The evolution of α/β barrel enzymes

Gregory K. Farber and Gregory A. Petsko

Of the enzymes whose structure is known, roughly one out of ten has an eight-stranded α/β barrel domain. Structural and chemical evidence suggests that all of these domains may have diverged from a common ancestor.

AS MORE PROTEIN CRYSTAL structures are solved, it is becoming obvious that the same folding motifs have been used over and over again for a variety of functions. This sense of *déjà vu* is overwhelming in enzyme structures. In this article, we propose to examine a large family of enzymes – those with eight-stranded α/β barrels. The question that we want to answer concerns the historical relationship of these enzymes. Is this group of enzymes related by divergent evolution from a single common ancestor, by convergent evolution to a stable fold, or by divergent evolution from several ancestors that possessed similar α/β barrel folds? The surprising conclusion of this study is that all 17 α/β barrel enzymes may have diverged from a common ancestor.

The archetypical α/β barrel protein, triose phosphate isomerase (TIM)[1] is illustrated in Fig. 1. The α/β enzymes have a domain with eight parallel β-strands surrounded by seven or eight α-helices. Each inner β-strand is connected to an outer α-helix, so to a first approximation the enzyme can be considered to be an $(\alpha/\beta)_8$ structure. Although this structural motif is called a barrel, the cross section of the barrel is usually elliptical rather than circular. In addition, enzymes with an α/β barrel domain often have other structures (α-helices, β-strands or entire domains) that precede, interrupt, or follow the barrel. The accompanying centerfold includes a list of abbreviations used for the 17 enzymes currently known to have this shape together with details about the structure of and the reactions catalysed by each enzyme (see Table I).

The first question that must be answered when constructing a family tree for α/β barrel enzymes concerns the number of times that this structural motif arose. For some families of proteins, evidence of a single common ancestor is easy to find. A classic example of divergent evolution seems to be the nucleotide binding domain (the so-called Rossmann fold). For this family, the same fold (a βαβ structure) performs the same function (nucleotide binding)[20]. In addition to similarities in topology and function, these domains also have some sequence similarity[20].

In contrast, subtilisin and chymotrypsin are a good example of convergent evolution to a similar active site and a similar enzymatic mechanism. These enzymes have very different overall folds and have no sequence similarity, yet they both catalyse the hydrolysis of peptide bonds. The active sites of both enzymes use a catalytic triad of His, Ser and Asp, but these residues come from completely different regions of the polypeptide chain in the two enzymes[21,22].

α/β barrels

The α/β barrel enzymes present a case different from either of the examples above. These enzymes all have one domain with approximately the same structural motif, but they have very different functions (see Table I). At first sight, it is hard to make any functional arguments in favor of divergent evolution from this data: the substrates for these enzymes have no similarities in size or functionality, and a wide range of cofactors is required.

Despite the apparent lack of functional similarity, there are three reasons to believe that these enzymes could have arisen from a common ancestor. The first reason concerns the large number of α/β barrels and the fact that they are all enzymes. There are ~350 protein structures known today. About half of these structures are enzymes. Therefore, the 17 α/β barrels account for 10% of all known enzymes. If these proteins are only related by convergent evolution to a stable fold, why are they all enzymes? One might have expected that at least one of them would only be a simple binding protein, yet all of them have catalytic function.

The second argument in favor of divergent evolution is the location of the active sites of these enzymes. Despite the chemical diversity of the reactions they catalyse, the active site is always found at the C-terminal end of

