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, cysteine or tyrosine residue in the polypeptide chain. The mechanism of covalent flavinylation is still obscure— as is the reason for covalent linkage. As an example of the diversity of flavoproteins, Figure 1 shows an alignment of the sequences for which gene products have been identified. The oxidative enzymes of the novel oxidoreductase family sharing a conserved FAD-binding domain (residues 1–250 and 501–506) provides the cofactor-binding site, whereas the cap domain (residues 271–499) forms the substrate-binding site. From this it is evident that the regions conserved in members of the novel oxidoreductase family come from inspection of the three-dimensional structure of VAO. VAO consists of two domains; the active site of the catalytic domain and the open reading frame products of vanillyl-alcohol oxidase (VAO)6. This fungal enzyme catalyses the oxidation of a variety of lignin-derived phenolic compounds7,8. We recently determined the crystal structure of VAO. VAO (Ref. 9), using the gapped-BLAST program10. We found that VAO shows sequence similarity to several other flavoenzymes of similar length, including p-cresol methylhydroxylase (bits = 314, E = 6 × 10−10), p-cresol dehydrogenase (bits = 57, E = 2 × 10−9), malic enzyme (bits = 43, E = 4 × 10−9), L-galactono-
lactone oxidase (bits = 36, E = 0.0); p-cresol-o-lactone oxidase shows significant sequence similarity to other flavoenzymes and uncharacterized open reading frame products. The N- and C-terminus of these proteins show most sequence conservation and were used for a more extensive search: a group of 54 homologous protein sequences, with representatives in all kingdoms, was found. Figure 1 shows an alignment of the sequences for which gene products have been identified. Except for alkylhydroxyacetonephosphate synthase and p-cresol-o-lactone oxidase, all the enzymes listed are known to contain FAD and catalyse redox reactions in a wide variety of metabolic pathways. A novel oxidoreductase family sharing a conserved FAD-binding domain.
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- \( \alpha \)-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, 3',5'-oligo- \( \alpha \)-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-\( \alpha \)-nicotine oxidase is homologous to Arg104 of VAO. In VAO, Arg104 lies close to the 8-\( \alpha \)-methyl group of the flavin ring, although this group is covalently linked to His422 (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 flavinylatation.

References