ELSEVIER

Evolutionary markers in the $(\beta/\alpha)_8$-barrel fold

M Cristina Vega, Esben Lorentzen, Anni Linden and Matthias Wilmanns*

Enzymes with the $(\beta/\alpha)_8$-barrel fold are involved in the catalysis of a wide variety of biochemical reactions. The active sites of these enzymes are located on the C-terminal face of the central β-barrel. Conserved amino acid sequence, as well as secondary, tertiary and quaternary structure patterns are providing a rich body of data to support the premise of a common ancestry of many members of the $(\beta/\alpha)_8$-barrel fold family of enzymes. Recent data indicate that there is at least one example of a bienzyme that functions as an ammonia channel, adding a new level of functional diversity to the $(\beta/\alpha)_8$-barrel fold. These proteins have become ideal tools that can be used in conjunction with directed evolution techniques to engineer novel catalytic activities.

Addresses
EMBL-Hamburg c/o DESY, Notkestrasse 85, D-22603 Hamburg, Germany
*e-mail: wilmanns@embl-hamburg.de

Current Opinion in Chemical Biology 2003, **7**:694–701

This review comes from a themed section on
Model systems
Edited by Wolf-D Woggon and Stefan Matile

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2003.10.004

Abbreviations

HisA	phosphoribosylformimino-5-aminoimidazole carboxamide ribonucleotide isomerase
HisF	imidazole glycerol phosphate synthase
HisH	glutaminase of the imidazole glycerol phosphate synthase
KGPDC	3-keto-L-gulonate 6-phosphate decarboxylase
OMPDC	orotidine 5′-monophosphate decarboxylase
PDB	Protein Data Bank
SCOP	Structural Classification of Proteins (database)
TrpA	α-subunit of tryptophan synthase
TrpF	N-(5′phosphoribosyl)anthranilate isomerase

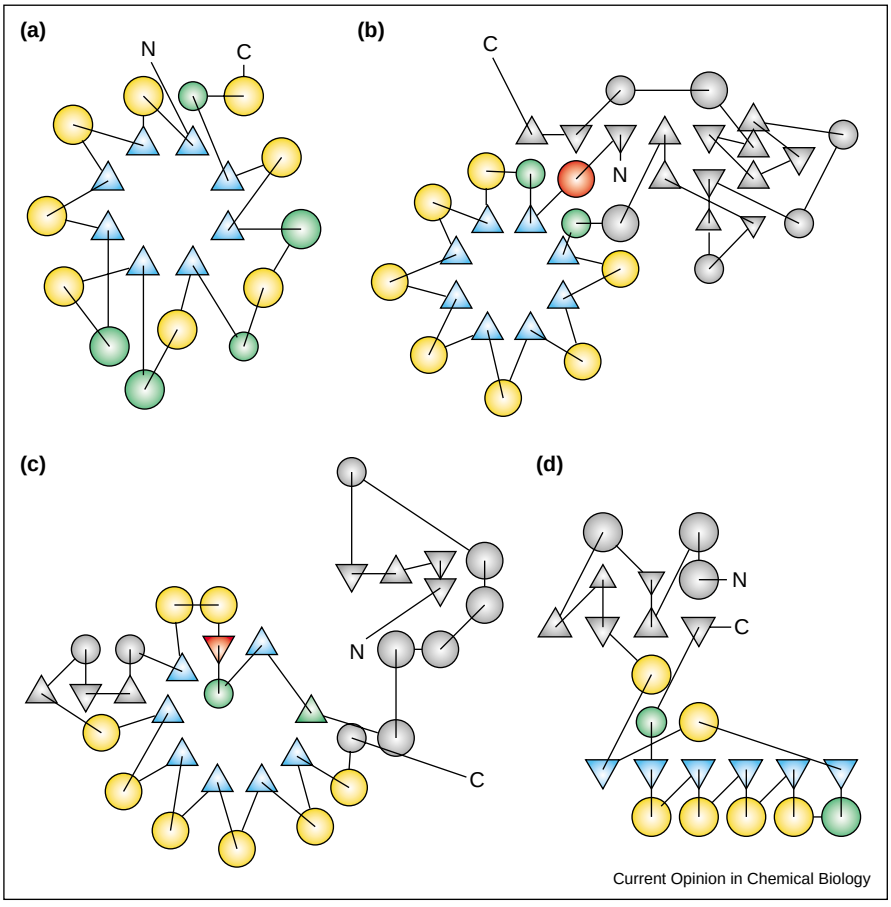
Introduction

Approximately 10% of all enzymes with known molecular structure are folded as $(\beta/\alpha)_8$-barrels, exceeding any other known fold in terms of overall number and functional diversity. A canonical $(\beta/\alpha)_8$-barrel consists of an inner ring of eight parallel β-strands that is wrapped by an outer wheel comprising typically eight α-helices. While the β-strands of the inner barrel are connected via a parallel β-sheet H-bond pattern, the interactions between the outer helices are less regular. These secondary structural elements generate an alternating (βα) pattern that facilitates reliable predictions of their fold from corresponding sequence patterns [1]. The Structural Classification of Proteins (SCOP) database (http://scop.mrc-lmb.cam.ac.uk/scop/, version 1.63, May 2003) lists 26 $(\beta/\alpha)_8$-barrel fold families. In the Class, Architecture, Topology and Homologous superfamily (CATH) database (http://www.biochem.ucl.ac.uk/bsm/cath/, version 2.4, January 2002), the class-3 (α and β) superfamily contains 28 $(\beta/\alpha)_8$-barrel families. Some proteins display deviations from the classical $(\beta/\alpha)_8$-barrel fold, including variations in the number of β-strands, their orientation (typically all parallel), local structural distortions and lack of barrel closure [2••,3]. A representative set of $(\beta/\alpha)_8$-barrel topology variants is shown in Figure 1.