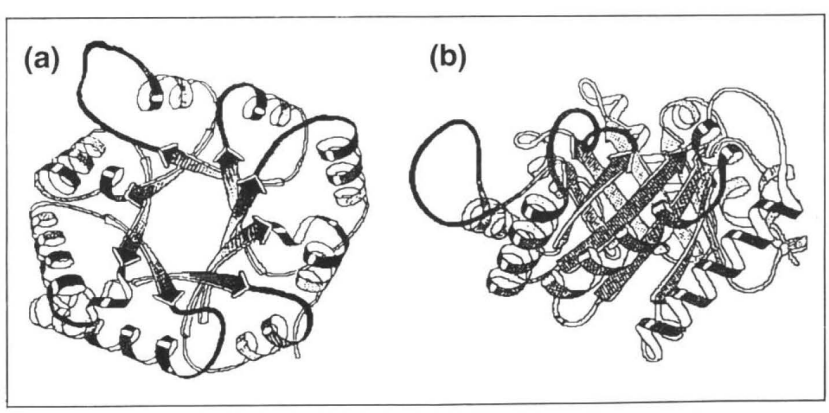


Figure 1
An α/β barrel enzyme. Triose phosphate isomerase (TIM), the first α/β barrel, is used to illustrate the general shape of these enzymes. (a) Viewed looking down the barrel axis. The C-terminal end of the barrel is closest to the reader. (b) Viewed perpendicular to the barrel axis. The C-terminal end of the barrel is toward the top of the page. These figures were modified, with permission, from Ref. 2.

G. K. Farber is at the Department of Chemistry, Pennsylvania State University, 152 Davey Laboratory, University Park, PA 16802, USA. **G. A. Petsko** is at Brandeis University, Rosensteil Center, 415 South Street, Waltham, MA 02254, USA.

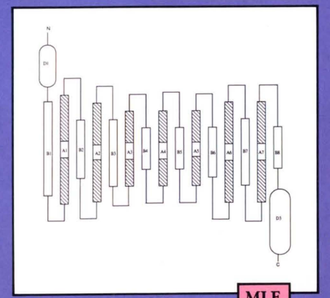
© 1990, Elsevier Science Publishers Ltd, (UK) 0376–5067/90/$02.00

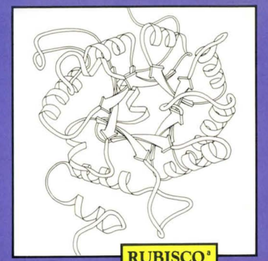
Petitioner Merck, Ex. 1142, p. 228
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00009

