NUCLEASE DOMAINE OF COLICIN E7 UNDER HEAVY METAL

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Introduction

Colicins are a group of protein antibiotics as toxins produced by strains of Escherichia coli in the extracellular medium during environmental stress as part of their defense system. [1] Colicin-producing bacteria are resistant to the action of their own colicin by a small immunity protein that binds and inactivates the cytotoxic domain of colicin. Colicins are classified into two groups according to the cell surface receptor. [1] Type E colicins bind to the protein product of the chromosomal gene btuB.[2] The ~15 kDa C-terminal DNase domain of colicin E7 is translocated into the cytoplasm, followed by hydrolysis of the chromosomal DNA of target cells.[4] NColE7 is a metal-dependent enzyme that binds a single divalent metal ion and it has three domains.[3] The core of NColE7 active site is the 32 amino acids HNH motif located at the C-terminus of the enzyme, which envelops the bound metal ion by three His residues. H544, H569, and H573 constitute the amino acids of the HNH motif and exhibit a characteristic secondary structure (Fig.1). The H545 histidine side-chain is suggested to participate in the generation of the OH^- ion nucleophile by promoting the deprotonation of the catalytic water molecule [5, 6]. Several crystal structures demonstrated that the metal ion can also bind a water molecule [7, 8], or a phosphate [5, 7, 9] or a sulphate [7,10] anion or the scissile phosphodiester bond of the substrate DNA [17,18] via the fourth binding site in the tetrahedral geometry. Previous and recent structural studies showed that the active site of colicin DNases is the HNH motif present also in homing endonucleases. This motif binds a single transition metal ion such as Zn^{2+} ion. Some environmental heavy transition metal ions may replace Zn^{2+} leading to change of the structure and catalytic properties of this enzyme. Ni^{2+} ion can remain from the purification procedure by immobilized metal ion chromatography through oligohistidine tags, Cu^{2+} is a strong Lewis acid and has high affinity to the His residues, and Cd^{2+} was selected as analogue of Zn^{2+}.

Experimental

The pGEX-6P-1 vector with an inserted mutant of NColE7-R447G- was constructed, NColE7-KGNK protein expressed and purified with N-terminal Glutathione-S-Transferase (GST) fusion according to previous described work [11]. After the protein purification, the GST tag was removed according to the described method in [11]. The buffer of the purified protein was exchanged to 20 mM N-2-hydrox-yethylpiperazine-N-2-ethane sulfonic acid (HEPES) pH 7.7 by an Amicon ultra 15 mL centrifugal filter. The purification steps were monitored via 15% (w/v) sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) using a mixture of 116, 66.2, 45, 35, 25, 18.4 and 14.4 kDa unstained proteins as a marker. Intact MS protein analysis was performed by an LTQ-Orbitrap Elite mass spectrometer coupled with a TriVersa NanoMate (Advion) chip-based electrospray ion source. MS measurements were performed in positive mode at 120,000 resolution, The protein concentration was 3.0 μM in 2.5 mM ammonium bicarbonate buffer (pH ~7.8) with various metal ions (added as $ZnCl_2$, $CuCl_2$,

$NiCl_2$ or $CdCl_2$) at different molar ratios. Circular dichroism (CD) spectra were recorded at room temperature utilizing a Jasso J-1500 CD spectrometer using the following parameters. Wavelength range: 280-180 nm; path length: 0.2 mm (Jasco cuvette); D.I.T.: 2 sec; bandwidth: 1.0 nm; scanning speed: 50 nm/min (continuous scanning mode); each spectrum was the average of three accumulated measurements. The concentration of the enzyme was 18.0 μM in 3-10 mM HEPES, pH 7.7. The measurements were carried out with Apo-enzyme and holo-enzyme with various metals salts ($ZnCl_2$, $CuCl_2$, $NiCl_2$ and $CdCl_2$) with various molar ratio. The catalytic activity of the NColE7-KGNK mutant protein was monitored against plasmid DNA (pUC119) as a substrate. The final concentration of the enzyme was 1.0 μM, while the DNA was 74 μM for base pairs in 20 mM HEPES, pH 7.7. The DNA cleavage was performed in apo and holo forms of different metal ions of Zn^{2+}, Cu^{2+}, Cd^{2+} and Ni^{2+} at various molar ratios. The reaction mixtures were incubated at 37 °C for various periods. Aliquots of 5 μL of the reaction mixture were taken four times and the reaction was stopped by adding 5 μL of 2% (w/v) SDS solution (1% (w/v) at final concentration). The products of the DNA cleavage assays were checked via agarose gel electrophoresis (AGE). The products were run in 1% (w/v) agarose gel containing 0.5 μg/mL ethidium bromide for the visualization of the DNA.