Most $(\beta/\alpha)_8$-barrel proteins function as enzymes, with a few exceptions, such as narbonin and concanavalin B [4]. These enzymes catalyze a diverse array of biochemical reactions [2••,5••]. Members of the $(\beta/\alpha)_8$-barrel fold are subsumed within five of the six general classes of catalytic activities according to the Enzyme Commission classification scheme, excluding only the ligases. Because of their repetitive amino acid sequence and structural features, it has been difficult to track a possible common evolutionary ancestry on the basis of sequence and structure comparative analyses only, triggering more than a decade-long debate on the convergent versus divergent general evolutionary roadway of these proteins [5••,6,7]. A general argument to support the premise of a broad, common ancestry may originate from the observation that the active sites of all $(\beta/\alpha)_8$-barrels are located on the C-terminal face of the central β-barrel, as there is no inherently obvious structural basis for preference of the C-terminal face over the N-terminal face.

It has been noted that a large proportion of the enzymatic reactions of some metabolic pathways, such as glycolysis or tryptophan biosynthesis, are catalyzed by $(\beta/\alpha)_8$-barrel proteins [8,9]. These observations have enabled the testing and validation of previous evolutionary models, including the 'retrograde pathway hypothesis' by Horowitz (1945), the 'patchwork evolution hypothesis' by Jensen (1976) and modifications thereof that have been reviewed recently [10,11]. These pathway-oriented considerations have been complemented by a series of investigations in an effort to derive common ancestry from mechanistic, biochemical associations [5••]. In their recent review, Gerlt and Raushel have summarized data providing evidence for the evolvement of 'new' enzymes based on a common chemical mechanism, a common ligand specificity as well as a common active site architecture. Recent sequence, structural and biochemical analyses of two $(\beta/\alpha)_8$-barrel enzymes of the histidine biosynthesis

Figure 1



Topology diagrams of a representative set of $(\beta\alpha)_8$-barrels. **(a)** Triose phosphate isomerase (PDB code: 1TIM); **(b)** Alanine racemase (PDB code: 1BD0), which forms a PLP binding barrel with a circular permutation of the representative fold; in this structure the first secondary structural element of the barrel is a helix (colored in red) converting it into a $(\alpha\beta)_8$-barrel. **(c)** Enolase (PDB code: 1E9I) with $\beta\beta\alpha\alpha(\beta\alpha)_6$ topology; strand β2, which is anti-parallel with respect to the remaining β-strands of the $(\beta\alpha)_8$-barrel, is colored in red. **(d)** Quinolinate phosphoribosyl transferase (PDB code: 1QPO) with only six β-strands, leading to a partially closed barrel. The $(\beta\alpha)_8$-barrel β-strands and α-helices are colored in cyan and yellow, respectively; additional secondary structural elements are in green; the secondary structural elements of other domains are in gray. This figure was prepared with the TOPS server (http://www.tops.leeds.ac.uk/).

pathway have led to the prediction that they may have evolved from smaller fragments such as $(\beta/\alpha)_4$-barrel units [12,13]. In the following contribution, we provide an overview of recent data revealing an association between $(\beta/\alpha)_8$-barrel enzymes with respect to their amino acid sequences, as well as secondary, tertiary and quaternary structures.

