INVITED REVIEW

Navigating the dynamic landscape of alpha-synuclein morphology: a review of the physiologically relevant tetrameric conformation

Heather R. Lucas*, Ricardo D. Fernández
Department of Chemistry, Virginia Commonwealth University, Richmond, VA, USA

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Abstract

N-acetylated α-synuclein (αSyn) has long been established as an intrinsically disordered protein associated with a dysfunctional role in Parkinson's disease. In recent years, a physiologically relevant, higher order conformation has been identified as a helical tetramer that is tailored by buried hydrophobic interactions and is distinctively aggregation resistant. The canonical mechanism by which the tetramer assembles remains elusive. As novel biochemical approaches, computational methods, pioneering purification platforms, and powerful imaging techniques continue to develop, puzzling information that once sparked debate as to the veracity of the tetramer has now shed light upon this new counterpart in αSyn neurobiology. Nuclear magnetic resonance and computational studies on multimeric αSyn structure have revealed that the protein folding propensity is controlled by small energy barriers that enable large scale reconfiguration. Alternatively, familial mutations ablate tetramerization and reconfigure polymorphic fibrillization. In this review, we will discuss the dynamic landscape of αSyn quaternary structure with a focus on the tetrameric conformation.

Correspondence to:
Heather R. Lucas, PhD,
hrlucas@vcu.edu.

orcid:
0000-0003-1207-7550
(Heather R. Lucas)

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Introduction

α-Synuclein (αSyn) is a major component of Lewy bodies and Lewy neurites, which are the pathological intracellular lesions associated with Parkinson's disease (PD), Alzheimer's disease, and dementia with Lewy bodies (Lippa et al., 1998; Braak et al., 2004; Wakabayashi et al., 2013; Longhena et al., 2019). Misfolded and aggregated αSyn also comprises inclusions linked to multiple system atrophy (Peng et al., 2018). Accumulation of these αSyn aggregates is a common denominator among all of these neurodegenerative diseases; the formation of oligomers is implicated as the toxic conformer that triggers pathogenesis. Other diagnostic hallmarks of PD are cell loss in the substantia nigra and other pigmented brain stem nuclei (Yazawa and Suzuki, 2014). The presence of functionally altered astrocytes and macrophages also accompany this loss. As the disease progresses, the neuropathic regions exhibit a consistent accumulation of αSyn oligomers and/or fibrils. Extracellular release and propagation of αSyn aggregates to recipient cells is thought to be a contributing factor to PD pathology (Danzer et al., 2009; Steiner et al., 2018; Hoffman et al., 2019). The PD pathosis is initially observed in the olfactory bulb; oligomers are consistently allocated at the dorsal motor nucleus of the vagus nerve in the medulla (Braak et al., 2004, 2006). PD thereafter spreads towards the brain stem, telencephalon, and cerebral cortex. Degeneration within the cerebral cortex often aligns with progression of dementia at advanced disease stages. It has also been suggested by some that PD pathology begins in the gut due to Lewy pathology in the enteric nervous system, pointing to a multi-organ degeneration pathway (Beach et al., 2010; Gelpi et al., 2014; Corbillé et al., 2016).

Historically, αSyn has been considered as an intrinsically disordered protein (IDP) in the healthy, physiological state, yet this natively unfolded protein is capable of undergoing structural recombination to form ordered protein arrays (Dunker et al., 2008; Mor et al., 2016). IDPs exist as dynamic protein ensembles with fluxional structures under physiological conditions that can adopt different conformations depending on their external stimuli, thus enabling the complex signaling and regulatory requirements of higher biological systems (Minde et al., 2017; Chen and Kriwacki, 2018). Under certain conditions, unfolded monomeric αSyn can alternatively misfold and self-assemble into a series of dysfunctional aggregates with a high degree of β-sheet structure through on- or off-pathway oligomerization and/or fibrillization routes (Tycko and Wickner, 2013; Wang et al., 2016; Ottolini et al., 2017). Multimeric αSyn morphology has been broadly studied providing irrefutable evidence of pathological character and dynamic co-existence with non-pathological structures. Tissue biopsies from mammalian sources identified an irreversible post-translational modification (PTM) of αSyn in which an acetyl group is covalently attached at the N-terminal methionine residue (Anderson et al., 2006). Incorporation of this functional group results in an alteration in the global landscape of αSyn, such as enhanced helicity (Fauvet et al., 2012a; Kang et al., 2012). No-

tably, biochemical strategies to mimic this native PTM have been accompanied by the characterization of a more structurally inert αSyn conformation, tetrameric αSyn, which was previously unknown (**Figure 1**).

