

Instantaneous Amyloid Fibril Formation of α -Synuclein from the Oligomeric Granular Structures in the Presence of Hexane

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ABSTRACT Amyloid fibrils found in various neurodegenerative disorders are also recognized as high-performance protein nanomaterials with a formidable rigidity. Elucidation of an underlying molecular mechanism of the amyloid fibril formation is crucial not only to develop controlling strategy toward the diseases, but also to apply the protein fibrils for future nanobiotechnology. α -Synuclein is an amyloidogenic protein responsible for the radiating filament formation within Lewy bodies of Parkinson's disease. The amyloid fibril formation of α -synuclein has been shown to be induced from the oligomeric granular species of the protein acting as a growing unit by experiencing structural rearrangement within the preformed oligomeric structures in the presence of an organic solvent of hexane. This granule-based concerted amyloid fibril formation model would parallel the prevalent notion of nucleation-dependent fibrillation mechanism in the area of amyloidosis.

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Amyloidosis is a clinical condition in which amyloid fibril formation derived from once soluble and innocuous proteins serves for a pathological signature as observed in various neurodegenerative disorders including Alzheimer's disease, Parkinson's disease (PD), and Prion disease (1–3). The amyloid fibrils have been also recognized as high-performance protein nanomaterials with a formidable rigidity (4,5). Elucidation of underlying molecular mechanism for the fibril formation, therefore, would provide us with not only controlling strategies toward the amyloidosis-related disorders but also biomaterials for future applications in the area of nanobiotechnology. The nucleation-dependent fibrillation has been widely recognized as a prevalent mechanism (6,7). By acting template, the nucleation centers accrete monomeric soluble proteins to the insoluble product of fibrils by directing structural transformation. In this article, however, we show an alternative fibrillation mechanism of concerted granular assembly to support the so-called stepwise polymerization model. α -Synuclein is an amyloidogenic protein responsible for the radiating filament formation in Lewy bodies of PD (8). Three separate missense mutations (A30P, E46K, and A53T) of its gene found in familial PD and deficits in motor activities of transgenic animals overexpressing the protein support the pathological role of α -synuclein (9,10). After a prolonged incubation in vitro, the protein known to exist in a 'natively unfolded' state turns into amyloid fibrils with discrete β -sheet conformation (11,12). Despite a great deal of effort, however, molecular mechanism for the fibril formation and toxic identity responsible for the neurodegeneration have not been unambiguously unveiled. In this respect, oligomeric species of α -synuclein have garnered much attention to assess not only the toxic cause but also the fibrillation process. The oligomeric species have been suggested to be a transient intermediate observed on the way to the eventual fibril formation (6,11). Alternatively, those species were also considered as an

off-pathway product independent of the fibrillation process (13,14). From the pathological point of view, they are suggested to be a toxic culprit for the neuronal degeneration by affecting membrane stability. We try to show that the oligomeric granular species act as a growing unit for the amyloid fibril formation by experiencing structural rearrangement within the preformed oligomeric structures in the presence of an organic solvent of hexane.

Accelerated amyloid fibril formation was observed with the oligomeric structures of α -synuclein in the presence of hexane (Fig. 1). The granular intermediates were collected at 12-h point during the lag phase of the fibrillation kinetics in which the stationary phase was reached in more than 60 h of prolonged incubation with a continuous shaking (Fig. 1 A). Homogeneous granular structures were revealed under atomic force microscope (AFM) with average height and width of 4.08 nm and 51.65 nm, respectively (see the Supplementary Material, [Data S1](#)). Dynamic light scattering experiment provided a hydrodynamic diameter of 49.04 nm for the granules (see the Supplementary Material, [Data S1](#)). The oligomeric species still retained mostly random structure as determined with circular dichroism spectroscopy although the minimum ellipticity at 195 nm became noticeably increased from that of soluble monomeric α -synuclein (Fig. 1 B). As hexane was added to a final concentration of 3%, the granules impressively turned into discrete amyloid fibrils within mere 5 min of vortexing. Shorter fibrils started to appear from 1% as shown with both AFM and transmission electron microscope (TEM) (Fig. 1, C and D). At 10% hexane, however, amorphous protein aggregates were obtained. On the other hand, the solvent did not affect the