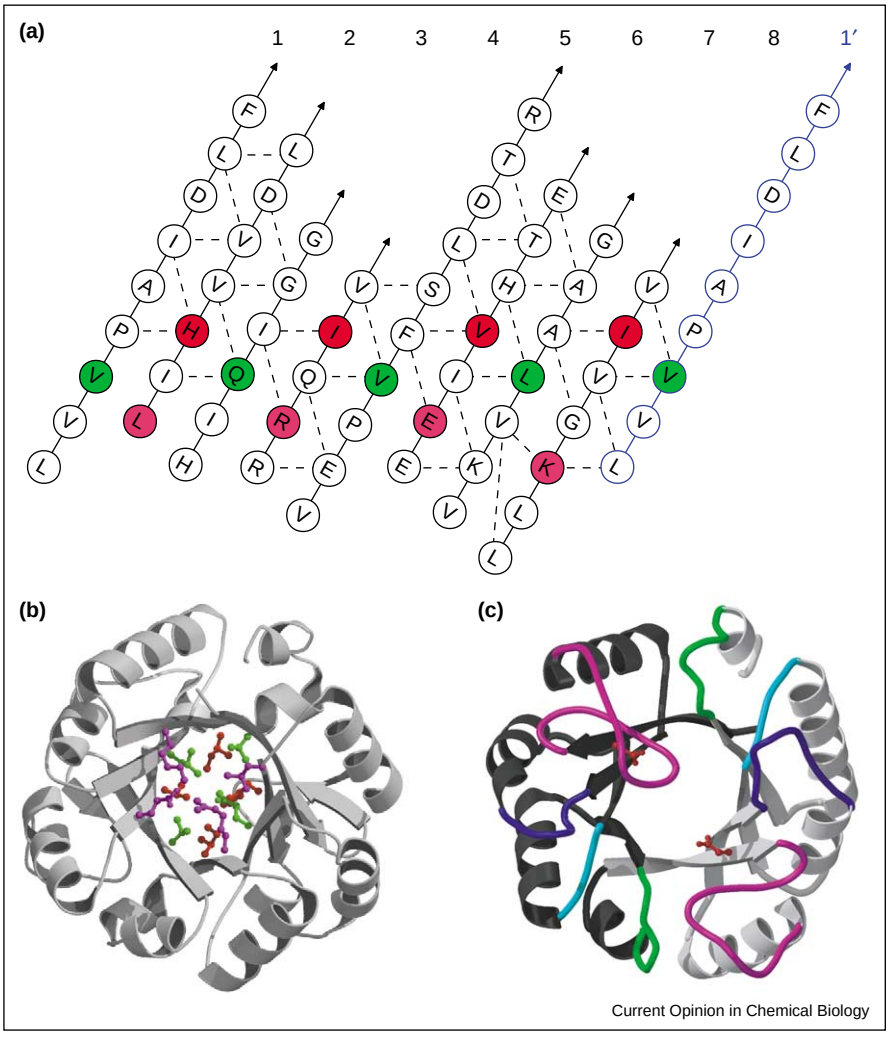
Sequence relations in (β/α)$_8$-barrels

Comparative analyses of the amino acid sequences of known $(\beta/\alpha)_8$-barrel folds do not reveal significant sequence similarity in general, precluding detection of an association by this criterion alone. However, there are several exceptions, mostly from small clusters of homologous $(\beta/\alpha)_8$-barrels that are associated with the catalysis of specific reaction mechanisms or are known to be members of the same or related pathways [14–18,19•]. Knowledge of their three-dimensional structures (see below), however, not only permits unbiased, structure-based sequence alignments, but also allows detection of conserved structural motifs not found in sequence comparisons.

Secondary structure relations in (β/α)$_8$-barrels

The eightfold repeat of (βα) units in the canonical $(\beta/\alpha)_8$-barrel fold generates a highly symmetrical arrangement of secondary structural elements. The structure of the inner parallel β-strand barrel is more constrained, with a few notable exceptions [2••], than the arrangement of the outer α-helical ring. The architecture of the inner β-barrel can be described geometrically as hyperboloid that, because of an approximate 36-degrees tilt with respect to

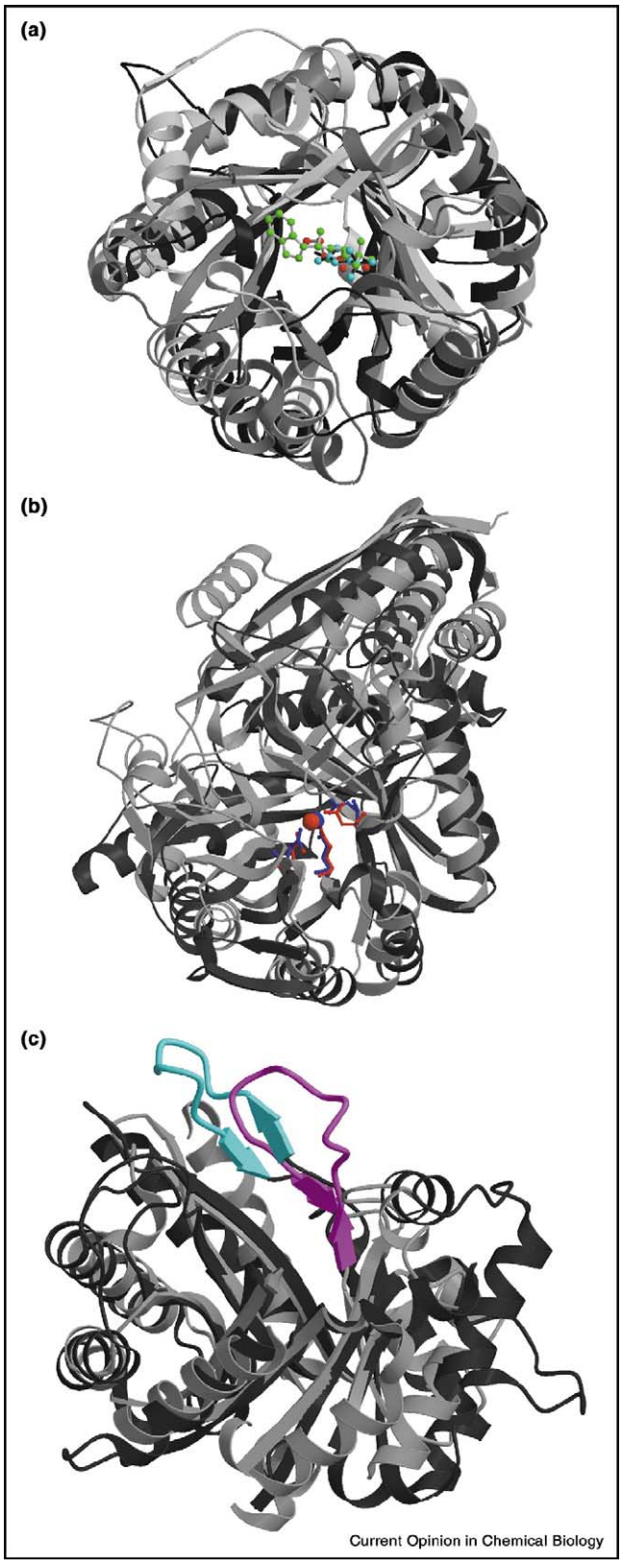
Figure 2



Inner β-barrel architecture in $(\beta\alpha)_8$-barrels, using the structural coordinates of HisA (PDB code: 1THF). **(a)** Schematic presentation of the β-strand arrangement in the β-barrel. Amino acids are designated by one-letter codes. The orientation of the β-strands is indicated by arrows. Inter-strand H-bonds are shown as dashed lines. The first strand β1 is shown twice (black, blue) to display the complete β-barrel H-bond pattern. Those residues that are involved in the layered packing of the hydrophobic core are shown in magenta (layer 1), green (layer 2) and red (layer 3). The layers are formed by alternating odd- and even-numbered β-strands. Part of the H-bond network between β-strands 6 and 7 in HisA is irregular. The β-barrel architecture forms residue layers that are tilted by approximately 36° with respect to the vertical axis, leading to a shift of eight residues after one turn. In the example, residue L1 of strands β1′ and F9 of β-strand β1 are connected by this type of layer, generating a shear number of eight. **(b)** Packing of the hydrophobic core inside the central β-barrel is accomplished by three residue layers that are either provided by odd-numbered β-strands (green) or by even-numbered β-strands (magenta, red). The colors are as in (a). View from the N-terminal face of the β-barrel. **(c)** Repetitive loop motifs and active site residues. Loops 1/5, 2/6, 3/7 and 4/8 are colored in magenta, blue, cyan and green, respectively. There are two catalytic aspartate residues in symmetrical positions (strands β1 and β5) that are colored in red. The N-terminal and C-terminal halves of the HisA $(\beta\alpha)_8$-barrel are shown by different gray shadings. View from the C-terminal active site face of the β-barrel. Further details are found in Lang *et al.* [12].

