OPTICAL IMAGING OF THE NATURE OF α-SYNUCLEIN AMYLOID FORMATION IN VITRO AND IN VIVO

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Misfolding and aggregation of amyloidogenic peptides lie at the root of many neurodegenerative diseases. Whilst aggregation can be readily studied in vitro by established biophysical techniques, a direct observation of the nature and kinetics of aggregation processes taking place in vivo has not yet been possible. We show using fluorescence lifetime imaging microscopy of a fluorescently tagged amyloidogenic protein that the degree of aggregation and the nature of the resulting oligomeric and fibrillar species are closely correlated with the value of the excited state lifetime of the attached fluorophore. This phenomenon therefore represents a powerful tool to monitor in real time the populations of different types of aggregates formed by amyloidogenic proteins. Indeed, for α-synuclein, a protein whose aggregation is linked to Parkinson's disease, we have been able to reveal the nature of the aggregated species and the kinetics of the self-association reactions taking place in vitro, in cells in culture and in living C. elegans, and directly correlate them with the appearance of a toxic phenotype. The capability of measuring the nucleation and growth of toxic amyloidogenic proteins in an aging animal provides a powerful new tool in the study of the pathology of several misfolding disorders. Our study confirms the importance of the molecular environment in which aggregation reactions take place, highlighting similarities as well as differences between the processes occurring in vitro and in vivo which may be crucial in the cause of disease.