OTR9 Effect of zinc on the structural stability of pneumococcal surface antigen A (PsaA)

Izabella Buty da Silva Corrêa[1], Ana Paula Dinis Ano Bom[1], Ana Paula Correa Argondizzo[1], Ariane Larentis[2], Marco Alberto Medeiros[1], José Godinho da Silva Junior[1]

[1] Bio-Manguinhos, Fiocruz, Rio de Janeiro, Brazil
[2] ENSP, Fiocruz, Rio de Janeiro, Brazil

Introduction: The *Streptococcus pneumoniae* is the most common human respiratory pathogen responsible for at least million of deaths worldwide annually. *S. pneumoniae* has two types of virulence determinants, the heterogenic capsule and the surface proteins. PsaA protein (pneumococcal surface antigen A) is a virulence factor of *S. pneumoniae* that belongs to the manganese and zinc bacterial transport system. The manganese PsaA binding has been associated with oxidative stress resistance which becomes a pivotal element in the proliferation and virulence of the bacteria. It has been shown that the excess of zinc promote bacteria toxicity since zinc inhibits the acquisition of manganese.

Objective: We have performed a conformational and stability analysis of PsaA recombinant protein in the presence (Zn-PsaA) or absence of zinc (free-PsaA), aiming to understand how zinc homeostasis impacts on the host protection and bacteria toxicity.

Methodology: In this study, PsaA was isolated and analyzed by SDS-PAGE-12%. The structural characterization of PsaA (0.08 mg/mL) was analyzed by fluorescence spectroscopy. We performed a test in the presence of increasing concentrations of zinc (5-500 µM) to determine the metal minimum concentration which induces a conformational change. The (Zn-PsaA or free-PsaA) protein stability was observed in the presence of different Urea (1–9 M), Guanidine (GdmCl) (1–7 M) or salt concentrations (25-500 mM). Moreover, the samples were analyzed in the pH range (2.6 at 8.0) or treated with temperatures from 25oC to 85oC.

Results: The experiments in the zinc presence demonstrated that the PsaA conformational changes started at 5 µM until 50 µM metal concentration. After 50 µM the structural modifications were stabilized. The free-PsaA and Zn-PsaA protein presented different unfolding profiles upon chemical denaturation. Conformational change for free-PsaA occurs at 1.0 M Urea or 0.5 M of GdmHCl. In contrast, the Zn-PsaA unfolding occurs in higher concentrations of Urea (4 M) or GdmHCl (1.5 M)**.** In the free-PsaA or Zn-PsaA heat denaturation process we verified conformational changes at 43ºC and 73ºC. It was demonstrated by light scattering that PsaA aggregation is inhibited by 50 µM zinc concentrations. To evaluate the PsaA hydrophobic surfasse

exposure we used bis-ANS probes, but these experiments showed no significant differences between the free or zinc bound protein. Moreover, the pH experiments showed a decrease of PsaA fluorescence intensity in acid pH when compared to neutral and basic pH, both in the zinc presence and absence. The free-PsaA, when submitted to salt concentrations, presented a higher center of mass variation when compared to Zn-PsaA.

Conclusion: Our data shows that free-PsaA has a lower stability if compared to the Zn-PsaA protein when they are subjected to physical or chemical denaturation. This study may contribute to elucidate the mechanism of colonization or inhibition of proliferation dependent of metal in the bacteria.

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