the barrel major axis, generates a shear number $S = 8$ [20] (Figure 2a). Furthermore, the eightfold β-barrel forms an arrangement of side chains in three to four layers within the core of the central β-barrel (Figure 2b). Each layer is perpendicular to the principal barrel axis and is established by an alternating pattern of residues from the four odd-numbered (1,3,5,7) or even-numbered (2,4,6,8) β-strands, imposing a fourfold symmetry pattern. Although rare in nature, circular permutated $(\beta/\alpha)_8$-barrel versions have been detected [21,22] and could be engineered [23]. The repetitive and symmetrical arrangement of secondary structural elements has facilitated several predictions of the $(\beta/\alpha)_8$-barrel fold from amino acid sequences with no known structure [1,24–26].

Figure 3



Conserved 3D structure motifs in (βα)$_8$-barrels. **(a)** Superposition of indole glycerolphosphate synthase (dark gray, PDB code: 1A53) from the tryptophan biosynthesis pathway, triose phosphate isomerase (light gray, PDB code: 1HG3) and fructose 1,6-bisphosphate aldolase (gray, PDB code: 1OK4) from the glycolysis pathway. In each of the three structures there is a substrate analog bound to the active site. They are colored in green, cyan and red, respectively. The phosphate groups of these compounds bind into the same binding pocket that is formed by the loops β7–α7 and β8–α8; **(b)** Superposition of 2-phosphoglycerate dehydratase (light gray, PDB code: 1E9I) and glucarate dehydratase (dark gray, PDB code: 1EC8). These two structures do not exhibit significant sequence similarities. Three structurally conserved residues and the active site magnesium ions are shown in red and blue, respectively. **(c)** Superposition of an eukaryotic (dark gray) and an archaeal (light gray) class I aldolase (PDB codes: 1J4E and 1OK4, respectively). The loop connecting β3 and α3 is involved in their oligomerization into tetramers and pentamers (forming a decamer with a second pentamer).

Common motifs in the 3D-structures of (β/α)$_8$-barrels

The general consensus is that the detection of a common (β/α)$_8$-barrel fold alone is insufficient evidence to prove a possible evolutionary association. Although the apparent restriction of the active sites to the C-terminal face of the inner parallel β-barrel does serve as an appealing argument for the general evolutionary ancestry of many, if not all, (β/α)$_8$-barrel enzymes, three-dimensional structural analyses of these enzymes are providing an abundance of common structural motifs, providing an impetus toward the detection of further evolutionary relations.

Common phosphate binding site

Approximately two-thirds of the established (β/α)$_8$-barrel enzyme families are utilizing substrates or cofactors that contain at least one phosphate group [2••]. Initially, it was recognized that three (β/α)$_8$-barrel enzymes from the tryptophan biosynthesis pathway use the same motif, comprising the loops connecting β7–α7 and β8–α8, to bind the phosphate groups of the respective substrates [9] (Figure 3a). The latter loop contains a short additional helix (α8′) whose N-terminal macrodipole moment provides additional binding potential. The phosphate binding site is considered to be the most conserved structural motif among (β/α)$_8$-barrel enzymes [2••].

Metal-assisted catalysis

Approximately one-half of the characterized (β/α)$_8$-barrel enzymes require the presence of metal ions for catalysis [2••]. Although the positions of catalysis-assisting metals are highly constrained in some enzymes from the enolase family [27,28], overall, there appears to be more structural versatility in terms of positions, types and number of metal centres [2••] than, for instance, for the conserved phosphate binding site (Figure 3b). A striking, recent example illustrates how modest changes in the active site architecture in a pair of closely related (β/α)$_8$-barrel enzymes, 3-keto-L-gulonate 6-phosphate decarboxylase (KGPDC) and orotidine 5′-monophosphate decarboxylase (OMPDC), lead to unrelated mechanisms of catalysis [19•]. These changes include

the conversion of a negatively charged residue into a positively charged one (KGPDC, E33; OMPDC, K33), thereby replacing the function of a magnesium ion in the active site of KGPDC with the side-chain amino group of K33 in OMPDC. Another interesting example is found in family-13 α-amylases with the $(\beta/\alpha)_8$-barrel fold. They contain a conserved calcium ion site in the close vicinity of the active site, probably for the purpose of stabilizing the active site conformation [29]. Interestingly, α-amylases from hyperthermophilic bacteria and archaea have developed distinct bi/tri-metal centres that superimpose, but are unrelated with respect to metal preference [30]. Comparative analyses of these enzymes indicate that structural adaptation to specific environmental conditions may have evolved independently of a $(\beta/\alpha)_8$-barrel progenitor.

