A Review of Atomic Force Microscopy in Parkinson's Research

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BIOGRAPHY

Paul Hansma received his BA from New College, Sarasoate, FL, and his MA and PhD from University of California, Berkeley. He came to the UCSB Physics Department in 1972 as an assistant professor, became an associate professor in 1976, and full professor in 1980. In 2000 he won the distinguished Biophysical Physics Prize from the American Physical Society.

ABSTRACT

Use of the atomic force microscope in Parkinson's disease research has increased due to the discovery that mis-sense mutations (A53T and A30P) in α-synuclein (α-syn) have been linked to early-onset Parkinson's disease in several families. α-syn, in fibrillar formation, has been identified as a component of the cytoplasmic inclusions known as Lewy bodies, which are characteristic of the Parkinson's disease brain. Three papers, on accelerated in-vitro fibril formation by a mutant α-syn linked to early-onset Parkinson's disease, α-syn membrane interactions and lipid specificity, and acceleration of oligomerization, not fibrillization, is a shared property of both α-syn mutations linked to early-onset Parkinson's disease, are examined for their use of atomic force microscopy in imaging α-syn fibril formation.

KEYWORDS

atomic force microscopy, α-synuclein, fibril, prefibril, oligomer, lipid, bilayer

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INTRODUCTION

Parkinson's disease (PD) is a mid to late onset disorder characterized clinically by resting tremor, bradykinesia, cogwheel rigidity, and postural reflex impairment. Pathologically, Parkinson's disease is distinguished from other neurological disorders by the loss of pigmented neurons in the substantia nigra, and the presence of associated characteristic ubiquitin and alpha-synuclein (α-syn) positive inclusion bodies (Lewy bodies, clear bodies). Predominant beliefs in the etiology of the disease have shifted from inheritance, to a post-influenza infectious etiology, to an environmental origin, and to multifactorial theories of gene-environment interaction. Although several theories have been proposed, there is no known cause of Parkinson's disease.

The presence of α-syn mutations in early onset PD suggests that accelerated fibrillization may contribute to the progression of the disease. However, whether the pathogenic species is the fibril or another pre-fibril formation is uncertain. Atomic force microscope images of α-syn (Fig 3) can distinguish fibril formation from other α-syn formations [1, 3-5]. The microscope consists of a very sharp tip attached to a sensitive spring cantilever, which is then run over the surface of a sample (Fig 1). The tip touches the surface of the sample, and responds to sub nanometer changes in the heights of surface features [6]. A laser-light beam is then reflected off the back of the spring onto a sensor that detects spring deflection [7] producing a topographic map of the sample with imaging software.

IN-VITRO FIBRIL FORMATION

Conway et al. [1] studied accelerated in-vitro fibril formation by a mutant α-syn linked to early-onset PD, and hypothesize that because amyloid beta-protein fibrillizes rapidly in early-onset familial Alzheimer's disease (FAD) [8] that an analogous process may occur with α-syn in PD. The group bacterially-expressed recombinant WT α-syn, and both A30P and A53T mutant proteins. Since all familial early-onset PD patients are heterozygotic for mutant α-syn, pure protein solutions were tested against 1:1 mixtures of WT and either A30P or A53T. Pure protein solutions were found to be indistinguishable from mixtures. Solutions were then incubated at certain temperatures and concentrations for a period of 2 to 9 weeks.

After a period of 3 weeks, spherical assemblies similar to protofibrils formed in the A30P solution, but there were no fibrils. In the A53T mutant a α-syn however, a substantial number of fibrils formed after two weeks (Fig 2). At slightly higher concentrations and temperatures, the A53T solution showed structural formation after only one day. The heterozygous WT/A53T was not as prolific, showing far fewer fibrils after two weeks. The A30P and WT solutions did show fibril formation, but not until after 9 weeks.

