Mapping protein aggregation landscapes by NMR

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Many human diseases are associated directly with aggregation of extracellular proteins, leading to formation of toxic oligomers and deposition of insoluble amyloid fibrils. For globular proteins, the process is initiated by local unfolding of the native structure to form aggregation-prone intermediates. Despite their key role in amyloidogenesis, influencing the kinetic partitioning between aggregation and refolding pathways, little is currently known about the structure of amyloidogenic intermediates because of their strong propensity to aggregate. Solution NMR provides a unique and powerful approach for mapping the kinetic aggregation landscape and characterizing the structure of transient intermediates. Applications of real-time ^{19}F NMR and CPMG relaxation dispersion to characterize the kinetic aggregation pathways of wild type and pathogenic variants of human transthyretin (TTR) will be described. Transthyretin amyloidosis is associated with neurodegenerative disease and cardiomyopathy. The early-onset familial diseases are associated with genetic mutations that destabilize the quaternary and/or tertiary structure of TTR whereas wild type TTR is linked to age-related amyloid disease. TTR amyloidosis is initiated by dissociation of the native tetramer followed by partial unfolding and aggregation of the monomer. Real-time ^{19}F NMR allows detailed kinetic and mechanistic characterization of the aggregation pathway of wild type and variant TTR at physiological concentrations (10 μM of TTR monomer)[1,2]. The measurements are highly efficient and enable visualization of intermediates and determination of kinetics, equilibrium constants, and free energies in a single experiment. Chemical shift analysis and ^{15}N and ^{1}H relaxation dispersion measurements on wild type and variant TTRs reveal differences in ground state structure and in the population of an excited state with altered structure in the subunit interfaces[3]. Relaxation dispersion studies of a monomeric TTR variant reveal μs-ms fluctuations in one of the β-sheets that appear to predispose the monomer for aggregation[4]. Overall, our experiments are providing important new insights into the molecular processes that cause the TTR tetramer to dissociate and partition into the downhill aggregation cascade that leads to amyloid formation.

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