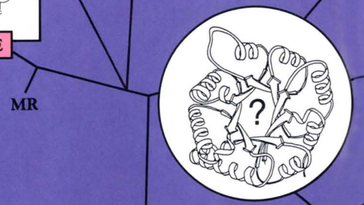
ELSEVIER TRENDS JOURNALS
June 1990
TIBS

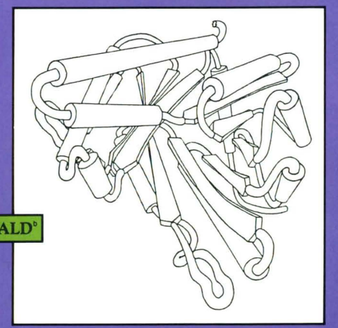
The evolution of α/β barrel enzymes

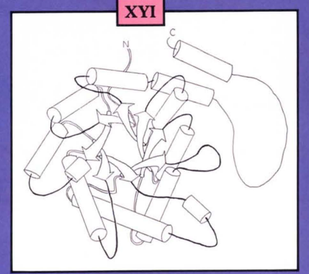
Gregory K. Farber and
Gregory A. Petsko











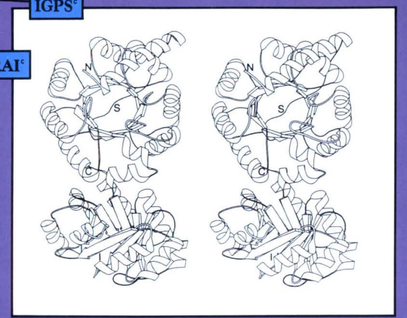


Table II. Sequence alignment of MR and MLE[a]

```
MR    MSEVLITGRL TRAV--NVPL AYPVHT-AVG TVGTAPLVLI DLATSAGV--   50
      *  ****            *  * *  *   *  *****     * **
MLE   MTSVLIE-RI EAIIVHDLPT IRPPHKLAMH TMQTQTLVLI RVRCSDGVEG

MR    VGHSYL---F AYTPVALKSL KQLLD-DMAA MIVNEPLAPV SLEAMLAKRF  100
      *     **   *  *   *   *  *  *    *   ***
MLE   IGEATTIGGL AYGYESPEGI KANIDAHLAP ALVGLPADNI N-AAMLK---

MR    CLAGYTGLIR MAAAGIDMAA WDALGKVHET PLVKLLGANA RPVQAYDSHS  150
      *    *     *  **  **  ** **     *   ****  *
MLE   -LDKLAKGNT FAKSGIESAL LDAQGKRLGL PVSELLGGRV RDSLEVAW-T

MR    LDHVKLATER AVTAAELGFR ---AVKTKIG TPALDQDLAV VRSIRQAVGD  200
      *    *     *  *   *   *   ****   *  ***   *  *    **
MLE   LASGDTARDI AEAQHMLEIR RHRVFKLKIG ANPLAQDLKN VVAIKRELGD

MR    DFGIMVDYNQ SLDVPAAIKR SQALQQEGVT WIEEPTLQHD YEGHQRIQSK  250
      *  **  **   *  **    *  *  *    *  **  *     *  *
MLE   SASVRVDVNQ YWDESQAIRA CQVLGDNGID LIEQPISRIN RSGQVRLMQR

MR    LNVPVQMGEN WLGPEEMFKA LSIGACRLAM PDAMKIGGVT GWIRASALAQ  300
      *    *     *  *    **   *    *   *   **    *   *  *
MLE   SPAPIMADES IESVEDAFSL AADGAASIFA LKIAKNGGPR AVLRTAQIAE

MR    QPGIPM--SS NLPQEI---- SAHLLAATPT ANW----LER LDLAGSVIEP  350
      *    **   *  *   ***            *    *    * *
MLE   AAGIALYGGT MLESSIGTLA SAHAFLTLRQ LTWGTELFGP LLLTEEIVDE

MR    TLTFEGGNAV IPDLPGVGII WREKEIGKYL V-                     382
                    **  **  *
MLE   PPQYRDFQLH IPRTPGLGLT LDEQRLARFA RR
```

[a] The alignment of MR[25] and MLE[26] was made using the program IALIGN at the Whitaker College Computational Facility at MIT. A mutation data scoring matrix was used, and gaps were assigned a penalty of 6. Total score=2479, 13 breaks 102 identities (28%). For 25 random runs: Mean = 2212.72. Standard deviation = 18.29. Alignment score = 14.56 SD.

