The TIM barrel — the most frequently occurring folding motif in proteins

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The TIM-barrel fold has now been found in 19 enzymes with different catalytic functions and different lengths of polypeptide chains. The domain organization of these chains are compared and discussed. For several of these chains, X-ray structures have recently been refined and active-site residues have been identified by studies of inhibitor complexes. Evolutionary implications of these results, including the first examples of evolutionarily related enzymes that catalyze different chemical reactions, are discussed.

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Introduction

For most α/β proteins, the active site is located at the carboxyl termini of the parallel β-strands [1]. Catalytic and substrate-binding residues frequently occur in loop regions that connect the carboxyl termini of the β-strands with the amino termini of the adjacent α-helix [2]. This is the case for both classes of α/β proteins: the open twisted-sheet structures, and the closed barrel structures. The open sheet structures exhibit considerable variability in the number of β-strands and in their topology whereas almost all α/β barrel structures have eight consecutive parallel β-strands surrounded by eight α-helices (Fig. 1).

The TIM barrel was first observed in chicken triosephosphate isomerase, hence its name [3]. It has since been found in 18 additional enzymes with different catalytic functions [4,5,6•,7,8,9••, 10–13,14••,15,16,17••–19••,20, 21]. In some of these enzymes, such as triosephosphate isomerase, the complete polypeptide chain (~250 residues) forms the barrel domain; in other enzymes with longer polypeptide chains, additional domains are present, usually with quite different structures.

Most of these polypeptide chains share no significant sequence homology, although the three-dimensional structures of their α/β-barrel domains are surprisingly similar. When the structures of two such domains are compared, it is usually found that ~150 C_α atoms superimpose within a root mean square value of 2.5 Å. The geometrical designs of these α/β-barrels have been analyzed [22], and the rules for packing side chains into the interior core of the barrel have been deduced [23].

During the past year, several new α/β barrel structures have appeared [17••–19••] and many of the earlier reported preliminary structures have been refined to high resolution [6•,9••,13,14••,21,24••,25•,26••]. Active-site residues have also been located by X-ray studies of inhibitor complexes [14••,24••,27••,28•–32•,33••]. In this review, I will mainly compare these structures and, in addition, discuss some evolutionary implications of these novel results. Short reviews on α/β-barrel structures have appeared previously [34–36].

Domain organization of α/β -barrel proteins

About one third of the known α/β-barrel proteins have their polypeptide chain arranged in a single barrel domain. They are triosephosphate isomerase [3], xylose isomerase [4], 2-keto-3-deoxy-6-phosphogluconate aldolase [5], fructose 1,6-bisphosphate aldolase [6•], glycolate oxidase [10], the α-subunit of tryptophan synthase [15] and adenosine deaminase [17••]. The length of the polypeptide chain of these proteins varies from 225 residues in phosphogluconate aldolase to about 400 in xylose isomerase. This variation results from excursions of the polypeptide chain both at the termini of the chain and in the loop regions. Long loop regions predominantly occur at the carboxyl termini of the β-strands, in other words within the β-loop-α units.

The framework region of ~150 residues that has similar structure in all α/β barrels comprises the eight β-strands and part of each of the eight α-helices of the α/β-barrels. The loop regions fall into two structurally distinct groups with different functions. The loops at the carboxyl termini of the β-strands, where the excursions occur, are structurally quite different and are functionally important, as they form most of the active site in all α/β-barrel enzymes. In contrast, the loops at the other end of the barrel, within the α-loop-β units, frequently belong to one of a few distinct structural loop motifs with conserved structure [37••,38]. These loops preserve specific hydrophobic interactions between the α-helix and β-strand of the corresponding α-loop-β unit.

The structural importance of the loop regions in the α-loop-β units has been corroborated by studies on a

