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A novel oxidoreductase family sharing a conserved FAD-binding domain

Flavoproteins play a key role in many biological processes, ranging from redox catalysis to DNA repair. Of the hundreds of flavoproteins isolated to date, most contain a dissociable flavin cofactor. However, in about 30 flavoenzymes, the isoalloxazine ring of the flavin group is covalently linked to a histidine residue in the polypeptide chain1–2. The exact mechanism of covalent flavinylation is still obscure – as is the reason for covalent binding3–5. We report here on a newly discovered family of oxidoreductases that share a conserved FAD-binding domain. The homologous enzymes are found in both eukarya and eubacteria, and are involved in a diverse range of redox reactions.

Recently, we solved the first crystal structure of an 8a-(3′-histidyl)-FAD-containing flavoprotein: vanillyl-alcohol oxidase (VAO)6. This fungal enzyme catalyses the oxidation of a variety of lignin-derived phenolic compounds17. We performed sequence comparisons with the recently determined primary structure of VAO (Ref. 9), using the gapped-BLAST program20. We found that VAO shows sequence similarity to several other flavoproteins of similar length, including p-cresol methyldihydrogenase6 (hits = 314, E = 6 × 10−15), p-lactate dehydrogenase21 (hits = 57, E = 2 × 10−14) and L-galactono-γ-lactone oxidase22 (hits = 36, E = 0.05). Moreover, representatives of all kingdoms, was evident that the regions conserved in these enzymes are represented by the homologous N-terminal part of 6-hydroxy-D-nicotine oxidase (HDNO), all 54 homologous sequences were identified using the position-specific iterative BLAST (PSI-BLAST) method. By screening the NCBI non-redundant protein database with the homologous N-terminal part of HDNO (bits 314, E = 0.016), all the enzymes listed in Table 1 are known to contain FAD and catalyse redox reactions in a wide variety of metabolic pathways.

Table 1

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<th>Percentage Similarity</th>
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<th>E-Value</th>
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<td>74.3</td>
<td>1077</td>
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</table>

Figure 1

Multiple alignment of 13 homologous sequences for which gene products have been identified. The gene products of all but fasciation enzyme (FASC) have been characterized. All (other) homologous sequences can be viewed at http://gcg.tran.wau.nl/local/Biochem.II.

The origin of the sequences is shown in the upper panel (b, bacterial; f, fungal; p, plant; a, animal), while the SWISS-PROT accession numbers are listed in the lower panel. Sequences were aligned using the ClustalW25 program and manual alignment. The number of residues present in gaps in the alignment (gapped-BLASTP) is indicated by parentheses. Conserved residues (predicted from the structure of VAO) are indicated in blue. The points of interaction between the FAD cofactor and specific conserved histidine residue to which FAD is bound in HDNO and BBE is shown in red. The points of interaction between the FAD cofactor and specific conserved histidine residue to which FAD is bound in HDNO and BBE is shown in red. The points of interaction between the FAD cofactor and specific conserved histidine residue to which FAD is bound in HDNO and BBE is shown in red. The points of interaction between the FAD cofactor and specific conserved histidine residue to which FAD is bound in HDNO and BBE is shown in red. The points of interaction between the FAD cofactor and specific conserved histidine residue to which FAD is bound in HDNO and BBE is shown in red. The points of interaction between the FAD cofactor and specific conserved histidine residue to which FAD is bound in HDNO and BBE is shown in red.
family (shown in green in Fig. 2) represent a conservation of this FAD-binding domain. It is apparent from the VAO structure that several conserved residues are involved in binding the FAD cofactor (Fig. 1). The intervening non-conserved region of about 240 residues exactly corresponds to the cap domain of VAO; the topology of this domain might therefore differ among the members of the family.

Interestingly, several of the VAO-related enzymes described above harbour a covalently bound FAD. For 6-hydroxy-

\( \text{nicotine oxidase and berberine bridge enzyme, the FAD-linking residue (shown in red in Fig. 1) is known.} \)

Remarkably, this histidine residue is conserved in 17 members of the family, which suggests that they are also covalently flavinylated.

Indeed, \( \text{d-arabino-1,4-lactone oxidase and hexose oxidase have been reported to contain a covalently bound flavin. Furthermore, the position of the flavinylated histidine residue in 6-hydroxy-\text{nicotine oxidase is homologous to Arg104 of VAO. In VAO, Arg104 lies close to the 8\text{-methyl group of the flavin ring, although this group is covalently linked to His42 (Ref. 6). Thus, if all sequences containing the conserved histidine residue truly represent covalent flavoproteins, then the percentage of covalent flavoproteins within this novel oxidoreductase family is relatively high. Moreover, in VAO and p-cresol methylhydroxylase, the flavin group is covalently linked to a residue in the cap domain. This indicates that, unlike the well-known Rossmann fold, this newly discovered, conserved FAD-binding fold favours covalent flavinylation.}

**References**


**Figure 2**

Ribbon diagram of a vanillyl-alcohol oxidase monomer from Penicillium simplicissimum. The covalently bound FAD is represented as a ball-and-stick model. The FAD-binding domain is shown in green and the cap domain is shown in red. The conserved regions at the N- and C-termini of the protein are shown as full-coloured ribbons. The figure was rendered using MOLSCRIPT.

**PROTEIN SEQUENCE MOTIFS**

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Ribbon diagram of a vanillyl-alcohol oxidase monomer from Penicillium simplicissimum. The covalently bound FAD is represented as a ball-and-stick model. The FAD-binding domain is shown in green and the cap domain is shown in red. The conserved regions at the N- and C-termini of the protein are shown as full-coloured ribbons. The figure was rendered using MOLSCRIPT.