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Structural Variation in Polyglutamine-rich Amyloid Fibrils Imaged By Multiple Electron Microscopy Methods

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Many neurodegenerative disorders such as Huntington's disease exhibit intracerebral deposition of aggregated polypeptides with abnormally extended tracts of tandem glutamine residues (polyQ) [1,2] These proteins are highly insoluble and form amyloid fibril-containing aggregates *in vivo* and *in vitr*o [3]. High-resolution structures for these fibrils would be of great value for investigating the molecular basis and the mechanism of progression of these diseases. However, despite overall progress in understanding other amyloids, analysis of polyQ fibrils has remained difficult. A major challenge has been their extreme insolubility whereby these polypeptides rapidly aggregate into higher-order complexes in which individual fibrils are barely distinguishable [4].

Aiming to produce fibrils suitable for structural analysis, we designed polyQ-containing peptides of suitable length (~30 amino acids) in which some L-lysine residues were included to enhance solubility and two D-lysines intended to interrupt the putative beta-strand by inserting reverse turns [5]. Two types of polypeptide were synthesized: polyQKd33 (33 residues) and polyQKd32 (32 residues), which has one less glutamine in the central part. Both peptides were found to be soluble at neutral pH and to assemble into well dispersed fibrils at pH 11-12 suitable for microscopic analysis. These fibrils were imaged by negative staining, cryo-EM, and electron diffraction from unstained air-dried specimens on Philips CM120 and CM200-FEG microscopes and by dark-field STEM of unstained freeze-dried specimens at the Brookhaven STEM facility [6].

When analyzed by electron diffraction, preparations of both fibrils showed a sharp ring at a spacing of $(0.47\ \text{nm})^{-1}$ (Fig. 1A, B), indicative of cross-beta structure, the hallmark of amyloid. However, the polyQKd32 fibrils were slightly but consistently thicker than polyQKd33 by both negative staining (4.7 ± 0.6 nm *vs*. 3.3 ± 0.6 nm; e.g. Figs 1C, D) and cryo-EM (3.9 ± 0.3 nm *vs.* 3.5 ± 0.2 nm; e.g. Figs. 1E, F and Figs. 1G, H). Furthermore, dark-field STEM imaging revealed the same trend in fibril diameters Figs. 1I, J), followed by mass-per-length analysis which revealed an accompanying increase in subunit packing density from ~1.0 to ~1.5 molecules per axial repeat (0.47 nm) of the cross-beta structure. Underlying these changes in overall fibril architecture is the insertion of a single additional glutamine residue in the central part of the polypeptide. These observations afford a basis for further more detailed structural analysis and for modeling the three-dimensional structures of these and other polyQ-containing fibrils.

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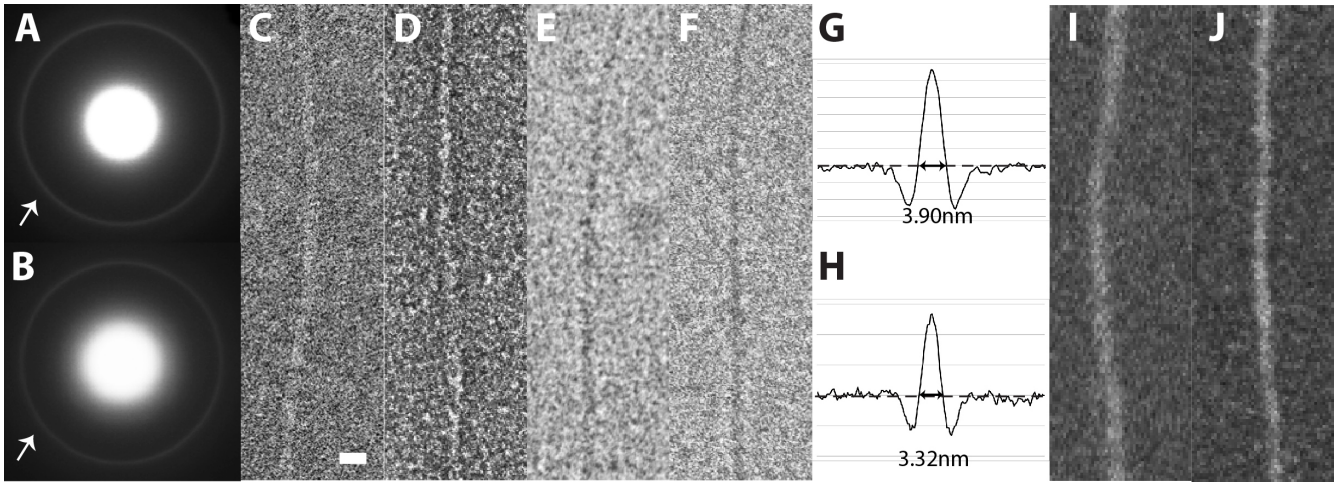

Figure 1: Structural analysis of polyQKd32 (A, C, E, G, I) and polyQKd33 (B, D, F, H, J) fibrils. Electron diffraction images (A, B) showed a ring (arrow) at a spacing of $(0.47\ nm)^{-1}$ using evaporated thallous chloride as a reference. Negative staining (C, D), cryo-EM images (E, F), and density profiles (G, H, average background level shown as a dotted line) calculated from cryo-EM images showed that polyQKd32 is slightly thicker than polyQKd33. STEM images (I, J) were used for mass per length analysis and fibril packing density determination. Scale bar = 10 nm.

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