Structure, function and evolution of the Archaeal class I fructose-1,6-bisphosphate aldolase

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Abstract

FBPA (fructose-1,6-bisphosphate aldolase) catalyses the reversible aldol condensation of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate to form fructose 1,6-bisphosphate. Two classes of FBPA, which rely on different reaction mechanisms, have so far been discovered, class I mainly found in Eucarya and class II mainly in Bacteria. Only recently were genes encoding proteins with FBPA activity identified in Archaea. Archaeal FBPA do not share any significant overall sequence identity with members of the traditional classes of FBPAs, raising the interesting question of whether they have evolved independently by convergent evolution or diverged from a common ancestor. Biochemical characterization of FBPAs of the two hyperthermophilic Archaea Thermoproteus tenax and Pyrococcus furiosus showed that the enzymes use a Schiff-base mechanism and thus belong to the class I aldolases. The crystal structure of the archaeal FBPA from T. tenax revealed that the protein fold, as for the classical FBPA I and II, is that of a parallel (βα)8 barrel. A substrate-bound crystal structure allowed detailed active-site comparisons which showed the conservation of six important catalytic and substrate-binding residues between the archaeal and the classical FBPA I. This observation provides further evidence that the two sequence families of proteins share a common evolutionary origin. Furthermore, structure and sequence analysis indicate that the class I FBPA shares a common evolutionary origin with several other enzyme superfamilies of the (βα)8 barrel fold.

Introduction

The cleavage and formation of FBP (fructose 1,6-bisphosphate) from GAP (glyceraldehyde 3-phosphate) and DHAP (dihydroxyacetone phosphate) by FBPA (fructose-1,6-bisphosphate aldolase; EC 4.1.2.13) constitute an important step in the Embden–Meyerhof–Parnas pathway. Two different classes of FBPA, which share no significant sequence identity, have so far been characterized. Although members of both classes adopt the (βα)8-(TIM)-barrel fold (where TIM is triosephosphate isomerase), they use different enzymic mechanisms. Class I FBPA (FBPA I) relies on the formation of a Schiff-base intermediate between the side-chain amino group of the active-site lysine and the substrate [1]. The classical FBPA I is mainly found in Eucarya and is from several crystal structures known to be homo-tetrameric [2–5]. Class II FBPA (FBPA II), on the other hand, uses divalent cations for catalysis. FBPA II is the major bacterial FBPA and forms a homodimer [6].

Activities of both class I and II FBPAs have been detected in several Archaea [7–9], but no genes encoding classical FBPA I or II were identified in fully sequenced archaeal genomes. This could either mean that novel FBPAs exist in Archaea or that the sequences of the classical FBPAs have diverged beyond recognition by conventional sequence-comparison techniques. The recent discovery of genes encoding archaeal FBPAs, the biochemical and structural characterization of the enzymes as well as their evolutionary relationship to classical FBPAs and to other enzymes of the TIM barrel fold will be reviewed and further discussed in this paper.

Archaeal FBPA constitutes a new sequence family of aldolases

The identification of archaeal FBPAs came with the characterization of an Escherichia coli gene product, which was originally mis-annotated as belonging to the family of dehydrins, a group of stress-response proteins found in plants (gene dhnA). When the gene product was biochemically characterized, it turned out to share all the properties of the classical FBPA I [10]. The striking observation that the five archaeal genomes completely sequenced at that time all contained at least one homologue suggested that this gene encoded the major archaeal type FBPA I [11]. Cloning, recombinant expression and subsequent biochemical characterization of the gene homologues from the crenarchaeote Thermoproteus tenax and the euryarchaeote Pyrococcus furiosus verified that they indeed code for FBPAs [12]. The observation that the enzymes were inhibited by sodium borohydride and that metals as well as EDTA did not show
any effect on activity demonstrated that the enzymes, like the classical FBPA I, function via a Schiff-base intermediate.

Sixteen out of the 20 archaeal genomes sequenced to date contain at least one gene homologue with only *Pyrobaculum aerophilum*, *Ferroplasma acidarmanus*, *Thermoplasma acidophilum* and *Thermoplasma volcanium* currently missing. These organisms could, however, possess genes encoding class I or II FBPAs which, due to low sequence identity to the known families, have not yet been identified. As most archaeal organisms possess at least one homologue of the *dhnA* gene, this third sequence family of FBPAs was designated the Archaeal type class I FBPA (FBPA IA) [12]. In addition to the archaeal members, the FBPA IA family comprises some bacterial genes present in the bacterial genome together with FBPA II, but no eucaryal FBPA IA has so far been discovered.

Although FBPA IA is a divergent family with overall sequence identity between members as low as 20%, homology is clearly indicated by several sequence tags including the Schiff-base-forming lysine and the common phosphate-binding site [12]. Furthermore, the common Schiff-base reaction mechanism, prediction of the TIM barrel fold and the location of functional residues would suggest a distant evolutionary relationship to the classical FBPA I [10–12].

