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An artificially constructed dimer through deformation of a short zinc-binding loop

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ABSTRACT

We have analyzed the crystal structure of the dimeric form of D-*glycero*-D-*manno*-heptose-1,7-bisphosphate phosphatase from *Burkholderia thailandensis* (*Bt*GmhB), catalyzing the removal of the phosphate at the 7 position of D-*glycero*-D-*manno*-heptose-1,7-bisphosphate. The crystal structure of *Bt*GmhB revealed a dimeric form caused by a disruption of a short zinc-binding loop. The dimeric *Bt*GmhB structure was induced by triggering the loss of Zn^{2+} *via* the protonation of cysteine residues at pH 4.8 of the crystallization condition. Similarly, the addition of EDTA also causes the dimerization of *Bt*GmhB. It appears there are two dimeric forms in solution with and without the disulfide bridge mediated by Cys95. The disulfide-free dimer produced by the loss of Zn^{2+} in the short zinc-binding loop is further converted to a stable disulfide-bonded dimer *in vitro*. Though the two dimeric forms are reversible, both of them are inactive due to a deformation of the active site. Single and triple mutant experiments confirmed the presence of two dimeric forms *in vitro*. Phosphatase assay results showed that only a zinc-bound monomeric form contains catalytic activity in contrast to the inactive zinc-free dimeric forms. The monomer-to-dimer transition caused by the loss of Zn^{2+} observed in this study is an example of reversal phenomenon caused by artificial proteins containing protein engineered zinc-finger motifs where the monomer-to-dimer transitions occurred in the presence of Zn^{2+}. Therefore, this unusual dimerization process may be applicable to designing proteins possessing a short zinc-binding loop with a novel regulatory role.

1. Introduction

Protein engineering is a key technique widely used in various interdisciplinary scientific areas. One of the main purposes of protein engineering is to construct artificial proteins performing useful or valuable functions. Well-known approaches achieve special functions by rational protein design or directed evolution [1]. In many cases, only a single mutation of a protein predicted by rational protein design can improve its function or help it obtain new properties. However, the prediction of single mutations capable of drastically influencing a protein's properties is quite limited. On the contrary, directed evolution employing random mutagenesis is often very useful for acquiring unpredictable protein functions. These methods have significantly influenced the progresses of biochemistry, biology, nanotechnology, and pharmacology. A recent example is a computational approach that had been designed to engineer peptides possessing specific protein-protein interaction capabilities [2]. However, careful analyses of natural phenomena still remain a good approach for learning how to generate new and better functions within the field of protein engineering.

Zinc fingers are unique protein motifs performing both structural and functional roles in biological molecules. Cysteine and histidine residues are co-players to chelate zinc ions. Zinc fingers can be classified into eight different groups [3], one of them being those with a short zinc-binding loop. D-*glycero*-D-*manno*-heptose-1,7-bisphosphate phosphatase (GmhB), catalyzing the removal of the phosphate at the 7 position of D-*glycero*-D-*manno*-heptose-1,7-bisphosphate, contains a short zinc-binding loop essential for structural stability and an indirect catalytic role. The GmhB family belongs to the haloacid dehalogenase (HAD) superfamily of hydrolases which is composed of phosphatases, epoxide hydrolases, and L-2-haloacid dehalogenases [4]. For phosphatase activity of GmhB, the stable zinc-binding state of the zinc-binding loop is critical to the spatial positioning of its catalytic residues. Therefore, the disruption of zinc-binding loops influences the catalytic activities of zinc-binding enzymes. The unnatural intra- or inter-disulfide bonds caused by the exposure of the free thiols of the zinc-binding cysteines often decreases protein solubility and/or triggers

