Soluble state high resolution atomic force microscopy study of Alzheimer's β -amyloid oligomers

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(Received 23 July 2009; accepted 29 September 2009; published online 6 November 2009)

We report here the direct observation of high resolution structures of assemblies of Alzheimer β -amyloid oligomers and monomers using liquid atomic force microscopy (AFM). Visualization of nanoscale features of $A\beta$ oligomers (also known as ADDLs) was carried out in tapping mode AFM in F12 solution. Our results indicate that ADDL preparations exist in solution primarily as a mixture of monomeric peptides and higher molecular mass oligomers. Our study clearly reveals that the size and shape of these oligomer aggregates exhibit a pronounced dependence on concentration. These studies show that *wet* AFM enables direct assessment of oligomers in physiological fluids and suggests that this method may be developed to visualize $A\beta$ oligomers from human fluids. © 2009 American Institute of Physics. [doi:10.1063/1.3251779]

Alzheimer disease (AD) is one of an increasing number of diverse disorders that have been shown to be due to neurotoxins formed from the abnormal protein folding and aggregation of physiological proteins.^{1–5} AD (Ref. 6) is a fatal progressive dementia characterized pathologically by protein-based hallmarks known as neurofibrillary tangles and amyloid plaques.

A molecular mechanism with implications for therapeutics and diagnostics is now emerging in which the specificity of AD for memory derives from disruption of plasticity at synapses targeted by toxic $A\beta$ oligomers (also known as ADDLs).^{7–9} ADDLs accumulate in AD brain and constitute long-lived alternatives to the disease-defining $A\beta$ fibrils deposited in amyloid plaques.

Physiological temperatures promote formation of 12mers and some large oligomers as well. Fractionation experiments have shown that ADDLs equivalent to 12–24mers can be abundant in aqueous, detergent-free solutions but when exposed to strong detergents such as sodium dodecylsulfate (SDS) break apart into monomers, trimers, and tetramers.⁹ It recently has been found that SDS-stable 12–24mers are also promoted by prostaglandin and levuglandin.¹⁰

In AD brain, ADDLs accumulate primarily as $A\beta$ 12mers (~54 kDa) (Ref. 5) and can be found in dotlike clusters distinct from senile plaques. Oligomers of equal mass have been reported to occur in transgenic mouse AD models where they emerge concomitantly with memory failure,^{11,12} consistent with ADDL inhibition of long-term potentiation (LTP).⁷ Previous structural studies of ADDLs using the dry atomic force microscope (AFM) have shown that they exist as globular proteins of varying mass.¹³ Figure 1 summarizes one model proposed for the formation of oligomers and fibrils and their effects on cells.

Structural studies of amyloids from AD brain have been done with circular dichroism,^{14,15} electron microscopy,^{16–18}

and so on. With the recent advancement in AFM especially for biological imaging, the study of detailed biological structures at high resolution in their natural ambient conditions became possible and created an enormous expectation for AFM as an ideal structural tool for biology.¹⁹ AFM applications for imaging protofibrils, fibrils, and oligomers have been used recently to study the aggregation and self-assembly dynamics of $A\beta$ both at ambient conditions and in fluids.^{20,21} However, a lack of resolution of AFM in a more physiological medium to study these oligomers has limited their use in characterizing small oligomeric states.

We report here the results of AFM imaging of both oligomers and monomers in wet ambient conditions that provides significantly higher resolution using our methodology that combines use of AFM and its very specific probe as well as surface chemistry previously used only for dry AFM.²² This methodology, which is based on a very high resolution silicon probe attached to silicon cantilever and low-force touch due to tapping mode at each pixel in the AFM field.

Oligomers are prepared according to a previously published protocol.²³ Briefly, solid amyloid β peptide ($A\beta_{1-42}$, American Peptide) is monomerized in hexafluoro isopro-

FIG. 1. (Color online) A model showing the sequence of schematic assembly of $A\beta_{42}$ monomers which can lead to the formation of $A\beta$ oligomers (dimers, tetramers, 12mers, etc., and fibrils).

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FIG. 2. (Color online) (a) Tapping mode image (in liquid) of APTES coated mica control sample showing featureless and uniform surface and (b) surface topography of oligomers taken using conventional SiN cantilever having pyramidal probes. Background noise and surface corrugation is dominant in the image.

panol, aliquoted, evaporated, and stored as a solid film at -80°C . The day before the experiment, the peptide film is resuspended in anhydrous dimethyl sulfoxide (Sigma-Aldrich) to make a 5 mM solution. The 5 mM peptide stock is then brought to 100 μM with the addition of F12 medium without phenol red (Caisson Laboratories). The solution is vortexed thoroughly and incubated for 24 h at $4-5^\circ\text{C}$. Following incubation, the solution is centrifuged at $14\,000\times g$ for 10 min in the cold. The supernatant, which comprises ADDLs, is transferred to a new tube and stored at 4°C .

