New Insights into Individual Amyloid Aggregates Structure by Infrared Nanospectroscopy

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Amyloids are insoluble proteins aggregates implicated in the onset of several neurodegenerative disorders, such as Alzheimer's, Huntington's and ataxia diseases. During aggregation, monomeric proteins undergo internal structural rearrangement forming amyloid fibrils with a universal cross β-sheet quaternary structure. This structure is independent by the monomeric initial native protein and it is the fingerprint of the onset of the related disease.
Infrared nanospectroscopy (nanoIR) is an innovative tool that exploits the combination of two techniques commonly used to study protein aggregation: Atomic Force Microscopy (AFM) and infrared spectroscopy (IR). The first can provide information on the morphology and mechanical properties of the species formed along the aggregation pathway, the second can characterize conformational changes in protein secondary structure. Although useful, these conventional techniques do not tell us separately if/at which time point misfolding occurs, nor what is the secondary structure of the *individual* species. Their combination, in infrared nanospectroscopy, enables a structural characterization at the nanoscale of the ultrastructural properties of amyloids through the acquisition of nanoscale chemical IR maps or spectra.
First, we could characterize at the individual aggregate scale the conformational rearrangements of proteins during their misfolding and aggregation. Furthermore, combining nanoIR with conventional AFM nanomechanical mapping, we correlated the secondary structure of amyloid intermediates and final aggregates to their nanomechanical properties. Our results directly demonstrated, for the first time at the *individual* amyloid species scale, that the increase of β-sheet content is a fundamental parameter determining amyloids intrinsic stiffness.[1,2] As next step, we structurally characterized single huntingtin amyloid fibrils as a function of the length of their mutated polyglutamine (polyQ) stretch. The results demonstrated that fibrils with higher polyQ content had a higher quality (i.e. intermolecular hydrogen bonds) of amyloidogenic β-sheet structure. Finally, it was shown that it exists a direct correlation between huntingtin polyQ stretching size and age of Huntington's disease onset. Thus, this data strongly suggests the structure improvement as one of the main factors causing toxicity above the polyQ pathogenic threshold for the disease onset.
Elucidating the structural properties of amyloidogenic proteins is essential for the unraveling of the molecular basis of their function in health and disease. Indeed, the improved structure could be more efficient in damaging cellular membranes, in sequestrating transcription factors, thus impairing the transcription of essential genes or could more easily overwhelm the ubiquitin-proteasome system, leading to its failure. The comprehension of these mechanisms is central to develop new pharmacological approach to neurodegenerative disorders.

References

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