

Dynamic Aspects of Amyloid Fibrils of α -Synuclein Related to the Pathogenesis of Parkinson's Disease

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Abstract

α -synuclein (α Syn) is a 140 amino acid protein of unknown function, abundant in presynaptic terminals of nerve cells. Filamentous aggregates (amyloid fibrils) of α Syn have been shown to be involved with the pathogenesis of Parkinson's disease, a progressive neurodegenerative disorder. Elucidation of the mechanism of amyloid fibril formation of α Syn is thus important for elucidation of the pathogenesis mechanism of this disease. Amyloid fibril formation is observed for many proteins including, for example, the amyloid- β peptide, the prion protein, and transthyretin. Extensive studies on amyloid fibril formation have characterized structural and kinetic properties of these proteins during fibril formation. Whereas involvement of unfolding/misfolding of the proteins with fibril formation implies that the dynamics of the proteins plays an important role in fibril formation, the dynamic aspects of fibril formation have not been explored very much. In this review, dynamic behavior of α Syn in the monomeric and fibril states is described, based on our recent study on the dynamics of α Syn using quasielastic neutron scattering, by which the dynamics of proteins can be directly measured. It was found that diffusive global motions of the entire molecules and segmental motions within the molecules are observed in the monomeric state but largely suppressed in the fibril state. On the other hand, the amplitudes of the local motions such as side chain motions were found to be larger in the fibril state than in the monomeric state. This implies that significant solvent space exists within the fibrils, which is attributed to α Syn molecule within the fibrils having a distribution of conformations. The larger amplitudes of the side chain motions in the fibril state than in the monomeric state imply that the fibril state is entropically favorable. Implications of this unusual dynamic behavior of α Syn fibrils are discussed in terms of possible clinical relevance.

Keywords: α -synuclein; Parkinson's disease; Amyloid fibril; Quasielastic neutron scattering

Introduction

Parkinson's disease (PD) is an age-related neurodegenerative disorder. Neuropathological hallmarks of PD are a prominent loss of dopaminergic neurons in the substantia nigra pars compacta and formation of the protein inclusions called Lewy bodies (LB) and Lewy neurites (LN) in neuronal somas and processes, respectively [1]. The major component in these inclusions is α -synuclein (α Syn), which is a 140 amino acid protein of unknown function, usually abundant in presynaptic terminals of nerve cells [2]. Missense mutations [3-5] and overexpression due to gene duplication or triplication [6-8] of this protein are known to cause autosomal dominant early-onset PD. α Syn is thus involved with the pathogenesis of PD. Moreover, formation of LB and LN is a common hallmark of a diverse group of neurodegenerative diseases referred to as synucleinopathies, including dementia with Lewy bodies and multiple system atrophy, indicating involvement of α Syn with these diseases [9]. Understanding the mechanism of the pathogenesis of PD and related synucleopathies thus requires elucidating how α Syn is involved with the pathogenesis. In particular, since α Syn exists as filamentous aggregates, or amyloid fibrils, in LB and LN [10], elucidating how formation of amyloid fibrils of α Syn is involved with the pathogenesis of these diseases is important.

Formation of amyloid fibrils is observed not only for α Syn but also for various disease-related proteins including, for example, the amyloid- β peptide (related to Alzheimer's disease), the prion protein (spongiform encephalopathies), amylin (type II diabetes) and transthyretin (senile systemic amyloidosis and familial amyloid polyneuropathy) [11]. Moreover, proteins not associated with any known diseases have also been shown to form amyloid fibrils *in vitro* [12], and even "functional amyloids" have been found [13]. These proteins are not related to each other at all, but the fibrils formed have common characteristics (uniform and unbranched fibers with diameters of 60-120 Å, stabilized by a so-

called cross- β structure) [14]. Fibril formation of these proteins *in vitro* is in general promoted under partially denaturing conditions such as low pH and heating: Partially unfolded proteins under such conditions are misfolded to form nuclei, the protofilaments grow subsequently on these nuclei, and the mature fibrils form possibly by lateral association of the protofilaments [15]. The fibrils are thus formed through some underlying common mechanism directly related to physicochemical properties of proteins. Elucidation of the mechanism of amyloid fibril formation has therefore profound implications not only for elucidation of the pathogenesis of various diseases as a basis for developing therapeutic strategies against these diseases but also for gaining insights into the generic properties of proteins related to protein folding and stability.

