OLIGOMERIZATION STATES OF LEUCYL AMINOPEPTIDASE FROM *Leptospira interrogans*

Álvares, A.C.M.[1], Silva, A.J.[1], Silva, H.A.[2], Barbosa, J.A.R.G., Santana, J.M.[2] and Freitas, S.M.[1]

[1]Department of Cellular Biology, Institute of Biology, [2] Department of Molecular Patology. University of Brasilia, Brasilia, Brazil.

The leucyl aminopeptidase of the *Leptospira interrogans* (LAPLi) adopts well-packed self-assembled state in hexameric form and clives the N-terminal region of proteins and peptides. Despite extensive biochemistry studies of LAP few data relating the functional activity of this enzyme with oligomeric state have been reported. In this work we present the expression, purification, oligomerization analysis and heterogeneous fluorescence quenching resolution of the LAPLi. These methods allows investigating the conformation changes of proteins correlated with oligomerization process and their functional importance.The enzyme was express in *E. coli* after inducing with 0.3 µM IPTG for 12 h and purified by affinity chromatography (His•Bind Kit). The oligomeric state of protein was monitored by dynamic light scattering spectroscopy (DLS) in different concentrations (14 - 100 µM) and temperature (25 - 60ºC). The fluorescence quenching assays (λ_{ex} = 295 nm, λ_{ex} = 332 nm) were performed with acrylamide at 25ºC and analyzed by Stern-Volmer approximation. DLS data showed that LAPLi oligomerization process is temperature-dependent. The hexameric form (330 kDa, hydrodynamic radius 6.8 nm) occurs only at 60ºC, in all studied concentrations. Stern-Volmer plot for the monomeric form presents a small upward concave toward the y-axis at concentration higher than 0.15 M of acrylamide, showing that two combined dynamic and static fluorescence quenching process are occurring. These results suggest the interaction of neutral quencher next to buried or exposed tryptophan in different environments. In order to explore this structural feature other charged quenchers and fluorescence lifetime measurements are been applied.

Keywords: leucyl aminopeptidase, oligomerization, fluorescence quenching.