Table I. Characteristics of 17 α/β barrel enzymes

Abbrev.	Enzyme name	E.C. number	Refs	Structural characteristics of α/β barrels[a]					Reactions catalysed		
				No. of domains	Location of domains	Other Secondary structure[b]	Secondary structure in loops	Major axis	Cofactor(s)	Substrates	Products
FCB2	flavocytochrome b_2	1.1.2.3	3	2	D1-β1	–	β4-H-H-α4 β6-H-α6 α6-H-β7 β8-H-α8	circular	FMN, heme	lactate	pyruvate
GO	glycolate oxidase	1.1.3.15	4	3	D1-β1 β4-D3-α4	–	β1-H-α1 β8-H-α8	circular	FMN	glycolate+O_2	glyoxylate+H_2O_2
TRI	trimethylamine dehydrogenase	1.5.99.7	5	3	α8-D2-D3	α0	β4-H-α4 β6-H-α6 β8-H-α8	β3	FMN, 4 Fe–4S	trimethylamine+H_2O	diethylamine+ formaldehyde
RUBISCO	ribulose-1,5-bisphosphate carboxylase/oxygenase	4.1.1.39	6,7	3	D1-β1 α8-D3	α0	β5-H-α5 α6-B-B-β7 α8-H	circular	Mg^{2+}	(a)CO_2+ribulose bisphosphate (b)O_2+ribulose bisphosphate	3-phospho-D-glycerate 3-phospho-D-glycerate + phosphoglycolate
MR	mandelate racemase	5.1.2.2	c	3	D1-β1 β8-D3	no α8	β6-H-α6	β1	Mg^{2+}	(*S*)-mandelate	(*R*)-mandelate
MLE	muconate cycloisomerase	5.5.1.1	8	3	D1-β1 β8-D3	no α8	–	β1	Mn^{2+}	5-oxo-2,5-dihydrofuran 2-acetate	*cis,cis*-hexadienedioate
AMYL	α-amylase	3.2.1.1	9,10	3	β3-D2-α3 α8-D3	–	β6-H-α6	β2	Ca^{2+}	amylose	glucose+amylose
CGT	cyclodextrin glycosyltransferase	2.4.1.19	11	5	β3-D2-α3 α8-D3-D4-D5	–	β1-H-α1 α1-H-β2 β6-B-β4 β6-H-a6	β2	Ca^{2+}	(a) 1,4α-D-glucopyranose polymer (G_n) (b) G_n + G_m	G_{m+x} + cyclodextrin (cG_x) (usually x=6,7 or 8) G_{n-y} + G_{m+y}
PK	pyruvate kinase	2.7.1.40	12	3	β3-D2-α3 β8-D3	–	β5-H-α5 β6-H-α6	β1	Mg^{2+},K^+	ATP+pyruvate	ADP+phosphoenolpyruvate
ENOL	enolase[d]	4.2.1.11	13	2	D1-β1	–	–	β1	Mg^{2+}	2-phospho-D-glycerate	phosphoenolpyruvate+H_2O
XYI	xylose isomerase	5.3.1.5	14	2	α8-D2	–	β2-H-α2 β4-H-α4 β6-H-α6	β1	Mg^{2+}	D-xylose	D-xylulose
PRAI	*N*-(5′-phosphoribosyl) anthranilate isomerase	5.3.1.16	15	1	none	–	β8-H-α8	β3	none	*N*-(5′-phosphoribosyl) anthranilate	1-(2-carboxyphenylamino)-1-deoxy-D-ribulose-5-phosphate
IGPS	indole-3-glycerol-phosphate synthase	4.1.1.48	15	1	none	α0	β8-H-α8	β3	none	1-(2-carboxyphenyl amino)-1-deoxy-D-ribulose-5-phosphate	C1-(3-indolyl)glycerol 3-phospate+CO_2+H_2O
TS	tryptophan synthase (α-subunit)	4.2.1.20	16	1	none	α0	β2-H-α2 β3-H-α3 β8-H-α8	β3	none	C1-(3-indolyl)glycerol 3-phosphate	indole+D-glyceraldehyde 3-phosphate
TIM	triose phosphate isomerase	5.3.1.1	1	1	none	–	β4-H-α4 β5-H-α5 α6-H-β6 β8-H-α8	β3	none	dihydroxyacetone phosphate	D-glyceraldehyde 3-phosphate
FALD	fructose bisphosphate aldolase	4.1.2.13	17	1	none	α0	β1-H-α1 β2-H-α2 β8-H-α8	β2	none	fructose1,6-bis-phosphate	D-glyceraldehyde 3-phosphate+ dihydroxyacetone phosphate
KDPG	2-keto-3-deoxy-6-phosphogluconate aldolase	4.1.2.21	18	1	none	α0	–	–	none	2-dehydro-3-deoxy-D-galactonate 6-phosphate	pyruvate+ D-glyceraldehyde 3-phosphate

[a] β4-H-α4 means that there is a small helix between β-strand 4 and α-helix 4. D stands for domain and B for β-strand.
[b] α0 means that the α/β barrel domain has an extra helix preceding the first β-strand.
[c] D. J. Neidhart, G. L. Kenyon, J. A. Gerlt and G. A. Petsko, unpublished.
[d] Enolase has been included in this family of α/β barrels despite a recent revision of the chain connectivity. This second version of enolase is a ββαα$(\alpha\beta)_6$ barrel rather than a $(\alpha\beta)_8$ barrel like the other 16 enzymes in this table[19].

