Minireview

The TIM-barrel fold: a versatile framework for efficient enzymes

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Abstract Recent studies on triosephosphate isomerase (TIM)-barrel enzymes highlight the remarkable versatility of the TIM-barrel scaffold. At least 15 distinct enzyme families use this framework to generate the appropriate active site geometry, always at the C-terminal end of the eight parallel β-strands of the barrel. Sequence and structure comparisons now suggest that many of the TIM-barrel enzymes are evolutionarily related. Common structural properties of TIM-barrel enzymes are discussed.

Key words: Enolase; Evolution; HisA; Triosephosphate isomerase; TrpF; Protein design

1. Introduction

In many protein structures parallel or antiparallel β-sheets can be detected. In an important subset of these proteins the β-sheet is folded into a closed β-barrel. In a closed β-barrel a complete ring of hydrogen bonds can be detected, such that each β-strand is hydrogen-bonded to two neighbouring strands, implying also that the first β-strand is hydrogen-bonded to the last β-strand. The number of strands in characterised barrel structures varies from four to 20 [1]. Most β-sheets in these β-barrels are antiparallel or mixed. A classic example is the antiparallel six-stranded β-sheet observed in each of the two domains of the chymotrypsin family of enzymes. The only exception is the classical TIM-barrel fold, which consists of an eightfold repeat of (βα) units, such that eight parallel β-strands on the inside are covered by eight α-helices on the outside. This fold, first seen in triosephosphate isomerase (TIM), is the topic of this review. The β-strands and α-helices will be numbered sequentially from the N-terminus as β1–β8 and α1–α8; the connecting loops are referred to as βα loops and αβ loops such that βα loop 1 follows after strand β1 and a αβ loop 1 follows after α1.

The TIM-barrel fold is the most common enzyme fold in the Protein Data Bank (PDB) database of known protein structures. It is seen in many different enzyme families, catalysing completely unrelated reactions [2]. Some of the most effective enzymes are TIM-barrel enzymes, for example TIM, which catalyses the interconversion of dihydroxyacetone phosphate and D-glyceraldehyde 3-phosphate at a diffusion rate limit [3]. Another example is orotidine 5′-monophosphate decarboxylase, which catalyses the synthesis of orotidine 5′-monophosphate with a rate enhancement that is larger than reported for any other enzyme [4]. The widespread occurrence of the TIM-barrel fold has raised much interest in the structural, enzymological and evolutionary properties of this fold.

Several reviews discuss on-going research aimed at a better understanding of the properties of TIM-barrel proteins [5–8]. Very recently an extensive review was published by Pujades and Palau [9]. Since then however, exciting new results on TIM-barrel proteins have emerged, in particular concerning (i) the overwhelming abundance of TIM-barrel enzymes in whole genome expression experiments [10], (ii) the structural properties [1], (iii) the evolutionary relationships [11,12], as well as (iv) the studies aimed at changing the catalytic properties of TIM-barrel enzymes [13,14]. This review will mainly discuss these very recent results.

2. Structural properties

The TIM-barrel domain by itself has typically about 250 residues, but it can be as small as 200 residues [15]. TIM-barrel enzymes can be small (only one domain), such as hevamine (monomeric, 273 residues [16]), or very large, such as β-galactosidase (tetrameric, 1023 residues per polypeptide chain [17], consisting of five domains). In β-galactosidase the catalytic TIM-barrel domain is in the middle (domain 3, residues 335–624), whereas domains 1 and 2 are located at the N-terminus and domains 4 and 5 are at the C-terminus.

Two key parameters of barrel structures are n (the number of strands in the barrel) and S (the shear number) (Fig. 1). All ($n = 8, S = 8$) barrels are TIM-barrels. For all TIM-barrels $n = 8$ and almost always $S = 8$ [1]. The TIM-barrel is the only barrel fold with a completely parallel sheet, except for the enolase family of structures, which has the topology $\beta\beta\alpha\alpha(\beta\alpha)_6$ instead of $(\beta\alpha)_8$ [18]. For the ($n = 8, S = 8$) barrels the β-strand side chains form layers. For these barrels each layer consists of four inwards pointing side chains (pointing into the β-barrel) and four outwards pointing side chains (pointing towards the α-helices). For example in Fig. 1, layer 2 residues from strands 1, 3, 5 and 7 point inwards and the residues from strands 2, 4, 6 and 8 point outwards. This property causes TIM-barrels to have fourfold symmetry (instead of eightfold symmetry), when viewed along the TIM-barrel axis [1,19]. Symmetry arguments therefore indicate that the smallest possible unit is the $\beta\alpha\beta\alpha$ unit. Structural [12] and sequence [20] studies of HisA and HisF (Fig. 2) show that the $(\beta\alpha)_8$ barrel of both of these enzymes is the result of a gene duplication