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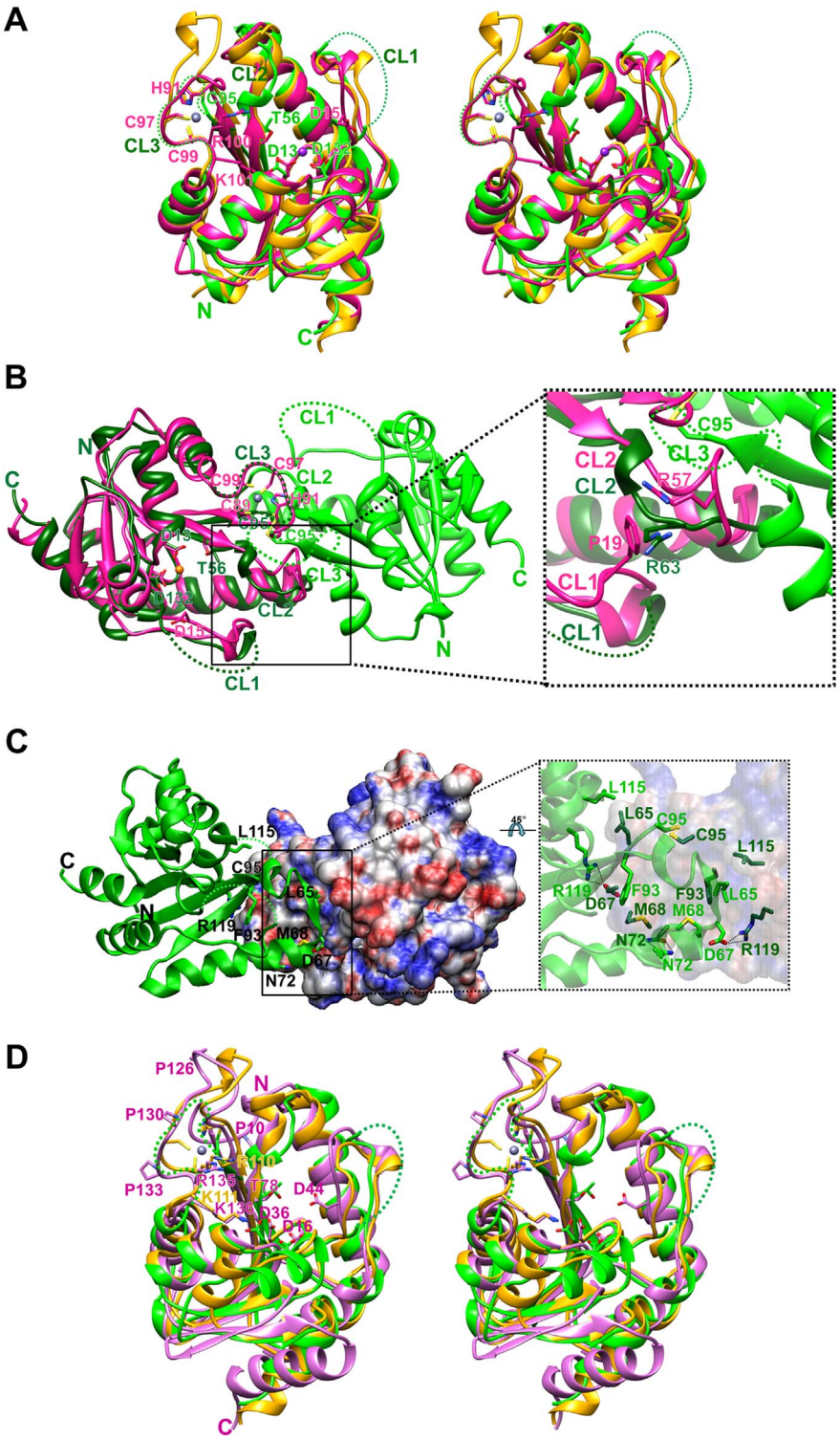

Fig. 1. (A) The stereodiagram of the superimposed crystal structure of *Bt*GmhB (green) with those of *Ec*GmhB (gold; PDB ID: 3L8E) and *Bb*GmhB (pink; PDB ID: 3L8H) are shown. The functionally important residues are shown and labeled in the stick model. The residues of *Bb*GmhB, Asp15, His91, Cys97, Cys99, Arg100, Lys101, correspond to the residues of *Bt*GmhB, Asp21, His97, Cys103, Cys105, Arg106, Lys107, respectively. The zinc (grey) and magnesium (violet) ions are drawn by spheres. *Ec*GmhB in this model only contains a Zn^{2+}. The dotted lines represent the missing loops of *Bt*GmhB. (B) The dimeric *Bt*GmhB (forest green and green) is compared with the *Bb*GmhB (pink). The zinc (grey) and magnesium (orange) ions are drawn by spheres. The catalytic loops, disulfide bond and metal binding residues are drawn and labeled according to where they belong. The solid box represents the area where the conformational change of CL2 from a loop to a 3_{10}-helix occurred. The zoomed view represents the area between Arg63 and Phe25 of *Bt*GmhB. The corresponding residues of *Bb*GmhB are Arg57 and Phe19, respectively. (C) The representation of the dimeric interface of *Bt*GmhB. The residues involved in dimerization are represented and labeled. One subunit is drawn with a ribbon model, and the other with an electrostatic surface model (red, negative; blue, positive; white, uncharged). The solid box represents the area around dimeric interfaces. The zoomed view represents the detailed view of dimeric contacts as discussed in the text. (D) The stereodiagram of the superimposed crystal structure of the *Bt*GmhB (green) with those of *Ec*GmhB (gold; PDB ID: 3L8E) and a HAD-like phosphatase from *M. Loti* (purple; PDB ID: 2O2X) are drawn. The proline residues stabilizing the large loop of 2O2X are labeled with their residue numbers, and they are drawn in a stick model. The residues of 2O2X, Arg135 and Lys136, and the residues of *Ec*GmhB, Arg110 and Lys111, correspond to the residues of *Bt*GmhB, Arg106 and Lys107, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

protein precipitation.

Zinc-binding motifs play an important role in protein-protein interactions (PPIs) through an intermolecular coordination of zinc ions that regulate protein assembly. Since PPIs exert a fundamental role in various biological processes such as protein sensing and signal transduction, protein engineered PPIs based on controllable leucine zipper, coiled-coil, EF-hand, and zinc-finger motifs have been performed [5]. Therefore, a discovery of noble PPI motifs is directly beneficial to the field of protein engineering.

In this study, we have refined the three-dimensional structure of GmhB from *B. thailandensis* E264 (*Bt*GmhB) of which the structure determination protocol using Global-Optimization-based and Template-based modeling of proteins (GOT) [6] was previously reported. *B. thailandensis* is a surrogate organism for studying the pathogenicity of *B. pseudomallei*. Melioidosis caused by *B. pseudomallei* is a serious invasive disease of animals and humans in tropical and subtropical regions. Its crystal structure analysis reveals an artificial dimer induced by a deformation of a short zinc-binding loop. Intriguingly, we discovered that the monomer-to-dimer transition detected in *Bt*GmhB is the reversal process of artificial proteins containing protein engineered zinc-finger