Aminopropyltriethoxysilane (APTES) was prepared by diluting 3 μl of glacial acetic acid into 50 ml double distilled water and adding 500 μl APTES (Sigma). Mica disks were then incubated with the APTES solution in a glass beaker for 15 min at RT with slow shaking. After rinsing six times with double distilled water, the disks were dried and baked at 100°C for 2 h in a glass petri dish. The disks were stored in desiccators until used. The solution to be tested (90–100 μl) was then carefully placed on the disk and allowed to incubate for 15–30 min before buffer (F12, 10 ml) was added to the dish, covering the mica.

AFM observations were carried out using the Veeco BioScope II. Imaging was performed under wet ambient conditions at 25°C using very sharp silicon probes (in tapping mode) attached to soft silicon nitride (SiN) cantilever with no reflecting coating on its back side (Spring constant $\sim 0.035\text{ N/m}$ and resonant frequency around 17 KHz). These especially designed probes were manufactured by Applied Nanostructures Inc. (model HYDRA6V200W).

When a 20 μM solution of ADDLs was brought into contact with the surface of APTES coated mica surface, AFM imaging using conventional SiN probe [Fig. 2] reveals soluble oligomers of $A\beta_{42}$ in F12 solution. It is clearly evident from the image that thermal noise due to laser spot heating in liquids as well as the pyramidal probes on SiN cantilever results in highly corrugated surface. Individual oligomer molecules are not resolved very well.

Figure 3(a) presents a wet AFM image (field size $2\text{ }\mu\text{m}\times 2\text{ }\mu\text{m}$) of soluble oligomers of $A\beta$ after <30 minutes deposition on the treated mica. The ADDLs appear as a field of globular structures with approximately

2–5 nm in height [as shown in cross-sectional profile in Fig. 3(b)]. The image resolution increases dramatically with these sharp probes with individual oligomers are resolved much well in comparison to conventional SiN probes. Moreover, the thermal noise in the images due to laser spot heating is eliminated from the image due to unique configuration of these probes as described in earlier sections.

Figure 4(a) shows a very high resolution image of $A\beta_{42}$ monomers (and oligomers) obtained by imaging an $A\beta_{42}$

FIG. 3. (Color online) (a) Liquid tapping mode image of $A\beta$ oligomers acquired in 20 μM solution of ADDLs in F12 solution. AFM examination of toxic ADDLs shows small globular structures approximately 2–4 nm in height and a distinct lack of fibrils. (b) Depicts cross-sectional profile.

FIG. 4. (Color online) (a) High resolution liquid tapping mode images of A β 42 monomers and oligomers. The molecule heights are in the range from 0.5–1 nm. For these images, the A β solution was imaged at a concentration of 1 μ M. (b) Section profile where the height of these monomers is around 0.5–1 nm.

solution at a concentration of approximately 1 μ M. These monomers have height of about 0.5 to 1 nm as seen in Fig. 4(b). These are the smallest particles that have been imaged in wet AFM and we interpret them as monomers.

High resolution tapping mode images of A β 42 monomers obtained in Fig. 4(a) are the result of several factors that includes the super sharp silicon probe on SiN cantilevers and surface chemistry of mica surface with APTES coating. The combination of these techniques allowed us the opportunity to image monomers with features down to 0.5 nm in F12. The mechanisms responsible for the binding of proteins to substrates may be due to electrostatic and Van der Waals forces.

Our study demonstrates that extremely high resolution images can be obtained from liquid AFM analysis using probes integrated with a very sharp silicon tip attached to a low stress SiN cantilever and combined with proper surface chemistry. In ADDLs research, it is most important to distinguish between the nontoxic monomer and potentially toxic oligomers. Furthermore, the question of which oligomeric

species is the causal agent in human toxicity is presently a matter of intense study. We may eventually be able to use this technique to determine the structure of ADDLs extracted from human brain.

This work was supported by grants from the National Science Foundation Award No. EEC-0647560 (WLK) and National Institutes of Health-National Institute on Aging Grant Nos. AG022547 and AG029460 (WLK). AFM imaging was performed in the NIFTI facility in the NUANCE center at Northwestern University, which is supported by NSF-NSEC, NSF-MRSEC, Keck Foundation, the State of Illinois, and Northwestern University.

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