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event indicating that the smallest evolutionary unit is the half-barrel, $(\beta\alpha)_4$. It is interesting to point out that this also agrees with two other observations with respect to the structure. Firstly, the most hydrophobic region of the TIM-barrel is not the inner part of the β-barrel, but the core region between the β-strands and α-helices [1]; in fact, in the $\beta\beta\alpha\alpha(\beta\alpha)_6$ enolase barrel a polar channel in the inner part of the β-barrel exists [18], such that a hydrogen-bonding network traverses through the complete molecule connecting the active site (at the C-terminal end) with the N-terminal end of the barrel (at the other side of the molecule). Secondly, the TIM-barrel enzyme methylmalonyl-CoA mutase exists in an open, unliganded form, such that one half of the barrel (strands 2–5) hinges away from the other half, allowing for the substrate to bind inside the β-barrel. In this structural switch all hydrogen bonds between strands β1 and β2 are broken [21]. In the closed, liganded conformation the substrate (acetyl-CoA) is bound in an extended conformation in the polar channel inside the β-barrel, extending from the C-terminal end to the N-terminal end.

The active sites of all TIM-barrel enzymes are at the C-terminal ends of the β-strands, implying that the geometry of the active sites is shaped by residues of the eight loops following after the β-strands. These βα loops are of variable length, but tend to be larger than the αβ loops, as for example in TIM [22] as well as in HisA and HisF [12]. In some enzymes these loops are very long and form individual domains; for example, in pyruvate kinase βα loop 3 (113 residues) is a separate domain [23]. Whereas the βα loops are important for the function, the αβ loops, at the backside of the molecule, are believed to be more important for the stability of the TIM-barrel proteins [24]. Modelling-based loop redesign experiments with TIM have indeed shown that the loops at the C-terminal end can be changed extensively without necessarily interfering with the stability of the fold [25]. Structural analysis [26–28] of the loops shows that preferred conformations exist for the αβ loops, but not for the βα loops. Two preferred conformations are observed, one for the odd-numbered strands and one associated with the even-numbered strands. These differences in sheet entry for adjacent strands correlate with the geometric feature that corresponding TIM-barrel residues are actually non-equivalent in the β-sheet structure, as they are displaced by one residue due to the tilt of the strands with respect to the barrel axis (Fig. 1).

The abundance of the TIM-barrel domain can be assessed in various ways; for example, with respect to (i) the list of known structures of distinct enzymes deposited in PDB, (ii) the gene composition of a genome, (iii) the composition of the transcriptome (the transcriptome is the set of genes weighted by transcript abundance). These analyses show the TIM-barrel fold to be a super-fold, associated with as many as 16 sequence families [1,2], 15 of which are enzymes (the only exception is narbonin [29]). Many of these enzymes are glycosyl hydrolases which hydrolyse a glycosidic bond [17], utilising an aspartate or glutamate as a catalytic residue [30,31]. As many as 10% of the enzymes may have the TIM-barrel fold; thus it could be the most common enzyme fold [11]. Similarly, transcriptome analysis of yeast genome expression studies shows the TIM-barrel to be the most common transcriptome fold: 8% of the expressed proteins have a TIM-barrel domain [10]. This is an enrichment as in the yeast genome the abundance of TIM-barrel sequences is 4%. A study of protein fold usage of several microbial genomes also indicates that the TIM-barrel fold is abundant in each of these genomes [32,33].

3. Evolutionary and functional relationships

The pairwise amino acid sequence similarity of TIM-barrel folds in non-homologous enzyme families is generally below the detectable level. Nevertheless, structure-based sequence alignments reveal the presence of physicochemically similar clusters of residues, which are observed to exist at equivalent topological positions and which therefore could direct, stabilise and determine the common TIM-barrel folding pattern [34]. Indeed, circular permutated sequence variants of a TIM-barrel enzyme (TrpF, Fig. 2) have been shown to fold as the wild-type; in these variants the wild-type N- and C-termini have been joined but discontinuities were made in βα loop 6 and αβ loop 6 [35], respectively.