Structural Properties and Characteristics of α -Synuclein

Amyloid fibril formation of α Syn has been extensively studied, and structural properties, kinetics of the fibril formation, and the factors that affect the kinetics have been characterized [16]. Bacterially expressed α Syn is intrinsically disordered in solution [17]. By many *in vitro* studies using this recombinant protein, the structural properties of α Syn in the

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monomeric state, intermediate oligomeric states, and the fibril state have been characterized [16]. In particular, recent crystallographic [18] and solid-state NMR spectroscopic [19] studies have shown the core structures of amyloid fibrils of α Syn at atomic resolution. Such structural information is obviously important for elucidating the mechanism of amyloid fibril formation of α Syn. However, involvement of the partial unfolding/(mis)folding in the process of fibril formation implies that the dynamics of the protein plays an important role. It is therefore important to characterize the dynamics of α Syn during fibril formation as well, particularly on the time scales of pico-to-nanoseconds, on which motions of polypeptide chains and their side chains occur [20], for full understanding of the mechanism of fibril formation. There were, however, rather few studies focusing on the dynamics of α Syn molecule [21-26], and direct comparison of the dynamics between different structural states was not reported.

Dynamic Behavior of α -Synuclein Characterized by Quasielastic Neutron Scattering

We recently reported a study comparing the dynamics of α Syn in the monomeric state with that in the fibril states [27]. We employed incoherent quasielastic neutron scattering (QENS) to measure the dynamics of α Syn on the picosecond time scale. QENS provides a unique tool to directly measure the dynamics of proteins on pico-to-nanosecond time scales and angstrom length scales [28]. QENS signals arise predominantly from the interactions between neutrons and hydrogen atoms because the scattering cross-section of hydrogen atoms is ~ 40 times larger than that of other atoms including deuterium (D). Since QENS measures the intensity of scattered neutrons as a function of both the energy transfer and the scattering angle, information on both frequency (corresponding to the energy transfer) and amplitudes (corresponding to the scattering angle) of the motions of hydrogen atoms can be obtained. The QENS measurements on protein samples in D_2O -solvent thus measure the dynamics of hydrogen atoms in the proteins. The motions of hydrogen atoms in proteins reflect the motions of larger groups, such as side chains and backbones of polypeptide chains, to which the hydrogen atoms are bound [28]. Because about half of the atoms in proteins are hydrogen atoms, and because they are pseudo-homogeneously distributed in the protein, QENS provides information on the average motion of the entire protein. QENS measurements can be performed whether the samples are in solutions, powders, or crystalline. The samples need not even be monodisperse. Direct comparison of the dynamics of the samples under various conditions is thus possible.

We carried out the QENS measurements on solution samples of α Syn in the monomeric and fibril states in D_2O -buffer. The QENS spectra of α Syn were extracted by subtracting the spectra of the D_2O -buffer from those of the samples, which contained small but non-negligible contribution from the signals from the D_2O -buffer. The QENS spectra of the proteins provide information on global motions related to those of the entire molecule and local motions such as the side chain motions. The information on the global motions is available as the apparent diffusion coefficients (D_{app}) of the proteins. The D_{app} values of α Syn in the monomeric state were found to be around $1-1.6 \times 10^{-6}$ cm²/s, which are about twice larger than the values of the translational diffusion coefficients of α Syn [29,30]. This was shown to be due to the contributions of rotational diffusion and additional internal motions such as segmental motions and long-range correlated motions, in addition to the translational diffusion. On the other hand, D_{app} in the

fibril state were found to be in the order of 10^{-8} cm²/s. This indicates that the global motions observed for α Syn in the monomeric state is largely suppressed in the fibril state, leaving only slow diffusive motion in a confined volume.