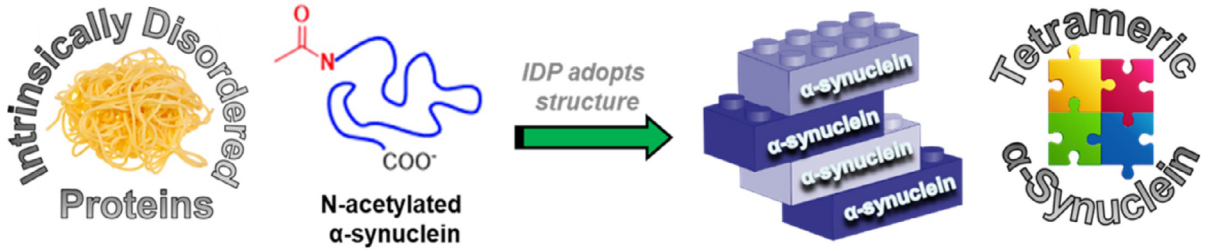

Figure 1 Representation of how the intrinsically disordered protein α-synuclein can adopt an ordered tetrameric structure.
IDP: Intrinsically disordered protein.

In recent years, tetrameric αSyn has been proposed as a novel target for therapeutic treatment of PD as a result of its ability to resist aggregation (Dettmer et al., 2015b). The pathway to tetramerization remains elusive, but its morphology is found to resist environmental conditions that are unfavorable for the unfolded monomer which can surrender to oligomerization and/or fibrillization. In the proximity of membranes rich in acidic phospholipids, monomeric αSyn adopts a helical structure (Trexler and Rhoades, 2009; Dikiy and Eliezer, 2014). This amphipathic α-helix region has been implicated to endorse the higher order tetrameric conformation (Rovere et al., 2018); however, evidence is still lacking. Helical αSyn multimers have also been suggested to regulate synaptic transmission and potentially be involved in the exocytic process and vesicle turnover (Burré et al., 2014; Wang et al., 2014). Novel purification methods from mammalian cells (Bartels et al., 2011b; Luth et al., 2015; Dettmer, 2018) and through bacterial cell lines (Wang et al., 2011; Trexler and Rhoades, 2012; Fernández and Lucas, 2018b) along with powerful computational studies (Gurry et al., 2013; Cote et al., 2018; Xu et al., 2018) have provided insight into the biophysical complexity of the elusive tetramer. Through continued cross-disciplinary research, the intricate relationship between the tetramer and the monomer will be clarified. In this review, we will highlight some of the molecular level details that define the structural diversity of multimeric αSyn, paying special attention to the newest conformer on the block, tetrameric αSyn. For this review, literature searches were performed on public databases (PubMed) using the terms "tetramer" and "synuclein"; articles that were published up until March 2019 were included.

Conformational Variance of α-Synuclein

αSyn is a structurally dynamic protein constituted of 140 amino acids, and it entails three characteristic regions (**Figure 2A**). The first 60 residues are known as the N-terminal amphipathic region; this segment demonstrates enhanced helical propensity under specific physiological and/or experimental conditions (Sung and Eliezer, 2018). The non-amyloid β-component (NAC) region comprises residues 61–95 and features the hydrophobic self-aggregation sequence designated as the NACore (Rodriguez et al., 2015). Between them, these two regions also house other key sequence features that will be discussed in more detail below, including a set of nine imperfect 11-residue repeats with a KTKEGV consensus core as well as pairs of β-branched amino acids (V, T) that influence protein secondary structure. A 10-residue stretch of amino acids just proximal to the NAC region is known as the "preNAC" segment (Rodriguez et al., 2015). The preNAC residues house many of the known familial mutations linked to PD and, like the NACore, this segment has been implicated in fibril formation. The third region is an acidic C-terminal region, composed of residues 96–140. Although αSyn as a whole demonstrates dynamic behavior, the C-terminal region in particular carries many characteristics of disordered proteins, including a relatively high net charge and a collection of proline residues that disrupt backbone hydrogen bonding, thus disfavoring secondary structural elements including α-helices and β-sheets. Indeed, the C-terminal region is expected to remain largely unfolded based on FoldIndex©, a predictive tool based on Uversky's algorithm for whether or not protein regions are intrinsically disordered (Uversky et al., 2000; Felder et al., 2005). The C-terminal region is also thought to be involved in mediating the interactions of αSyn with other neuronal proteins, contributing to an αSyn interactome with the diversity typical of IDPs (Kim et al., 2002; Lassen et al., 2016). The STRING database, which enables the mapping of protein-protein association networks through published experimental evidence, literature reports, and bioinformatic predictions, was used to illustrate the nearest ten associations (**Figure 2B**) (Gable et al., 2018).