AFM was used to compare in-vitro (A53T, A30P, and WT) fibrils with those found in Lewy bodies. The group found the dimensions and morphology of their samples to be similar to those in Lewy bodies in brain slices of PD patients, and those in patients with diffuse Lewy body disease (LBD). In-vitro A53T, A30P and WT fibrils were 8-10 nm in height (by AFM), and around 10 nm in width (by electron microscopy). Some A53T and A30P fibrils were around 5 nm and contained a helical periodicity. Reported dimensions of fibrils extracted from the brains of LBD and multiple systems atrophy (MSA) patients are between 5 and 18 nm wide, and of similar structure.

In conclusion, Conway and coworkers find that accelerated α-syn fibril formation may result from the A53T and A30P mutations, thus causing early-onset PD.

ACCELERATION OF OLIGOMERIZATION

In another study led by Conway et al. [3], on acceleration of oligomerization, not fibrillization, is a shared property of both α-synuclein mutations linked to early-onset Parkinson's disease: implications for pathogenesis and therapy, they investigated whether nonfibrillar α-syn oligomers, instead of fibrils, are involved in PD pathogenesis.

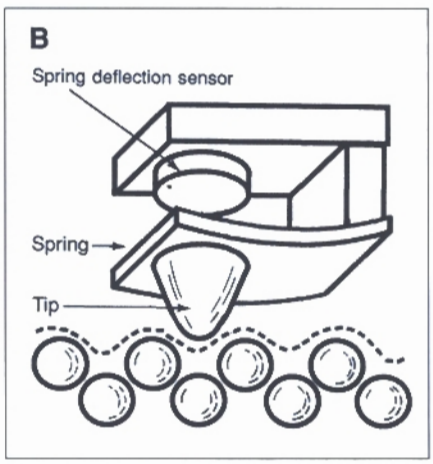

Figure 1:
Schematic diagram of the force sensor for an AFM. The tip, shown as a rounded cone, follows a path (dashed line) along the topography of the sample surface. Graphic originally in Hansma et al. [7] and is reproduced with the permission of the copyright holder.

This study follows a similar procedure of the last: solutions of WT, A53T and A30P are incubated for a period of 66 days, and fibrils were periodically removed from the supernatant, filtered, and quantified. Unfolded monomeric α-syn was separated from fibril α-syn. Rates of disappearance of unfolded monomeric α-syn were then contrasted with rates of appearance of fibril forms.

In comparing the kinetic activity of the WT and two mutant forms of α-syn, it was found that A53T monomeric form was consumed more rapidly, and fibrils were formed more rapidly than either the WT or A30P. However, in the A30P solution, the monomeric form of α-syn disappeared slightly more rapidly than WT, but fibrillized more slowly than WT. This suggested that the A30P solution formed an intermediary such as a prefibril oligomer.

Intermediary prefibril oligomers were detected with AFM imaging. Fibrils were separated from aliquots by sedimentation, and supernatant was subjected to gel filtration. After the monomeric protein was separated out, the remaining volume eluate was concentrated, and adsorbed onto atomically smooth mica for microscopy. Three related (of similar sizes) but discrete oligomers were observed: spheres, chains and rings (Fig 4).

It is not known if oligomers progress from sphere to chain to fibril, as they do in Aβ protofibrils in Alzheimer's disease, but this progression is suggested. Using this as an analogy, the group proposes that the α-syn chain "may be a direct precursor to the fibril; thus, its circularization may prevent fibril formation".

In conclusion the group suggests the possibility that fibrils are inert (or less toxic), and prefibrilar oligomers may be the pathogenic species. There has been some support for the idea that α-syn fibrils have a neuroprotective effect [9-11], and the mechanism proposed here supports that idea.