Conserved active sites

Although $(\beta/\alpha)_8$-barrel enzymes are involved in a diverse array of catalyzed reaction mechanisms, there are groups among these enzymes that share remarkable active site similarities. Aldolases that are folded as $(\beta/\alpha)_8$-barrels exhibit a range of different substrate specificities and display, in part, unrelated catalytic mechanisms. Class I aldolases from eucarya and archaea, specifically catalyzing the cleavage of fructose 1,6-bisphosphate, have diverged considerably in sequence, precluding the detection of significant sequence similarity [31]. However, superposition of the structures of an eukaryotic and archaeal fructose 1,6-bisphosphate aldolase has revealed that they share at least six active site residues located at structurally invariant positions, thereby indicating common ancestry [32•]. One of the most widely studied groups of $(\beta/\alpha)_8$-barrel enzymes is the enolase family, which, according to present data, is associated with 11 known different catalytic activities and numerous others yet to be defined [5••]. While the canonical enolase $(\beta/\alpha)_8$-barrel comprises a conserved pair of acid/base catalysts plus an active site magnesium ion, several modified active site topologies have evolved to contend with specific mechanistic requirements of other members of the same family [33]. Other examples of enzymes with conserved active sites have been well illustrated in a recent review [5••].

Repeated motifs

Structural comparison of two $(\beta/\alpha)_8$-barrel enzymes from the histidine biosynthesis (HisA, HisF) pathway has revealed a common twofold repeat structure, each comprising a $(\beta\alpha)_4$ unit [12] that show native-like biophysical properties if purified separately [13]. The twofold repetitive structure is reflected by structure-based sequence similarities, two phosphate binding motifs (loops 3/4 and loops 7/8), a symmetric arrangement of catalytic residues on loops 1 and 5, and a twofold repetitive loop structure on the C-terminal face of each of the two $(\beta/\alpha)_8$-barrels (Figure 2c). Interestingly, a twofold repetitive arrangement of catalytic acid/base residues has also been found in class I aldolases [32•]. A recent study was carried out on two differentially regulated isoforms of the DHAP synthase $(\beta/\alpha)_8$-barrel, yielding catalytically active chimeras [34••].

Related quaternary structural arrangements

Many of the known $(\beta/\alpha)_8$-barrel structures are oligomeric. Recently, it has been shown that the structural dimers of two decarboxylases (OMPDC, KGPDC) of the ribulose phosphate binding family (SCOP nomenclature) can be superimposed, demonstrating that a common quaternary arrangement may serve as further indication for common ancestry [19•]. Interestingly, even OMPDC and other dimeric $(\beta/\alpha)_8$-barrels, such as triose phosphate isomerase, display related quaternary arrangements that form an active site interface. In other families, such as fructose 1,6-bisphosphate aldolases, different decameric and tetrameric arrangements have been found. Although they lead to unrelated quaternary structures, it has been noticed that their assembly is generated by a topographically identical loop [32•] (Figure 3c). In another group of oligomeric $(\beta/\alpha)_8$-barrel enzymes, first observed in phosphoenolpyruvate mutase [35], swapping of the C-terminal barrel helix α8″ appears to be critical for oligomeric assembly. The latest addition to this class of enzymes has been the structure of ketopantoate hydroxymethyltransferase from the pantothenate biosynthesis pathway [28].

$(\beta/\alpha)_8$-Barrels as part of molecular machines

The structure of the tryptophan synthase αββα complex, the terminal enzyme complex of tryptophan biosynthesis, has provided a spectacular example of how a $(\beta/\alpha)_8$-barrel enzyme (TrpA) may be involved in a complex reaction requiring compound tunneling [36,37]. While the active site loop structure of the $(\beta/\alpha)_8$-barrel TrpA subunit is critical for submitting its product indole into the tunnel linking the TrpA and TrpB active sites, TrpA does not provide the structural framework for tunneling. Recent molecular structures of the glutaminase-imidazole glycerol phosphate synthase (HisH-HisF) complex, however, indicated, for the first time, how a $(\beta/\alpha)_8$-barrel (HisF) may directly provide the scaffold for compound (ammonia) tunneling [38•,39•,40]. In these complexes, the glutaminase subunit (HisH) docks onto the N-terminal face of the HisF $(\beta/\alpha)_8$-barrel, thereby suggesting a model for ammonia tunneling along the central β-barrel axis of the HisF $(\beta/\alpha)_8$-barrel towards the synthase active site on the C-terminal β-barrel face of HisF. Recent structural data indicate that at least one conserved residue on the N-terminal face of the HisF $(\beta/\alpha)_8$-barrel (Q123) is essential for glutaminase catalysis ([38•], MC Vega and M Wilmanns, unpublished data), supporting previous mutagenesis data [41]. The presence of catalytic residues and a cluster of charged residues providing the gate for the suggested ammonia tunnel may explain previous observations of an unusually high number of conserved

residues on the N-terminal face of the HisF (β/α)$_8$-barrel [12]. The FMN binding domain of glutamate synthase, which uses ammonia that is generated by glutamine hydrolysis as well, is also folded as (β/α)$_8$-barrel. In contrast to the glutaminase-imidazole glycerol phosphate synthase complex, the ammonia channel in glutamate synthase seems to be formed by the interface from different domains not directly involving the (β/α)$_8$-barrel core [42•].

Knowledge-based engineering of novel catalytic activities in (β/α)$_8$-barrels

The present sequence, structural and biochemical data provide an increasingly emerging body of unambiguous evidence for a common evolutionary ancestry for enzymes that are folded as (β/α)$_8$-barrels. Several possible evolutionary scenarios have been widely discussed [5••,43], although available data on their phylogenetic association with implications for family classifications are not yet fully consistent [2••,18] and, therefore, must be interpreted with caution [5••]. Recent support for a common progenitor for two (β/α)$_8$-barrel enzymes comes from a genetic study in *Mycobacterium tuberculosis* and *Streptomyces coelicolor*, providing evidence that in some microbial organisms, two similar isomerase reactions in histidine biosynthesis (HisA) and tryptophan biosynthesis (TrpF) [44] may be catalyzed by a bifunctional common ancient-like enzyme [45•]. In a previous directed evolution experiment in *Escherichia coli*, it was shown that one amino acid replacement is sufficient to swap the substrate specificities of these two enzymes [46]. Directed evolution techniques have also been applied to (β/α)$_8$-barrel members of the enolase family, generating enzymes with indiscriminate substrate specificities but requiring different reaction mechanisms [47•], and similar experiments for future studies have been proposed [48]. Using the same technique, the enantioselectivity of the reaction catalyzed by *N*-acetylneuraminic acid aldolase from *E. coli* could be converted, demonstrating the potential of the (β/α)$_8$-barrel fold to tinker specific chemical reactions [49].

