PROPERTIES OF TEM-1 β-LACTAMASE UNDER HEAVY METAL POLLUTION CONDITIONS

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Introduction

β-Lactamases protect bacterial carboxy-transpeptidases from β-lactam antibiotics by hydrolyzing the β-lactam ring before reaching its targets [1]. Two systems of β-lactamase classification have been used; sequence information based system was introduced by Ambler [2], while the more recent activity based system was invented by Bush-Jacoby-Medeiros [3]. Accordingly, four classes are termed A, B, C, and D recognized by difference in hydrolytic activity. The active site of classes A, C, and D contains a Ser-Xaa-Xaa-Lys motif, therefore these are called serine β-lactamases. Class B is a heterogeneous group of zinc metalloenzymes, called metallo-β-lactamases [5]. The Ser-Xaa-Xaa-Lys motif employs Ser as nucleophile hydrolyzing β-lactams by forming covalent acyl-enzyme intermediate, while metallo-β-lactamases utilize Zn(II) activated water molecules as the nucleophile for hydrolysis of β-lactams [4]. As a source of development of bacterial antibiotic resistance class A TEM-1 β-lactamase is a frequent target of scientific research. Although TEM-1 β-lactamase is not a metalloenzyme, we could purify this protein by chelating to Ni(II)-loaded sepharose column[5]. This suggested that the enzyme can bind metal ions, which may influence its structure and catalytic activity. Therefore, our aim was to purify the enzyme and study the effect of metal ions, potentially present as heavy metal pollutants in the environment.

Experimental

The purity of TEM-1 β-lactamase was determined using 12-15% (w/v) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) with a mixture of seven unstained proteins (116, 66.2, 45, 35, 25, 18.4, and 14.4 kDa) as molecular markers (Thermo Scientific #26610), while the concentration of TEM-1 β-lactamase was determined by Cary 3500 UV–Vis double beam, multicell spectrophotometer at 25 °C controlled temperature (Agilent Technologies) in the 210–300 nm wavelength interval in a quartz cuvette (Hellma) with 1 cm path length. CD spectra were recorded at room temperature by a Jasco J-1500 CD spectrometer. The parameters were adjusted as described in the following. Wavelength range: 300 – 180 nm; path length: 0.2 mm (Hellma quartz cuvette); D.I.T.: 2 sec; bandwidth: 1.0 nm; scanning speed: 50 nm/min (continuous scanning mode). Intact protein mass measurement and top-down protein analysis was carried out on a Nanomate TriVersa (Advion) chip-based nanoelectrospray ion source coupled to a linear trap quadrupole Orbitrap Elite mass spectrometer. The protein concentration was 2.0 μM in each individual sample which contained 0, 2, 5 and 10 equivalents of $NiCl_2$. The samples were buffered at pH 7.8 with a solution containing 50 mM ammonium bicarbonate/NH_3 (aq). TEM-1 β-lactamase activity was assessed by UV absorption spectrometry via hydrolysis of series ampicillin concentrations (230 μM and 930 μM) as substrate (SERVA Electrophoresis GmbH) in 1 ml 20 mM Tris-HCl, pH 7.5.

Results and discussion

TEM-1 β-lactamase was purified by immobilized Ni(II) ion affinity chromatography, based on its metal ion binding sites without denaturation or fusing affinity tags [5]. There are several amino acids (His, Glu, Asp, Ser) in the protein behaving as potential binders to NTA-Ni(II)-resin (Fig. 1). The investigation of the Ni(II) binding of the purified enzyme by mass spectrometry, we could detect up to three bound metal ions in the presence of increasing Ni(II) excess. However, the main peak in the mass spectra of Ni(II) TEM-1 β-lactamase was assigned to the monometalated enzyme at each applied metal-to-enzyme ratio. Protein binding to two Ni(II) ions appeard at 5:1 ratio and its intensity slightly increased at 10:1 Ni(II):protein molar ratio. Traces of the protein bound to three Ni(II) were also detected. TEM-1 β-lactamase may coordinate more strongly to the first Ni(II) through H151, H156 His pair at ~3.5 Å distance. The next binding site is weaker and the third site barely binds the metal ion. Interaction of TEM-1 β-lactamase with Ni(II) was also proven by the successful purification by chelating to Ni(II)-loaded sepharose column. Only a slight change was observed in the CD spectra on increase of the Ni(II) content. TEM-1 β-lactamase most likely could bind more than one Ni(II) ion, but with low affinity and without a significant change of the secondary structure. One Ni(II) can bind to H151 and H156 or H94, and H110, or H24 and H285. Excess of Hg(II) caused a more significant change in the CD spectrum, but still not a large change in the secondary structure. Hg(II) may bind to C75 and C121, which are close to the active center of the enzyme. Sulphur donor groups of soft character from methionines (Fig. 1) close to the active centre may also support binding of Hg(II). The specific activity of the enzyme decreased by ~50% in presence of Hg(II). Future mass spectrometric measurements are planned to reveal the Hg(II) binding sites.

