

# Sequence similarities in $(\alpha/\beta)_8$ -barrel enzymes revealed by conserved regions of $\alpha$ -amylase

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The parallel  $(\alpha/\beta)_8$ -barrel is a frequently occurring protein-folding motif. Although the arrangement of secondary structural elements along the barrel is very similar in different  $(\alpha/\beta)_8$ -barrel enzymes, there is a very low mutual amino acid sequence homology among the enzymes, contributing in part to the hazy view of their evolution. Here an approach to identifying at least the rough of evolutionarily conserved  $(\alpha/\beta)_8$ -barrel sequence is presented. Based on the idea that highly conserved sequence regions of a particular enzyme should be more or less conserved in the sequences of the other evolutionary related enzymes, five sequence similarities of ten different  $(\alpha/\beta)_8$ -barrel enzymes were revealed, using the five conserved regions of the amino acid sequence of the  $\alpha$ -amylase  $(\alpha/\beta)_8$ -barrel as the templates.

$\alpha$ -Amylase;  $(\alpha/\beta)_8$ -Barrel enzyme; Conserved region; Sequence similarity; Evolutionary relationship

## 1. INTRODUCTION

The daunting number of known protein amino acid sequences (over 17,000) in comparison with about a 40-times lower number of known protein tertiary structures [1], is fortunately weakened by the existence of families of proteins that have similar folding patterns [2]. One of them, the parallel 8-folded  $(\alpha/\beta)_8$ -barrel, first recognized in chicken muscle triosephosphate isomerase [3], has been observed in the structure of roughly every tenth enzyme solved to date [4]. Almost all  $(\alpha/\beta)_8$ -barrel structures have 8 consecutive parallel  $\beta$ -strands surrounded by 8  $\alpha$ -helices [5]. The motif enables the enzyme to catalyze quite different biochemical reactions [6] and the  $(\alpha/\beta)_8$ -barrel enzyme family covers all types of enzyme nomenclature except for ligases.

Two evolutionary histories have been explored for  $(\alpha/\beta)_8$ -barrel enzymes [5]. The first one is based on a proposal by Gilbert [7] that proteins evolved by different combinations of exons coding for small functional or structural units, and has been documented by a comparison of the intron–exon arrangement in triosephosphate isomerase from different species [8]. In the second case Farber and Petsko [4] have elucidated the reasons favoring the more probable divergent evolution of  $(\alpha/\beta)_8$ -barrel enzymes from a common ancestor in contrast with convergent evolution. Despite these ef-

forts the challenging question of how the  $(\alpha/\beta)_8$ -barrel fold has arisen remains unanswered.

Due to very low mutual sequence homology [4,5], a comparison of all the  $(\alpha/\beta)_8$ -barrel sequences is not yet available. If there is any evolutionary relationship among them, however, at least the stretches of the  $(\alpha/\beta)_8$ -barrel's ancestors or descendant amino acid sequences should be identified by using the conserved regions of a particular  $(\alpha/\beta)_8$ -barrel enzyme as structural templates where some similarities have been protected or selected throughout the enzyme family. This report reports 5 such sequence similarities of 10 different  $(\alpha/\beta)_8$ -barrel enzymes. They were revealed in the parts of their  $(\alpha/\beta)_8$ -barrels that correspond with five conserved regions of the  $(\alpha/\beta)_8$ -barrel of  $\alpha$ -amylase.

## 2. MATERIALS AND METHODS

$\alpha$ -Amylase (EC 3.2.1.1) from porcine pancreas (AMYL) was used as the template  $(\alpha/\beta)_8$ -barrel enzyme in this study. Its amino acid sequence, as well as the arrangement of  $\alpha$ -helices and  $\beta$ -sheets along the  $(\alpha/\beta)_8$ -barrel superstructure, were taken from the literature [9,10].

Five conserved amino acid regions ([1], Š. Janeček, submitted for publication) were found to form approximately the same parts of the  $(\alpha/\beta)_8$ -barrels of different  $\alpha$ -amylases. Their locations in the AMYL  $(\alpha/\beta)_8$ -barrel were then used in the search for sequence similarities among the amino acid sequences of the other 10  $(\alpha/\beta)_8$ -barrel enzymes.