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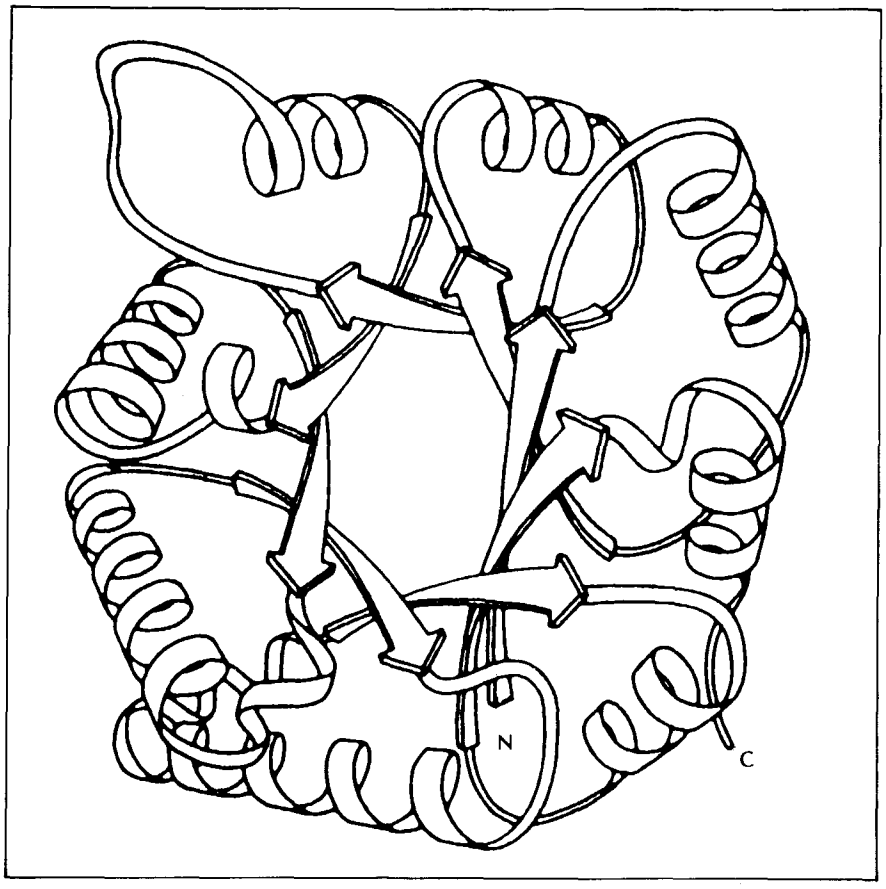

Fig. 1. Schematic drawing of the TIM-barrel motif of eight parallel β-strands surrounded by eight α-helices. N, amino terminus; C, carboxyl terminus. Adapted from [50].

recombinant $(\alpha\beta)_9$ barrel, which contains a tandemly duplicated αβ unit within the polypeptide chain. This chain folds as a regular $(\alpha\beta)_8$ barrel with the additional αβ unit forming an excursion within a loop at the carboxyl terminus of the β-strands and not within an α-loop-β unit (R Urfer and K Kirschner, personal communication).

Several of the known barrel enzymes have an extra amino-terminal domain. In yeast flavocytochrome b_2, where the barrel domain has sequence homology to spinach glycolate oxidase [39••], this extra domain is a cytochrome which in turn is homologous to microsomal cytochrome b_5 [11]. Clearly, these similarities reflect domain shuffling events during evolution. Other barrel enzymes with an extra amino-terminal domain include ribulose 1,5-bisphosphate carboxylase [13,14••], muconate lactonizing enzyme [20] and the homologous enzyme mandelate racemase [19••], enolase [21] and cellobiohydrolase II [18••]. Some members of this group contain α/β barrels in which the regular framework structure of the $(\alpha/\beta)_8$ barrel is modified or distorted.

In the case of muconate lactonizing enzyme and mandelate racemase, the barrel contains only seven α-helices; the polypeptide chain forms a hairpin β-structure after β-strand 8 instead of forming α-helix 8 of the barrel. The amino-terminal domain of these enzymes is built up from an antiparallel β-meander followed by three α-helices. A similar amino-terminal domain is present in enolase which has a more distorted α/β barrel structure. The first two β-strands in the enolase barrel form an antiparallel hairpin which is followed by two antiparallel α-helices before the chain enters β-strand 3 of the barrel. Thus, instead of a regular TIM-barrel structure, the enolase barrel folds into a $\beta\beta\dot{\alpha}\dot{\alpha}(\beta\alpha)_6$ structure. The barrel in cellobiohydrolase II is even more distorted with one β-strand missing altogether. The absence of this β-strand creates a crevice at the active site which, in combination with two loop regions, forms a tunnel that encloses the substrate. The structure of the amino-terminal domain, which is linked to the barrel domain by a flexible glycosylated region, has been determined separately by two-dimensional NMR [40].

