Université franco-allemande
Deutsch-Französische Hochschule

Molecular and structural bases of dysfunction of selenoprotein N in diverse forms of congenital muscular dystrophies

Vanessa Dacleu

Selenoproteins are proteins containing a selenocysteine residue (U) in their amino acid sequence. Twenty-five proteins constitute the human selenoproteome. Among them is Selenoprotein N or SelenoN; mutations in the SELENON gene can lead to a group of congenital dystrophies now designated as SELENON-related myopathies. SelenoN is a 72 kDa membrane and glycosylated protein of the endoplasmic reticulum. It handles in its amino acid sequence a redox motif SCUG like the one of thioredoxin reductases, and an EF-hand domain which is a calcium binding site. Recent studies showed the implication of SelenoN in muscle development and maintenance, and position its function at the crossroad between oxidative stress control and calcium homeostasis. However, its catalytic function remains elusive. The research project presented in this thesis concerns the crystallization, characterization and comparison of one bacterial and the zebrafish SelenoNs. Bioinformatics analyses revealed that the two proteins share 37% degree of identity and a common domain which corresponds to a thioredoxin fold of unknown function which includes the redox motif SCUG. From the biophysical characterization, both recombinant proteins are found to be naturally well-folded and enriched in α-helical domains. The bacterial SelenoN which handles an additional C-terminal thioredoxin domain is an extended monomer whereas zebrafish SelenoN is a compact dimer. Biochemical characterization indicated that Ca2+ binding mediates zSelenoN oligomerization. Initial crystals of the zSelenoN in its deglycosylated form were obtained. Bacterial SelenoN crystallization yielded crystals belonging to two different space groups with different cell parameters. An initial partial model covering the C-terminal thioredoxin domain of the bacterial SelenoN was obtained at 2.3Å. Together, these results lay a foundation for the structure-function studies of SelenoN. Conditions for recombinant bacterial and zebrafish SelenoNs expression, purification and crystallization were optimized and strategies for solving the structure are being proposed.

Keywords : Selenoprotein, SelenoN, X-ray diffraction