It is not clear if a common evolutionary origin can be assigned to all TIM-barrel families. Based upon extensive analysis of sequence families, Copley and Bork [11] favour the idea of divergent evolution from a common ancestor for most of the TIM-barrel enzyme families involved in pathways of the central metabolism. In any case, based on recent directed evolution selection experiments it is clear that small sequence changes can change the function. For example Sterner and colleagues [14] found that one point mutation is sufficient to convert the HisA protein into a protein with TrpF activity. Both HisA and TrpF catalyse an Amadori rearrangement (Fig. 2), which is the irreversible isomerisation of an aminoaldose into an aminoketose. HisA and TrpF catalyse reactions of the histidine and tryptophan biosynthesis pathways, respectively. The subsequent steps in these biosynthetic pathways are catalysed by HisF and TrpC, respectively (Fig. 2). HisA, HisF, TrpF and TrpC are TIM-barrel enzymes. HisA, TrpF and TrpC occur as monomeric, monodomain

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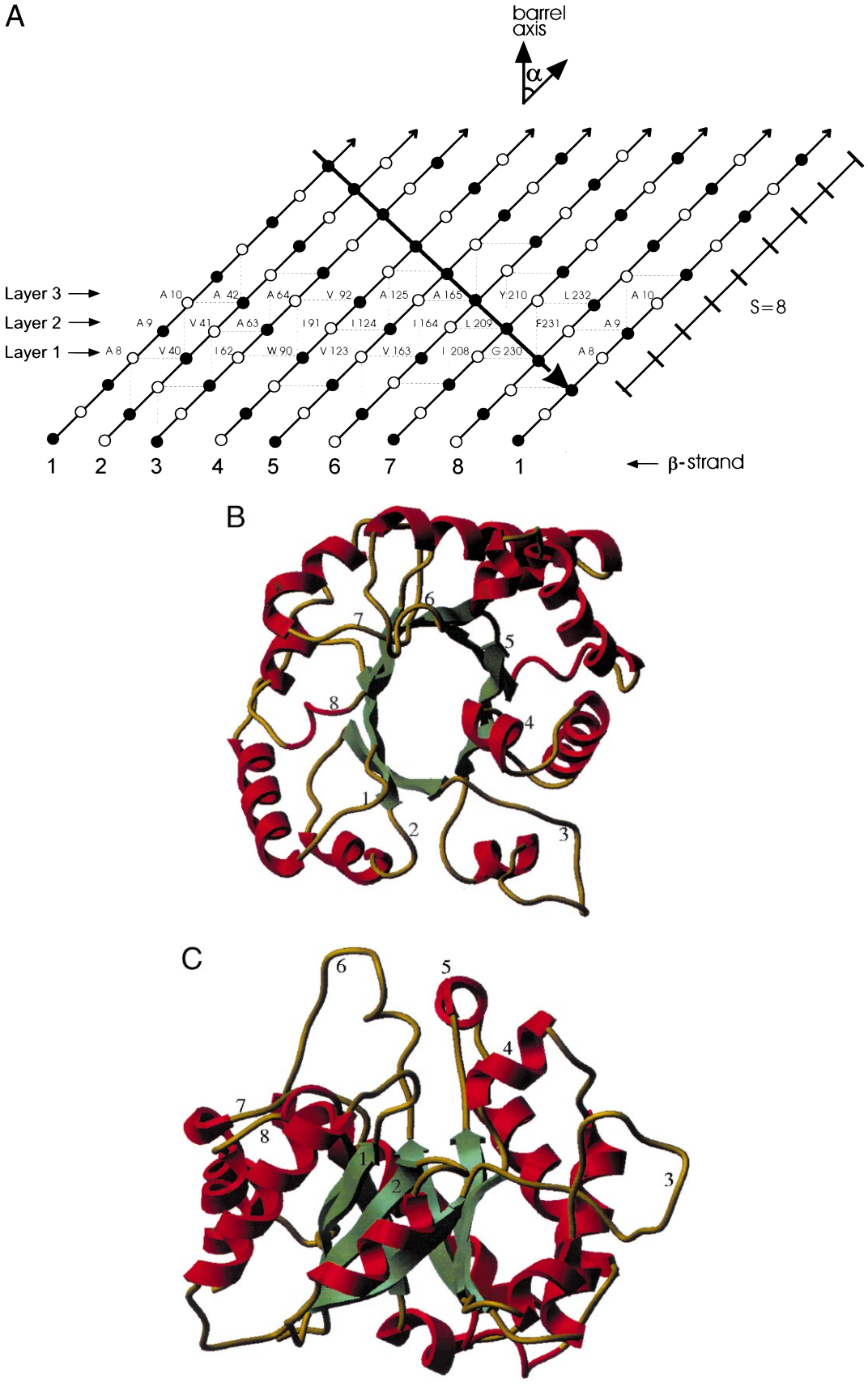
Fig. 1. Geometrical features of the TIM-barrel fold, illustrated using trypanosomal TIM (5TIM in PDB) as an example. A: Schematic drawing of the central β-sheet, when unrolling the barrel. The radius of the cylinder shaped by the eight β-strands is approximately 7 Å. The shear number (S) is 8; S is a measure of the extent to which the β-sheet is staggered. S is determined by moving around the β-barrel to corresponding β-sheet residues, as indicated by the arrow, until the first strand is reached again; due to the tilt of the β-strands with respect to the barrel axis the endpoint is displaced with respect to the starting point [43–46]. This displacement is the shear number. The tilt of the β-strands with respect to the barrel axis, α, is approximately 35°. The open and closed circles refer to residues pointing, respectively, towards the exterior and interior of the barrel, as deduced from [1]. The hydrogen-bonding interactions of the β-sheet residues, calculated by DSSP [47], are indicated by the dotted lines. The residues with inwards pointing side chains (closed circles) are hydrogen-bonded with the preceding strand (at the left) and the residues with the outward pointing side chains (open circles) are hydrogen-bonded to the following strand (at the right). B: Top view (along the barrel axis) of the TIM-barrel, into the active site. The helix of βα loop 4 positions the catalytic residue His-95 and the 3_{10}-helix of βα loop 8 anchors the phosphate moiety of the substrate. C: Side view of the TIM-barrel. The longest loops are respectively βα loop 3 (15 residues, important for dimerisation [48]), βα loop 4 (12 residues, important for positioning the catalytic histidine, His-95 [3]) and βα loop 6 (13 residues, this loop closes on binding of substrate [3]). Panels B and C were made with ICM [49].