The local motions were found to be regarded as diffusive motions in a confined space for both the monomeric and fibril states. The activation energy of these motions in both states was comparable to that of the motions of water molecules, indicating that these motions involve hydrogen-bond fluctuations to water molecules. This implies that the side chains of α Syn are surrounded by water molecules even in the fibril state. The amplitudes of the local motions can be characterized by the radius of a confined sphere, in which the atoms undergo diffusive motions [31]. The radius of the confined sphere was found to be larger in the fibril state (4.9-7.5 Å, depending on the temperature) than in the monomeric state (3.5-4.4 Å). This is surprising because association of the protein molecules usually restricts their motions as, for example, observed for F-actin, a filamentous polymer formed by the actin molecules (the actin molecules in F-actin show less flexibility than non-polymerizable G-actin [32,33]). Large solvent-filled space within the fibrils is required to make such behavior possible. Such space should exist to accommodate the α Syn molecules, which are disordered even in the fibrils except for the core structure, into the fibrils.

Implications of the Dynamic Behavior of α -Synuclein Fibrils

This unusual behavior of the fibrils has important implications. The radius of the confined sphere of the side chain motions can be related to the conformational entropy of the proteins [34]. The larger radius in the fibrils than in the monomers implies the larger conformational entropy of the fibrils than that of the monomers. Estimation of the conformational entropy change by fibril formation, taking account of this relationship, the conformational entropy of the backbone, the entropy loss due to fibril formation, and the hydration entropy, suggested that the entropy increases with fibril formation. Comparison of this entropy change with the enthalpy change by fibril formation suggested that fibril formation is an entropy-driven process. Thus, once a potential barrier is overcome, fibril formation could proceed naturally. The rate-limiting step of fibril formation would then be the kinetics involved to overcome the potential barrier.

Various factors affect the rate of fibril formation of α Syn, including external triggers such as agitation and environmental conditions such as ionic strength, metal concentration, pH, temperature, and molecular crowding [16]. Moreover, the mutants of α Syn related to familial PD, such as A30P and A53T, have an increased propensity to form fibrils [35]. Such external triggers and environmental conditions appear to be factors that affect the potential barrier against the fibril formation. Considering the fact that sporadic PD is an age-related late-onset disease whereas the familial PD, in which the mutations above were identified, is an early-onset disease, the onset of the symptom of PD may depend critically on this kinetic step to overcome the potential barrier.

The unusual dynamic behavior observed for α Syn fibrils is also observed for amyloid fibrils of other proteins such as concanavalin A [36] and hen egg-white lysozyme in water-ethanol mixtures as a model system of amyloid fibril formation [37]. This "abnormal" dynamics may well be relevant to the general mechanism of amyloid fibril formation. A possibility that fibril formation proceeds naturally is in concert with a study [38] suggesting that amyloid fibrils are thermodynamically

more stable than the functional native states. A therapeutic strategy for preventing fibril formation should then include increasing the kinetic barrier for fibril formation by possibly controlling the dynamics of the target protein. This kind of strategy has indeed been employed for the drug development for the transthyretin amyloidosis [39]. Elucidating how the dynamics of a protein can be controlled requires characterization of the dynamic behavior of the protein, to which QENS can make substantial contribution.

Conclusion

The dynamic properties of α Syn are important for elucidating the mechanism of fibril formation of α Syn, as well as the structural properties. Our recent study using QENS characterizes the dynamic behavior of α Syn in the monomeric and fibril states. It is shown that α Syn in the monomeric state undergoes global motions including not only translational and rotational diffusion of the entire molecule but also segmental motions within the molecule, which are largely suppressed in the fibril states. It is also shown that the local motions such as the side chain motions have larger amplitudes in the fibril state than those in the monomeric state. This behavior implies that fibril formation is an entropy-driven process, which further implies that once a potential barrier of fibril formation is overcome, fibril formation could proceed naturally. A possible strategy for preventing fibril formation would then be increasing this kinetic barrier by possibly controlling the dynamics of the proteins.

