FINE STRUCTURE OF THE AMYLOID-BETA PEPTIDE (1-42) FIBRILS WITH ATOMIC FORCE MICROSCOPY

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Alzheimer's disease (AD) is characterized by the presence of extracellular and cerebrovascular senile amyloid plaques and by the intraneuronal deposition of neurofibrillary tangles (NFTs). Amyloid plaques are mainly composed of amyloid-beta peptide (AβP), a 39 to 42-residue fragment that is cleaved from the amyloid precursor protein (APP). Among the different forms of AβP, the 42-residue fragment ($A\beta P_{1-42}$) is the most aggregative and thus, the most difficult to work with. In this work we have used the atomic force microscopy (AFM) [1] to determine the fine structure of $A\beta P_{1-42}$ fibrils. All the studies have been performed in liquid environment to mimic physiological conditions. AFM has several advantages over other techniques when imaging biological samples. It is a noninvasive technique which enables to work in solution and thus, the obtained images represent a more trustworthy structure in contrast to, for example, the negative staining used in electron microscopy studies, which may affect the morphology of the fibrils.

When $A\beta P_{1-42}$ preparations were imaged on freshly cleaved graphite, small fibrillar species termed 'protofibrils' were observed covering the surface (Fig. 1). However, these protofibrillar species were not seen on mica, suggesting that they are essentially hydrophobic. These protofibrils are believed to be precursors of the final amyloid fibers [2]. In addition we have been able to resolve the substructure of $A\beta P_{1-42}$ fibrils (Fig. 2A), that consist of various protofilaments which are about 5-9 nm wide each (Fig. 2B). Other authors [3-5] have described the possible supramolecular assembly of protofilaments in different amyloid family proteins. However, this substructure has not been previously observed in $A\beta P_{1-42}$ fibers with AFM.

AD pathology has been also related to 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 like those found in the characteristic hallmarks of AD, i.e. 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 chains of proteoglycans. We have also studied the effect on the process of $A\beta P_{1-42}$ fibrillogenesis of different carbohydrates such as glucose, fructose, heparan sulphate, and chondroitin sulphate.

Although the causative role of AβP in AD has not been definitively established, a more detailed knowledge of the structure of AβP fibrils and the relationship with other molecules may provide new insights into the mechanism of fibrillogenesis and therefore, lead to new therapeutics for the treatment of AD.

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Figures:

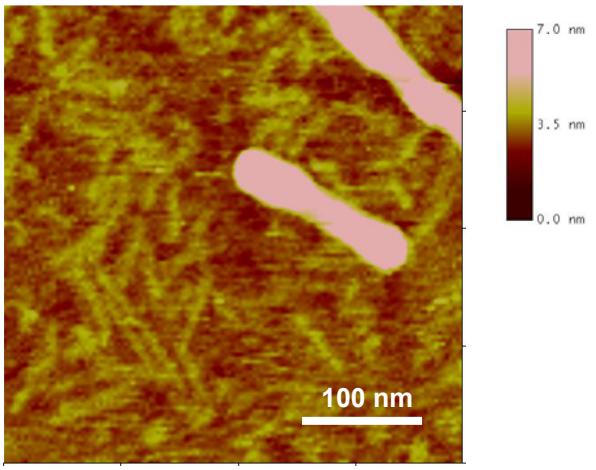

Figure 1: AFM height image of an $A\beta_{1-42}$ preparation on graphite. Next to the large amyloid fibers, protofibrils can be seen covering the surface.

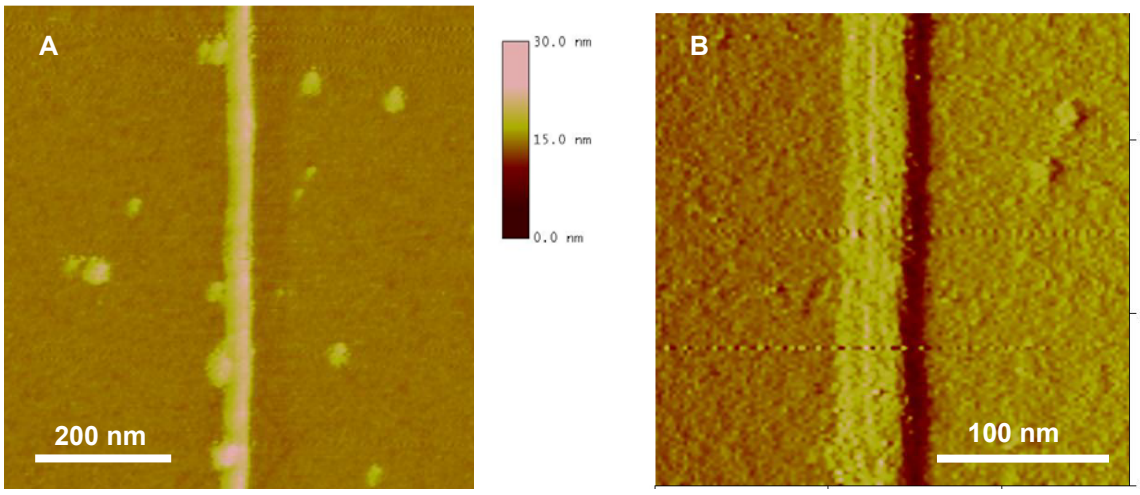

Figure 2: $A\beta_{1-42}$ preparation imaged on graphite. (A) AFM height image of a fibril. (B) AFM amplitude image of the same fibril, revealing the internal substructure.