### Three-dimensional structure of the archaeal FBPA I

The first FBPA IA crystal structure was recently solved from the hyperthermophilic crenarchaeote *T. tenax* (Tt-FBPA) at 1.9 Å resolution [13]. Like the classical FBPA I and II, the FBPA IA monomer adopts the ubiquitous fold of a parallel (βα)8 barrel. The quaternary structure of the FBPA IA protein was from size-exclusion chromatography and ultracentrifugation suggested to exist as homo-octamers, decamers or even higher oligomers [10,12,14]. In the crystal structure, however, Tt-FBPA forms homo-decamers consisting of two identical rings of pentamers (Figure 1). The protein complex has a molecular mass of 280 kDa and has an overall doughnut shape with a diameter of about 100 Å and a height of 75 Å. As the buried surface area in pentamer formation is 26% of the total accessible surface area of the monomer, the pentamer is a highly stable structure. The decamer, in contrast, is loosely attached with only 2% of the accessible surface area of the pentamer engaged in pentamer–pentamer interactions. This supports the observation that the protein in solution exists in equilibrium between two oligomeric states [12]. Pentamers assemble with the N-terminal of the barrels facing each other and with the C-terminal active...
Figure 2 | Ball-and-stick representation of the active sites of the archaeal FBPA I from *T. tenax* (light colours) and the eukaryotic FBPA I from rabbit (dark colours) after the active-site residues have been structurally superimposed

Both enzymes contain the substrate DHAP covalently bound in the active site. The residues are labelled according to the Tt-FBPA sequence.

sites pointing away from the interface. The pentamer interface is highly hydrophobic with 68% of the involved atoms being non-polar. Although no structure is available of an archaeal FBPA I from a mesophilic organism for detailed comparison, it seems likely that the large predominantly hydrophobic pentamer interface could be an important determinant in ensuring thermostability of the protein.

The quaternary arrangement of the FBPA IA structure is thus distinct from the tetrameric FBPA I, but the detailed comparison of active sites do support the notion of a common evolutionary origin for the two types of aldolases (see below).

**The classical and archaeal FBPAs I are homologous**

The evolutionary history of FBPA I, FBPA IA and FBPA II is not completely evident. With the crystal structure of the FBPA IA from *T. tenax* solved, it is clear that all three sequence families of FBPA adopt the TIM barrel fold. However, there is consensus that sharing the TIM barrel fold is not in itself enough to warrant common ancestry between proteins [15–17]. The low complexity and repetitive structure of the TIM barrel might imply that it has arisen more than once during evolution.

More evidence is thus needed to unravel the evolution of proteins of the TIM barrel fold. If two proteins of the same function are homologous, it is expected that many of the functionally important residues would be conserved during evolution. In order to identify active-site residues, a crystal structure of the FBPA IA in complex with the substrate DHAP was solved at 2.1 Å resolution [13]. This structure can then be compared with a classical FBPA I from rabbit, also in complex with DHAP, which was previously solved at 2.6 Å resolution [18]. As the active sites are in the same conformation, this provides an excellent possibility for comparisons of catalytic and substrate-binding residues. Six active-site residues are seen to be structurally conserved when FBPA I and FBPA IA are superimposed (Figure 2). The six residues superimpose well with an RMSD (root mean square deviation) of 1.1 Å and a *P* value of $3 \times 10^{-4}$ [13], where *P* is the probability of finding the given similarity by chance (*P* = 1 being random) [19]. The conserved residues include the Schiff-base-forming Lys-177 (numbering according to the *T. tenax* FBPA sequence), the general base Asp-24, the substrate binding Ala-22 as well as a common phosphate-binding site, which in FBPA I/IA comprises Gly-204, Gly-231 and Arg-232 [13]. This phosphate-binding site is also found in many other TIM barrel enzymes utilizing phosphorylated substrates and might represent an evolutionary marker for common ancestry [15,20]. The highly conserved active site between FBPA I and FBPA IA is a very good indication that they share a common ancestor.

In contrast, the class II FBPA do not share a common active site with the class I enzymes. The Schiff-base-forming lysine found in the class I enzymes is absent in class II aldolases and the metal co-ordinating residues of FBPA II are missing in FBPA I. They do, however, share the common phosphate-binding site located between β7 and β8 [15,20]. Furthermore, the fact that the family of deoxyribose 5-phosphate aldolases...
(DeoC) shows similarity to both classes of FBPAs, and thus might represent an intermediate form between FBPA I and II, supports the notion of common ancestry [11,21].

**Comparison with other TIM barrel enzymes**

The crystal structure of Tt-FBPA allowed a more detailed comparison with structures of other enzyme families of the TIM barrel fold. As structure in general is more conserved than sequence, thorough structural comparisons and structure-based sequence alignments can help, in cases where standard sequence-alignment tools fail, to determine distant evolutionary relationships.

In a structural comparison with all TIM barrel proteins of known structure, an extraordinarily high similarity between the Tt-FBPA monomer and those of the functionally different thiamin phosphate synthase [22] and glycolate oxidase [23] is found. Both proteins belong to the superfamily of FMOP (FMN-dependent oxidoreductases and phosphate-binding enzymes) [16]. Tt-FBPA superimposes with thiamin phosphate synthase with an RMSD of 1.9 Å and a structure-based sequence identity of 25% for 122 out of 226 residues ($P = 5.8 \times 10^{-11}$) and with glycolate oxidase with an RMSD of 2.1 Å and a structure-based sequence identity of 27% for 133 out of 250 residues ($P = 2.4 \times 10^{-13}$). These results indicate, in agreement with the findings of Nagano et al. [16], that the FMOP superfamily is homologous to the superfamily of aldolases.

Of all the structures currently available in the protein data bank [24], the Tt-FBPA monomer is, from an overall structural point of view, most similar to the structures of TIMs of hyperthermophilic archaeal organisms [13]. TIM, the enzyme giving the name to the $(\beta\alpha)_8$-(TIM)-barrel fold [25], catalyses the interconversion between GAP and DHAP and forms a superfamily different from that of the aldolases [13]. The Tt-FBPA and the Tt-TIM monomers superimpose with an RMSD of 1.7 Å for the 149 out of 224 residues found as structurally equivalent. Thirty out of the 149 residues are found to be identical (20%) in a structure-based sequence alignment (Figure 3). The low likelihood ($P = 3.7 \times 10^{-8}$) of finding such a similarity by chance [26] provides good evidence that the superfamilies of aldolases and TIMs share a common ancestor.

**References**


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