Tata Institute of Fundamental Research
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Webinar

Biophysical characterization of structure-function differences in the apolipoprotein E isoforms

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Apolipoprotein E (apoE) is a major protein constituent of lipoproteins in the human body. Human apoE has three isoforms, apoE2, apoE3 and apoE4, differ from each other by single amino acid at two positions, viz. 112 and 158, where apoE3 contains a cysteine and an arginine residue respectively, apoE2 contains cysteine and apoE4 contains arginine residues at both the positions. ApoE4 is the strongest genetic risk factor in Alzheimer's disease, whereas apoE3 is considered normal. It was hypothesized that domain interactions between N-terminal domain (NTD) and C-terminal domain (CTD) differs between apoE3 and apoE4, whereas apoE4 shows domain interactions, apoE3 lacks it. But solution NMR structure and HDX-MS experiments reported earlier show that domain interactions are also present in apoE3 and differs between apoE3 and apoE4. The main goal of my thesis is investigations and quantification of domain interactions in apoE.

My work shows that free energy of domain interactions is almost the same for apoE3 and apoE4. But the stability of apoE strongly depends on the mutations at 112th position in apoE. ApoE4 shows least stability whereas C112I shows highest. Domain interactions measured by inter-domain FRET also show strong dependence on mutations at 112th position. Interdomain FRET shows highest stability for apoE4 and C112T, intermediate for C112 (apoE3) and lowest for C112I. Enhanced inter-domain FRET and low cooperativity of unfolding of apoE4 lead to the possibility of presence of intermediate state in apoE4. Widest distribution of m/z spectra obtained from kinetics of HDX-MS shows a higher population of intermediate states is apoE4. Population of the intermediate state shows strong dependence on mutations at the 112th position. Finally, enhanced bis-ANS fluorescence and protease susceptibility assay show that apoE4 possesses the highest population of molten globule (MG)-like states in physiological pH. Enhanced interdomain FRET and bis-ANS fluorescence of apoE4 is correlated. Also, mutations at 112th alters the population of MG-like states in apoE. Functional differences among isoforms might involve the population of MG-like states in native conditions.

Tuesday, Oct 13th 2020

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