A
barrel axis
α
Layer 3
Layer 2
Layer 1
A 10 A 42 A 64 V 92 A 125 A 165 Y 210 L 232 A 10
A 9 V 41 A 63 I 91 I 124 I 164 L 209 F 231 A 9
A 8 V 40 I 62 W 90 V 123 V 163 I 208 G 230 A 8
S=8
1 2 3 4 5 6 7 8 1
β-strand
B
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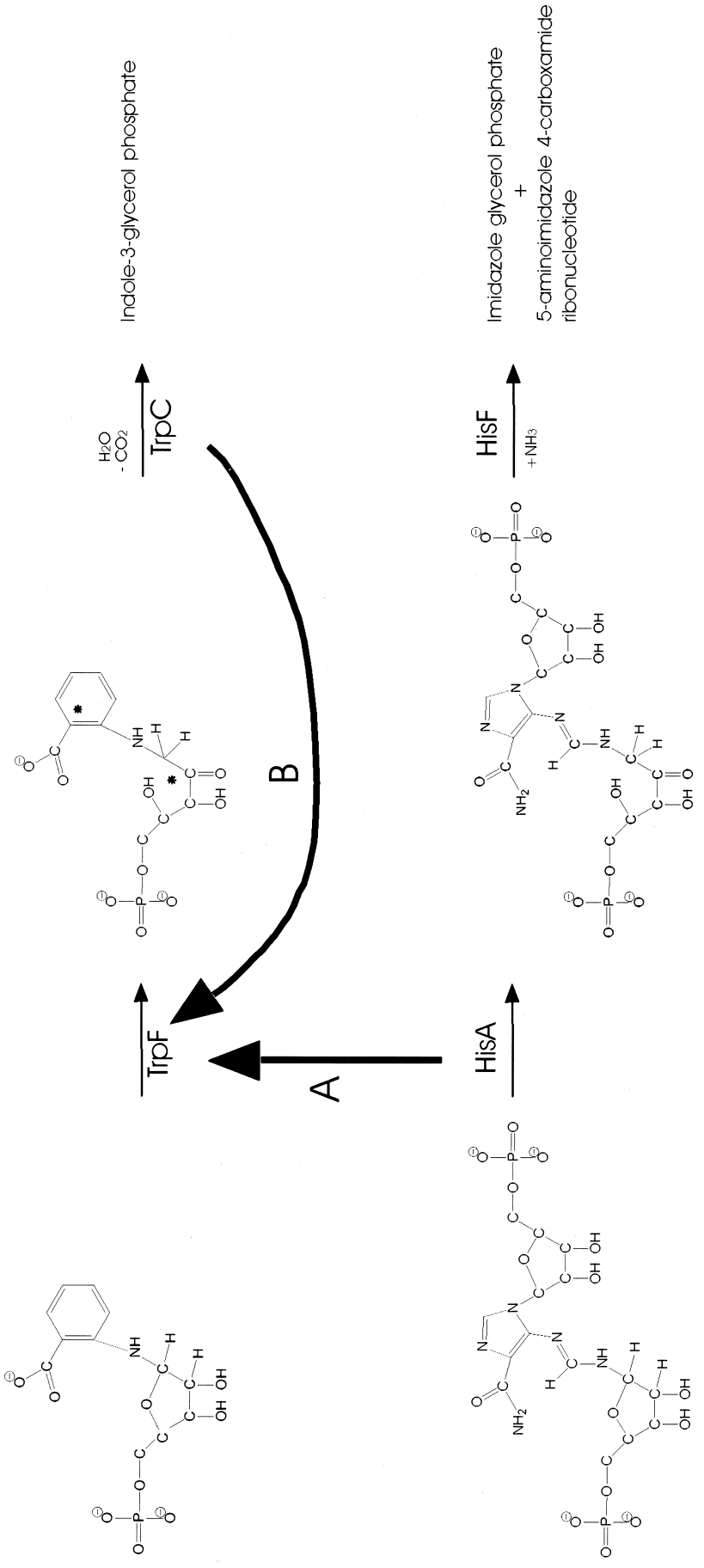
Indole-3-glycerol phosphate
Imidazole glycerol phosphate
+
5-aminoimidazole 4-carboxamide
ribonucleotide
H2O
-CO2
TrpC
HisF
+NH3
B
TrpF
A
HisA

