DOI: 10.20396/revpibic2720192296

XXVII Congresso de Iniciação Científica Unicamp
16 a 18 de outubro de 2019 - Campinas | Brasil

In vitro penicillinase activity of β-lactamase OXA-143(P227S): pH effect

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Abstract

Antibiotic resistance is a global threat. The Class D β-lactamase OXA-143 has been described as an efficient penicillinase, oxacillinase, and carbapenemase. This Class of enzymes has an unusual lysine (K84) that in the carbamylated form acts as a general base. Recent studies performed in our laboratory showed that P227S is a positive mutation within the evolutionary pathway of the OXA-143 sub-family towards a more efficient carbapenemase. Once the mutant OXA-143(P227S) is an evolved version of OXA-143 it is important to analyze the effect of the buffer for the kinetic in vitro assays. Therefore, as the carboxylated lysine acts as a general base in the proposed covalent catalysis mechanism, in this study, we performed assays to investigate the effect of the pH in the structure and catalytic efficiency of the mutant OXA-143(P227S). Our results show a direct pH dependence on penicillinase activity, with an optimal range from pH 7 to 9. In addition, our findings also suggest the exchange of two hydrogens during the reaction with ampicillin. Furthermore, it was also observed a direct correlation between the catalytic efficiency in this pH range and the enzyme thermal stability.

Key words:

Antibiotic resistance, Carbapenem-hydrolyzing Class D β-lactamase, enzyme kinetics.

Introduction

Bacterial resistance is a global threat and the production of β-lactamases, enzymes capable of hydrolyzing ß-lactam antibiotics, is the main mechanism of resistance against this class of antibiotics. Carbapenem-hydrolyzing Class D ß-lactamases (CHDLs) confer the clinically challenging Gram-negative ROD genus *Acinetobacter baumannii,* bacterial resistance to carbapenems (“*last resort”* antibiotics). OXA-143 was isolated in Brazil and first described in 2009 as an efficient penicillinase, oxacillinase, and carbapenemase. The P227S mutation is localized in the conserved loop *β*5-*β*6 and has been described as a case of evolutionary convergence for homologous OXA enzymes OXA-160 and OXA-225.[1,2,3] Recent studies performed in our laboratory showed that P227S is also positive mutation within the evolutionary pathway of the OXA-143 sub-family towards a more efficient carbapenemase. In this study, we focus our investigation on the effect of the pH in the structure and catalytic efficiency of the *mutant* OXA-143(P227S).

Results and Discussion

Enzymatic assays against ampicillin were performed varying the pH from 4.0 to 11.0 and all buffers had controlled ionic strength of 0.15 M. The reactions followed Michaelis-Menten equation. The logarithm of the catalytic efficiency (k_{cat}/Km) *versus* pH presented as a bell-shaped curve distribution (Figure 1). This pattern indicates the exchange of two hydrogens during reaction at pH=pK_{E1}=6.8 and pH=pK_{E2}=9.0. Furthermore, Figure 1 shows that the optimal condition to work with this enzyme is in the pH range from 7.4 to 9.0.

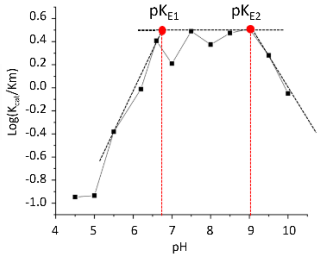

Figure 1. Ampicillin degradation by OXA-143(P227S): log(k_{cat}/Km) *versus* pH.

Regarding how the pH affects the enzyme secondary structure, our circular dichroism results indicate that the OXA-143(P227S) has a similar alpha-helix percentage in all pH conditions, except for pH 4. In this condition, the enzyme was unstable and presented a random coil profile as can be observed in Figure 2a (black squares). In general, it was observed an increase of molar ellipticity and melting temperature with pH (Figures 2a and b**)**, indicating that the mutant OXA-143(P227S) is more stable in neutral and alkaline conditions. The absence of structure at pH 4 is the most probable explanation for the lack of enzymatic activity under acidic condition.

a) **b)**

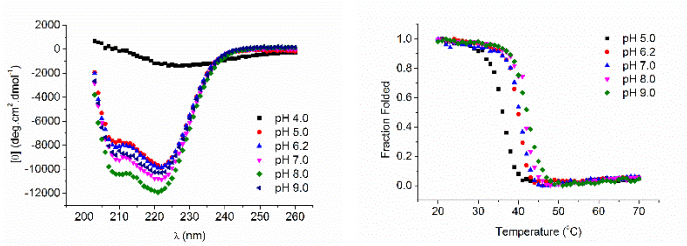

Figure 2. a) CD spectra of OXA-143(P227S) secondary structure at 20 °C and **b)** Thermal induced unfolding of OXA-143(P227S) monitored at 222 nm.

Conclusions

Our results indicate that the mutant OXA-143(P227S) has an optimal catalytic efficiency in the range of pH 7 to 9. In addition, the results suggest the exchange of at least 2 hydrogen atoms during the enzymatic reaction. Furthermore, the pH has a direct effect on the enzyme structure and stability.

Acknowledgement

FAEPEX (519.292, 3095/1 e 2391/18); CAPES; FAPESP; IQ-UNICAMP.

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