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Electron diffraction and microscopy studies of an amyloid forming peptide

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Amyloid aggregates have been linked to a number of animal and human pathological conditions, such as Alzheimer's disease, Parkinson's, type II diabetes mellitus and prion diseases. However, although amyloid aggregates have been known for more than 50 years, little is known about the structure of these aggregates or the process that leads to their formation.

The seven residue peptide GNNQQNY from the N-terminal region of the yeast prion protein Sup35, forms both amyloid fibers and highly ordered microcrystals (Fig. 1) upon contact with water. At relatively low concentrations (~10mg/ml) the peptide forms microcrystals, whereas at high concentrations (~50mg/ml), fiber formation occurs. The fibers show the typical green birefringence of amyloid aggregates when complexed with Congo red, and X-ray diffraction patterns of these fibers display the ~4.7Å meridional reflection, as well as the ~10Å equatorial reflection, characteristic of a cross-beta structure. The microcrystals of the peptide, which have largest dimension ~1μm diffract electrons to ultra high resolution (<0.5Å spacing) (see Fig. 2). The sharp 4.86Å layer line spacing along the long axis of the crystals (with no trace of a 9.72Å spacing) establishes that the peptide chains form parallel β-sheets.

Two distinct forms of the crystals have been observed to occur, depending on the previous history of the peptide. In one case, the peptide crystallizes on an orthorhombic unit cell with a $\boldsymbol{P2_12_12_1}$ space group and unit cell dimensions ranging from |**a**|=22.7-21.2, |**b**|=39.9-39.3, |**c**|=4.89-4.86Å and α=β=γ=90 degrees for the wet to dried state. The second crystal form has a monoclinic unit cell with |**a**|=23.2, |**b**|=37.7 and |**c**|=4.75Å, with α=β=90^o, and γ=82.25^o.

The two crystal forms of the peptide are presumably related to the fiber form, and provide a model system to characterize the structure and stability of the elusive cross-beta amyloid conformation. The relative intensities of the Bragg reflections determined from the electron diffraction data of the orthorhombic crystals suggest that the four peptides in the unit cell are likely to be aligned closely parallel to the **a**-axis, with the cross-beta sheets connected in two pairs related by the crystal 2-fold screw axes (Fig. 3). However, other packing schemes are feasible for the same unit cell dimensions and crystal symmetry and have yet to be ruled out. A considerable amount of electron diffraction data has been collected using a slightly convergent beam to restrict irradiation to a relatively small region of the microcrystals (~1μm). Some microcrystals have allowed us to collect up to ~30 diffraction patterns, and from these tilt series we have been able to map intensities in reciprocal space to spacings the order of 2Å.

Comparison of the cell volume with that calculated for the four peptides implies that water may occupy only 10-15% of the volume in the hydrated state for both crystal forms. This nearly anhydrous packing can account for the insolubility of the crystalline aggregates of this hydrophilic peptide, since the activation energy for rehydration may be extremely high. Water excluding packing of paired cross-beta peptide segments in thin protofilaments may be a general characteristic of the wide variety of anomalously stable pathological amyloid assemblies, and the relatively similar

conditions under which the two crystal forms can be produced, might help establish a structural basis for the strain differences observed in prion diseases.

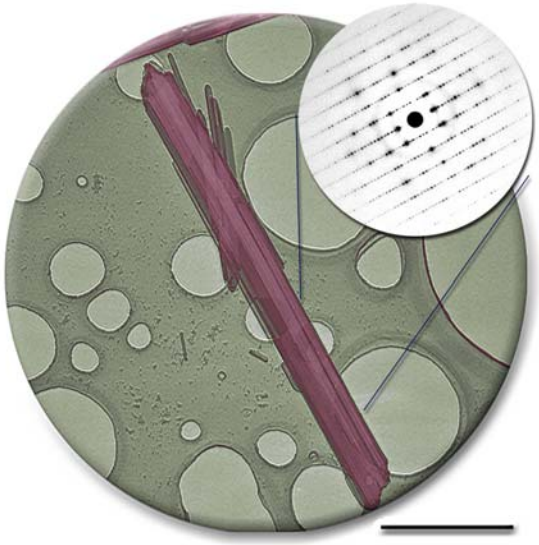

Figure 1. Electron micrograph of a microcrystal of GNNQQNY. The bar represents 0.5mm, and the insert shows the electron diffraction pattern of the region indicated. The electron diffraction data has been collected using a slightly convergent beam.

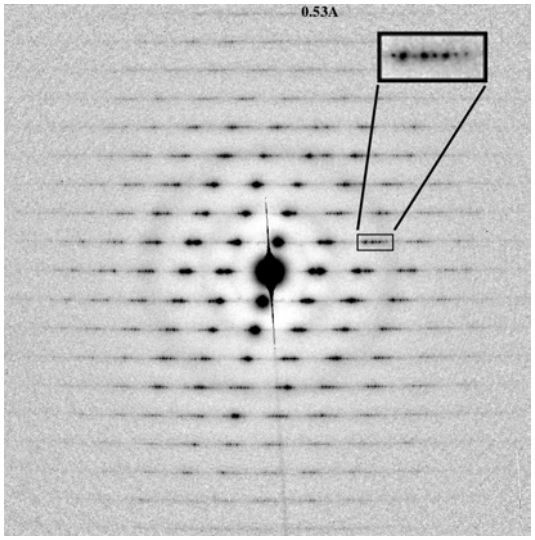

Figure 2. Electron diffraction pattern of GNNQQNY showing ultra-high resolution data. The pattern also shows the presence of Laue zones.

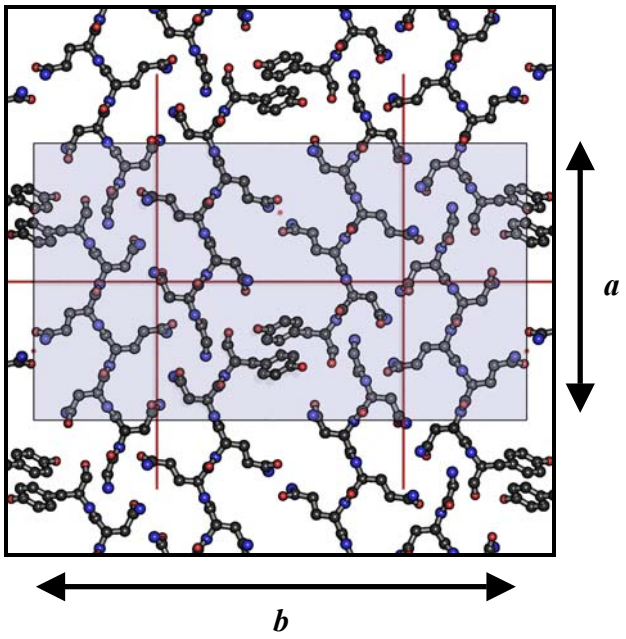

Figure 3. Possible view of the packing of the peptide in the orthorhombic unit cell. There must be four molecules per unit cell, related by screw axes parallel to the unit cell edges. The unit cell is represented by the shaded area in the figure.

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