Conclusions

Proteins with the (β/α)$_8$-barrel architecture present the largest fold family of enzymes with known molecular structure. There is an overwhelming and increasingly emerging body of evidence that most of them, if not all, share one common evolutionary ancestor. Proteins with the (β/α)$_8$-barrel fold provide an ideal framework to study almost any conceivable biochemical reaction, including reactions that are coupled with other enzymatic activities. Hence, they represent excellent tools that can be used in conjunction with directed evolution techniques to explore novel catalytic activities.

Update

Recently, the yeast cytosine deaminase crystal structure has been reported [50]. Unlike the bacterial enzyme with (β/α)$_8$-barrel topology, the yeast cytosine deaminase shows a mixed (α/β) topology, suggesting independent evolution. In addition, the structure of ketopantoate hydroxymethyl transferase from *E. coli* in a decameric arrangement of (β/α)$_8$-barrels has been published [51].

Acknowledgements

We thank Areti Malapetsas for editing the manuscript. MW acknowledges support of the Deutsche Forschungsgemeinschaft in relation to the topics discussed in this review (Wi 1058/5-3).

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Pickett SD, Saqi MA, Sternberg MJ: **Evaluation of the sequence template method for protein structure prediction. Discrimination of the (β/α)$_8$-barrel fold**. *J Mol Biol* 1992, **228**:170-187.

2. •• Nagano N, Orengo CA, Thornton JM: **One fold with many functions: the evolutionary relationships between TIM barrel families based on their sequences, structures and functions**. *J Mol Biol* 2002, **321**:741-765.

The sequence, structural and functional diversity of (βα)$_8$-barrels is thoroughly reviewed, mostly from a bioinformatics perspective. The review contains excellent illustrations on various conserved (βα)$_8$-barrel motifs.

3. Evans JC, Huddler DP, Jiracek J, Castro C, Millian NS, Garrow TA, Ludwig ML: **Betaine-homocysteine methyltransferase: zinc in a distorted barrel**. *Structure* 2002, **10**:1159-1171.

4. Hennig M, Jansonius JN, Terwisscha van Scheltinga AC, Dijkstra BW, Schlesier B: **Crystal structure of concanavalin B at 1.65 Å resolution. An 'inactivated' chitinase from seeds of *Canavalia ensiformis***. *J Mol Biol* 1995, **254**:237-246.

5. •• Gerlt JA, Raushel RM: **Evolution of function in (β/α)$_8$-barrel enzymes**. *Curr Opin Chem Biol* 2003, **7**:252-264.

Several scenarios for the evolution of (βα)$_8$-barrels are reviewed mostly from a biochemical viewpoint, particularly considering preservation of reactions mechanisms, ligand (substrate) specificity and active site architecture. Data from the enolase, amidohydrolase, OMP decarboxylase and aldolase superfamilies are summarized and discussed. The review contains numerous key references.

6. Farber GK, Petsko GA: **The evolution of α/β barrel enzymes**. *Trends Biochem Sci* 1990, **15**:228-234.

7. Reardon D, Farber GK: **The structure and evolution of α/β barrel proteins**. *FASEB J* 1995, **9**:497-503.

8. Erlandsen H, Abola EE, Stevens RC: **Combining structural genomics and enzymology: completing the picture in metabolic pathways and enzyme active sites**. *Curr Opin Struct Biol* 2000, **10**:719-730.

9. Wilmanns M, Hyde CC, Davies DR, Kirschner K, Jansonius JN: **Structural conservation in parallel β/α barrel enzymes that catalyze three sequential reactions in the pathway of tryptophan biosynthesis**. *Biochemistry* 1991, **30**:9161-9169.

10. Rison SC, Thornton JM: **Pathway evolution, structurally speaking**. *Curr Opin Struct Biol* 2002, **12**:374-382.

11. Schmidt S, Sunyaev S, Bork P, Dandekar T: **Metabolites: a helping hand for pathway evolution?** *Trends Biochem Sci* 2003, **28**:336-341.

12. Lang D, Thoma R, Henn-Sax M, Sterner R, Wilmanns M: **Structural evidence for evolution of the β/α barrel scaffold by gene duplication and fusion**. *Science* 2000, **289**:1546-1550.

13. Hoecker B, Beismann-Driemeyer S, Hettwer S, Lustig A, Sterner R: **Dissection of a (βα)$_8$-barrel enzyme into two folded halves**. *Nat Struct Biol* 2001, **8**:5-7.

14. Belfaiza J, Parsot C, Martel A, de la Tour CB, Margarita D, Cohen GN, Saint-Girons I: **Evolution in biosynthetic pathways: two enzymes catalyzing consecutive steps in methionine biosynthesis originate from a common ancestor and possess a similar regulatory region**. *Proc Natl Acad Sci USA* 1986, **83**:867-871.

15. Alifano P, Fani R, Lio P, Lazcano A, Bazzicalupo M, Carlomagno MS, Bruni CB: **Histidine biosynthetic pathway and genes: structure, regulation and evolution**. *Microbiol Rev* 1996, **60**:44-69.

16. Janecek S: **Invariant glycines and prolines flanking in loops the strand beta 2 of various $(\alpha/\beta)_8$-barrel enzymes: a hidden homology?** *Protein Sci* 1996, **5**:1136-1143.

