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Crystal structure of toxin HP0892 from *Helicobacter pylori* with two Zn(II) at 1.8 Å resolution

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1 Introduction

Toxin-antitoxin (TA) systems have been shown to help bacteria and archaea survive in conditions of environmental stress by modulating the global level of biological processes such as translation and DNA synthesis. In healthy survival conditions, the antitoxin forms a tight complex with the toxin, neutralizing the cytotoxic effect of a toxin, and the complex remains in a stable dormant stage. However, a change in temperature, oxidative stress conditions or nutritional deprivation give rise to stress conditions, which trigger the host proteases, enabling them to degrade the structurally unstable antitoxin more rapidly than the toxin. The unbound or free toxin in the cell causes cytotoxic activity resulting in the inhibition of cellular processes, leading to cell death. This process is commonly known as post-segregational killing. Although the first proposed role of these TA systems was to arrest cell growth, enabling bacteria to survive in unfavorable environmental conditions, many other functions have also been reported, such as gene regulation, persistence and programmed cell death. Because of the direct involvement of TA systems in cell death, these systems have been targeted in the search for alternative antibiotics against multidrug-resistant bacteria. Given the vast activity of these TA systems, very little is known about the TA systems of *H. pylori*. For instance, the involvement of general acid and/or general base residues in a trigonal bipyramidal transition state, which is required for efficient RNase activity, as seen in *E. coli* RelE toxins, has not been discussed in detail for HP0892. The present study was undertaken to investigate the effect of metal ions on toxins, specifically the HP0892 toxin from HP0892-HP0893 toxin-antitoxin system, and its interaction with the substrate-binding residues. In addition, we investigated the binding of zinc metal ions with the HP0892 toxin through NMR perturbation, circular dichroism and isothermal titration calorimetry (ITC). Taken together, the data from these experiments reveal the location of binding sites of zinc ions and the nature of their interaction with the HP0892 toxin.

2 Experiment

The expression plasmid of wild type HP0892 and its mutants H86A, E58A, E58A/H60A were constructed using vector pET21a(+). The crystals of metal (zinc) bound wild type HP0892 were grown using hanging-drop vapour-diffusion method. 1 μl of protein solution was mixed with an equal volume of crystallization buffer on a siliconized cover slip, and the mixture was equilibrated over 500 μl of reservoir solution at 4 °C. Zinc-bound HP0892 was crystallized in 20% w/v polyethylene glycol (PEG) 3350 and 0.2 M lithium sulfate monohydrate in 30 days. The cubic-shaped crystals grew to the largest dimension of 0.1 x 0.1 x 0.1 mm. A suitable cryoprotectant was determined to be the mother liquor supplemented with 20% v/v glycerol, and the crystals were flash-frozen in liquid nitrogen before data collection. Crystals of zinc-bound HP0892 belong to the space group P21 with unit cell parameters of a = 36.01, b = 47.03, and c = 52.60, and $\alpha = \gamma = 90.0°$, and $\beta = 110.0°$. Two monomers of zinc-bound HP0892 are present in each asymmetric unit, with a calculated crystal volume per protein weight (V_M) of 2.01 $Å^3Da^{-1}$ and a solvent content of 38.8%. Two zinc atoms in each monomer of zinc-bound HP0892 was located.

3 Results and Discussion

The apo HP0892 (NMR structure, published earlier) (green color, Fig. 1) was compared with the zinc-bound crystal structure of HP0892 (blue color, Fig. 1, this paper), and the reorientation was observed for His47, Glu58, His60 and His86. The observed residues were also seen perturbed in NMR perturbation experiments. The isothermal calorimetry studies were carried out on wild type HP0892 and on the mutational proteins H86A HP0892, E58A HP0892 and on a double mutational protein E58A/H60A HP0892, and the binding affinity of zinc with the protein was monitored. All of the results showed the involvement of His47, Glu58, His60 and His86. Through the structural difference between the apo and the metal-bound state, and using a homology modeling tool, the involvement of the metal ion in mRNase active site could be identified. The most catalytically important residue, His86, reorients itself to exhibit RNase activity. His47, Glu58, and His60 are involved in metal binding where Glu58 acts as a general base and His47 and His60 may also act as a general acid in enzymatic activity. Glu58 and Asp64 are involved in substrate binding and specific sequence recognition. Arg83 is involved in phosphate binding and stabilization of the transition state, and Phe90 is involved in base packing and substrate orientation.

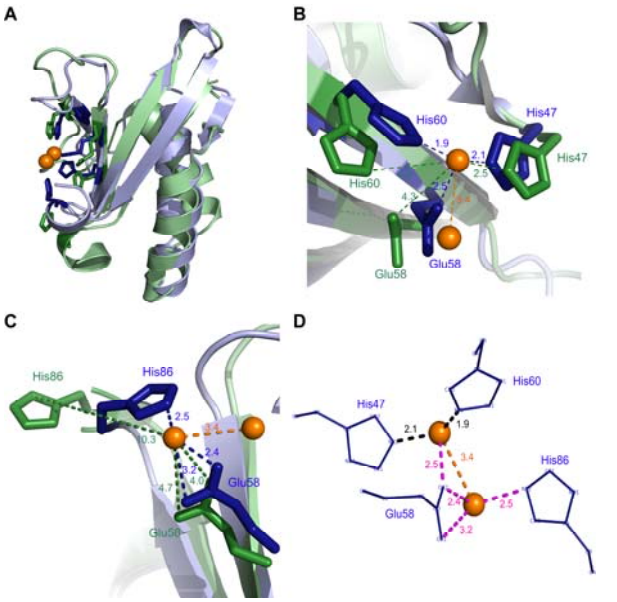

Fig. 1: Zinc-bound state of HP0892. (A) The apo- and zinc-bound HP0892 were superimposed for comparison. Both structures show a similar fold. (B and C) His47, Glu58, His60, and His86 in the zinc-bound (blue, zinc in orange) and in apo-states (green). The interaction of one zinc ion is shown in (B), and the interaction of second zinc ion is shown in (C). The distances of the interacting atoms are shown in corresponding colors. (D) Simplified and magnified image of the side chains of all interacting groups HP0892 with the zinc ions. The distances are divided into two color groups for ease of understanding.

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References

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