First publ. in: Trends in Biochemical Sciences 23 (1998), 2, pp. 56-57

A hydrophobic sequence motif common to N-hydroxylating enzymes

The first committed step in the biosynthesis of various bacterial and fungal siderophores (low-molecular-weight iron chelators that are produced in response to iron deficiency) of the hydroxamate type, such as aerobactin, alcaligin and ferrichrome, involves N-hydroxylation of a primary amino group. This reaction is catalyzed at the expense of NADPH by a family of FAD-dependent enzymes. Some of the siderophores act as virulence factors[1]. A similar reaction is carried out by a family of mammalian flavin-containing dimethylaniline monooxygenases. In the latter case, the substrates are tertiary and secondary diet-derived alkylamines and, as such, these enzymes play a role in the degradation of xenobiotics. Deficiency in this enzyme activity was recognized as the cause of the inheritable 'fish-odor syndrome' (trimethylaminuria) which is characterized by an increased excretion of malodorous trimethylamine[2].

A BLAST search[3] in the NCBI non-redundant database (as of 5 August 1997) and sequence alignment with CLUSTALX (Ref. 4) and MACAW (Ref. 5) of four siderophore biosynthetic enzymes from *Escherichia coli* (aerA; iucD)[6,7], *Pseudomonas aeruginosa* (pvdA)[8], *Bordetella bronchiseptica* (alcA)[9] and *Ustilago maydis* (sid1)[10] and about 30 sequences of flavin-containing mammalian monooxygenases (nine representative sequences are shown in Fig. 1) revealed three dominant areas of similarity (Fig. 1a, b and c). As expected, all proteins contained two nucleotide-binding folds; the N-terminal fold was assigned as the FAD and the one towards the centre as the NADP binding site (Fig. 1a and b, respectively)[11,12]. The FAD-binding site of the mammalian monooxygenases has the typical fingerprint sequence GXGXXG, whereas the siderophore biosynthetic enzymes (alcA, iucD, pvdA and sid1, see Fig. 1) exhibit an exchange of the last glycine to proline. This quite unusual replacement is unique among FAD-dependent enzymes and it was assumed to be the cause of the weak binding of FAD to lysine N^6-hydroxylase (EC 1.14.13.-)[12]. Similarly, alcA and pvdA possess an alanine and sid1 a serine instead of the last glycine in the putative NADP-binding site.

The third and new sequence similarity was discovered in the C-terminal part of the proteins (Fig. 1c). The similarity starts with a highly conserved aspartate and is followed by eight hydrophobic amino acids. The core region consists of the sequence L/FATGY and ends with a proline after four variable amino acids. An exception was found in the two ornithine N^5-hydroxylases