In one of the barrel enzymes with two domains, phosphoribosyl anthranilate isomerase:indole-glycerol phosphate synthase from *Escherichia coli* [16], both domains are α/β barrels which catalyze different chemical reactions. In a different bacterium, *Bacillus subtilis*, these two enzymatic functions reside on two separate polypeptide chains which are homologous to the corresponding regions of the *E. coli* enzyme. This system illustrates that, in many cases, domain organization in a polypeptide chain reflects different ways of organizing the genome rather than functional demands on the protein structure. This argument is corroborated by a recent structure determination of a recombinant *E. coli* indoleglycerol phosphate synthase domain [41]. The domain forms trimers with interaction areas between the monomers that involve the same residues that form the interactions between the synthase and isomerase domains in the bifunctional protein chain.

Cyclodextrin glycosyltransferase has the longest polypeptide chain of these TIM barrel enzymes, with 684 amino acid residues that are arranged in five domains [9••]. The α/β barrel domain, domain A, is interrupted after β-strand 3 by an excursion of ~70 residues that form domain B. Domain C is an eight-stranded antiparallel β-barrel formed by ~90 residues. The topology of this β-barrel is identical to that of a γ-crystallin domain [42], with two adjacent Greek-key motifs [1], but the actual structure is different. Domain C is followed by two additional domains of the antiparallel β-type, the first of which has the immunoglobulin fold.

The structure of amylase [8] is very similar to that of domains A, B and C of cyclodextrin glycosyltransferase, which is reflected in weak sequence identity between these enzymes. Pyruvate kinase [7] and methylamine dehydrogenase [12] also have two domains in addition to the α/β barrel domain. These additional domains have, however, totally different structures from those of amylase. In methylamine dehydrogenase, both extra domains are carboxy-terminal to the α/β-barrel domain and their structures are of the α/β open-sheet type and both are similar to the FAD- and NADPH-binding domains of the glutathione reductase superfamily [43]. The second domain of pyruvate kinase is inserted after β-strand 3 of the α/β barrel, as occurs for amylase, but the structure of this domain is quite different. The carboxy-terminal domain in pyruvate kinase is of the α/β open-sheet type with yet another fold.

Evolutionary aspects of α/β-barrel proteins

The α/β-barrel structure provides one of the most clear cut examples of the distinction within a protein structure between those amino acids that provide a stable framework and those that participate in function. One would

Table 1. Amino acid residues involved in metal binding, catalytic function and phosphate binding in α/β barrel enzymes.

	Metal-binding site			Phosphate-binding site	
Enzyme	I	II	Catalytic function	I	II
Xylose isomerase	E(5), E(6), D(7), D(8)	H(6), E(6), D(7)	K(5), H(2), D(2), H(6)	–	–
Ribulose-bisphosphate carboxylase	D(2), E(2), K*(2)		K(6), S(7)	R(5), H(5), H(6)	NH(7), K(1), NH(helix 8′)
Adenosine deaminase	H(1), H(5), D(8)		E(5), H(6), D(8)	–	–
Enolase	D(3), E(4), D(5)		E(2), H(7), K(8)	K(6), R(7)	–
Pyruvate kinase	E(5), C = O(6)	K(5), R(6)	K(3), R(2)	R(6)	
Triosephosphate isomerase			H(4), E(6)	–	NH(7), K(1), NH(helix 8′), NH(6)
Fructose-bisphosphate aldolase				K(4), R(4)	K(1), R(1), R(8)
Glycolate oxidase			Y(3), K(5), H(6)	–	NH(7), R(7), NH(helix 8′), R(8)
Tryptophan synthase			E(2), D(2)	–	NH(7), S(8), NH(helix 8′)
Phosphoribosyl-anthranilate isomerase				–	NH(7), NH(helix 8′)
Indole-3-glycerol phosphate synthase				–	NH(7), K(1), NH(helix 8′)
Amylase			D(4), E(5), D(7)	–	
Cellobiohydrolase II			D(2), D(3)	–	

The amino acid type (using the one-letter code) and the loop position (in parentheses) are given for each residue. Thus, K(5) refers to a lysine residue in the loop region that connects β-strand 5 to α-helix 5 in the α/β barrel. Helix 8′ is a short helix within loop 8 which is frequently involved in phosphate binding. K*(2) for ribulose-bisphasphate carboxylase denotes a carbamylated lysine residue in loop 2.

expect that modification of residues within the loop regions of the β-loop-α units could change the enzymatic function without affecting the stability of the fold. It is therefore not surprising that one finds in nature pairs of enzymes with α/β-barrel structures that exhibit clear sequence identity (and therefore are evolutionarily related) but catalyze different chemical reactions. Such pairs are rarely found but are very interesting because they represent one route by which evolution can generate new function.