Fig. 2. Reactions catalysed by TrpF, TrpC, HisA and HisF. TrpF and TrpC catalyse two subsequent reactions in the biosynthetic pathway of tryptophan. TrpF is a phosphoribosylanthranilate isomerase (PRAI); TrpC is an indole-3-glycerol-phosphate synthase (IGPS), which catalyses a carbon–carbon ring-forming reaction in which the labelled C atoms (*) become covalently bonded. HisA (catalysing an isomerisation) and HisF (catalysing a ring closure reaction in which imidazole-3-glycerol phosphate is formed) catalyse two subsequent steps in the histidine biosynthetic pathway. HisA and TrpF catalyse the same isomerisation reaction but with different substrate specificity; the substrate of HisA is a much larger molecule with two phosphate moieties. This reaction is an Amadori rearrangement by which a substituted 1-amino-ribose-5-phosphate is isomerised into a 1-amino-ketose-5-phosphate. The successful directed evolution experiments are indicated by additional arrows, labelled 'A' and 'B'. 'A' refers to the conversion of HisA into TrpF [14] and 'B' refers to the conversion of TrpC into TrpF [13].
←

enzymes of approximately 250 residues. The conversion of the functionality of HisA into TrpF concerns a change of substrate specificity. HisA and TrpF have no detectable sequence similarity [14]. New variants of HisA were made by random mutagenesis of the HisA gene; subsequently the new variants were selected using an *Escherichia coli* strain in which the TrpF gene was deleted. The obtained HisA variant has significant TrpF activity [14]. Fersht and coworkers [13], using the TIM-barrel enzyme TrpC, have shown that not only substrate specificity, but also the catalytic step itself can be changed by the directed evolution approach. In their experimental study TrpC was changed into TrpF by an extensive directed evolution experiment. TrpC and TrpF have significant sequence identity (22%), but catalyse a different reaction; they form a metabolically linked suprafamily [36]. First the sequences of βα loop 1 and βα loop 6 of TrpC were randomised (also allowing for deletions and insertions) because βα loop 1 and βα loop 6 are known from the structure to be important for catalysis in both TrpC and TrpF. Variants with improved TrpF catalytic activity were screened in an *E. coli* strain devoid of TrpF activity. Subsequently several other directed evolution steps, including DNA shuffling, were carried out. Remarkably, a new variant was eventually found with better catalytic properties, in terms of k_{cat}/K_m, than the wild-type TrpF. This new variant has 28% sequence identity with TrpF and 90% sequence identity with TrpC, but it does not have TrpC activity.

