STRUCTURAL CHARACTERIZATION OF THE PROTEIN BEX3
Raymundo, D. P.¹; Sampaio, L. A. G.¹; Cabral, K. M. S.¹; Silva, V. S.¹; Cordeiro, Y.²; Cirauqui, N.²; Almeida, M. S.¹
¹Instituto de Bioquímica Médica, UFRJ, Rio de Janeiro, Brazil
²Faculdade de Farmácia, UFRJ, Rio de Janeiro, Brazil

BEX3 (Brain expressed X-linked 3) is an eutherian-specific protein that is encoded by a gene located in a region of the X chromosome that contains genes related to the evolution of the neocortex. BEX3 plays a role in the extrinsic and intrinsic pathways of apoptosis by interacting with the intracellular domain of p75
\textsuperscript{NTRDD} and Smac, respectively. During this study, we have performed a structural characterization of BEX3. Nuclear Magnetic Resonance spectroscopy, Tryptophan intrinsic fluorescence, Proteinase K and Circular Dichroism showed that BEX3 presents α-helix and intrinsically disordered regions. Furthermore, molecular modeling experiments suggested that BEX3 exists in two antiparallel subunits (coiled-coil). Atomic Force Microscopy, crosslinking, X-ray scattering at low angle (SAXS) and Thioflavin T data revealed that BEX3 form a soluble oligomer and has a hydrophobic core. Some studies have described that three conserved hydrophobic residues in the Nuclear Export Signal motif, namely L94, L97 and L99, are important for nuclear export, auto association, and interaction with p75
\textsuperscript{NTRDD} and pro-apoptotic activity. We characterized a triple mutant of BEX3, L94A, L97A and L99A and showed that it is monomeric (Gel filtration, SDS-PAGE and Thioflavin T) and the three-dimensional structure is very similar to the native protein (Circular Dichroism, Tryptophan intrinsic fluorescence). Bis-ANS and ThT experiments also suggest that the mutant form has no tendency to form aggregates. We discuss the possible physiological role of the oligomers on the activity of BEX3 as a mechanism to avoid aggregation and degradation.

Keywords: Intrinsically Disordered Protein, Protein aggregation, Protein misfolding.
Support: CNPq and FAPERJ.