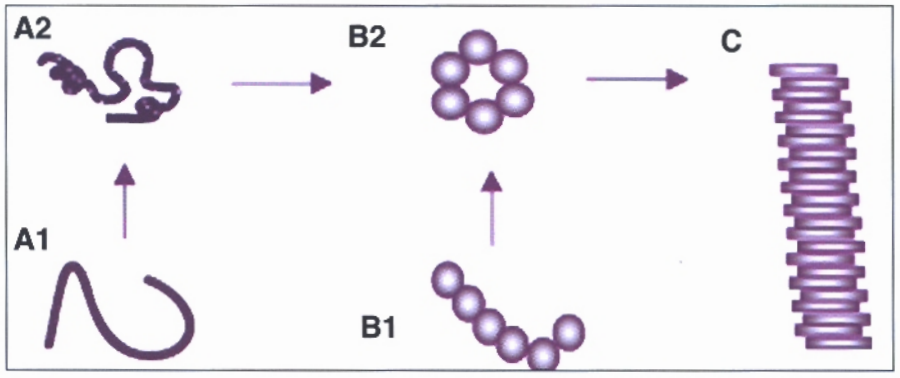

Figure 2:
Visual representation of α-synuclein fibril formation. (A1) Natively unfolded protein. (A2) Protein with cation interaction. (B1) Protofibrillar chain. (B2) Circularized protofibrils. Protofibrils are also referred to as prefibrillar oligomers. (C) α-syn fibrils.

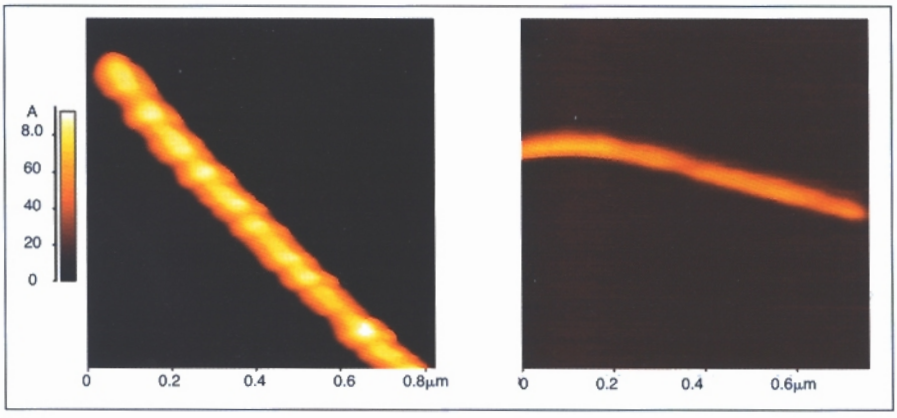

Figure 3:
AFM imaging of α-synuclein fibril.

LIPID BILAYER ACTIVITY

Taking another approach to discovering the pathogenesis of α-syn in PD, Jo et al.[2] have investigated whether α-syn interferes with the lipid bilayer activity in cells. Their paper begins with an evaluation of the structure of α-syn, diagramming the amphipathic α-helical domain at the amino-terminal that may be responsible for membrane-protein interactions. In addition to this, there is a hydrophobic fragment in the central region of α-syn, and an acidic region at the carboxyl-terminal region rich in glutamate, which may also play a role in lipid membrane interactions.

They further their suggestion that α-syn plays a role in lipid transport and synaptic membrane biogenesis by presenting four pieces of evidence. First, the amphipathic α-helical domain bears a similarity to class A_2 apolipoproteins, which reversibly bind and carry lipid molecules [12]. Second, α-syn has shown to be a "highly specific inhibitor of phospholipase D_2 which hydrolyzes phosphatidylcholine to produce phosphatidic acid and diacylglycerol" [13]. Therefore, the amount of α-syn expressed may alter the cleavage of certain membrane lipids. Third, α-syn co-localizes with synaptic vesicles [14-16]. Lastly, α-syn has been reported to "regulate the size of presynaptic vesicular pools" [17].

Using thin-layer chromatography (TLC), the group examined if there was a specific lipid ligand for α-syn. They found α-syn to bind to acidic phospholipid vesicles, and that "this binding was significantly augmented by the presence of phosphatidylethanolamine (PE)". AFM, in tapping mode, was used to examine the interaction of α-syn with these lipids (Fig 5).

