Evidence for Intramolecular Antiparallel β-Sheet Structure in α-Synuclein Fibrils Aggregated Under Physiological Conditions

Steven J. Roeters*, Aditya Iyer, Vinod Subramaniam and Sander Woutersen

Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands, * sroeters@gmail.com
Nanoscale Biophysics Group, FOM Institute AMOLF, Science Park 104, 1098 XG Amsterdam, The Netherlands

The aggregation of alpha-synuclein into amyloidic fibrils in the presence of varying NaCl concentrations is studied with 2D-IR spectroscopy and complemented with spectral calculations to assign the spectra. We find different fibril structures depending on whether the protein is aggregated in < 25 mM or >25 mM NaCl buffer solution.

The structure of the amyloidic fibrils formed by alpha-synuclein (αS) is not yet fully resolved, although detailed knowledge of the monomer structure, the way monomers form fibrillar hydrogen-bonded β-sheets, and how these sheets are stacked together, is essential understanding the etiology and progression of Parkinson's disease. Here, we present evidence from AFM and UV-CD, XRD, and amide-I 1D- and 2D-IR spectroscopy that αS fibrillized in low ionic-strength buffers ([NaCl] < 25 mM) are significantly different from fibrils grown in higher ionic-strength buffers. The observations for low-salt fibrils are consistent with an extended conformation of αS molecules, forming hydrogen-bonded fibrillar sheets that are loosely packed in a parallel fashion. For high-salt fibrils (including those prepared in circumstances mimicking the physiological situation) the measurements are consistent with more tightly-packed αS molecules that have an antiparallel intramolecular conformation, that form two hydrogen-bonded protofibrils (each composed of five hydrogen-bonded sheets), twisting around each other to form a fibril. We find that the high-frequency mode in the IR spectra is fundamentally different from the mode to which it is commonly assigned. The high sensitivity of the fibril structure to the ionic strength might form the basis of different pathologies: even small changes in the ionic strength (25 → 50 mM NaCl) are sufficient to result in very different fibril structures, so that the different environments experienced by αS molecules in different cells and at different moments are likely to lead to structurally different αS fibrils.