

## **OTR9 - STRUCTURAL CHARACTERIZATION OF PNEUMOCOCCAL SURFACE ANTIGEN A (PsaA) OF STREPTOCOCCUS PNEUMONIAE**

Izabella Sodré Buty da Silva<sup>1</sup>, Ana Paula Dinis Ano Bom<sup>1</sup>, Ana Paula Correa Argondizzo<sup>1</sup>, Ariane Leite Larentis<sup>1</sup>, Marco Alberto Medeiros<sup>1</sup>, José Godinho da Silva Junior<sup>1</sup>

<sup>1</sup> Bio-Manguinhos, Oswaldo Cruz Foundation, Technological Development, Rio de Janeiro, Brazil

**Objectives:** *Streptococcus pneumoniae* bacteria is responsible for many severe diseases in humans. Pneumococcal surface antigen A (PsaA) is a virulence factor of *S. pneumoniae* and a potential candidate for development of a protein-based vaccine against this pathogen. In this context the present work was done aiming to study PsaA stability in presence of denaturing agents such as urea, guanidine hydrochloride (GdmCl), different temperatures and pH in the presence or absence of Zinc.

**Methods:** Recombinant PsaA crude preparation was submitted to ion-exchange chromatography in Hitrap DEAE Sepharose FF. The isolated PsaA fraction was analyzed by SDS-PAGE-12%, Fluorimetry and Circular Dichroism. Fluorescence measurements were carried out using the excitation wavelength fixed at 280 nm, and the emission spectrum was recorded from 295 nm to 415 nm. The Circular Dichroism (CD) spectra were monitored from 200 nm to 260 nm, averaged over 3 scans at a speed of 50 nm / min. If not mentioned, all experiments were performed at pH 8.0.

**Results:** Protein homogeneity was confirmed by denaturing gel electrophoresis (MW 37,500). Circular Dichroism data showed a partial reduction of secondary structure in 1 M. urea. Decrease of fluorescence spectral area was already observed in 0.25 M urea. Partial loss of protein secondary structure and conformational changes were detected at concentrations of 0.75 M GdmCl and 0.50 M urea. PsaA thermal denaturing process showed changes in secondary and tertiary structures at 45°C, despite the partial secondary structure to be still maintained at temperatures until 85°C. PsaA fluorescence intensity

decreasing was more observed in acid pH than neutral and basic pH. On the hand the protein solution in presence of Zinc showed conformational changes since 5  $\mu\text{M}$  until 50  $\mu\text{M}$  metal concentration. It was demonstrated by light scattering that PsaA aggregation is inhibited by 0.25  $\mu\text{M}$  – 500  $\mu\text{M}$  Zinc concentrations.

**Conclusion:** All data related to PsaA conformational analysis here obtained are significant in the aim to improve the structural understanding of this protein as a potential vaccine antigenic target.