ATOMIC FORCE MICROSCOPY STUDY OF THE FIBRILLOGENESIS OF TAU PROTEIN

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Alzheimer's disease (AD) is a senile dementia defined by the presence of two aberrant structures, the senile plaques, an extracellular deposition of insoluble amyloid fibers consisting of the β-amyloid peptide, and the neurofibrillary tangles (NFTs), the result of the intraneuronal accumulation of the microtubule-associated protein Tau. In AD Tau is abnormally hyperphosphorylated; in this situation, Tau separates from the microtubules, loses its regulatory function, precipitates into the paired helical filaments (PHFs) and forms the intracellular NFTs.

It is known the effect in AD pathology of the non-enzymatic reaction of glucose to form advanced glycation endproducts (AGEs). AGEs are sugar-derived protein modifications able to irreversibly crosslink long-lived proteins including the characteristic hallmarks of AD, i.e. β-amyloid plaques and NFTs. AGE formation starts with the reaction of the amino groups of proteins, particularly the side chains of lysine, arginine and histidine, with reducing sugars, such as glucose, fructose, the carbohydrate units of glycoproteins, and the glycosaminoglycan (GAG) chains of proteoglycans. Intracellular AGEs crosslink cytoskeletal proteins and render them insoluble (Dukic-Stefanovic et al., 2001).

The study of Tau fibrillogenesis has been done mainly with PHFs extracted from the brain of AD patients (Moreno-Herrero,F et al. 2004) because the formation of filaments from full-lenght recombinant Tau in vitro has consistently failed. By contrast, incubation of recombinant Tau with sulphated GAGs such as chondroitin sulphate B (CSB) and with RNA has been observed to stimulate the formation of PHFs (Goedert et al. 1996)

The aim of this work is the in vitro study of the effect of AGEs and AGE inhibitors on the fibrillogenesis of recombinant Tau, using atomic force microscopy (AFM). AFM visualization on mica of recombinant Tau incubated for 48 hours reveals the globular morphology described by other authors (Hasegawa et al., 1997; Figure 1A). A similar result is observed when the same conditions are maintained in the presence of 10 mM CSB (Figure 1B). The visualization on highly oriented graphite (HOG) of Tau samples containing CSB concentrations that range from 500 μM to 20 mM reveal fiber-like structures (Figure 2). These AFM studies are complemented with transmission electron microscopy and with polyacrilamide gel electrophoresis analysis of Tau incubated in different combinations of AGEs and AGE inhibitors.

In summary, we show that the AFM is a good technique for imaging the grow of tau fibrils when incubated with sulphated GAGs. Moreover, we show a different behavior when incubated the protein samples on mica or on highly oriented graphite.

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References:

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Figures:

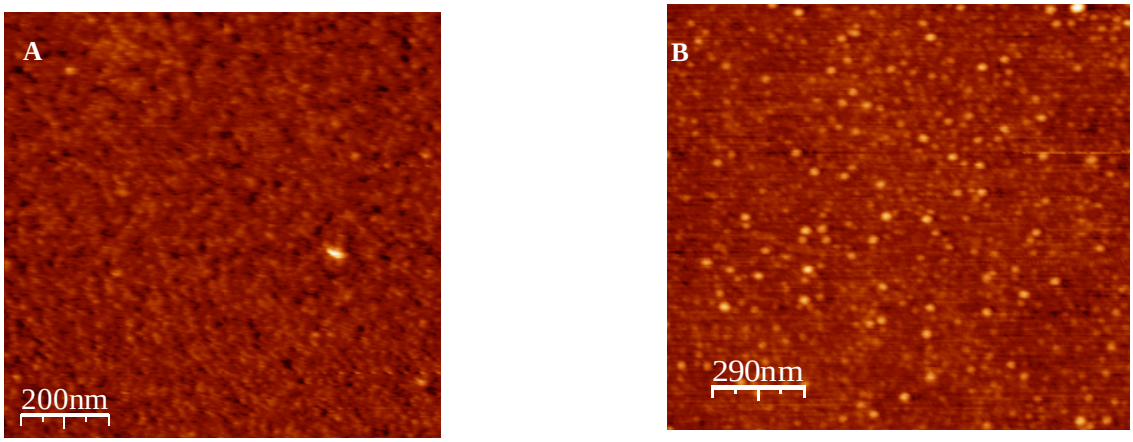

Figure 1. AFM images taken in air of Tau protein (2 μM) for 3 days. A. Incubated alone on mica. B. Incubated with chondroitin sulfate (10 mM) in the same conditions. The false color height scale is 5 nm.

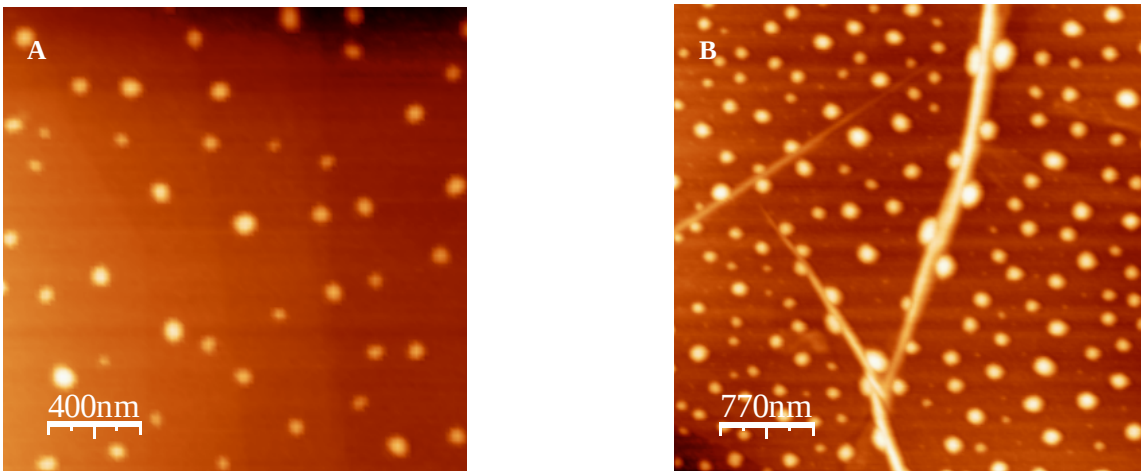

Figure 2. AFM images taken in air of Tau protein (2.5 μM) incubated for 3 days on HOG. A. Incubated alone. B. Incubated with chondroitin sulfate (500 μM). The false color height scale is 15 nm.