTPPEHLDRTDITKAA 179
F--------------R--A--------------------------D--EK----E-- 180

LGVLRQTRVKTVWLVGRRGPLQVAFTIKELREMIQLPGARPILDPVDFLGLQDKIKELPR 239
--A---S------I-----------------------T--M---A-------R---AA- 240

PRKRLTELLLRTATDKPGPAEAARQASASRAWGLRFFRSPQQVLPSPDGRRAAGARLAVT 299
-----M--------E---VE----R----------------------------I----- 300

RLEGVDEATRAVPTGDMEDLPCGLVLSSIGYKSRPVDPSVPFDGKLGVIPNVEGRVMDVP 359
----IG----------V-----------------I-------P----V--M----V-M- 360

GLYCSGWVKRGPTGVIATTMTDSFLTGQMLLQDLKAGLLPSGPRPGYAAIQALLSSRGVR 419
---------------T-----------I--------H--------S-F-K---D----W 420

PVSFSDWEKLDAEEVARGQGTGKPREKLVDPQEIVRLLGH 459
--------------S---AS-------L----ML----- 460
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Figure 2. Amino acid sequences of human (35) and bovine (11, 12, 32) adrenodoxin reductase. The first 29 residues are the sequences of the amino termini that are cleaved upon entry of the enzyme into mitochondria. For the rest of the sequences, the numbering on the left side starts with the first residue of the amino terminus of the mature enzyme. The human enzyme sequence is aligned with the bovine enzyme sequence with a single gap of one amino acid at position 30. The positions that correspond to the Rossman fold type FAD and NADP binding site fingerprint sequences (12) are marked with an asterisk.

The sequence of adrenodoxin reductase shows no similarity to the sequences of microsomal P450 reductase (30), plant ferredoxin reductase (16), and putidaredoxin reductase of the bacterial P450cam (18). This was a surprising finding because both adrenodoxin reductase and plant ferredoxin reductase bind NADP and ferredoxin, and *a priori* some similarity between these enzymes was expected. In contrast, even though microsomal P450 reductase does not interact with a ferredoxin, it shows sequence homology with plant ferredoxin reductases (30). The structure of spinach ferredoxin reductase does not show any homology with the structure of glutathione reductase (17). Thus, ferredoxin reductase, microsomal P450 reductase and some other enzymes represent a separate group of structurally related flavoenzymes (17). The consensus sequence of FAD and NADP binding sites does not appear in this group of enzymes (12).

5. Regulation of expression

The molar concentration of adrenodoxin reductase is many fold lower than those of adrenodoxin and mitochondrial P450s in both the adrenal cortex and corpus luteum (10, 25, 37). Similarly, the level of adrenodoxin reductase mRNA is about 10-fold lower than that of P450scc (11). This correlation between the relative levels of the enzymes and their mRNAs indicates that the relatively low level of adrenodoxin reductase is a reflection of its mRNA level, and is not a result of lower translational efficiency of its mRNA (11).

The levels of mitochondrial P450s in steroidogenic tissues is regulated by specific trophic hormones as part of the physiological mechanisms regulating the steroid output of these tissues (reviews: 13, 23, 40). The level of adrenodoxin reductase is correlated with the level of adrenodoxin and P450scc in both adrenal cortex and corpus luteum in vivo (11). To understand the mechanism of hormonal regulation of these enzymes we studied the kinetics of ACTH induction of the three mitochondrial P450 system enzymes and their mRNAs in bovine adrenal cortex cells in primary culture (15). When these cells are grown to confluence in the absence of ACTH, the levels of the enzymes drastically decrease. ACTH increases the levels of all three enzymes and their mRNAs (15, 40). P450scc and adrenodoxin reductase show similar time courses at both protein and mRNA level. But, adrenodoxin mRNA and protein show significantly earlier increases than the other two enzymes, indicating differences in the regulation of the enzymes (15).

In analogy to the regulation observed in steroidogenic tissues, the levels of some mitochondrial P450 system enzymes in other tissues may also be regulated by the relevant physiological factors. The study of these processes is difficult because of the low levels of these enzymes in non-steroidogenic tissues (Table 1). Nonetheless, there is evidence that in kidney the levels and activity of these enzymes may be affected by vitamin D deficiency (22).

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