In-situ AFM revealed that WT α-syn disrupted the POPC/POPS lipid bilayer. Over the period of several hours, holes formed and expanded in the bilayer. "Small aggregates and putative fibrils" were also detected against the mica surface. With the use of phase imaging and AFM it was detected that the aggregates are "likely aggregated α-syn or possibly α-syn-lipid complexes."

Lipid binding in mutant A53T α-syn was compared with WT through the use of in-situ AFM. AFM imaging from WT α-syn show more aggregation and fibrils than A53T (Fig 6). These results suggest that the A53T mutant α-syn may not bind lipids as well as WT. The group hypothesized that this difference in

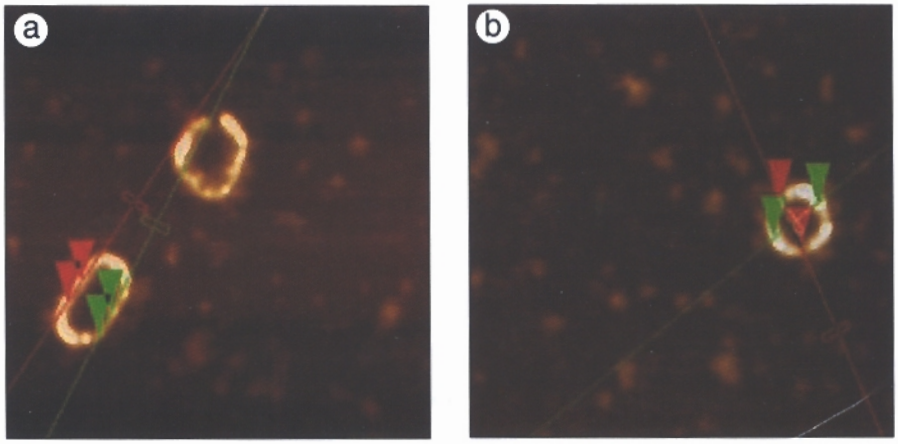

Figure 4:
AFM of two ring types: circle and ellipse. The arrows indicate points of maximum height: (a) 4nm; (b) 3.6-4.1nm (green); 2.1 and 2.2nm (red). HFW 400 nm and 300 nm respectively. Images originally in Conway et al. [3] and are reproduced with the permission of the copyright holder.

binding arises because A53T β-sheet structure does not bind as effectively as the monomeric α-helical structure. In conclusion, the authors suggest that part of the normal functioning of α-syn is the binding of lipids. Interestingly, it is noted in the background of this paper that the A30P mutations were unable to bind lipid vesicles [18]. This provides further evidence that α-syn normally binds lipids.

CONCLUSIONS

Clearly there are several uses for atomic force microscopy in Parkinson's disease research. α-syn aggregation and lipid binding are two areas that have benefited from microscopy, but there are perhaps other uses for the microscope. One group – Laney, Garcia, Parsons, and Hansma – has used AFM to image cholinergic synaptic vesicles in shark brains [19]. Their creative use of tapping-mode AFM has enabled them to create force plots and maps of entire biological samples. These maps provide information about the size, elasticity, and adhesive properties of the sample. This technique could provide important information about how proteins and neurons function in response to mechanical stress. If accurate information could be drawn from dopaminergic neurons in PD brains, new therapeutic and etiologic ideas may develop. Potentially, this could lead to an exciting new phase of Parkinson's disease research.

