THE EFFECT OF THE METAL IONS ON THE CATALYTIC ACTIVITY OF TEM-1 β-LACTAMASE

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Abstract
The kinetic parameters of TEM-1 β-lactamase promoted ampicillin hydrolysis were revisited. The hydrolytic process was described by the kinetic analysis of the set of catalytic progress curves recorded at multiple wavelengths, including various concentrations of the enzyme and substrate. The primary hydrolysis product of ampicillin was converted to a second product, needed also to be considered in the evaluation process. Thus, instead of the usual Michealis-Menten formalism, the full set of the stoichiometric and rate equations were used in the calculations. We showed that the catalytic activity of TEM-1 β-lactamase was slightly decreased by the gradual increase of concentration of Hg(II). These catalytic progress curves could be fitted allowing the change of the active enzyme concentration, yielding the same kinetic parameter values as in the absence of Hg(II). However, we have found that Ni(II) and Cd(II) ions slightly promoted the enzyme activity. We suggest the binding of these metal ions to the β-lactam ring of antibiotics activating the substrate for the nucleophilic attack by the enzyme.

Introduction
Some bacteria are resistant to *β*-lactam antibiotics due to the expression of enzymes like TEM-1 β-lactamase. The enzyme consists of 286 amino acids, but 24-286 residues were detected by the X-ray diffraction method reflecting the mature form of the enzyme [1,2]. Ser70 is employed as a nucleophile hydrolyzing *β*-lactams while metallo-*β*-lactamases utilize a Zn(II)-activated water molecule as a nucleophile. TEM-1 β-lactamase is not a metallo-enzyme, but it contains several putative metal ion binding sites in its amino acid side-chains: three histidine pairs, two cysteines forming disulfide bridge, nine methionine residues, and other negatively charged side chains such as Asp, Glu residues. The success of the purification procedure by immobilized Ni(II) ion affinity chromatography proved that the protein can bind e.g., Ni(II) in its native form. In other words, the three pairs of His residues on the TEM-1 β lactamase surface allowed for binding to the borderline transition metal ion such as Ni(II) [3]. The two Cys residues of the mature TEM-1 β-lactamase close to the active center are involved in a structural disulfide bridge [4] that rarely interacts with metal ions, with the exception of Hg(II) [5]. In addition, TEM-1 β-lactamase the sulfur donor atoms in the thioether groups of Met residues can also bind Hg(II) ion and more weakly Cd(II) and Ni(II) [6-9]. The catalytic activity of the enzyme was assessed in the absence and presence of metal ions like Ni(II), Cd(II), and Hg(II) against the ampicillin substrate. Interaction of TEM-1 β-lactamase with some metal ions Ni(II), Hg(II), and Cd(II) is our target of scientific research due to developing efficient antibiotics, as well as their environmental effects.

Experimental

Two buffers (20 mM Tris-HCl, pH 7.5; and 50 mM phosphate buffer, pH 7.0) were applied in the process of determination of kinetic parameters The purified TEM-1 β-lactamase or metallized TEM-1 β lactamase catalyzed the hydrolysis of ampicillin in the substrate concentrations range of 230 µM – 930 µM. The final concentration of purified enzyme was between 0.24 - 2.4 nM with and without metal ions ($HgCl_2$, $Hg(ClO_4)_2$, $NiCl_2$, $CdCl_2$) at various protein:metal ion molar ratios. The kinetic reactions were monitored using a Cary 3500 UV-Vis double beam 51 multi-cell and controlled temperature spectrophotometer at two temperatures (25 ºC and 30 ºC) at multiple wavelengths between 210 – 235 nm, with 1.0 cm path length quartz cuvette (Hellma). The kinetic calculations were performed using ChemMech program. This program was developed specifically to fit more experimental curves simultaneously in a single run. The absorbance versus time curves can be calculated by the Beer-Lambert equation with an appropriate set of molar absorbance values. The experiments were supplemented by cell culture investigations at various metal ion concentrations.

Results and discussion

Complexation of TEM-1 β-lactamase by a metal ion may cause changes in the catalytic efficiency of the enzyme in the hydrolysis of β-lactam molecules. No significant changes were detected in the absorbance at 235 nm for the ampicillin solution without the enzyme but in the presence or absence metal ions upon incubation for several hours. The primary hydrolysis product was further converted into second product depending on the pH and temperature.