17. Souchet M, Legave M, Jullian N, Bertrand HO, Bril A, Berrebi-Bertrand I: **Structure of the human glycogen-associated protein phosphatase 1 regulatory subunit hGM: homology modeling revealed an $(\alpha/\beta)_8$-barrel-like fold in the multidomain protein**. *Protein Sci* 1999, **8**:2570-2579.

18. Copley RR, Bork P: **Homology among α-β_8 barrels: implications for the evolution of metabolic pathways**. *J Mol Biol* 2000, **303**:627-641.

19. • Wise E, Yew WS, Babbitt PC, Gerlt JA, Rayment I: **Homologous $(\beta/\alpha)_8$-barrel enzymes that catalyze unrelated reactions: orotidine 5′-monophosphate decarboxylase and 3-keto-L-gulonate 6-phosphate decarboxylase**. *Biochemistry* 2002, **41**:3861-3869.

The structures of KGPDC and OMPDC are compared, revealing close relations of two $(\beta\alpha)_8$-barrels that catalyze unrelated reactions, thus presenting a case study for 'opportunistic' divergent evolution.

20. Lasters I, Wodak SJ, Alard P, van Cutsem E: **Structural principles of parallel beta-barrels in proteins**. *Proc Natl Acad Sci USA* 1988, **85**:3338-3342.

21. MacGregor EA, Jespersen HM, Svensson B: **A circularly permuted alpha-amylase-type α/β-barrel structure in glucan-synthesizing glucosyltransferases**. *FEBS Lett* 1996, **378**:263-266.

22. Jia J, Huang W, Schorken U, Sahm H, Sprenger GA, Lindqvist Y, Schneider G: **Crystal structure of transaldolase B from *Escherichia coli* suggests a circular permutation of the α/β barrel within the class I aldolase family**. *Structure* 1996, **4**:715-724.

23. Luger K, Hommel U, Herold M, Hofsteenge J, Kirschner K: **Correct folding of circularly permuted variants of a $\beta\alpha$ barrel enzyme *in vivo***. *Science* 1989, **243**:206-210.

24. Wilmanns M, Eisenberg D: **Inverse protein folding by the residue pair preference profile method: estimating the correctness of alignments of structurally compatible sequences**. *Protein Eng* 1995, **8**:627-639.

25. Hansson M, Gough SP, Brody SS: **Structure prediction and fold recognition for the ferrochelatase family of proteins**. *Proteins* 1997, **27**:517-522.

26. Rigden DJ, Jedrzejas MJ, de Mello LV: **Identification of catalytic barrel domains in seven further glycoside hydrolase families**. *FEBS Lett* 2003, **544**:103-111.

27. Hasson MS, Schlichting I, Moulai J, Taylor K, Barrett W, Kenyon GL, Babbitt PC, Gerlt JA, Petsko GA, Ringe D: **Evolution of an enzyme active site: the structure of a new crystal form of muconate lactonizing enzyme compared with mandelate racemase and enolase**. *Proc Natl Acad Sci USA* 1998, **95**:10396-10401.

28. Chaudhuri BN, Sawaya MR, Kim C-Y, Waldo GS, Park MS, Terwilliger TC, Yeates TO: **The crystal structure of the first enzyme in the pantothenate biosynthetic pathway, ketopantoate hydroxymethyltransferase, from *M. tuberculosis***. *Structure* 2003, **11**:753-764.

29. Henrissat B, Davies G: **Structural and sequence-based classification of glycoside hydrolases**. *Curr Opin Struct Biol* 1997, **7**:637-644.

30. Linden A, Mayans O, Meyer-Klaucke W, Antranikian G, Wilmanns M: **Differential regulation of a hyperthermophilic alpha-amylase with a novel (Ca, Zn) two-metal center by zinc**. *J Biol Chem* 2003, **278**:9875-9884.

31. Siebers B, Brinkmann H, Doerr C, Tjaden B, Lilie H, van der Oost J, Verhees CH: **Archaeal fructose-1,6-bisphosphate aldolases constitute a new family of archaeal type class I aldolase**. *J Biol Chem* 2001, **276**:28710-28718.

32. • Lorentzen E, Pohl E, Zwart P, Stark A, Russell RB, Knura T, Hensel R, Siebers B: **Crystal structure of an archaeal class I aldolase and the evolution of $(\beta\alpha)_8$ barrel proteins.** *J Biol Chem*, in press.

Comparison of a eukaryotic and an archaeal class I aldolase reveals that their actives sites are conserved despite lack of significant sequence similarity. Symmetric arrangement of catalytic residues is suggestive of an ancestry of half barrels.

33. Babbitt PC, Mrachko GT, Hasson MS, Huisman GW, Kolter R, Ringe D, Petsko GA, Kenyon GL, Gerlt JA: **A functionally diverse enzyme superfamily that abstracts the alpha protons of carboxylic acids**. *Science* 1995, **267**:1159-1161.

34. •• Hartmann M, Schneider TR, Pfeil A, Heinrich G, Lipscomb WN, Braus GH: **Evolution of feedback-inhibited β/α barrel isoenzymes by gene duplication and a single mutation**. *Proc Natl Acad Sci USA* 2003, **100**:862-867.

Replacement of a single residue is sufficient to swap feedback inhibition of two DAPH synthase isoforms either by phenylalanine or by tyrosine. Using a new structure of the two isoenzymes, the residue is mapped into the hinge of two half-barrel entities. Engineered catalytic active half-barrel chimeras of the two isoenzymes support ancestry from a $(\beta\alpha)_4$ half-barrel unit.

35. Huang K, Li Z, Jia Y, Dunaway-Mariano D, Herzberg O: **Helix swapping between two alpha/beta barrels: crystal structure of phosphoenolpyruvate mutase with bound Mg^{2+}-oxalate**. *Struct Fold Des* 1999, **7**:539-548.