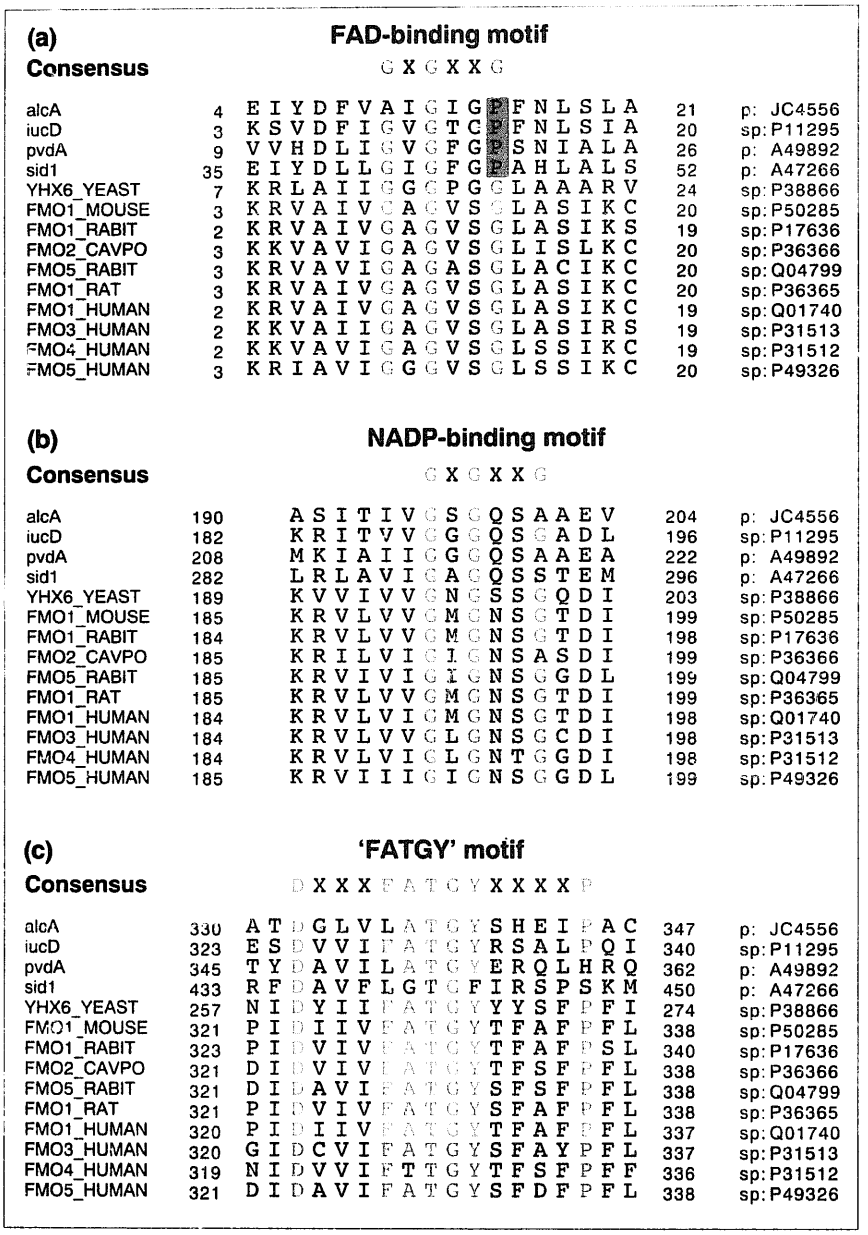

Figure 1

Multiple sequence alignment of N-hydroxylating siderophore biosynthetic enzymes and mammalian N-hydroxylating dimethylaniline monooxygenases. The sequences were aligned using the programs CLUSTALX[4] and MACAW[5]. **(a)** FAD-binding motif. **(b)** NADP-binding motif. **(c)** 'FATGY' motif. Protein names are given on the left and accession numbers from SWISS-PROT (sp) and PIR (p) on the right. The numbers before and after the sequence show the position of the displayed regions in their respective sequences. The first row gives the consensus sequence that is shared by the majority of the sequences. The consensus box is shaded in yellow. Absolutely conserved residues are highlighted in red. Residues where the chemical character is conserved are highlighted in blue. The green shaded area illustrates the exchanged glycine to proline in the FAD-binding motif in the siderophore biosynthetic enzymes. Aligned sequences: alcA, alcaligin biosynthesis enzyme (*Bordetella bronchiseptica*); iucD, L-lysine N^6-hydroxylase (*Escherichia coli*); pvdA, L-ornithine N^5-oxygenase (*Pseudomonas aeruginosa*); sid1, L-ornithine N^5-oxygenase (*Ustilago maydis*); YHX6_YEAST, hypothetical 42.4 kDa protein (*Saccharomyces cerevisiae*); FMO1_MOUSE, hepatic flavin-containing monooxygenase 1 (FMO 1) (*Mus musculus*) (mouse); FMO1_RABIT, hepatic flavin-containing monooxygenase 1 (FMO 1) (*Oryctolagus cuniculus*) (rabbit); FMO2_CAVPO, pulmonary flavin-containing monooxygenase 2 (FMO 2) (*Cavia porcellus*) (guinea pig); FMO5_RABIT, hepatic flavin-containing monooxygenase 5 (FMO 5) (*Oryctolagus cuniculus*) (rabbit); FMO1_RAT, hepatic flavin-containing monooxygenase 1 (FMO 1) (*Rattus norvegicus*) (rat); FMO1_HUMAN, fetal hepatic flavin-containing monooxygenase 1 (FMO 1) (human); FMO3_HUMAN, hepatic flavin-containing monooxygenase 3 (FMO 3) (human); FMO4_HUMAN, hepatic flavin-containing monooxygenase 4 (FMO 4) (human); FMO5_HUMAN, hepatic flavin-containing monooxygenase 5 (FMO 5) (human).