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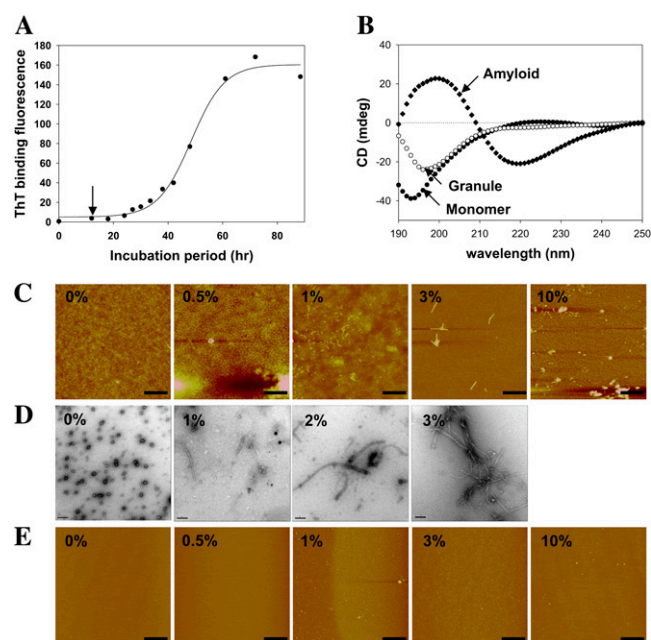


FIGURE 1 (A) Amyloid fibrillation kinetics of α -synuclein; granule collection at 12 h (arrow). (B) Circular dichroism spectra of monomers, granules, and amyloid fibrils. (C) AFM and (D) TEM images of the granules treated with hexane at final concentrations shown on the panels. (E) AFM images of the monomers treated with hexane. Scale bars in AFM and TEM represent 1 μ m and 200 nm, respectively.

monomeric protein at any concentrations to 10% (Fig. 1 E). The hexane-induced fibril was indistinguishable from the agitation-induced fibril obtained at the stationary phase (Fig. 1 A) with respect to their rugged surface appearance (Fig. 2 A). Its saw-like surface structure may indicate that the fibrillation could be derived from lateral association among the

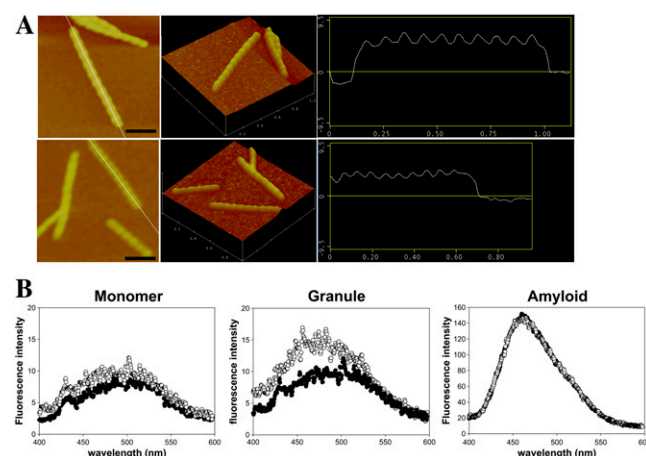


FIGURE 2 (A) AFM images of hexane- (top) and agitation-induced (bottom) fibrils; their surface appearance. (B) ANS binding fluorescence spectra of the monomer, the granule, and the amyloid in the presence (○) and absence (●) of 5% hexane. Scale bar, 0.25 μ m.

preformed granular species as they are structurally distorted or altered by the solvent. As a matter of fact, our ANS binding study showed that the granular species experienced a significant structural rearrangement as the ANS binding fluorescence at 475 nm was enhanced (Fig. 2 B, middle) whereas the ANS binding to monomeric α -synuclein was not influenced by the hexane treatment (Fig. 2 B, left).

To assess the granular assembly process, the preformed oligomeric species were incubated in the presence and absence of 5% hexane, which was compared with another fibrillation kinetics obtained by replacing a half of the granules with monomeric α -synuclein (Fig. 3 A). At the early stages of fibrillation (12-h time point), the hexane treatment augmented the fibrillation of the granule-only (1 mg) preparation by ~ 2 -fold compared to the 1:1 mixture between granules (0.5 mg) and monomers (0.5 mg), indicating that the hexane-dependent fibrillation was proportional to the quantity of granules (Fig. 3 B). Even after a prolonged incubation, the monomer-containing granular preparation did not reach to the fibrillation level achieved by the granule-only preparation regardless of the presence of hexane (Fig. 3 A). To prove the assembly of granular units, the 1:1 mixture was subjected to centrifugation to