References

[a] **RUBISCO** Drawing by Jane Richardson, Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, USA
[b] **FALD** Sygnsch, J., Beaudry, D. and Allaire. M. (1987) *Proc. Natl Acad. Sci. USA* 84, 7846–7850
[c] **IGPS, PRAI** Priestle, J. P., Grütter, M. G., White, J. L., Vincent, M. G., Kania, M., Wilson, E., Jardetzky, T. S., Kirschner, K. and Jansonius, J. N. (1987) *Proc. Natl Acad. Sci. USA* 84, 5690–5694

Petitioner Merck, Ex. 1142, p. 229
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00009

the β-barrel (see Fig. 1). In most cases, the active site residues are located in the loops which connect a β-strand with the following α-helix. In a few cases (e.g. xylose isomerase [XYI] and fructose bisphosphate aldolase [FALD]) these residues are deeper in the interior of the barrel, but the active site is always in the C-terminal half of the barrel domain. Why should this occur? After all, since these enzymes catalyse a wide range of reactions there is no functional reason for the active site to be at this end of the molecule. The probability of this happening by chance is 1 in 1072 ($1:2^{17}$). This very unlikely occurrence can be explained if we assume that a common ancestral α/β barrel enzyme had its active site at the C-terminal end of its barrel. Although the descendants of this molecule have diverse functions, they all continue to use the same location for catalysis.

The third and most convincing argument for divergent evolution comes from a more detailed study of the crystal structures of the α/β barrel enzymes. If these enzymes have diverged from one or more common ancestors, some structural patterns should be present. These patterns, if present, should enable us to construct a family tree for the α/β barrel enzymes. Families proposed on the basis of a structural analysis will be most convincing if, in addition to structural similarities, they contain common functional features or sequence homology.

To look for these familial relationships, several different structural features of the enzymes were examined: the length of helices and sheets that make up the barrel, the number and location of extra domains, the location of extra helices or strands of β-sheet, and the location of the long axis of the ellipse of the barrel. Some of these features have been summarized in Table I. Cartoons showing these features were made for all 17 enzymes. The cartoon for MLE is shown in the centerfold as an example. (Plain rectangles represent β-sheets, striped rectangles represent α-helices and rectangle lengths are proportional to the number of amino acids.)

Only fairly rough structural criteria were chosen for two reasons. The first is simply the current state of these crystal structures. Few of these structures have been refined at high resolution. Therefore, it seems useless to make very detailed comparisons. In addition, many of these structures have not yet been deposited in the Protein Data Bank. Therefore, in some cases, the only way to view the structure was by looking at stereo pictures in journals. The second reason for using these criteria is that we are trying to look for similarities in enzymes that are only distantly related. If there are structural families they will probably be most apparent using these 'low resolution' criteria.

Structural families

Based on these criteria, it is possible to organize the 17 α/β barrel enzymes into four different structural families (see the 'family' tree in the centerfold. The lengths of the branches in this tree represent structural similarity). The first of these families is composed of flavocytochrome b_2 (FCB2), glycolate oxidase (GO), trimethylamine dehydrogenase (TRI) and ribulose-1,5-bisphosphate carboxylase/oxygenase (RUBISCO). Both FCB2 and GO have barrels that are nearly circular, and they both have an additional domain which blocks the N-terminus of the β-barrel. These enzymes are clearly very closely related[3]. TRI is related to these two enzymes, but not as closely. Instead of an entire domain covering the N-terminus of the barrel, TRI only has a single α-helix. In addition, the cross section of the TRI barrel is elliptical with the major axis near β-strand three. These three enzymes all have a small helix between β-strand eight and α-helix eight. This helix points toward the phosphate group of the FMN cofactor in all three enzymes. RUBISCO is assigned to this family based on its circular cross section and on its similarities to TRI. Both of these enzymes have an α-helix covering the N-terminus of the barrel. In addition, the long C-terminal excursion after the last α-helix of RUBISCO is similar to the structure between the last helix and the second domain of TRI.