36. Hyde CC, Ahmed SA, Padlan EA, Miles EW, Davies DR: **Three-dimensional structure of the tryptophan synthase alpha 2 beta 2 multienzyme complex from *Salmonella typhimurium***. *J Biol Chem* 1988, **263**:17857-17871.

37. Miles EW: **Tryptophan synthase: a multienzyme complex with an intramolecular tunnel**. *Chem Rec* 2001, **1**:140-151.

38. • Chaudhuri BN, Lange SC, Myers RS, Chittur SV, Davisson VJ, Smith JL: **Crystal structure of imidazole glycerol phosphate synthase: A tunnel through a $(\beta/\alpha)_8$ barrel joins two active site**. *Structure* 2001, **9**:987-997.

The structure of the bifunctional glutaminase imidazole glycerolphosphate synthase from yeast suggests passage of ammonia via a channel across the $(\beta\alpha)_8$-barrel of the imidazole glycerolphosphate synthase.

39. • Douangamath A, Walker M, Beismann-Driemeyer S, Vega MC, Sterner R, Wilmanns M: **Structural evidence for ammonia tunneling across the $(\beta\alpha)_8$ barrel of the imidazole glycerol phosphate synthase bienzyme complex**. *Structure* 2002, **10**:185-193.

The structure of the heterodimeric complex of a glutaminase subunit and the imidazole glycerolphosphate synthase subunit from the hyperthermophile *Thermotoga maritima* suggests channeling of ammonia via a tunnel across the $(\beta\alpha)_8$-barrel of the imidazole glycerolphosphate synthase, thus confirming and complementing data from Chaudhuri *et al.* [38•].

40. Omi R, Mizuguchi H, Goto M, Miyahara I, Hayashi H, Kagamiyama H, Hirotsu K: **Structure of imidazole glycerol phosphate synthase from *Thermus thermophilus* HB8: open-closed conformational change and ammonia tunneling**. *J Biochem (Tokyo)* 2002, **132**:759-765.

41. Klem TJ, Chen Y, Davisson VJ: **Subunit interactions and glutamine utilization by *Escherichia coli* imidazole glycerol phosphate synthase**. *J Bacteriol* 2001, **183**:989-996.

42. • Van den Heuvel RH, Svergun DI, Petoukhov MV, Coda A, Curti B, Ravasio S, Vanoni MA, Mattevi A: **The active conformation of glutamate synthase and its binding to ferredoxin**. *J Mol Biol* 2003, **330**:113-128.

Using X-ray crystallography and X-ray small angle scattering methods a model is proposed to explain how the activity of glutamate synthase is regulated by changes in the quaternary structure.

43. Hoecker B, Juergens C, Wilmanns M, Sterner R: **Stability, catalytic versatility and evolution of the $(\beta\alpha)_8$-barrel fold**. *Curr Opin Biotechnol* 2001, **12**:376-381.

44. Henn-Sax M, Thoma R, Schmidt S, Hennig M, Kirschner K, Sterner R: **Two $(\beta/\alpha)_8$-barrel enzymes of histidine and**

tryptophan biosynthesis have similar reaction mechanisms and common strategies for protecting their labile substrates. *Biochemistry* 2002, **41**:12032-12042.

45. Barona-Gomez F, Hodgson DA: **Occurrence of a putative**
• **ancient-like isomerase involved in histidine and tryptophan biosynthesis**. *EMBO Rep* 2003, **4**:296-300.
Orthologous genes from *M. tuberculosis* and *S. coelicolor* can complement *E. coli* auxotrophes for HisA and TrpF, thus indicating that their gene products are bifunctional, and suggesting the presence of an ancient-like HisA/TrpF isomerase in *M. tuberculosis* and *S. coelicolor.*

46. Juergens C, Strom A, Wegener D, Hettwer S, Wilmanns M, Sterner R: **Directed evolution of a $(\beta\alpha)_8$-barrel enzyme to catalyze related reactions in two different metabolic pathways**. *Proc Natl Acad Sci USA* 2000, **97**:9925-9930.

47. Schmidt DM, Mundorff EC, Dojka M, Bermudez E, Ness JE,
• Govindarajan S, Babitt PC, Minshull J, Gerlt JA: **Evolutionary potential of $(\beta/\alpha)_8$-barrels: functional promiscuity produced by single substitutions in the enolase superfamily**. *Biochemistry* 2003, **42**:8387-8393.
Using an *o*-succinylbenzoate synthase auxotroph *E. coli* strain, directed evolution experiments were carried out using the genes of the related L-Ala-D/L-Glu epimerase and muconate lactonizing enzyme II, resulting in $(\beta\alpha)_8$-barrel enzyme versions with relaxed substrate specificities.

48. Cheon YH, Kim HS, Han KH, Abendroth J, Niefind K, Schomburg D, Wang J, Kim Y: **Crystal structure of D-hydantoinase from *Bacillus stearothermophilus*: insight into the stereochemistry of enantioselectivity**. *Biochemistry* 2002, **41**:9410-9417.

49. Wada M, Hsu CC, Franke D, Mitchell M, Heine A, Wilson I, Wong CH: **Directed evolution of *N*-acetylneuraminic acid aldolase to catalyze enantiomeric aldol reactions**. *Bioorg Med Chem* 2003, **11**:2091-2098.

50. Ireton GC, Black ME, Stoddard BL: **The 1.14 Å crystal structure of yeast cytosine deaminase: evolution of nucleotide salvage enzymes and implications for genetic chemotherapy**. *Structure* 2003, **11**:961-972.

51. von Delft F, Inoue T, Saldanha SA, Ottenhof HH, Schmitzberger F, Birch LM, Dhanaraj V, Witty M, Smith AG, Blundell TL, Abell C: **Structure of *E. coli* ketopantoate hydroxymethyl transferase complexed with ketopantoate and Mg^{2+}, solved by locating 160 selenomethionine sites**. *Structure* 2003, **11**:985-996.