HisA and HisF have a sequence identity of approximately 25% [13]. Studies of HisA and HisF have revealed that these TIM-barrels have an internal twofold repeat pattern in their sequence and fold [13,20], indicating that these proteins evolved from a common ancestor. This ancestor itself was the result of a gene duplication followed by gene fusion of a gene encoding a phosphate-binding half-barrel folding unit. HisA and HisF are the only examples of TIM-barrel enzymes in which such a repeat pattern has been found. This repeat pattern is such that the main chain atoms of the N-terminal half (in HisA residues 2–117) can be superimposed on the C-terminal half (in HisA residues 118–241) with a rms deviation below 2 Å and sequence identity of approximately 20% for the comparison of the four half-barrels. The repeat pattern is also present in the distribution of loop lengths and loop functionalities. For example in all four half-barrels the first loop is rather long, and for HisF it is known that βα loops 3 and 4 and βα loops 7 and 8 of, respectively, the N-terminal and C-terminal half bind a phosphate. As seen in Fig. 2, the substrates of both HisA and HisF have two phosphate moieties, suggesting that the ancestral $(\beta\alpha)_4$ unit of HisA and HisF was a phosphate binding protein. This evolutionary scenario is further supported by the finding that the N-terminal and C-terminal half-barrel domains of HisF have now been shown to be folding units. When expressed separately the HisF-N and HisF-C units can be obtained as inactive homo-oligomers; when expressed jointly fully active heterodimers are obtained [37]. In at least one other TIM-barrel enzyme the N-terminal and C-terminal halves have been recognised as separate evolutionary units [38], suggesting that at least in some cases the TIM-barrel has been formed by modular construction from different half-barrel units [39].

As mentioned above, all ($n=8, S=8$) barrel proteins have an eight-stranded parallel β-sheet, except for the $\beta\beta\alpha\alpha(\beta\alpha)_6$ enolase barrel. It is interesting to point out that the enolase family of structures clearly belongs to a larger family of structurally related enzymes that include mandelate racemase and muconate-lactonising enzyme, which both exhibit the classical TIM-barrel topology [40]. These three enzyme families catalyse a similar reaction, in which the α proton of a carboxylic acid is abstracted. Comparison of the active sites shows that the chemistry of this reaction is catalysed in the same way but not necessarily by uniquely conserved side chains. Firstly it is seen that the type of catalytic residue and three-dimensional position in the active site is conserved, but not the relative position in the sequence. For example, Lys-396 in enolase is functionally (but not in sequence alignment) equivalent to Lys-164 in mandelate racemase. Secondly, even more unusual is the conservation of the position of the catalytic residue in the active site geometry, but not its identity; for example, the base in enolase is Lys-345 whereas the equivalent base in mandelate racemase is His-297 [40].

4. Concluding remarks

The versatility of the TIM-barrel scaffold is remarkable. This concerns its modular use in single-domain monomeric enzymes as well as its occurrence as the enzymatic domain in much larger multi-domain, multi-subunit enzymes. It also concerns its use for at least 15 different enzymatic functions. Can common features of TIM-barrel proteins be recognised? All TIM-barrel proteins have a known enzymatic function, with only one exception, which is narbonin. All TIM-barrel enzymes have their active site at the C-terminal end of the β-strands. Many TIM-barrel enzymes have flexible βα loops that are used to bind substrate and to bury the catalytic site from bulk solvent. Electrostatic calculations have highlighted a common, distinct electrostatic field pattern determined predominantly by the backbone atoms, which generates a positive potential at the C-terminal end of the barrel near the active site region [41]. This correlates with the known preference of TIM-barrel folds to bind negatively charged substrates, in particular phosphate-containing molecules [11]. In the SCOP database six TIM-barrel family enzymes are mentioned to have an equivalent phosphate binding site, between βα loop 7 and βα loop 8 [42]. The TIM-barrel enzymes HisA and HisF act on substrates with two phosphate moieties; the structures of these enzymes show twofold symmetry, indicating that the ancestral protein evolved from a fusion of two

phosphate binding half-barrel modules. Perhaps some other TIM-barrel proteins have also been constructed from half-barrel units [39], introducing a new level of complexity for establishing the evolutionary relationships of these proteins. Recent directed evolution experiments have shown that the substrate specificity and the catalytic specificity of TIM-barrel enzymes can be changed by random mutagenesis, in particular of the βα loops. This indicates that in the future new TIM-barrel enzymes with completely novel catalytic activities could become available from structure-based, directed evolution experiments.

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