Structural Basis for the Single Active Site to Catalyze Two Distinct Reactions in a Primitive Enzyme FBPA/P

The bifunctional enzyme fructose 1,6-bisphosphate aldolase/phosphatase (FBPA/P) is distributed among primordial organisms such as hyperthermophiles and catalyzes two consecutive reactions of gluconeogenesis: the aldol condensation of dihydroxyacetone phosphate and glyceraldehyde-3-phosphate to fructose 1,6-bisphosphate, and the hydrolysis of FBP to fructose-6-phosphate. X-ray crystallographic analysis revealed that conversion between aldolase and phosphatase forms of the enzyme is based on the toggle switch-like motions of three mobile loops in the active site. This is the first example of an enzyme that catalyzes two different reactions in a single active site.

An enzyme called fructose 1,6-bisphosphate aldolase/phosphatase (FBPA/P) catalyzes two consecutive reactions of gluconeogenesis: (1) the aldol condensation (aldolase reaction) of dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate to fructose 1,6-bisphosphate (FBP), and (2) the hydrolysis (phosphatase reaction) of FBP to fructose-6-phosphate and phosphate. FBPA/P is distributed among hyperthermophilic archaea and deeply branched bacteria [1].

FBPA/P was initially identified as a novel class of fructose 1,6-bisphosphatase (class V FBPase), essential for gluconeogenesis of thermophilic organisms [2, 3]. In 2004, the first structure of class V FBPase (ST0318) from a hyperthermophilic archaeon *Sulfolobus tokodaii* was determined as a complex with FBP (PDB ID: 1UMG) [4].

In 2010, the discovery that archaeal FBP aldolase is identical with class V FBPase, thereby designated as a bifunctional enzyme FBPA/P, raised the serious question as to how Class V FBPase can act as FBP aldolase, because Lys232 (K232) essential for the aldolase reaction is far away (~17Å) from the substrate binding site in the 1UMG structure [1]. In 2011, the crystal structure of the ST0318 protein in complex with DHAP was determined (PDB ID: 3R1M) [5], using the AR-NW12A beamline.

The overall structures of both 1UMG and 3R1M are similar, with eight subunits stacking back-to-back, and the active site is located at the subunit interface within the ring-shaped tetramer (Fig. 1). Detailed structural comparison revealed a conformational difference in the active site arising from the toggle switch-like motions of three mobile loops termed lid (blue), Schiff-base (yellow), and C-terminal (pink) loops. The structures 1UMG and 3R1M represent 'phosphatase form' and 'aldolase form', respectively.

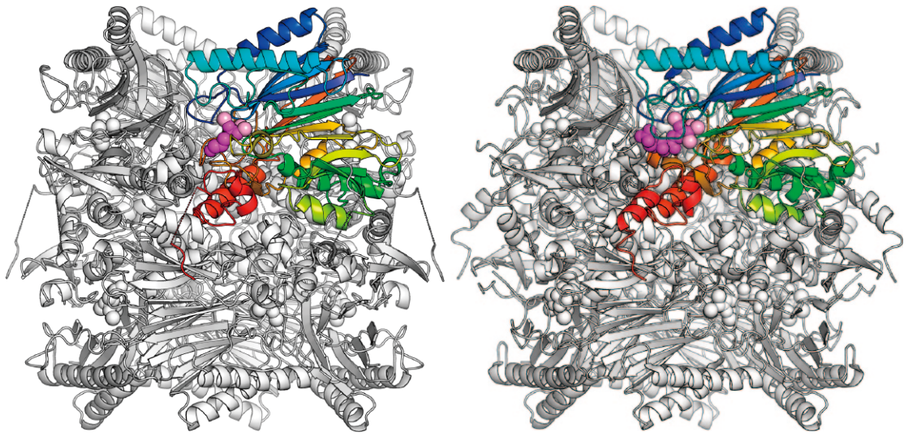

Figure 1
Octameric structure of aldolase (left) and phosphatase (right) forms of FBPA/P. One subunit is shown in rainbow colors. Ligands at one active site are shown as purple (DHAP and FBP) and pink (Mg^{2+}) spheres.

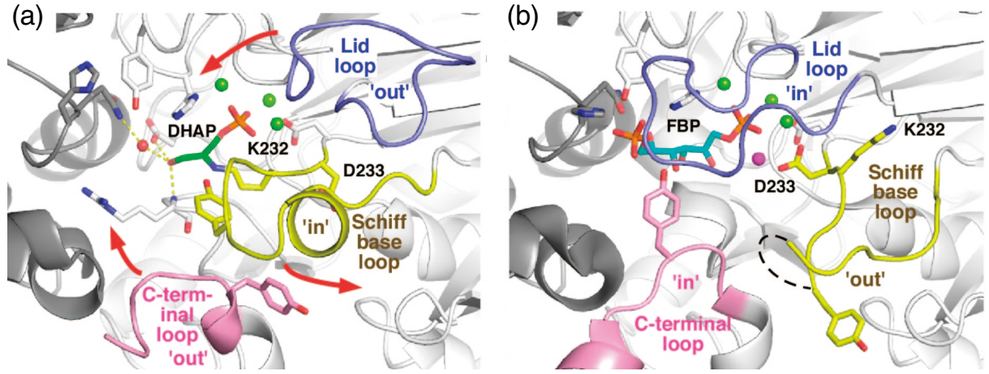

Figure 2
Active site of FBPA/P. (a) Aldolase form and (b) phosphatase form.

Figure 2 compares the active site of the two forms. A large structural change is observed at the three mobile loops. In the aldolase form [Fig. 2(a)], a critical lysine residue (K232) forms a covalent Schiff base with DHAP (green). Three Mg^{2+} ions (green) are also bound. During the structural change from the aldolase to phosphatase form, the Schiff-base loop moves from 'in' to 'out' conformation. In contrast, the lid and C-terminal loops move from 'out' to 'in'. In the phosphatase form [Fig. 2(b)], a FBP molecule (cyan) is bound in an open-keto (linear) form. An additional Mg^{2+} ion (magenta) is bound in this form, and this fourth Mg^{2+} ion is essential for the phosphatase reaction. The 'in' to 'out' conformational change in the Schiff-base loop flips the D233 side chain, thereby creating the binding site for the fourth Mg^{2+}. An independent group also reported a similar result, using another FBPA/P enzyme from a hyperthermophilic archaeon, *Thermoproteus neutrophilus* [6].

The organisms that possess FBPA/P occupy the closest branches to the root of the phylogenetic tree of life, and they thus may retain some of the physiological features of early life forms. FBPA/P may represent an ancestral, simple gluconeogenic system that was present in primordial chemolithoautotrophic organisms. This study is the first to elucidate the molecular mechanism of a "true" bifunctional enzyme. Our findings suggest the possible existence of undiscovered enzymes that also catalyze multiple chemical reactions at a single site.

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BEAMLINE

AR-NW12A

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