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Enoyl-acyl-carrier-protein reductase and *Mycobacterium tuberculosis* InhA do not conserve the Tyr-Xaa-Xaa-Xaa-Lys motif in mammalian 11β- and 17β-hydroxysteroid dehydrogenases and *Drosophila* alcohol dehydrogenase

In the last few years, there has been an explosive increase in the number of sequences of oxidoreductases in the short-chain alcohol dehydrogenase family [1,2]. These enzymes also have been called *sec*-alcohol dehydrogenases, a functional definition based on the properties of their substrates, which are either secondary alcohols or ketones [3]. The diversity of this family can be seen from examination of over 60 different sequences from bacteria, plants, invertebrates and vertebrates that are contained in the Swiss Protein Database release 30. These enzymes are involved in all aspects of cell biochemistry and physiology, including metabolism of sugar, synthesis or degradation of fatty acids, synthesis of polyketides by soil bacteria, and synthesis or degradation of glucocorticoids, oestrogens, androgens, and prostaglandins E_2 and $F_{2\alpha}$ in humans, which is an important regulatory mechanism for these hormones.

Sequence analysis [1–5] has revealed that these enzymes contain a highly conserved pentapeptide of the form Tyr-Xaa-Xaa-Xaa-Lys, at about residue 150 in a 250-residue dehydrogenase. The tyrosine and lysine residues, which are absolutely conserved, are central to the Prosite Signature for this protein superfamily. These two residues are likely to be important in catalysis. For *Drosophila* alcohol dehydrogenase, the tyrosine's deprotonated phenolic group is proposed to catalyse hydride transfer, with the positively charged lysine residue lowering the pK_a of the tyrosine's phenolic group from 10.0 to about 7.6 [6]. Lysine also binds the 2′-hydroxyl group of the ribose associated with the nicotinamide moiety on NADH [7,8]. Support for the importance of this tyrosine and this lysine comes from mutagenesis studies with *Drosophila* alcohol dehydrogenase [6,9], human 15-hydroxyprostaglandin dehydrogenase [10] and human 11β-hydroxysteroid [11] and 17β-hydroxysteroid [12] dehydrogenases.

Although the signature motif is found in over 60 enzymes, we have uncovered a group of enzymes that, as shown below, belong to this protein superfamily, yet lack either the conserved tyrosine or lysine. Some of these enzymes also have significant differences in the nucleotide-binding domain at the N-terminus, suggesting novel aspects of catalysis.

Figure 1 shows these enzymes aligned with several homologues that contain the Prosite Signature motif and the canonical nucleotide-binding domain. The Signature tyrosine is replaced by methionine in *Escherichia coli* enoyl-acyl-carrier-protein reductase (EnvM, FabI) [13], *Mycobacterium tuberculosis* InhA [14], *Brassica napus* enoyl-acyl-carrier-protein reductase [15] and an *Anabaena* ORF (open reading frame) [16], by serine in rat [17] and human [18] 2,4-dienoyl-CoA reductase, and by valine in *Saccharomyces cerevisiae* sporulation-specific protein (SPX19) [19]. The Signature lysine is replaced by methionine in *Drosophila* P6 [20].

As shown in Figure 1, *E. coli* EnvM, *M. tuberculosis* InhA, the

E. coli 7α-hydroxysteroid DH	11 G K C A I I T G A G A	G I G K E I A	155 N M T S Y A S S K A A A S H L V R
Human 15-hydroxy-prostaglandin DH	5 G K V A L V T G A A Q	G I G R A F A	147 Q Q P V Y C A S K H G I V G F T R
Streptomyces hydrogenans 20β-hydroxysteroid DH	6 G K T V I I T G G A R	G L G A E A A	148 L T S S Y G A S K W G V R G L S K
Human 11β-hydroxysteroid DH-1	34 G K K V I V T G A S K	G I G R E M A	179 M V A A Y S A S K F A L D G F F S
Human 11β-hydroxysteroid DH-2	82 T R A V L I T G C D S	G F G K E T A	228 C L G A Y G T S K A A V A L L M D
Human 17β-hydroxysteroid DH-1	2 R T V V L I T G C S S	G I G L H L A	151 F N D V Y C A S K F A L E G L C E
Human 17β-hydroxysteroid DH-2	82 Q K A V L V T G G D C	G L G H A L C	228 R L A S Y G S S K A A V T M F S S
Human 17β-hydroxysteroid DH-3	48 G Q W A V I T G A G D	G I G K A Y S	194 L Y S M Y S A A K A F V C A F S K
Pig 17β-hydroxysteroid DH-4	9 G R V V L V T G A G G	G L G R T Y A	160 G Q A N Y S A A K L G L L G L S N
Rat 2,4-dienoyl-CoA reductase	59 G K V A F I T G G G T	G L G K A M T	206 F V M P S S S A K S G V E A M N K
Human 2,4-dienoyl-CoA reductase	59 G K V A F I T G G G T	G L G K G M T	206 F V V P S A S A K A G V E A M S K
B. napus enoyl-ACP reductase	91 G K R A F I A G I A D D N G Y G W A V A		271 Y G G G M S S A K A A L E S D T R
E. coli enoyl-ACP reductase (EnvM)	6 G K R I L V T G V A S K L S I A Y G I A		155 N Y N V M G L A K A S L E A N V R
S. cerevisiae Sporulation protein (SPX19)	27 G K V A F V T G G A G T I C R V Q T E A		175 F Q G H V G A A K A G I D A L A K
Anabaena ORF	12 G K N A L V T G I A N N R S I A W G I A		163 N Y N V M G V A K A G L E A S V R
M. tuberculosis InhA	7 G K R I L V S G I I T D S S I A F H I A		157 A Y N W M T V A K S A L E S V N R
Drosophila P6 protein			147 A F S V Y A A A M H G I L G F T R