The second structural family is composed of α-amylase (AMYL), cyclodextrin glycosyltransferase (CGT), enolase (ENOL), mandelate racemase (MR), muconate cycloisomerase (MLE), pyruvate kinase (PK) and XYI. The major axis of the barrel for all of these enzymes is near β-strand one. Within this large family, several subfamilies can be distinguished. One subfamily contains MLE and MR. Both of these enzymes are missing the final α-helix of the barrel. The space that would have been occupied by this helix is taken by the two other domains. In addition to substituting for the missing helix, these domains partially cover the C-terminal end of the β-barrel. CGT, AMYL and PK are the members of a second subfamily. These enzymes are composed of three or more domains. In this subfamily, a domain that is inserted between β-strand three and α-helix three covers part of the C-terminal end of the barrel. In addition, these enzymes all possess a domain after α-helix eight which blocks the N-terminus of the barrel. In either of its chain tracings, ENOL falls somewhere between these two subfamilies. Its one extra domain does cover the C-terminal end of the barrel. The final member of this family, XYI, appears to be only distantly related to the other six enzymes.

The third family is composed of *N*-(5'-phosphoribosyl)anthranilate isomerase (PRAI), indole-3-glycerol-phosphate synthase (IGPS), tryptophan synthase (TS) and TIM. These enzymes are all composed of a single domain. Their barrels all have the major axis near β-strand three. In addition, these enzymes all have a small helix between β-strand eight and α-helix eight. In TIM, this helix points toward the phosphate group of the substrate. A similar role has been postulated for this helix in TS[16].

The fourth family consists of the two single domain enzymes, FALD and 2-keto-3-deoxy-6-phosphogluconate aldolase (KDPG), that have an α-helix preceding the α/β barrel. As in TRI and Rubisco, these helices block the N-terminal end of the barrel. There are only two members of this final family, and it is possible that the aldolases are distant relatives of one of the other structural families.

The surprising feature of these structural families is that they make chemical and metabolic sense. All of the α/β barrel enzymes that require FMN (FCB2, GO, TRI) are in the same family. With the exception of Rubisco, all of the enzymes that require a divalent metal ion (AMYL, CGT, ENOL, MR, MLE, PK, XYI) also end up in the same family. It has been suggested that metabolic pathways can evolve by gene duplication followed by specialization[23]. The structural families presented here support this view. Three of the four final steps in tryptophan biosynthesis (PRAI, IGPS, TS) are catalysed by α/β barrels from the same family. ENOL and PK, which are consecutive enzymes in glycolysis, are also in the same family. The possible exceptions to this 'rule' are FALD and TIM, which are not currently

Petitioner Merck, Ex. 1142, p. 233
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00009

in the same family although TIM follows FALD in glycolysis. However, since the evidence for the aldolases as a separate family is weak, it is possible that this pair is not an exception.

The case for divergent evolution from a common ancestor would be strengthened by sequence homology within the proposed structural families. Although work comparing all of these sequences is not yet complete, three clear examples of sequence homology have been found. FCB2 and GO show considerable sequence homology[24]. MR and MLE show extensive sequence identity (Table II)[25,26]. The crystal structures of MR and MLE are also so similar that it seems likely that they have diverged from each other recently. The final pair of structures that have sequence homology are AMYL and CGT[27]. All of these pairs of enzymes belong to the same structural family. No convincing examples of sequence homology have yet been found between enzymes that are not in the same family.

