Probable circular permutation in the flavin-binding domain

Sir—Liepinsh *et al.*[1] have recently proposed an evolutionary link between electron transport and proteolysis based upon a structural relationship of the FMN binding protein (FMN-bp) from *Desulfovibrio vulgaris* (Miyazaki F) to chymotrypsin and related proteases. Having examined the sequence and structure of FMN-bp, I found no evidence that FMN-bp may be descended from a so far hypothetical single-domain precursor of the protease superfamily.

Firstly, unlike the serine proteases which have evolved into a vast and widespread sequence family, FMN-bp is an orphan and therefore is not likely to be of such an ancient origin. Secondly, the FMN-bp fold belongs to a different class of the β-sheet barrels than the fold of chymotrypsin domains. The β-sheet barrels are classified by two integer numbers: n, the number of strands, and S, the shear number[2,3]. In the FMN-bp barrel, n = 6 and S = 10, whereas, in both chymotrypsin barrels, n = 6 and S = 8 (defined as in ref. 3, the shear number is always even due to the periodicity of β-structure). The distinction in shear number is not always distinguished by present automated methods for structural similarity searches. It has only a minor effect on the number of spatially superimosable main chain atoms and their root-mean-square (r.m.s.) deviation; however it results in significant differences in the packing of side chains in the barrel interior and the overall barrel geometry[3].

I argue here instead that FMN-bp probably has a recent origin and evolved from the ferredoxin reductase[4] superfamily of flavoenzymes. Inspection of β-barrel folds in the SCOP database[5], has readily identified this superfamily as a putative parent family for FMN-bp. Known structures of this superfamily also include phthalate dioxygenase reductase, nitrate reductase, cytochrome b_5 reductase and flavodoxin reductase[6], cytochrome P450 reductase[7] and flavohemoglobin[8]. The FAD-binding domain of reductases adopts a β-barrel fold of the same class as the FMN-bp barrel (n = 6, S = 10). The topologies of the FAD-binding domain and FMN-bp are different, but they can be related by a circular permutation (Fig. 1). (To convert the topology of FAD-binding domain into the FMN-bp topology, the end of strand 6 to the beginning of strand 1 would have to be connected with a peptide linker, while the connection between strands 2 and 3 would have to be cut to create the new termini). This permutation also will bring the flavin-binding sites of FMN-bp and FAD-binding domain into the same topological location. The binding modes for FMN and the equivalent part of FAD are very similar (Fig. 1): the cofactor molecule bridges the gap between two strands (strands 4 and 5 in the FAD-binding domain and their counterparts strands 2a and 3 in FMN-bp). The isoalloxazine ring and ribityl chain hydrogen bond to free main chain groups in these strands and make non-polar contacts to side chains in the barrel interior. The phosphate group binds at the N terminus of α-helix that caps each barrel.

Structural evidence accumulated over the past years suggests that circularly permuted proteins can and do occur naturally[9]. It is further supported here by the structural and functional similarity between FMN-bp and the FAD-binding domain that are very likely to share a common ancestor. The FMN-bp fold probably has been created by a recent circular permutation event in one of the FAD-binding domains, therefore its superficial structural similarity to the serine protease fold is convergent rather than divergent.

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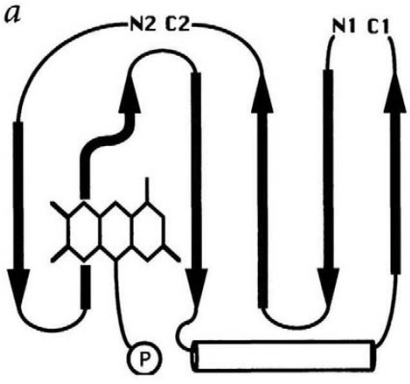

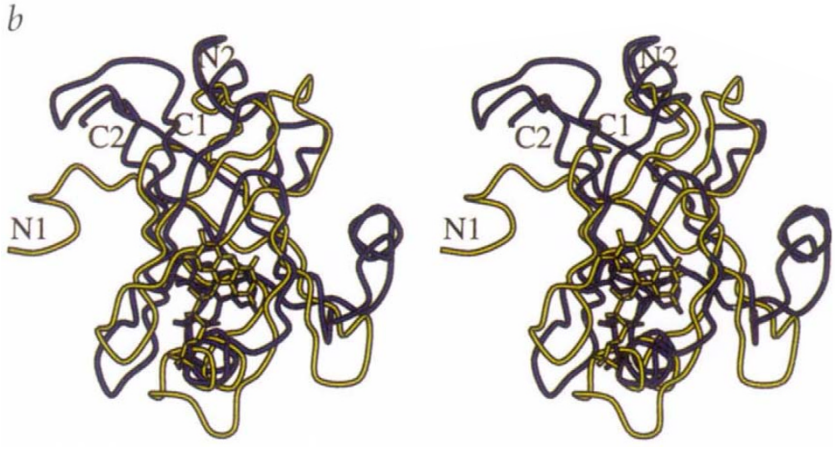

Fig. 1 ***a***, Topology diagram of the flavin-binding β-barrel fold. Arrows represent β-strands and cylinder represents the capping α-helix. In the FAD-binding domain, the protein chain begins at N1, continues through the gap between C2 and N2 and ends at C1,whereas, in the FMN-binding protein, it begins at N2 and ends at C2. The position of the flavin-binding site is indicated by simplified picture of FMN. ***b***, Stereo view showing a superposition of the backbones of the FAD-binding domain from phthalate dioxygenase reductase (yellow; PDB entry 2PIA, residues 1–100) and FMN-bp (blue; 1AXJ, coordinates courtesy of G. Otting). In this superposition, 65 pairs of Cα atoms in all of the six barrel strands and the capping helix give an r.m.s. deviation of 2.3 Å. The FMN molecules bound to each structure shown in stick representation.