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References

1. Yasuda T, Nakata Y, Mochizuki H (2013) α -Synuclein and neuronal cell death. *Mol Neurobiol* 47: 466-483.
2. Maroteaux L, Campanelli JT, Scheller RH (1988) Synuclein: A neuron-specific protein localized to the nucleus and presynaptic nerve terminal. *J Neurosci* 8: 2804-2815.
3. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, et al. (1997) Mutation in the α -synuclein gene identified in families with parkinson's disease. *Science* 276: 2045-2047.
4. Krüger R, Kuhn W, Müller T, Woitalla D, Graeber M, et al. (1998) Ala30Pro mutation in the gene encoding α -synuclein in Parkinson's disease. *Nat Genet* 18: 106-108.
5. Zarranz JJ, Alegre J, Gómez-Esteban JC, Lezcano E, Ros R, et al. (2004) The new mutation, E46K, of α -synuclein causes Parkinson and Lewy body dementia. *Ann Neurol* 55: 164-173.
6. Singleton AB, Farrer M, Johnson J, Singleton A, Hague S, et al. (2003) A-Synuclein locus triplication causes Parkinson's disease. *Science* 302: 841.
7. Farrer M, Kachergus J, Forno L, Lincoln S, Wang DS, et al. (2004) Comparison of kindreds with parkinsonism and α -synuclein genomic multiplications. *Ann Neurol* 55: 174-179.
8. Singleton A, Gwinn-Hardy K, Sharabi Y, Li ST, Holmes C, et al. (2004) Association between cardiac denervation and parkinsonism caused by α -synuclein gene triplication. *Brain* 127: 768-772.
9. Spillantini MG, Crowther RA, Jakes R, Cairns NJ, Lantos PL, et al. (1998) Filamentous α -synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. *Neurosci Lett* 251: 205-208.
10. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) A-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. *Proc Natl Acad Sci U S A* 95: 6469-6473.
11. Rambaran RN, Serpell LC (2008) Amyloid fibrils: Abnormal protein assembly. *Prion* 2: 112-117.
12. Dobson CM (2001) The structural basis of protein folding and its links with human disease. *Philos Trans R Soc Lond B Biol Sci* 356: 133-145.
13. Fowler DM, Koulov AV, Balch WE, Kelly JW (2007) Functional amyloid--from bacteria to humans. *Trends Biochem Sci* 32: 217-224.
14. Sunde M, Blake CCF (1998) From the globular to the fibrous state: Protein structure and structural conversion in amyloid formation. *Quart Rev Biophys* 31: 1-39.
15. Dobson CM (2003) Protein folding and misfolding. *Nature* 426: 884-890.
16. Breydo L, Wu JW, Uversky VN (2012) α -synuclein misfolding and Parkinson's disease. *Biochim Biophys Acta* 1822: 261-285.
17. Weinreb PH, Zhen W, Poon AW, Conway KA, Lansbury PT Jr (1996) NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry* 35: 13709-13715.
18. Rodriguez JA, Ivanova MI, Sawaya MR, Cascio D, Reyes FE, et al. (2015) Structure of the toxic core of α -synuclein from invisible crystals. *Nature* 525: 486-490.
19. Tuttle MD, Comellas G, Nieuwkoop AJ, Covell DJ, Berthold DA, et al. (2016) Solid-state NMR structure of a pathogenic fibril of full-length human α -synuclein. *Nat Struct Mol Biol* 23: 409-415.
20. Henzler-Wildman K, Kern D (2007) Dynamic personalities of proteins. *Nature* 450: 964-972.
21. Ulmer TS, Bax A, Cole NB, Nussbaum RL (2005) Structure and dynamics of micelle-bound human α -synuclein. *J Biol Chem* 280: 9595-9603.
