BL-5A, 17A, NW12, NE3A/2011G088, 2012G672

X-ray crystallographic structure of alkaline phosphatase derived from a moderate halophilic *Halomonas* sp.593

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1 Introduction

Halophilic proteins have unique structural characteristics: high content of acidic residues creating negatively charged surface, high reversibility of tertiary structure and activity even in high salt concentration, which make it possible to create potent adhesives for rare metal ions and harmful metal ions. As part of our structure-function studies for halophilic proteins [1,2], we succeeded in crystallization of several halophilic proteins using divalent metal ions as additives. Here, we describe a successful example of structural determination of alkaline phosphatase from *Halomonas* sp.593 (HaAP).

2 Experiment

Before crystallization, HaAP was dialyzed against 50 mM Tris–HCl (pH 8.0) containing 1 M NaCl and 2 mM $SrCl_2$. A sitting drop was prepared by mixing 0.3 µl each of the protein solution and the reservoir solution, and the resulting drop was equilibrated against 70 µl of reservoir solution. Cubic shaped crystals having diffraction-quality were obtained from Crystal Screen I No.6 (0.2 M $MgCl_2$, 0.1 M Tris–HCl (pH 8.5), 30% w/v PEG4,000) containing 15.0 mg/ml protein at 293 K.

Diffraction datasets were taken at BL-5A, 17A, NW12 and NE3A beamlines. Initial phase information for HaAP was obtained by the molecular replacement (MR) method using the program *MOLREP* in which the structure of alkaline phosphatase from *Vibrio* sp. G15-21 (PDB ID: 3E2D) was used as a search model. The modelling and refinement were carried out using programs *CNS 1.21*, *REFMAC5* and *Coot*. Determined structure of HaAP was published in PDB as 3WBH.

3 Results and Discussion

The crystal structure of HaAP was determined to 2.1 Å resolution with an R-factor of 17.7% (freeR 22.5%) in space group $P2_1$ and unit cell parameters a = 52.7 Å, b = 147.0 Å, c = 58.3 Å, = 105.2° (Figure 1a). One asymmetric unit includes two HaAP chains (A and B) comprising 497 residues per chain, 93 waters, six Mg^{2+}, four Zn^{2+}, and two Cl^-. Chains A and B in the asymmetric unit of the HaAP crystal are related by a non-crystallographic two-fold axis.

From the detailed structural investigation of HaAP, we found that the negative charge density at the molecular surface of HaAP (2.8E-03 e/A^2) is higher than those of alkaline phosphatases from other halophiles (0.6E-03 ~ 1.9E-03 e/A^2) from *E. coli* (non-halophile, 0.4E-03 e/A^2) having known X-ray structures (PDB ID: 3E2D, 2X98, 2IUC and 1ED9). From this result, it can be considered that HaAP may interact with various metal ions including rare metal ions and harmful metal ions.

We further clarified the relationship between the structure and the enzymatic function of HaAP. It is known that halophilic proteins usually lose the enzymatic activity according to the decrease in salt concentration. This is because the electrostatic repulsion caused between the side chains of acidic amino acids destabilizes a tertiary structure of halophilic protein. However, HaAP exceptionally maintains the enzyme activity in a wide salt concentration range (1 ~ 4M NaCl). The reason was partly explained by the structural study that HaAP has 37 internal hydrophobic amino acids, the number of which is larger than those of alkaline phosphatases from other halophiles (24 ~ 26) and from *E. coli* (non-halophile, 27). Moreover, we also found that HaAP has a hydrophobic cluster that may induce a substrate (phosphoester) to the active site (Figure 1b). From these observations, we concluded that highly denseness with both acidic and hydrophobic amino acids in HaAP structure is responsible for the unique structural and functional adaptation in a wide salt concentration range.

The structural information is useful to create protein absorbents for rare metals and harmful metals.

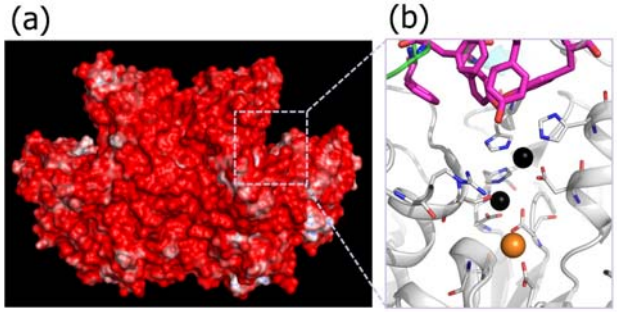

Fig. 1: (a) The molecular surface of HaAP. Negatively charged residues are colored in red. (b) The enlargement of residues in a hydrophobic cluster (bold magenta sticks) and in the catalytic site (thin grey sticks) of HaAP. Zn^{2+} and Mg^{2+} are shown by spheres colored black and orange, respectively.

References

[1] Arai S. et al., *Protein Sci.* **21**, 498 (2012).
[2] Arai S. et al., *Acta Crystallogr.* D, **70**, 811 (2014).

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