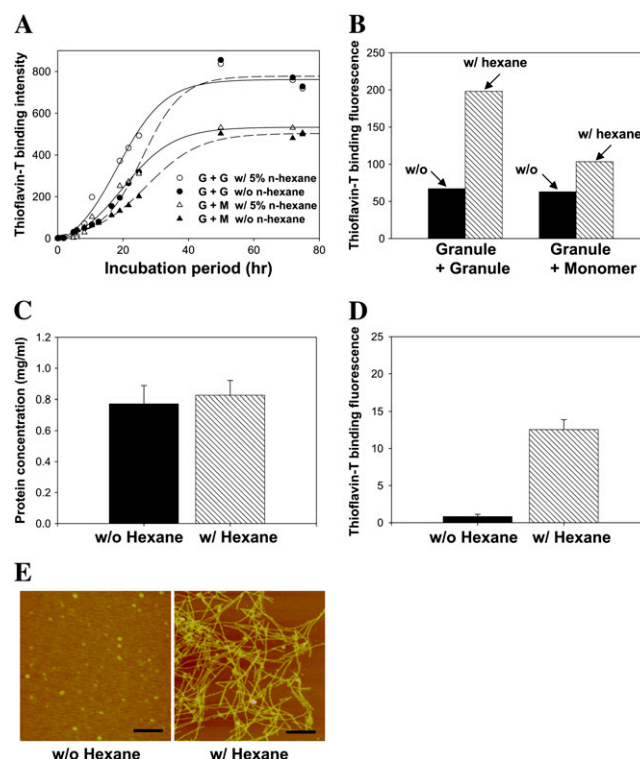
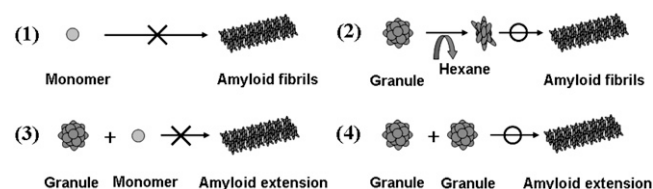


FIGURE 3 (A) Amyloid fibrillation kinetics of α -synuclein prepared in 1:1 mixtures of granule + granule or granule + monomer in the presence and absence of 5% hexane. (B) Thioflavin-T binding fluorescence of the aggregation mixtures collected at 12 h. (C) Protein content in the supernatants and (D) thioflavin-T binding fluorescence of the precipitates for the 1:1 mixture of granule + monomer with and without the 5% hexane-treatment. (E) AFM images of the precipitates with (right) and without hexane (left). Scale bar, 1 μ m.



SCHEME 1 Amyloid fibril formation of α -synuclein in the presence of hexane.

separate supernatant and precipitate in the presence or absence of the hexane treatment at 5%. Monomer level was evaluated in the supernatant with BCA protein assay whereas the fibril formation was monitored with thioflavin-T binding fluorescence and AFM. The monomer level in the hexane-treated sample was 0.83 mg/ml that was very much comparable to the amount obtained without the hexane treatment (Fig. 3 C). The precipitate contained a considerable level of amyloid fibrils as judged by the thioflavin-T binding fluorescence along with the AFM image (Fig. 3, D and E). In the absence of hexane, however, no fibrils except the granules were found in the precipitate (Fig. 3 E). Taken together, the amyloid fibrils have been shown to be produced exclusively from the granules without any involvement of the monomeric species.

In the presence of hexane, the amyloid fibrils were produced from the preformed granules, not from the monomers, through the structural distortion/alteration imposed by the organic solvent (Scheme 1). Subsequent lateral association of the energized granular species is responsible for the final suprastructure formation. Therefore, a concerted association mechanism of the preformed oligomeric species of α -synuclein has been proposed, in which the structural distortion induced by hexane could be considered as a prerequisite for the accelerated amyloid fibril formation. Exposure of the interfaces previously involved in intramolecular interactions within the preformed structures would lead to new engagement for intermolecular interactions between the distorted granules by acting as a growing unit in the presence of hexane.

The mechanism proposed in this report as the granule-based concerted amyloid fibril formation would provide a conceptual framework for which therapeutic strategies could be developed to take care of various amyloidosis-related diseases. In addition, the novel assembly mechanism from the preformed granular structures could shed light on our understanding of suprastructure formation and producing protein-based nanomaterials for applications in the area of biotechnology.

SUPPLEMENTARY MATERIAL

To view all of the supplemental files associated with this article, visit www.biophysj.org.

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