Mandelate racemase and muconate lactonizing enzyme constitute one such pair [19••]. Mandelate racemase catalyzes the interconversion of two enantiomers, whereas muconate lactonizing enzyme catalyzes a cycloisomerization by addition to a double bond. The two enzymes are nevertheless evolutionarily related, with a sequence identity of ~30%. Glycolate oxidase, flavocytochrome b_2 and mandelate dehydrogenase [39••,44••] constitute another set of evolution-related enzymes that perform different biological functions. In this case, a few mutations in the active site have fine tuned these enzymes to operate on different substrates.

Is there any evolutionary relationship between those α/β barrels that show no sequence homology? Sixteen such α/β barrel structures are now known; this is a fairly large proportion of the ~200 unique protein structures that are known. No evolutionary relationship among these 16 barrels would imply that this fold has occurred independently in almost 10% of all proteins and, as far as we know, with the active site positioned at the same place every time. Such a history has an extremely low probability. It is therefore not surprising that many attempts have been made to find evidence for evolutionary relations among these proteins. On balance, it is fair to say that none of these attempts have convincingly demonstrated any such relationships.

Two different evolutionary histories have been explored. One of these is based on a proposal by Gilbert [45] that functional proteins evolved by different combinations of exons that coded for small functional or structural units. A comparison of the intron–exon arrangements in triosephosphate isomerase from different species provided some evidence that exons are structural modules or building blocks for the α/β-barrel structure [46]. An analysis of the intron arrangement in several genes for different α/β proteins favored α-loop-β units as the building blocks [47]. Evolution of α/β proteins from α-loop-β building blocks also explains why the active site always occurs at the carboxyl termini of the β-strands. The recent observations that α-loop-β units form specific structural motifs but β-loop-α units do not, is consistent with such an evolutionary history [37••].

The second evolutionary history assumes the existence of one or a very limited number of primordial barrel structures from which all present day barrels have evolved by divergent evolution. This assumption explains the conservation of the position of the active site in these barrels. By examining side-chain packing inside the α/β barrel, Lesk *et al.* [23] identified two classes of packing arrangements and suggested that these two classes might have evolved from separate precursor proteins. However, this argument is not convincing in the light of recent experiments which have shown that cyclically permuted α/β barrels fold properly [48].

Farber and Petsko [36] have arranged the known α/β-barrel proteins into four distinct families essentially on the basis of different geometries of the barrels and different arrangements of the loop excursions and additional domains. Some functional relationship exists among members of each individual family as a result but, from a functional point of view, these enzymes can be arranged in several different and equally valid ways.

Wilmanns *et al.* [49] have suggested an evolutionary relationship for the three α/β barrels involved in three successive steps of the tryptophan biosynthetic pathway: phosphoribosyl anthranilate isomerase, indoleglycerol phosphate synthase and the α-subunit of tryptophan synthase. This suggestion is based on the presence of a common phosphate-binding site and on sequence similarity resulting from structural alignment. These authors further suggest that several other α/β barrels that also contain the same phosphate-binding site might belong to the same evolutionarily related family.

In Table 1 I have compiled published information on which residues are involved in the different functions of TIM barrels in order to examine if there is a consistent pattern which might reveal evolutionary relationships. Most of this information was extracted from recent studies of inhibitor complexes [14••,24••,27••,28•–32•, 33••]. Five of these enzymes require metal ions for activity. These metal ions all bind in the active site in quite similar positions relative to the barrel structure, close to the barrel axis. They are all bound to protein side chains from loop regions in the β-loop-α units. If these metal-binding sites have evolved from a common metal-binding precursor, one would expect that the same loop regions are still involved in metal binding, at least to some extent. This is not the case — there is an almost random distribution of loop regions that provide ligands to the metal atoms. Therefore, involvement of metals in the catalytic action of these enzymes must have occurred through convergent evolution.

Similar diversity is found among the residues involved in the catalytic function and in phosphate binding, with the exception of the common phosphate-binding site that was discussed above. This site is formed by main-chain NH groups from loop 7 and from a small helix, helix 8′, in loop 8. This is the only common detailed feature in the active sites of these α/β barrel enzymes. However, the probability that convergent evolution has produced this phosphate-binding site using the same loop regions and no other loops in at least six different α/β barrels is very low. The presence of this common phosphate-binding site is the strongest evidence obtained so far for some kind of divergent evolutionary history for these α/β-barrel enzymes.

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