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Discovery of the cesium and strontium binding sites on β-Lactamase from a moderate halophile *Chromohalobacter* sp.560

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1 Introduction

Halophilic proteins derived from extreme halophiles and extracellular and periplasmic fractions of moderate halophiles have highly acidic protein surfaces owing to the abundant content of acidic amino acids that may interact with metal ions. The PDB coordinates of halophilic proteins include various types of metal atoms such as Na, Mg, K, Ca, Fe, Zn, Sr, and Cd, which contribute to the enzyme activity and structural stability of halophilic proteins. Because various metal ion binding sites exist on halophilic proteins, we proposed that the metal ion binding sites with an affinity to harmful metals and rare metals could be identified using X-ray crystallographic analysis in the presence of those metal ions. If metal ion binding sites with a specific affinity to harmful metals or rare metals can be found in halophilic proteins using X-ray crystallographic analysis, the structures can be used as a scaffold for constructing artificial binding sites for harmful or rare metals that could potentially serve as protein-based metal adsorbents. Adsorbents are particularly needed for Sr^{2+} and Cs^{+} because removal of radioactive Sr and Cs that leaked out from the Fukushima first Nuclear Power Plant is one of the most important social problems in Japan. To identify metal ion binding sites for Sr^{2+} and Cs^{+} on a protein molecule, we determined the X-ray crystal structure of β-Lactamase from a moderate halophile *Chromohalobacter* sp.560 (HaBLA) in this study.

2 Experiments

The wild-type HaBLA (WT-HaBLA) has Asn-Gly sequences at the 288-289th and 321-322nd residues, which cause deamidation. Because deamidation often causes heterogeneity of protein molecules in crystals and deteriorates the diffraction resolution, the N288Q-N321Q mutant HaBLA (NQ-HaBLA) was prepared and used as the standard HaBLA in this study.

The WT-HaBLA crystal was grown in 50 mM HEPES buffer (pH 7.0) containing 0.2M NaCl, 0.2 M Mg formate, 30% w/v polyethylene glycol (PEG) 3,350 (originated from PEG/ION screen I, solution No.20), and 47 mg/ml protein at 293 K. The NQ-HaBLA crystals were grown in 0.1 M MES-NaOH buffer (pH 6.5) containing 0.2 M Ca acetate hydrate, 18% w/v PEG 8,000 (originated from Crystal Screen I, solution No.46) and 30 mg/ml protein at 293 K. Moreover, the obtained NQ-HaBLA crystals were soaked in solutions containing Sr^{2+} and/or Cs^{+} in order to test their affinities for these ions. Diffraction datasets were taken at BL-5A, 17A, NW12A beamlines.

3 Results

The crystallization of WT-HaBLA and NQ-HaBLA required divalent metal ions (at least either of Mg^{2+} or Ca^{2+}). Both WT-HaBLA and NQ-HaBLA crystallized isomorphically, and both contain three HaBLA molecules in an asymmetric unit. The tertiary structures were determined to 1.8 Å resolution for NQ-HaBLA and 3.0 Å resolution to WT-HaBLA in $P3_1$ space group using X-ray crystallography. The unit cell parameters were a=b=116.2Å, c=68.0Å, α=β=90°, γ=120° for WT-HaBLA and a=b=114.9Å, c=67.6Å, α=β=90°, γ=120° for NQ-HaBLA. Based on the chelating coordination and the strength of the electron density, the locations of at least five divalent metal ions (Ca^{2+} or Sr^{2+}) and two Cs^{+} were discovered on a NQ-HaBLA molecule (Figure 1). To further distinguish Ca^{2+} / Sr^{2+} and Na^{+} / Cs^{+} in the crystal structure, now we are attempting to detect Sr^{2+} and Cs^{+} in the NQ-HaBLA crystal using the anomalous diffraction data.

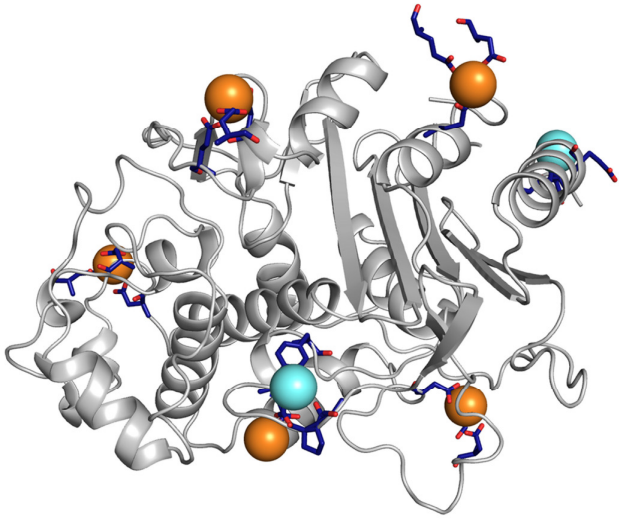

Figure 1: Sr^{2+} and Cs^{+} binding structure of NQ-HaBLA, in which Sr^{2+} and Cs^{+} observed around three NQ-HaBLA molecules in an asymmetric unit are integrated into the single molecule of NQ-HaBLA. The orange and blue balls show Sr^{2+}, and Cs^{+}, respectively. The residues recognizing Sr^{2+} and Cs^{+} are shown by sticks.

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