The catalytic activity of TEM-1 β-lactamase was slightly reduced by the gradual increase the Hg(II) ion concentration. However, this led to the same parameter values as in the absence of Hg(II) ion. This suggests that only the V_{max} values decreased when molar ratio of the Hg(II) ion and the enzyme increased, i.e., that Hg(II) ions did not affect the substrate binding property of the enzyme in a competitive manner, but they affected the concentration of the catalytically effective enzyme, thereby blocking a fraction of the enzyme molecules.

Ni(II), as a borderline transition metal ion, and Cd(II), which has a moderately soft character, probably could bind to the His residue pairs that are positioned on the surface of the enzyme. The experiment containing 10× excess of Ni(II), and Cd(II) as compared to the apo-enzyme itself, revealed that in contrast to the Hg(II) containing catalytic system, the presence of Ni(II) or Cd(II) ions slightly promoted the catalytic activity of TEM-1 β-lactamase (**Fig. 1**).

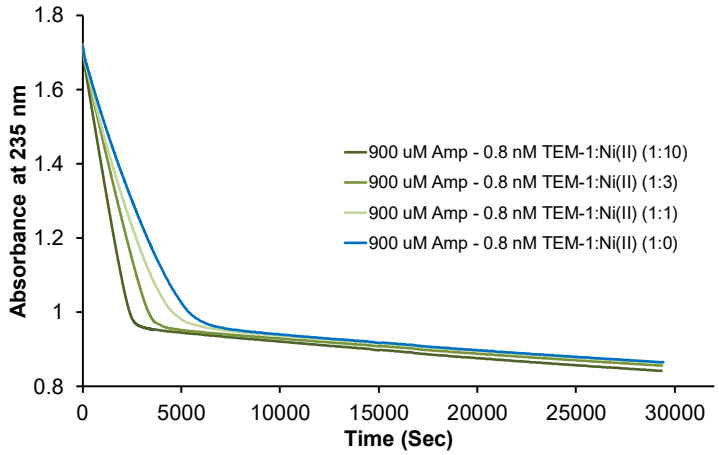

Figure 1. The effect of Ni(II) on the hydrolytic process of ampicillin catalyzed by TEM-1 β-lactamase in 50 mM phosphate buffer (pH 7.0) at 30ºC.

These His residue pairs are far away from the active center, so this could not account for a direct effect of Ni(II) and Cd(II) on substrate-enzyme complex in the active center. On the other hand, these metal ions are also supposed to bind ampicillin close to the β lactam ring via nitrogen and oxygen donor groups [11;12]. It is well known that the Lewis acid property of metal ions may promote the hydrolysis processes of the β-lactam ring by a different mechanistic pathway [13;14]. Therefore, we suppose that the hydrolytic efficiency may be enhanced by Ni(II) or Cd(II) ions, by binding to the antibiotics with a concomitant deactivation or activation of the substrate for the nucleophilic attack by Ser70 residue of the enzyme [11;15]. At the same time, Hg(II) is supposed to bind to the sulfur donor atom of a soft character (**Fig. 2**)

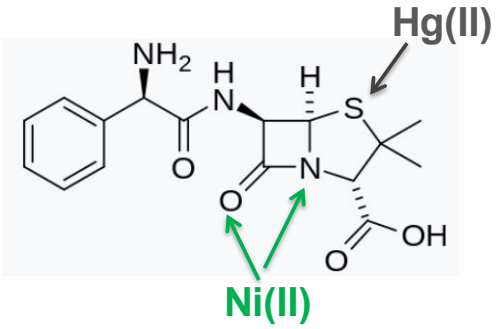

Figure 2. The proposed binding sites of metal ions to ampicillin.

The above results were well supported by the cell culture experiments, which also reflected the activation of the enzyme by Ni(II), and inhibition by Hg(II).

Conclusion

TEM-1β-lactamase activity was monitored by spectrophotometry via hydrolysis of ampicillin as a substrate. Continuous decrease of the measured values was experienced upon recording the absorbances for an extended period suggesting that the primary hydrolysis product was further converted into different molecules depending on the pH and temperature. Ni(II) and Cd(II) slightly promoted the enzyme activity but it was slightly reduced by the gradual increase of Hg(II). We suggested the coordination of the metal ions to antibiotic doner groups. This coordination in case of Ni(II) and Cd(II) occurs through the beta lactam O and N donor atoms, which causes activation of the substrate. Hg(II) shall bind to the S donor atom. The observed changes in the hydrolytic activity in the presence of Hg(II) could not be explained only by the metal ion interaction with the substrate, but complexation with the methionine S atoms close to the active center shall also be considered. Hg(II) in fact, influences the concentration of the catalytically effective enzyme possibly by blocking a fraction of the enzyme molecules.

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