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TEM ANALYSIS OF β-AMYLOID FIBRILLOGENESIS: NEW STRATEGY-OLD PROBLEM

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The brains of patients with Alzheimer's disease (AD) contain plaques composed of proteolytic fragments of 40-43 amino acids (a.a.) known as β-amyloid (Aβ) precursor protein. The N-terminus (1-17) is hydrophilic while the central region (17-21) is hydrophobic and the C-terminus (29-43) is very hydrophobic. Self-assembled paracrystalline fibrils form insoluble structures that maintain a characteristic β-peptide structure containing a.a. 10-35. Solid state NMR and small angle neutron scattering (SANS) provided inter-strand distance, solubility and single stranded characteristics of Aβ(10-35)-PEG.[1,2] Uranyl acetate negative-stain TEM provided fibril morphology of the solubilized Aβ(10-35)-PEG. [1,2] In order to further study the fine structure of these fibrils, a shorter peptide Aβ (10-21) and it's mutant Aβ (10-21)E11N (asparagine for glutamine at the 11a.a. position)) has been analyzed with phosphotungstenic acid (PTA) negative stain for TEM. Low dosage strategies for TEM were developed to minimize structural damage.

Samples of Aβ(10-21) E11N and Aβ(10-21) were dissolved in ddH_2O at a concentration of 3mg/ml, pH adjusted to 5.6 with NaOH and allowed to self assemble for 6 days. 2% PTA (EMS# 19500) was prepared fresh in CO_2 free water and used immediately. PTA was used at low pH (~2) and at pH 5.6 (K-PTA). A drop of protein was suspended on a 200-mesh carbon coated copper grid for 30 sec, then blotted dry. PTA droplets were placed onto parafilm and the grids were inverted onto the drop for 30 sec., removed and blotted dry with filter paper. Specimens were imaged in a JEOL 1210 TEM equipped with a LaB_6 filament operated at 90 kV. Specimens were photographed at 25-50 kX onto standard Kodak 4489 film in 2.2 sec. Negatives were recorded digitally with an Agfa Duoscan T-2500 high resolution (10,000 element CCD) scanner, 2000or 4000 pixels per inch. Areas of interest were selected, enlarged electronically and processed using Photoshop 5.5.

Radiation damage created in a 200 kX recording by a 90 kV source seriously impaired the quality of fibril lamination observed within a 20 nm wide supramolecular twist of PTA stained Aβ(10-21)E11N (Fig.1). Recordings made at 30 kX of the same specimen, when scanned and enlarged, revealed high fidelity multilamellar features of the supramolecular amyloid ribbon (Fig. 2). High magnification prints were made demonstrating twisted pairs with individual fibril widths in the 5-nm range (Fig. 3).

The current work describes methods for molecular level resolution of amyloid fibrils. An ongoing effort to understand fibrillogenesis of β-amyloid necessitates correlation of neutron scattering data with TEM measurements and these measurements are consistent with SANS results. Information on fibril self-assembly into supramolecular protein ribbons may help allude to factors involved in AD.

References

1. T.S. Burkoth et al., J. Am. Chem. Soc. 120:6 (1998) 7655.
2. T.S. Burkoth et al., J. Am. Chem. Soc. 120:30 (2000) 7883.

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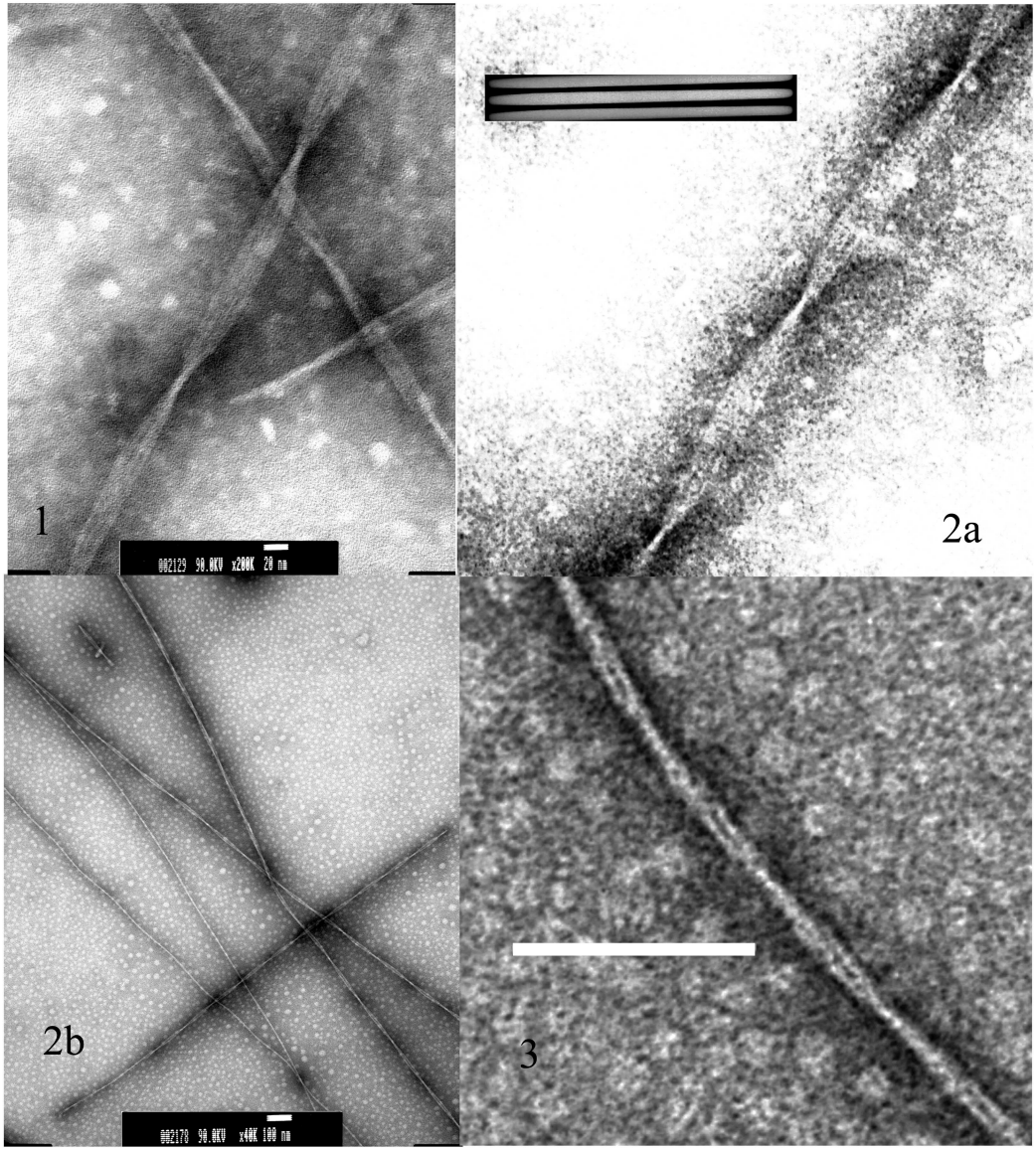

FIG. 1. Radiation damaged amyloid fibril imaged at 200kX. Bar = 20 nm.
FIG. 2a. Amyloid fibril enlarged from a 2000ppi image recorded at 30 kX. Bar = 200 nm.
FIG. 2b. Amyloid fibrils diplay twists at 40 kX. Bar = 100 nm
FIG. 3. Single twisted pair of amyloid fibers enlarged from a 4000ppi image recorded at 25kX. Bar = 100 nm.

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