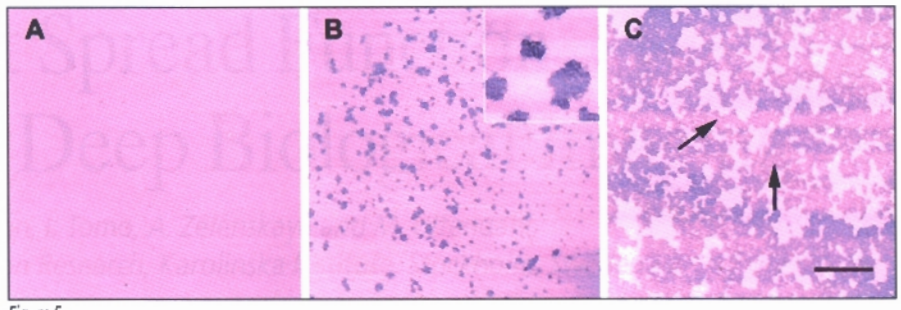

Figure 5:
AFM of POPC/POPS lipid bilayer disrupted by WT α-syn. (A) Bilayer. (B) 30 min after adding the WT α-syn. (C) 9 h after adding WT α-syn. Bar = 2 μm. Images from Jo et al.[2] and are reproduced with the permission of the copyright holder.

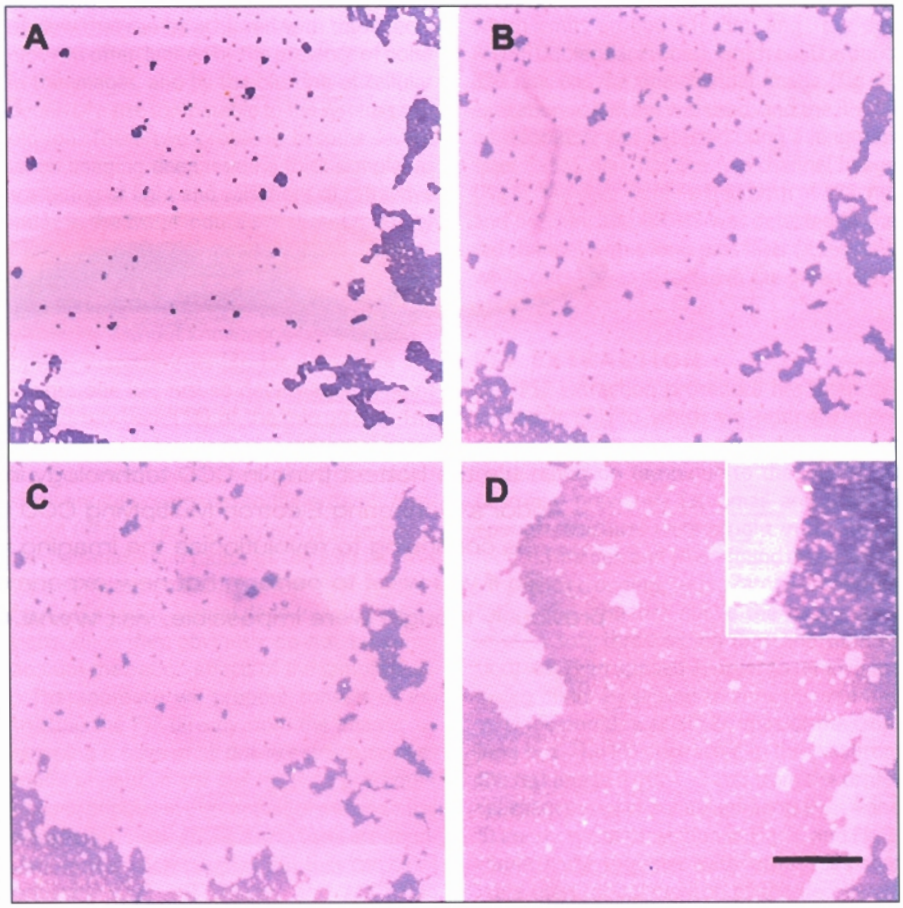

Figure 6:
AFM of POPC/POPS lipid bilayer disrupted by A53T mutant α-synuclein. (A) Bilayer. (B) 30 min after adding A53T α-syn. (C) 11 h post addition. (D) 15 h post addition. Bar = 2 μm. Images from Jo et al. [2], and are reproduced with the permission of the copyright holder.

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