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

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Abstract

α-synuclein (αSyn) is a 140 amino acid protein of unknown function, abundant in presynaptic terminals of nerve cells. Filamentous aggregates (amyloid fibrils) of αSyn 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 αSyn 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 αSyn in the monomeric and fibril states is described, based on our recent study on the dynamics of αSyn 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 αSyn 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 αSyn 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 (αSyn), 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. αSyn 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 αSyn with these diseases [9]. Understanding the mechanism of the pathogenesis of PD and related synucleopathies thus requires elucidating how αSyn is involved with the pathogenesis. In particular, since αSyn exists as filamentous aggregates, or amyloid fibrils, in LB and LN [10], elucidating how formation of amyloid fibrils of αSyn is involved with the pathogenesis of these diseases is important.

Formation of amyloid fibrils is observed not only for αSyn 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 αSyn 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 αSyn is intrinsically disordered in solution [17]. By many *in vitro* studies using this recombinant protein, the structural properties of αSyn 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 αSyn at atomic resolution. Such structural information is obviously important for elucidating the mechanism of amyloid fibril formation of αSyn. 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 αSyn 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 αSyn 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 αSyn in the monomeric state with that in the fibril states [27]. We employed incoherent quasielastic neutron scattering (QENS) to measure the dynamics of αSyn on the picosecond time scale. QENS provides a unique tool to directly measure the dynamics of proteins on pico-to-nanosecond time scales and ångstrom 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 αSyn in the monomeric and fibril states in D_2O-buffer. The QENS spectra of αSyn 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 αSyn in the monomeric state were found to be around 1-1.6 × 10^{-6} cm^2/s, which are about twice larger than the values of the translational diffusion coefficients of αSyn [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} cm^2/s. This indicates that the global motions observed for αSyn 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 αSyn 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 αSyn 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 αSyn, 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 αSyn 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 αSyn 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 αSyn are important for elucidating the mechanism of fibril formation of αSyn, as well as the structural properties. Our recent study using QENS characterizes the dynamic behavior of αSyn in the monomeric and fibril states. It is shown that αSyn 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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