The enzymes were chosen based on the proposed organization of the whole  $(\alpha/\beta)_8$ -barrel enzyme family into 4 different structural sub-families [4]; one representative from each family was considered here. Needed structural details, such as amino acid sequences and composition of secondary structure in individual  $(\alpha/\beta)_8$ -barrels, were taken from the literature, and the enzymes were abbreviated as follows: GO, spinach glycolate oxidase [12]; RBCO, ribulose-1,5-bisphosphate carboxylase/oxygenase from *Rhodospirillum rubrum* [13,14]; MR, mande-

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Table I  
Five conserved amino acid sequence regions of ( $\alpha/\beta$ )<sub>8</sub>-barrel enzymes

Region	Enzyme <sup>a</sup>	Numbering	Sequence <sup>b</sup>	Secondary structure <sup>c</sup>
I	AMYL	96-101	DAVINH	$\beta$ 3, loop 3
	GO	124-129	RFFQLY	$\beta$ 3, loop 3
	RBCO	227-232	LFSANI	$\beta$ 3, loop 3
	MR	193-198	MVDYNQ	$\beta$ 3, loop 3
	MLE	183-188	HVVAIK	$\beta$ 3
	CGT	135-140	DFAPNH	$\beta$ 3, loop 3
	XYI	87-92	MATTNL	$\beta$ 3, loop 3
	PRAI	309-314	GVFRNH	$\beta$ 3, loop 3
	IGPS	111-116	ILCKDF	$\beta$ 3, loop 3
	TIM	60-65	TVGAQN	$\beta$ 3, loop 3
	FALD	102-107	IILGIK	turn <sup>d</sup> , $\beta$ 3
	II	AMYL	165-169	LLDLA
GO		131-135	YKDRN	loop 3, $\alpha$ 3
RBCO		233-237	TADDP	loop 3, $\alpha$ 3
MR		199-203	SLDVP	loop 3, $\alpha$ 3
MLE		191-195	LGDSA	loop 3, $\alpha$ 3
CGT		197-201	LADFN	loop 3
XYI		98-102	FKDGG	loop 3, $\alpha$ 3
PRAI		316-320	IADV	$\alpha$ 3
IGPS		117-121	IIDPY	loop 3, $\alpha$ 3
TIM		83-87	IKDVG	$\alpha$ 3
FALD		127-131	LDDLA	loop 3, $\alpha$ 3
III		AMYL	193-201	GFRLDASKH
	GO	153-161	ALTVDTPLR	$\beta$ 4, loop 4
	RBCO	259-267	ALLVDGYVA	$\beta$ 4, loop 4
	MR	219-227	WIEEPTLQH	turn, $\beta$ 4, loop 4
	MLE	210-218	AIRACQVLG	$\beta$ 4, loop 4, $\alpha$ 4
	CGT	225-233	GIRVDVVKH	$\beta$ 4, loop 4
	XYI	123-131	DLAVELARK	$\beta$ 4, loop 4
	PRAI	330-338	AVQLHGNEE	$\beta$ 4, loop 4
	IGPS	135-143	ILMLSVLDD	$\beta$ 4, loop 4, $\alpha$ 4
	TIM	90-98	WVILGHSE	$\beta$ 4, loop 4
	FALD	148-156	RCVLKIGKN	$\beta$ 4, loop 4
	IV	AMYL	233-236	EVID
GO		230-233	KGVI	$\beta$ 5, loop 5
RBCO		287-290	HRAG	$\beta$ 5, loop 5
MR		247-250	ENWL	loop 5
MLE		235-238	SGQV	$\beta$ 5, loop 5
CGT		257-260	EWFL	$\beta$ 5, loop 5
XYI		180-183	EPKP	$\beta$ 5
PRAI		363-366	ETLP	loop 5
IGPS		163-166	EVS	$\beta$ 5, loop 5
TIM		129-132	ETLE	$\beta$ 5, loop 5
FALD		189-192	EVL	$\beta$ 5, loop 5
V		AMYL	295-300	FVDNHD
	GO	283-288	FLDGGV	$\beta$ 7, loop 7
	RBCO	366-371	IISGGM	$\beta$ 7, loop 7
	MR	293-298	PMSHL	$\beta$ 7, loop 7
	MLE	285-290	VLRTAQ	$\beta$ 7, loop 7
	CGT	323-328	FIDNHD	loop 7
	XYI	249-254	SGIKYD	$\beta$ 7, loop 7
	PRAI	403-408	LLAGGL	$\beta$ 7, loop 7
	IGPS	212-217	ISESGI	$\beta$ 7, loop 7
	TIM	206-211	IYGG	$\beta$ 7, loop 7
	FALD	269-274	FLSGGQ	$\beta$ 7, loop 7

<sup>a</sup> Full names of the enzymes and their sources are given in section 2.

<sup>b</sup> Amino acids are shown in the one-letter code.

<sup>c</sup> Elements of secondary structure in loops are not specified.

