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Notes and comments

The $(\alpha/\beta)_8$-barrel structural domains of α-amylase and old yellow enzyme are discontinued by a similar excursion at the same place of the barrels: Does it mean they are evolutionarily related?

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Introduction

The family of proteins adopting parallel $(\alpha/\beta)_8$-barrel folding motif in their structures has become an attractive subject for evolutionary studies (Farber and Petsko, 1990, Janeček and Baláž, 1993). Almost all of the $(\alpha/\beta)_8$-barrels are enzymes involved in various kinds of biochemical reactions. Despite the fact that these proteins contain common $(\alpha/\beta)_8$-barrel domain or some disrupted version of it (for a recent review, see Reardon and Farber, 1995), their amino acid sequences, in general, exhibit no detectable sequence similarities. Only a few examples of clear evolutionary relatedness between several pairs of $(\alpha/\beta)_8$-barrels have been found, e.g. α-amylase and cyclodextrin glycosyltransferase (MacGregor and Svensson, 1989) or glycolate oxidase and flavocytochrome b_2 (Lindqvist et al., 1991).

The efforts to join the seemingly unrelated subfamilies of $(\alpha/\beta)_8$-barrels sequentially have not led, however, to unambiguous success, mainly when the approaches were based on a production of the amino acid sequence alignments that would be structurally satisfactory. Such alignments of $(\alpha/\beta)_8$-barrel sequences (Pickett et al., 1992, Sergeev and Lee, 1994) are perfect from the structural point of view but they do not necessarily result in the homologies. These could be hidden in $(\alpha/\beta)_8$-barrels due to their very long evolutionary history. During development the homologous amino acid residues from the primordial barrel(s) might have adopted different functional roles (Janeček and Baláž, 1995). This theory is anchored in a simple idea that a conserved sequence region of an $(\alpha/\beta)_8$-barrel enzyme should be more or less

conserved also in the equivalent part of structure of the other $(\alpha/\beta)_8$-barrel enzymes owing to their mutual evolutionary relatedness (Janeček, 1993). This has recently been illustrated (Janeček and Baláž, 1995) by the catalytic Glu230 of α-amylase located near the C-terminus of the fifth β-strand of its $(\alpha/\beta)_8$-barrel that has functionally related structural equivalents in more than ten other $(\alpha/\beta)_8$-barrel enzymes, i.e. the glutamates might have adopted different functional roles.

This study presents an indication of evolutionary relatedness of two different $(\alpha/\beta)_8$-barrel subfamilies, starch hydrolases and flavin mononucleotide-dependent enzymes with α-amylase (EC 3.2.1.1) and old yellow enzyme (EC 1.6.99.1) as representatives, respectively. The $(\alpha/\beta)_8$-barrel of α-amylase (Matsuura et al., 1984, Swift et al., 1991) as well as of all starch hydrolases and related enzymes (for a review, see Janeček, 1994) is interrupted between the third β-strand and the third α-helix by a long loop that forms, in fact, a small distinct domain. Similar segment of the polypeptide chain protruding out in the structurally equivalent part of the old yellow enzyme's $(\alpha/\beta)_8$-barrel (Fox and Karplus, 1994a, 1994b) evoked an idea that these structural excursions from the regular barrel domains could be a joining feature remaining from one of the primordial barrel structures. This view can be supported by the comparison of catalytic amino acid residues of α-amylase and old yellow enzyme localised also in the structurally eqivalent parts of their $(\alpha/\beta)_8$-barrels.

Materials and methods

The information concerning the amino acid sequences and three-dimensional structures of α-amylase from *Aspergillus oryzae* and old yellow enzyme from *Saccharomyces cerevisiae*, respectively, were retrieved from the SwissProt Sequence Data Base: accession numbers P10529 and Q02899, from the Brookhaven Protein Data Bank: PDB-entries 6TAA and 1OYA (kindly provided by Dr. P. A. Karplus, Cornell University, Ithaca) and from the relevant literature (Matsuura et al., 1984, Swift et al., 1991) and (Fox and Karplus, 1994a, 1994b).

The segments of polypeptide chains of α-amylase and old yellow enzyme forming the excursions protruding out of their $(\alpha/\beta)_8$-barrel domains between the third β-strand and the third α-helix were aligned using the program CLUSTAL (Higgins et al., 1992). The residues forming secondary structural elements were manually tuned, where applicable.

For structural comparison of peptide segments of α-amylase and old yellow enzyme containing the functionally important amino acid residues, the program HYPERCHEM (Autodesk, Inc.) was used.

