Quantitative AFM morphometry of non-fibrillar α-synuclein aggregation products induced by the chaperone-like protein 14-3-3η

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Introduction

Idiopathic and familial forms of Parkinson's disease (PD) are associated with the abnormal neuronal accumulation of α synuclein (aS) leading to the formation of β-sheet-rich aggregates called Lewy Bodies (LBs). A connection between PD and the 14-3-3 chaperone like protein family was recently proposed, based on the fact that several 14-3-3 isoforms interact with PD-associated proteins such as parkin, LRRK2 and aS [1]. Moreover, 14-3-3 proteins show structural similarity with aS [2] and were found to be components of LBs in human PD [3]. The 14-3-3η (Eta) isoform in particular has been found associated with aS in parkinsonian brains, but not in healthy ones. In spite of the above, literature regarding the Eta/aS interplay is sparse.

Recent results [4] demonstrate that while Eta is unable to bind monomeric aS, it strongly interacts with oligomeric aS aggregates occurring during the pathological early aggregation stages of aS amyloidogenesis, drastically diverting the aggregation process even when present in sub stoichiometric amounts relative to aS.

Herein, we characterize the alternative aS non-fibrillar products (NFPs) induced by Eta via atomic force microscopy (AFM) imaging, then perform quantitative morphometry on a large pool of images to infer information about the mechanism of interaction between aS and Eta.

Materials and Methods

Protein expression and purification: aS and Eta were expressed and purified as described elsewhere [4].

Aggregation assays: Prior to aggregation, monomeric aS solutions were ultra filtered with a 100 kDa cut-off Vivaspin (Sartorius) filter to remove oligomeric aggregates. Eta was added to the aggregation mixtures to afford specific Eta:aS stoichiometric ratios of 1:4, 1:7, 1:12, 1:20, 1:24, 1:30, and 0. All aggregation experiments were carried out at 37°C in PBS supplemented with 0.05% (w/v) Sodium Azide and 5 mM DTT (when not stated differently), and providing a constant agitation at 1000 rpm. The sample volume was 200 μL and the reaction vessel a PCR vial.

Atomic force microscopy imaging was performed in PeakForce tapping mode with Scanasyst-Air probes (Bruker, Mannheim, Germany) on a Nanoscope V system equipped with a Multimode head and a type E piezoelectric scanner (Bruker, Mannheim, Germany). 10 μl of sample were deposited on freshly cleaved mica (RubyRed Mica Sheets, Electron Microscopy Sciences, Fort Washington, USA) and left to adsorb for 5 min at room temperature (~20 °C). The mica surface was then rinsed with ~500 μL of MilliQ H_2O (Millipore Simplicity) at the same temperature and dried with dry nitrogen. In most experiments, the sample was diluted ~10 times with PBS then equilibrated at RT for 10' prior to deposition in an attempt to minimize overlap of individual aS aggregates on the surface.

Results and Conclusions

While all aggregation experiments resulted in the formation of aS amyloid aggregates (Figure 1A, left column), only those obtained at an aS/Eta molar ratio of 30 or above revealed the canonical characteristics of mature aS fibrils. Fibril diameter distributions were converted into fibril section area per unit length (Figure 1A, right column). The integral of the equivalent disc area distribution calculated in the 0-75 nm^2 (and 75-300 nm^2) interval was used as an estimation of the total volume of NFPs (and fibrillar products) to generate the plot shown in Figure 1B. Our assumption is that the packing density of monomeric aS included into all aggregates is the same, thus the volumes calculated as described above are directly proportional with the amount of monomeric aS converted into the relevant aggregate type. The plots were fitted with a Langmuir isotherm (R^2=0.99) resulting in a reduced affinity constant (rK_d) of 26.6 aS

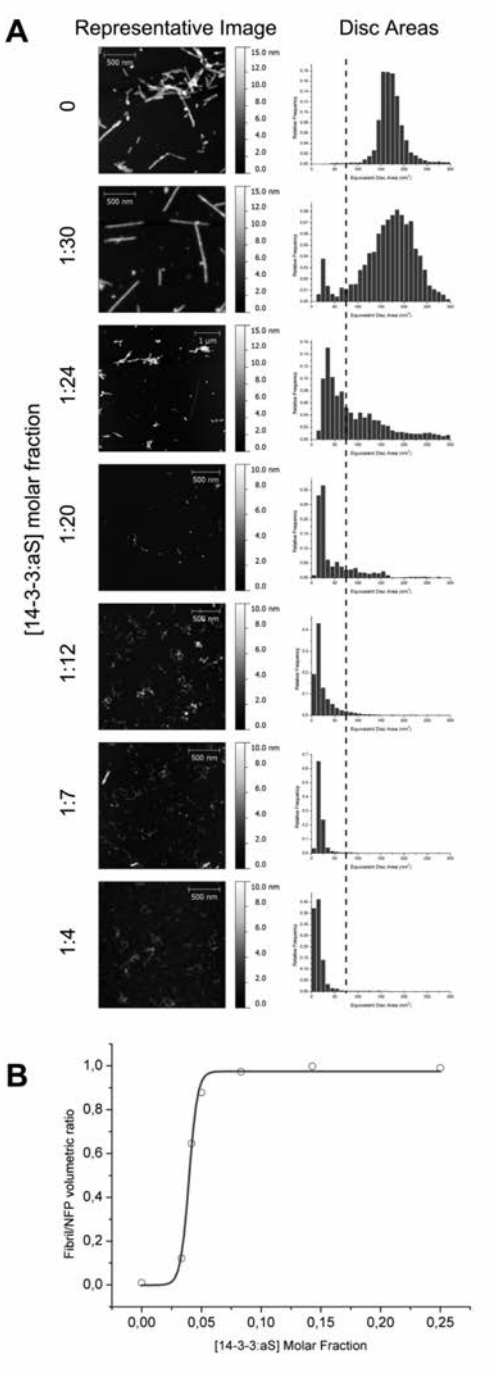

Figure 1. (A) AFM representative images (left column) and equivalent disc area distributions (right column) of the aS-NFPs induced by Eta at various relative stoichiometries. (B) Plot of relative molar fraction of starting monomeric aS trapped into NFPs (circles) VS [Eta:aS] molar ratios. The corresponding Langmuir isotherm fit is shown as a solid grey line.

equivalents per Eta homodimer. The fact that the dose-dependent repartition of monomeric aS between fibrils and NFPs can be fitted by a Langmuir isotherm showing almost no deviation from ideal behavior strongly suggests that the binding partner of Eta during aS aggregation is a specific, relatively homogeneous class of "critical" early oligomeric aggregates.

References

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