Results and Discussion

The interaction of the NColE7-KGNK mutant protein with various transition metal ions was monitored via circular dichroism (CD) spectroscopy and MS spectrometry. As well the effect of transition metal ions on the catalytic activity of the enzyme was investigated in aqueous solutions. The Zn^{2+} ion is the native metal ion for the enzyme, but we used other transition metal ions in this work, like Ni^{2+} ion because it is the most common component of the immobilized metal ion affinity chromatography (IMAC) technique for protein purification, Cu^{2+} ion is a strong Lewis acid which could strongly compete for histidine side-chains, and Cd^{2+} ion has a d^{10} electron system in analogy to Zn^{2+} ion. The binding of Zn^{2+} ion to the apo-enzyme causes a slight but significant change in the CD spectrum, reflected in a red shift of about 2 nm and, this agreed with previous measurements. [11,12] However, addition of one equivalent of non-endogenous metal ions like Ni^{2+}, Cu^{2+}, or Cd^{2+} ions to the apo-protein caused very similar changes in the CD spectra to that related to the effect of Zn^{2+} ion. In the mass spectra two main peaks were detected upon measuring the Apo-NColE7-KGNK protein without the addition of metal ions: one with ~20% relative intensity related to that of the mono-metallated KGNK with Zn(II) and one with ~80% relative intensity. This means that the apo-enzyme easily acquires metal ions from buffers/reagents/containers/sample holders applied during the experiments. The apo-enzyme coordinated the added Zn^{2+} ions, as well as the non-endogenous metal ions (Ni^{2+} and Cd^{2+}) in the active center in mono-metallized enzyme. However, one equivalent of Cd^{2+} ions could not saturate the available Apo-protein fully; a small peak appeared related to the Apo-enzyme. In addition, Ni^{2+} ions could bind to the apo-enzyme, but they could not replace the Zn^{2+} ions, and there was ternary complexes including two different metal ions bound to the protein. Cd^{2+} ions proved to be weaker interacting agents than Ni^{2+} ions. It was difficult to prove whether the Cu^{2+} ion could replace the Zn^{2+} ion in the apo-enzyme via MS because of the small difference in the atomic weight.

The DNase activity of NColE7-KGNK was studied using pUC119 plasmid DNA as a substrate via agarose gel electrophoresis. DNA substrate mainly contains the super-helical form of the plasmid; during the cleavage first, the open circular form appears as a consequence of single-strand nicks. Then, linear DNA is formed upon double-strand cleavage. The DNA solution may contain a residual concentration of Zn^{2+} ions. The addition of one equivalent of Zn^{2+} ions, surprisingly resulting in a less active enzyme compared to the “apo” form. Supplementing one equivalent of Cu^{2+} ions to the protein provided similar behavior to that of Zn^{2+} ions. More

efficient DNA cleavage is observed in the presence of Cd^{2+} ions than with Zn^{2+} or Cu^{2+} ions and less than that of the apo-protein in the absence of added metal ions. The enzyme in the presence of Ni^{2+} ions showed much higher activity than the apo-protein and in presence of Zn^{2+} used as a control. Nevertheless, it could not replace the Zn^{2+} ion for the active center.

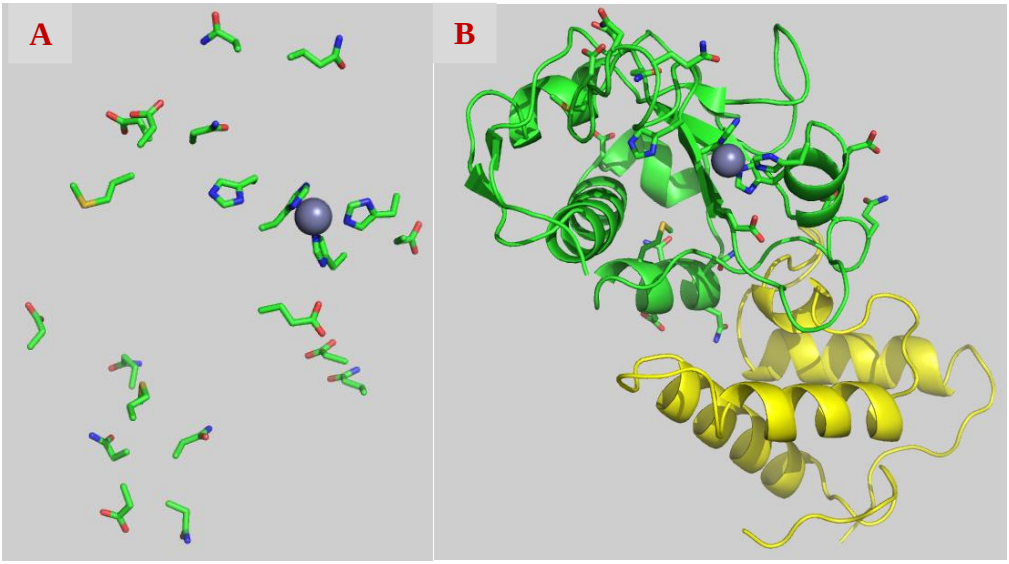

Fig. 1: Sequence and structure of the *E. coli* NColE9 (PDB code: 7CEI). A: The relative positions of the side-chains of the HNH motif and putative metal ion binding residues. B: The cartoon representation of the crystal structure of NColE9-Im9 complex with the putative metal ion binding sites were detected by the X-ray diffraction method.

Conclusion

The colicin E7 was studied in this project in the presence of non-endogenous metal ions such as Ni(II), Cu(II) and Cd(II), which may originate from environmental pollution. The purpose of use of the mutated NColE7 is the decreased activity to monitor the catalytic process. CD and mass spectrometric results revealed that all of the metal ions used in this study bound to the active center of the enzyme in the absence of Zn(II). In another word, they could not compete with Zn(II) in binding to the active site. It was detected that the enzyme is very active in the presence of tiny amount of metal ion. On the other hand, there is very high activity of the enzyme in the presence of Ni(II) and Cd(II) ions which could not be inhibited by the excess of the metal ion, but it was inhibited by Zn(II). These results suggest that the presence of these non-endogenous metal ions like Ni(II) and Cd(II) ions in the environment may lead to highly efficient toxic bacteria that can digest the other bacterial cells faster from the native one leading to biological contamination.

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