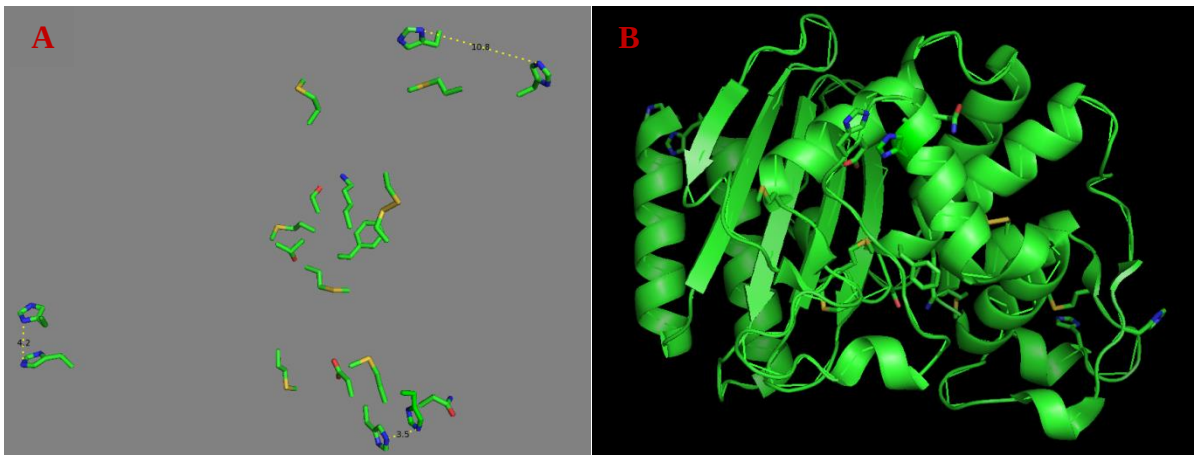

Fig. 1: Sequence and structure of the *E. coli* TEM-1 β-lactamase (PDB code: 1zg4) [6]. A: The relative positions of the side-chains of the SXXK motif and putative metal ion binding residues. B: The cartoon representation of the crystal structure of TEM-1 β-lactamase with the putative metal ion binding sites and the SXXK motif shown by sticks, 24–286 residues were detected by the X-ray diffraction method.

Conclusion

The metal binding sites of TEM-1 β-lactamase can be a good candidate to change the chemical structure of the enzyme. The CD spectra of metalized TEM-1 β-lactamase with Ni(II) and Cd(II) only slightly differed from that of the free protein suggesting that the available binding sites can coordinate to Ni(II) or Cd(II) individually without change in the secondary structure. Nevertheless, the affinity towards Ni(II) and Cd(II) significantly decreases with the binding of

the first, second and third metal ion, as confirmed by mass spectrometric measurements. Excess of Hg(II) caused a more significant change in the spectrum, but still not a large change in the secondary structure. At the same time, the presence of Hg(II) caused significant inhibition the efficiency of enzyme in hydrolysis of β-lactams by ~ 50%. This result suggests that the heavy metal ions increase the toxicity of the antibiotics against bacteria, which effect may be further enhanced by the toxic property of the metal ions themselves.

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