Interaction of Huntingtin Exon-1 peptides with lipid-based micellar nanoparticles probed by solution NMR and Q-band pulsed EPR

Alberto Ceccon,[1] Thomas Schmidt,[1] Vitali Tugarinov,[1,*] Samuel A. Kotler,[1] Charles D. Schwieters,[2] and G. Marius Clore,[1,*]

[1]Laboratory of Chemical Physics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-520. [2]Imaging Sciences Laboratory, Center for Information Technology, National Institutes of Health, Bethesda, MD 20892-5624.

Huntington's disease is a fatal neurodegenerative disease arising from the presence of 36 or more CAG repeats within exon 1 of the Huntingtin (htt) gene, resulting in expansion of the polyQ domain that lies immediately downstream of the 16-residue N-terminal amphiphilic sequence (htt^{NT}) of the huntingtin protein. The presence of a long polyQ stretch results in the rapid formation of polymorphic fibrils, the rate of which is modulated by the presence of flanking regions (htt^{NT} and the proline rich domain C-terminal to the polyQ sequence). Although the length of the polyQ domain is directly related to the severity of disease, the aggregation schemes of mutant exon 1 can be further complicated by additional factors including binding to surfaces (including lipid membranes) and the presence of reactive oxygen species (ROS). Recent atomic force microscopic (AFM) studies have emphasized the role of htt^{NT} in modulating the membrane-associated oligomerization on supported lipid bilayers. Nevertheless, the molecular details of how membrane association can possibly impact polyglutamine nucleation as well as the existence of transient oligomeric membrane-associated states involved in the early-stages of amyloid fibril formation are currently missing. In this paper, we have characterized the interaction of two such peptides, $htt^{NT}Q_7$ and $htt^{NT}Q_{10}$ comprising the N-terminal amphiphilic domain of huntingtin followed by 7 and 10 glutamine repeats, respectively, with lipid micelles as membrane-mimic using NMR chemical exchange saturation transfer (CEST), circular dichroism and pulsed Q-band EPR. Exchange between free and micelle-bound $htt^{NT}Q_n$ peptides occurs on the millisecond time scale with a Kd ~ 0.5-1 mM. Upon binding micelles, residues 1-15 adopt a helical conformation. A structure of the bound monomer unit is calculated from the backbone chemical shifts of the micelle-bound state obtained from CEST. Pulsed Q-band EPR shows that a monomer-dimer equilibrium exists on the surface of the micelles and that the two helices of the dimer adopt a parallel orientation, thereby bringing two disordered polyQ tails into close proximity which may promote aggregation upon dissociation from the micelle surface through high local concentration effects. Interestingly, oxidation of Met_7 to a sulfoxide due the presence of ROS reduces the binding affinity ~3-4 fold, increases the length of the helix by a further two residues and inhibits aggregation.