Early evolution

We have made a case to support no more than four families of α/β barrels so far. Is it possible to distinguish between four primordial α/β barrel enzymes and a single ancestral enzyme? One might still argue that the fact that the active sites are all on the C-terminal end of the barrel suggests a common ancestor. However, the odds of this happening by chance have been reduced to only 1 in 8 ($1{:}2^4$). Multiple ancestors, while unlikely, cannot be ruled out on this basis alone.

In addition, it would be nice to have a mechanism to account for the dramatic structural changes between the four families. Recent work from Kirschner's laboratory has provided a possible solution to both of these problems[28]. Using PRAI, they showed that it was possible to make cyclic permutations of the gene and end up with a properly folded, functional enzyme. Such cyclic permutations can easily account for the fact that different families have the major axis of the ellipse at different β-strands. This work also suggests one mechanism by which a single ancestral enzyme could have diverged into the four families of enzymes described above.

Conclusion

Using crystal structure data, α/β barrel enzymes can be classified into no more than four different families. Evidence has been presented that all of these enzymes might have diverged from a common ancestor. These families are also useful as a guide for further experiments in understanding how enzymes within a family work. For example, without considering the structural families, the only obvious α/β barrel enzyme to use as a guide for understanding xylose isomerase is triose phosphate isomerase, since both TIM and XYI catalyse aldose/ketose isomerizations. However, the work presented here shows that TIM and XYI do not belong to the same structural family. Consideration of these two enzymes in the light of this analysis suggests that comparing XYI with MR may give a deeper understanding of both enzymes.

Lesk *et al.* have attempted to analyse the structural features of three α/β barrel enzymes[29]. Their conclusion was that α/β barrel enzymes can be placed into two broad classes based on side-chain packing considerations. These two classes have substantially different packing arrangements, so the authors suggest that they are related by convergent evolution to a stable fold, since point mutations would not seem to allow interconversion between the two classes. However, Lesk *et al.* do agree that gene permutations, like those demonstrated in Kirschner's laboratory, would provide a mechanism for divergent evolution from a common ancestor.

Evolutionary arguments are always *ad hoc*, and we do not believe that the crude structural arguments presented here are conclusive proof of divergent as opposed to convergent evolution. We welcome alternative explanations and other methods of comparing these structures. One reason for suggesting these different structural families is that they provide a framework for classifying and understanding new α/β barrel structures. Further evidence for or against this family tree must await the discovery of new α/β barrel proteins. Since one out of ten enzymes has this motif, we will not have long to wait.

Acknowledgements

We would like to thank Dr Georg Schultz for a preprint of his paper on the CGT structure. We would also like to thank Drs David Eisenberg, Adrian Goldman, Elias Lolis, David Neidhart, Lukasz Lebioda, J. Jansonius and Craig Hyde for providing coordinates. This work was supported by grant GM26788 to GAP. GKF was a Richard Lounsbery Fellow of the Life Sciences Research Foundation at the University of Wisconsin.

