BIOPHYSICAL STUDY AND SAXS-DERIVED LOW RESOLUTION MODEL OF PRION PROTEIN (PRP) COMPLEXED WITH THE CO-CHAPERONE HOP/STI-1.

Romano S. A[*]., Coreiro Y. [†], Lima L. M. T. R. [†], Foguel D. [‡], Linden R[*].

[*]Instituto de Biofísica Carlos Chagas Filho, [†]Faculdade de Farmácia , [‡]Instituto de Bioquímica Médica from Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil.

Prion diseases are spongiform encephalopathies related to de cellular prion protein (PrP^c), abundantly expressed in the central nervous system. During the course of the disease, PrP^c is transformed in a pathological isoform, PrP^{Sc}, leading to neurodegeneration through accumulation of this abnormally folded protein. Numerous studies have focused on the pathological properties of PrP^c, however, its cellular function is still unclear. Lately, our group has identified and studied PrP^c binding proteins, showing that PrP^c participates in a multi-protein complex resident in the cellular surface that it is involved in cell-cell and cell-extracellular matrix interactions that modulate diverse aspects of cellular processes. One of the binding proteins, known as heat shock organizing protein or stress-inducible protein 1 (hop/STI-1), induces trophic and neuroprotective signals through the recruitment of neuronal PrP^c. The present work aims at studying the biophysical and biochemical properties of the PrP:hop/STI-1 complex *in vitro*, aiming to gain insight into the molecular mechanisms that underlie the PrP^c cellular function. The formation of the complex was evidenced by fluorescence anisotropy of fluorescein-tagged PrP incubated with hop/STI-1. The data obtained strongly suggests a 1:1 stoichiometry, and the complex dissociation constant was calculated. Analysis of far UV Cicular Dichroism (CD) spectra showed that the complex suffers a small but significant loss of secondary structure content when compared to the sum of the monomers spectra. This loss of secondary structure was particularly strong for PrP, who suffered a 30% decay of it's α-helical CD signal, suggesting a stretching of helical structures and a possible exposure of residues that could be relevant for the signalling capabilities of the complex. This structural rearrangement hypothesis was further supported by stationary state intrinsic fluorescence spectroscopy, since binding between PrP and a peptide containing hop/STI-1 binding site massively diminishes the PrP intrinsic fluorescence spectrum. In order to obtain information about the overall structure of the complex, Small Angle X-ray Scattering (SAXS) data was collected, allowing us to obtain the Radius of Gyration and Maximum Intermolecular Distance of the molecule. Furthermore, it was possible to obtain a low resolution *ab initio* model of the PrP:hop/STI-1 complex. Since the structure of the tetratricopeptide repeat (TPR) domains of hop/STI-1 were previously resolved, as is the case for the globular domain of PrP, we were able to superimpose these domains on the SAXS model of the complex, confirming the 1:1 stoichiometry and gaining important insight into the structural organization of the PrP:hop/STI1 complex. Importantly, the present model constitutes the first empirical representation of a complex involving PrP and a proteic physiological ligand.