Figure 1 Alignment of enzymes containing the Tyr-Xaa-Xaa-Xaa-Lys motif in hydroxysteroid dehydrogenases with homologues that lack either tyrosine or lysine

The AMP-binding domain at the N-terminus of *sec*-alcohol dehydrogenases contains a hydrophobic pocket with a sequence of Thr-Gly-Xaa-Xaa-Xaa-Gly-Xaa-Gly. The boxed tyrosine and lysine residues are thought to be essential for catalysis. Abbreviation: DH, dehydrogenase.

Table 1 ALIGN comparison of *E. coli* 7α-hydroxysteroid dehydrogenase with various proteins

Similarity between proteins was analysed with the ALIGN program [21]. For the analysis reported here, 2500 random permutations were used for statistical analysis and the Dayhoff matrix was used with a bias of 6 and a gap penalty of 8. The alignment score is the number of S.D. by which the maximum score for the real sequences exceeds the average maximum score for the random. The probability of getting a score of 9 S.D. by chance is 10^{-19}. The similarity extends over about 240 out of 255 residues of *E. coli* 7α-hydroxysteroid dehydrogenase.

Protein	ALIGN comparison score (S.D.)
Rat 2,4-dienoyl-CoA reductase	16.4
S. cerevisiae sporulation-specific protein (SPX19)	14.5
Anabaena ORF	13.5
E. coli enoyl-acyl-carrier-protein reductase (EnvM)	12.2
B. napus enoyl-acyl-carrier-protein reductase	9.3
M. tuberculosis InhA	8.2

Brassica napus enzyme and *Anabaena* ORF, and yeast SPX19, have differences from the canonical sequence in the cofactor-binding domain, including an insertion of two residues and poor conservation of the second and third canonical glycine residues.

Bergler et al. [13] aligned the sequence of *E. coli* EnvM with those of several *sec*-alcohol dehydrogenases, but did not quantitatively determine the similarity. In view of the unusual sequence in the Signature domain and the N-terminus, we decided to confirm this similarity with an ALIGN analysis [21] of these proteins with *E. coli* 7α-hydroxysteroid dehydrogenase, a *sec*-alcohol dehydrogenase, containing the Signature motif and the canonical nucleotide-binding domain [22]. Table 1 summarizes the results of the ALIGN analyses. Most comparison scores are over 9 S.D., sufficient to support the common ancestry of these proteins. The most divergent protein, InhA, was clearly shown by Banerjee et al. [14] to be homologous to EnvM. Rat et al. [20] showed that *Drosophila* P6 is a close homologue of *Drosophila* alcohol dehydrogenase.

Despite the lack of the Signature tyrosine residue, three of the proteins have been shown to have enoyl-CoA reductase activity [15,17,23]. At this time, their catalytic mechanism is unknown. However, Bergler et al.'s studies with EnvM [23] provide an important insight: EnvM does not bind NAD^+ in the absence of substrate, consistent with EnvM's N-terminal sequence. Nor does radioactive diazaborine, which appears to mimic the substrate, bind to EnvM in the absence of NAD^+. However, NAD^+ binds to EnvM in the presence of diazaborine. This requirement for both cofactor and diazaborine for their binding to EnvM differs from that of *Drosophila* alcohol dehydrogenase, where NAD^+ binds first, inducing a conformation change that facilitates substrate binding [24,25].

InhA is of much interest because it is a target for drugs such as isoniazid and ethionamide that control *M tuberculosis*. InhA is thought to be involved in synthesis of mycolic acid, a fatty acid that is part of the bacterial cell wall. Bacterial strains with resistance to isoniazid are becoming more widespread in the general population and in those with AIDS, contributing to the rapid proliferation of tuberculosis. The sequence divergence of InhA from human hydroxysteroid and hydroxyprostaglandin dehydrogenases and their homologues could diminish the binding of isoniazid and ethionamide to these enzymes, which would decrease side effects of these drugs in humans. This also would be true for drugs that act on EnvM in *Salmonella* and *E. coli*. This makes InhA and EnvM good targets for controlling their bacterial hosts. Understanding the catalytic mechanism of InhA, EnvM and their close homologues is likely to provide important insights for designing drugs to control *M. tuberculosis*, *Salmonella*, *E. coli* and other bacterial diseases, as well as for understanding the catalytic mechanism of the over 60 *sec*-alcohol dehydrogenases that contain the Prosite Signature motif.

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