References

1 Banner, D. W., Bloomer, A. C., Petsko, G. A., Phillips, D. C., Pogson, C. I., Wilson, I. A., Corran, P. H., Furth, A. J., Milman, J. D., Offord, R. E., Priddle, J. D. and Waley, S. G. (1975) *Nature* 255, 609–614
2 Richardson, J. S. (1981) *Adv. Protein Chem.* 34, 167–339
3 Xia, Z-X., Shamala, N., Bethge, P. H., Lim, L. W., Bellamy, H. D., Xuong, N-H., Lederer, F. and Mathews, F. S. (1987) *Proc. Natl Acad. Sci. USA* 84, 2629–2633
4 Lindqvist, Y. and Brändén, C-I. (1985) *Proc. Natl Acad. Sci. USA* 82, 6855–6859
5 Lim, L. W., Shamala, N., Mathews, F. S., Steenkamp, D. J., Hamlin, R. and Xuong, N-H. (1986) *J. Biol. Chem.* 261, 15140–15146
6 Chapman, M. S., Suh, S. W., Crumi, P. M. G., Cascio, D., Smith, W. W. and Eisenberg, D. S. (1988) *Science* 241, 71–74
7 Schneider, G., Lindqvist, Y., Brändén, C-I. and Lorimer, G. (1986) *EMBO J.* 5, 3409–3415
8 Goldman, A., Ollis, D. L. and Steitz, T. A. (1987) *J. Mol. Biol.* 194, 143–153
9 Matsuura, Y., Kusunoki, M., Harada, W. and Kakudo, M. (1984) *J. Biochem.* 95, 697–702
10 Buisson, G., Duée, E., Haser, R. and Payan, F. (1987) *EMBO J.* 6, 3909–3916
11 Hofmann, B. F., Bender, H. and Schulz, G. *J. Mol. Biol.* (in press)
12 Muirhead, H., Clayden, D. A., Barford, D., Lorimer, C. G., Fothergill-Gilmore, L. A., Schiltz, E. and Schmitt, W. (1986) *EMBO J.* 5, 475–481
13 Lebioda, L. and Stec, B. (1988) *Nature* 333, 683–686
14 Farber, G. K., Petsko, G. A. and Ringe, D. (1987) *Protein Eng.* 1, 459–466
15 Priestle, J. P., Grütter, G., White, J. L., Vincent, M. G., Kania, M., Wilson, E., Jardetzky, T. S., Kirschner, K. and Jansonius, J. N. (1987) *Proc. Natl Acad. Sci. USA* 84, 5690–5694
16 Hyde, C. C., Ahmed, S. A., Padlan, E. A., Miles, E. W. and Davies, D. R. (1988) *J. Biol. Chem.* 263, 17857–17871
17 Sygusch, J., Beaudry, D. and Allaire, M. (1987) *Proc. Natl Acad. Sci. USA* 84, 7846–7850
18 Mavridis, I. M., Hatada, M. H., Tulinsky, A. and Lebioda, L. (1982) *J. Mol. Biol.* 162, 419–444
19 Lebioda, L., Stec, B. and Brewer, J. M. (1989) *J. Biol. Chem.* 264, 3685–3693
20 Rossmann, M. G., Liljas, A., Brändén, C-I. and Banaszak, L. J. (1975) in *The Enzymes* (Boyer, P. D., ed.), Vol. 11, p. 62–102, Academic Press
21 Wright, C. S., Alden, R. A. and Kraut, J. (1969) *Nature* 221, 235–242
22 Birktoft, J. J. and Blow, D. M. (1972) *J. Mol. Biol.* 68, 187–240
23 Horowitz, N. H. (1945) *Proc. Natl Acad. Sci. USA* 31, 153–157
24 Cederlund, E., Lindqvist, Y., Söderlund, G., Brändén, C-I. and Jörnvall, H. (1988) *Eur. J. Biochem.* 173, 523–530
25 Ransom, S. C., Gerlt, J. A., Powers, V. M. and Kenyon, G. L. (1988) *Biochemistry* 27, 540–545
26 Aldrich, T. L., Frantz, B., Gill, J. F., Kilbane, J. J. and Chakrabarty, A. M. (1987) *Gene* 52, 185–195
27 Kimura, K., Kataoka, S., Ishii, Y., Takano, T. and Yamane, K. (1987) *J. Bacteriol.* 169, 4399–4402
28 Luger, K., Hommel, U., Herold, M., Hofsteenge, J. and Kirschner, K. (1989) *Science* 243, 206–210
29 Lesk, A. M., Brändén, C-I. and Chothia, C. (1989) *Proteins* 5, 139–148

Petitioner Merck, Ex. 1142, p. 234
Merck Sharp & Dohme LLC v. Halozyme Inc.
PGR2025-00009