Results and discussion

It has been recently pointed out that the $(\alpha/\beta)_8$-barrel protein family may have diverged so far that no detectable sequence homologies appear among its members (Janeček and Baláž, 1995). Therefore, in the search for some evolutionary joining features among different $(\alpha/\beta)_8$-barrel enzymes, any trace seeming to be relevant

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                <-β->  <α>                          <α>        <β->  <-β-->
AMY  119  VANHMGYDGAGSSVDYSVFKPFSSQDYFHPFFIQNYEDQTQVEDCWLGDNTVSLPDLDTT  179
          *    ..    .      . . * . .      . *.. . ..          *.   *
OYE  117  VLGWAAFPDNLARDGLR-YDSASDNVF------MDAEQEAKAKK-------ANNPQHSLT  162
                 <-α-->     <β>              <---α--->          <β>
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Fig. 1. Amino acid sequence alignment of the polypeptide excursions from the $(\alpha/\beta)_8$-barrels of α-amylase (AMY) and old yellow enzyme (OYE). Asterisks and dots signify the identical residues and conservative substitutions, respectively. Gaps are indicated by hypens. The secondary structure elements are bolded and indicated above and under the alignment for α-amylase and old yellow enzyme, respectively.

should not be neglected. One of these evolutionary traces could be just the excursion from the regular $(\alpha/\beta)_8$-barrel domains of α-amylase and old yellow enzyme.

The excursions protrude from their barrels between the third β-strand and the third α-helix (Matsuura et al., 1984; Fox and Karplus, 1994a) forming a small distinct domain in both cases. A little is known about its function. It determines, for instance, several functional and stability properties that distinguish the barley α-amylase isozymes (Rodenburg et al., 1994). The amino acid sequence alignment of these segments of the polypeptide chains of α-amylase and old yellow enzyme is shown in Fig. 1. There are only five identical residues in the alignment but, more importantly, these positions together with the additional 14 conservative substitutions yielded a degree of sequential similarity (the ratio of the sum of identical and similar residues calculated using the number of residues of the smaller enzyme) as high as 41.4%. It is worth mentioning that this value for two sequentially and structurally homologous, clearly related $(\alpha/\beta)_8$-barrel enzymes, α-amylase and cyclodextrin glycosyltransferase, was found to be 34.4% (their alignment is not shown). Moreover, the secondary structural elements from the excursions of α-amylase and old yellow enzyme mostly match quite well (Fig. 1). And finally, similar excursions are present in the $(\alpha/\beta)_8$-barrels of cyclodextrin glycosyltransferase and oligo-1,6-glucosidase that are closely related to α-amylase (Jespersen et al., 1993) as well as of trimethylamine dehydrogenase that is closely related to old yellow enzyme (Fox and Karplus, 1994b).

To support the possibility presented above regarding the evolutionary relatedness of these two enzymes, their catalytic amino acid residues were investigated. Remarkably, these residues of old yellow enzyme, His191 and Asn194, are located near the C-terminus of the β4-strand (Fox and Karplus, 1994a) where, in the α-amylase structure, Asp206 is positioned, one of the three catalytic residues of α-amylase (Matsuura et al., 1991). This Asp206 is oriented equivalently with the catalytic histidine of old yellow enzyme (Fig. 2). The fact that the backbones of α-amylase and old yellow enzyme behind the β4-strand segments do not fit each other, should not be surprising at all since the enzymes are wholly different, one is a starch hydrolase and the other ranks among flavin mononucleotide-dependent enzymes.

The presence of the other functionally important amino acid residues of α-amylase at or around its strands β4 should be also taken into account, such as Lys209 and His210 (both at the substrate binding site (Matsuura et al., 1984)) as well as

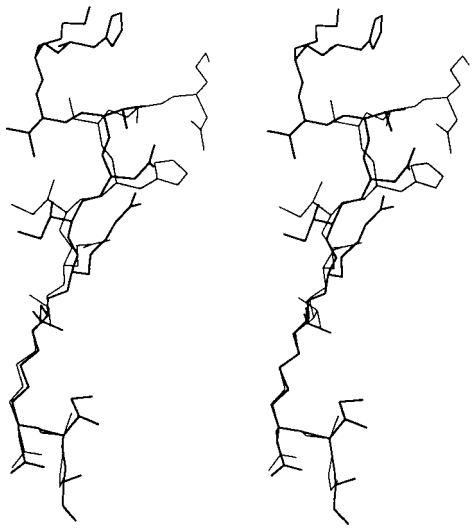

Fig. 2. β4-Strands of α-amylase (thick lines) and old yellow enzyme (thin lines) are shown overlapped. Corresponding sequences: α-amylase: 199_SIDGLRIDTVKH; old yellow enzyme: 184_GADGVEIHSANG. Asp206 of α-amylase and His191 and Asn194 of old yellow enzyme are involved in catalysis of the respective enzymes.

Arg204 (its equivalents in the α-amylase from pig pancreas and *Bacillus licheniformis* are involved in the chloride binding site (Qian et al., 1993; Machius et al., 1995)). And since the β4-strand of α-amylase belongs to one of its highly conserved sequence regions (Janeček et al., 1995), these findings are consistent with the theory on mutual conservation of sequence regions of $(\alpha/\beta)_8$-barrel enzymes located in the equivalent parts of their barrel structures (Janeček, 1993).

To summarise, the presented proposal concerning the possible evolutionary relatedness of α-amylase and old yellow enzyme due to the similarity of their outside $(\alpha/\beta)_8$-barrel excursions located in the third loop as well as the similarity between their β4-strands comprising their catalytic amino acid residues, could make a new connection in the family of all $(\alpha/\beta)_8$-barrels. It does not answer the question how these enzymes evolved, but it can support the possibility of a far, divergent evolution road which in the absence of data to the contrary seems more reasonable than a convergence to the sturdy barrel structure (Doolittle, 1995).

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