<sup>d</sup> Turn means a stretch preceding a  $\beta$ -strand.

late racemase from *Pseudomonas putida* [15,16]; MLE, muconate lactonizing enzyme from *Pseudomonas putida* [17,18]; CGT, cyclodextrin glycosyltransferase from *Bacillus circulans* [19]; XYI, xylose isomerase from *Streptomyces olivochromogenes* [20]; PRAI, phosphoribosylanthranilate isomerase from *Escherichia coli* [21]; IGPS, indoleglycerol-phosphate synthase from *Escherichia coli* [21]; TIM, yeast triosephosphate isomerase [22,23]; FALD, fructose-1,6-bisphosphate aldolase from *Drosophila melanogaster* ([24,25], K. Piontek, personal communication).

### 3. RESULTS AND DISCUSSION

This is the first report giving several sequence stretches of different  $(\alpha/\beta)_8$ -barrel enzymes that might be conserved during evolution. The stretches were deduced from the assumption that the highly conserved regions in the amino acid sequence of one  $(\alpha/\beta)_8$ -barrel enzyme should be more or less conserved in the other enzymes with this folding motif because they are evolutionarily related.

The results from the search for sequence similarities are shown in Table I. Although the similarities do not exhibit a strict homology, there are mostly semiconservative substitutions of amino acid residues among the compared enzymes. The fact that these regions with a high degree of similarity are localized in nearly the same locations of the individual  $(\alpha/\beta)_8$ -barrels (last column in Table I) is of special importance. Moreover, the regions containing only 30 amino acid residues, i.e. less than 10% of the average length of an  $(\alpha/\beta)_8$ -barrel enzyme, include about 50% of all the residues involved in the catalytic functions of the enzymes studied here (Table II).

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Table II

Amino acid residues involved in catalytic function in  $(\alpha/\beta)_8$ -barrel enzymes found in five sequence similar regions

Enzyme <sup>a</sup>	Catalytic residues <sup>b</sup>	In five conserved regions
AMYL	D197 <sup>c</sup> , D300	D197 (III) <sup>d</sup> , D300 (V)
GO	Y24, Y129, H254, R257	Y129 (I)
RBCO	K166, K158, K329, S368	S368 (V)
MR	K166, H297	H297 (V)
MLE <sup>e</sup>	—	—
CGT	D229, E257, D328	D229 (III), E257 (IV), D328 (V)
XYI	E180, H219, D286	E180 (V)
PRAI	C260, Q332, D379	Q332 (III)
IGPS	S60, K114, E163, N184, R186	K114 (I), E163 (IV)
TIM	H95, E165	H95 (III)
FALD <sup>f</sup>	K107, K146, R148, K229	K107 (I), R148 (III)

<sup>a</sup> Full names of the enzymes and their sources are given in section 2.

<sup>b</sup> Residues involved in catalysis were extracted from literature given also in section 2.

<sup>c</sup> Amino acid type (using the one-letter code) and the position in the sequence of the particular enzyme are shown.

<sup>d</sup> The number of conserved regions (in parentheses).

<sup>e</sup> Residues have not been determined.

<sup>f</sup> Residues have been proposed only as the active site residues.

$(\alpha/\beta)_8$ -barrel enzymes (binding of phosphate), Wilmanns et al. [26] found some local sequence similarity for GO, RBCO, PRAI, IGPS, TIM,  $\alpha$ -subunit of tryptophan synthase (TS<sub>a</sub>) and flavocytochrome *b*<sub>2</sub> (FCB2). It should be pointed out that a part of that similarity [26] fell down in the fifth conserved region proposed here, where the alignments of amino acid sequences from both studies were almost identical.

Although the comparison of all the  $(\alpha/\beta)_8$ -barrel enzymes sequences is not yet available [4], several groups, such as AMYL and CGT [27], MR and MLE [28], GO and FCB2 [29], and PRAI, IGPS and TS<sub>a</sub> [26], have been shown to have sufficient sequence and, eventually, structural identity. Therefore, these groups of  $(\alpha/\beta)_8$ -barrel enzymes are considered to have evolved by divergent evolution, either from the same ancestor or, in part, from each other. Despite the lack in overall homology in the  $(\alpha/\beta)_8$ -barrel enzyme family [4,5], the existing structural similarities almost certainly arise from the stringent requirements of the  $(\alpha/\beta)_8$ -barrel fold [30].

From the results of this study, however, it might be concluded that, independent of the type of evolution (divergent or convergent), the rough amino acid sequence of the ancestor or the descendant  $(\alpha/\beta)_8$ -barrel or, at least, a substantial part of it, can be designed simply by putting together the highly conserved regions of the particular  $(\alpha/\beta)_8$ -barrel enzymes. The possibility of constructing an evolutionarily conserved amino acid sequence which will adopt the parallel 8-folded  $(\alpha/\beta)_8$ -barrel might be useful not only in the elucidation of evolutionary relationships in the  $(\alpha/\beta)_8$ -barrel enzyme family, but also in the prediction of further enzymes with this folding motif prior to their X-ray crystallographic studies.

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