22. Heise H, Hoyer W, Becker S, Andronesi OC, Riedel D, et al. (2005) Molecular-level secondary structure, polymorphism, and dynamics of full-length α -synuclein fibrils studied by solid-state NMR. *Proc Natl Acad Sci USA* 102: 15871-15876.
23. Sung YH, Eliezer D (2007) Residual structure, backbone dynamics and interactions within the synuclein family. *J Mol Biol* 372: 689-707.
24. Wu KP, Kim S, Fela DA, Baum J (2008) Characterization of conformational and dynamic properties of natively unfolded human and mouse α -synuclein ensembles by NMR: Implication for aggregation. *J Mol Biol* 378: 1104-1115.
25. Ahmad B, Chen Y, Lapidus LJ (2012) Aggregation of α -synuclein is kinetically controlled by intramolecular diffusion. *Proc Natl Acad Sci U S A* 109: 2336-2341.
26. Bousset L, Brewee C, Melki R, Migliardo F (2014) Dynamical properties of α -synuclein in soluble and fibrillar forms by quasi elastic neutron scattering. *Biochem Biophys Acta* 1844: 1307-1316.
27. Fujiwara S, Araki K, Matsuo T, Yagi H, Yamada T, et al. (2016) Dynamical behavior of human α -synuclein studied by quasielastic neutron scattering. *PLoS ONE* 11: e0151447.
28. Smith JC (1991) Protein dynamics: comparison of simulations with inelastic neutron scattering experiments. *Quart Rev Biophys* 24: 227-291.
29. Bodner CR, Dobson CM, Bax A (2009) Multiple tight phospholipid-binding modes of α -synuclein revealed by solution NMR spectroscopy. *J Mol Biol* 390: 775-790.
30. Wang Y, Benton LA, Singh V, Pielak GJ (2012) Disordered protein diffusion under crowded conditions. *J Phys Chem Lett* 3: 2703-2706.
31. Volino F, Dianoux AJ (1980) Neutron incoherent-scattering law for diffusion in a potential of spherical symmetry: General formalism and application to diffusion inside a sphere. *Mol Phys* 41: 271-279.
32. Fujiwara S, Plazanet M, Matsumoto F, Oda T (2008) Differences in internal dynamics of actin under different structural states detected by neutron scattering. *Biophys J* 94: 4880-4889.
33. Fujiwara S, Plazanet M, Matsumoto F, Oda T (2011) Internal motions of actin characterized by quasielastic neutron scattering. *Eur Biophys J* 40: 661-671.
34. Fitter J (2003) A measure of conformational entropy change during thermal protein unfolding using neutron spectroscopy. *Biophys J* 84: 3924-3930.
35. Li J, Uversky VN, Fink AL (2001) Effect of familial parkinson's disease point mutations A30P and A53T on the structural properties, aggregation and fibrillation of human α -synuclein. *Biochemistry* 40: 11604-11613.

36. Schirò G, Vetri V, Frick B, Militello V, Leone M, et al. (2012) Neutron scattering reveals enhanced protein dynamics in concanavalin A amyloid fibrils. J Phys Chem Lett 3: 992-996.
37. Fujiwara S, Yamada T, Matsuo T, Takahashi N, Kamazawa K, et al. (2013) Internal dynamics of a protein that forms the amyloid fibrils observed by neutron scattering. J Phys Soc Jpn 82: SA019.
38. Baldwin AJ, Knowles TP, Tartaglia GG, Fitzpatrick AW, Devlin GL, et al. (2011) Metastability of native proteins and the phenomenon of amyloid formation. J Am Chem Soc 133: 14160-14163.
39. Johnson SM, Connelly S, Fearn C, Powers ET, Kelly JW (2012) The transthyretin amyloidosis: From delineating the molecular mechanism of aggregation linked to pathology to a regulatory-agency-approved drug. J Mol Biol 421: 185-203.

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