(Fig. 1c; pvdA and sid1) where this proline is not conserved. Although some variance is observed, exchanges are conservative in nature, that is, serine to threonine or isoleucine to valine. This $D(X)_3(L/F)ATGY(X)_1P$-motif was found to be rather hydrophobic in the mammalian monooxygenases[11], and using the procedure of Kyte and Doolittle[13] (a window size of 15 amino acids) this was also confirmed for the siderophore biosynthetic enzymes. In fact, in lysine N^6-hydroxylase it is the region of the highest hydrophobicity.

A pattern scan using the expasy server (http://expasy.hcuge.ch/sprot/scnpsit2.html) in SWISS-PROT and TREMBL databases showed that the $D(X)_3(L/F)ATGY(X)_1P$, $D(X)_3(L/F)ATGY$ or $(L/F)ATGY(X)_1P$ motif is only present in: mammalian flavin-containing dimethylaniline monooxygenases; L-lysine N^6-hydroxylase (*Escherichia coli*); L-ornithine N^5-oxygenase (*Pseudomonas aeruginosa*); alcaligen biosynthesis enzyme; and a hypothetical 42.4 kDa yeast enzyme, which, according to a BLAST search and CLUSTALX sequence alignment, is similar to mammalian dimethylaniline monooxygenases. A search using the sequence 'FATGY' alone shows that this pentamer occurs in nearly 25 additional proteins, which are not related to flavin-containing N-hydroxylating enzymes, for example, many DNA-binding proteins like zinc-finger proteins and viral DNA polymerases.

Based on the discovery of the $D(X)_3(L/F)ATGY(X)_1P$-motif in N-hydroxylating enzymes (Fig. 1c), we propose that this motif is part of a substrate-binding site with the L/FATGY core providing a hydrophobic pocket. The highly conserved aspartate, on the other hand, could serve as a proton-abstracting base to render the amino group more reactive in the reaction with the hydroxylating flavin species, that is, the flavin-4a-hydroperoxide[12].

References

1 Weinberg, E. D. (1984) *Physiol. Rev.* 64, 65–102
2 Mitchell, S. C. (1996) *Perspect. Biol. Med.* 39, 514–526
3 Altschul, S. F. *et al.* (1990) *J. Mol. Biol.* 215, 403–410
4 Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994) *Nucleic Acids Res.* 22, 4673–4680
5 Schuler, G. D., Altschul, S. F. and Lipman, D. J. (1991) *Proteins* 9, 180–190
6 De Lorenzo, V., Bindereif, A., Paw, B. H. and Neilands, J. B. (1986) *J. Bacteriol.* 165, 570–578
7 Gross, R., Engelbrecht, F. and Braun, V. (1984) *Mol. Gen. Genet.* 196, 74–80
8 Visca, P., Ciervo, A. and Orsi, N. (1994) *J. Bacteriol.* 176, 1128–1140
9 Kang, H. Y., Brickman, T. J., Beaumont, F. C. and Armstrong, S. K. (1996) *J. Bacteriol.* 178, 4877–4884
10 Mei, B., Budde, A. D. and Leong, S. A. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 90, 903–907
11 Lomri, N., Gu, Q. and Cashman, J. R. (1992) *Proc. Natl. Acad. Sci. U. S. A.* 89, 1685–1689
12 Macheroux, P., Plattner, H. J., Romaguera, A. and Diekmann H. (1993) *Eur. J. Biochem.* 213, 995–1002
13 Kyte, J. and Doolittle, R. F. (1982) *J. Mol. Biol.* 157, 105–132

Note added in proof

A recently cloned and characterized L-ornithine N^5-oxygenase from *Burkholderia cepacia* (accession number: AF013993) also contains the unusual putative FAD binding site 'GXGXXP' (Fig. 1a) and a 'DXXXLATGY'-motif (Fig. 1c) as described here for the hydroxylases.

MATTHIAS STEHR AND HANS DIEKMANN

Institut für Mikrobiologie, Universität Hannover, Schneiderberg 50, D-30167 Hannover, Germany.

LILIANA SMAU, OLIVER SETH AND SANDRO GHISLA

Fakultät für Biologie, Universität Konstanz, D-78567 Konstanz, Germany.

MAHAVIR SINGH

Gesellschaf. für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-38124 Braunschweig, Germany.

PETER MACHEROUX

Institut für Pflanzenwissenschaften, ETH-Zürich, Universitätstr. 2, CH-8092 Zürich, Switzerland.
Email: peter.macheroux@ipw.biol.ethz.ch