Classical biochemistry pedagogy teaches that protein sequence dictates protein structure, and protein structure dictates protein function. In contrast to canonical proteins possessing well-defined three-dimensional structures, IDPs often function from a transient or dynamic structural state, and it is becoming increasingly clear that this once-overlooked class of proteins plays a critical role in many biological pathways (Oldfield and Dunker, 2014; Minde et al., 2017). IDPs can reconfigure structurally in response to environmental conditions, such as thermal fluctuations, solvent exposure, pH changes, or chemical dyshomeostasis. While

well-defined folded proteins tend to favor a single physiologically relevant structure as it represents the lowest energy state, IDPs have multiple local energy minima and can thus access multiple folded or unfolded states, which can be conformationally steered through external phenomena (**Figure 2C**) (Burger et al., 2014). Like many IDPs, αSyn displays a broad plasticity in conformational distribution, enabling a versatile structural continuum and the possibility of diverse functional roles that would not be feasible from a single well-ordered structure.

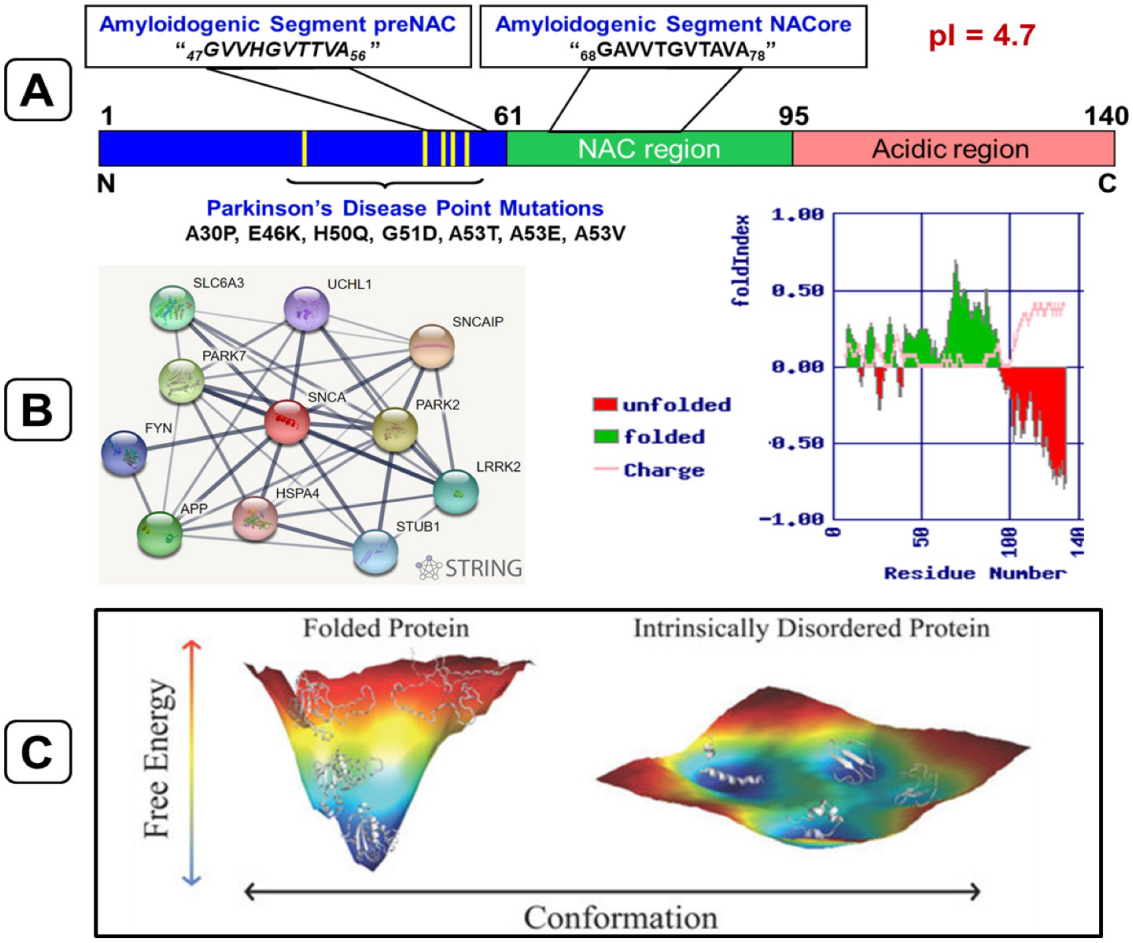

Figure 2 Demonstration of the unique structure, biophysical characteristics, and features of α-synuclein.
(A) Simplified depiction of the three regions of α-synuclein that pinpoints the amyloidogenic segments and Parkinson's disease-relevant genetic point mutations. (B) Data obtained from publicly available computational algorithms, including the STRING database and FoldIndex©. (C) Exemplary energy landscape of a folded protein versus an intrinsically disordered protein; diagram is modified from Burger et al. (2014). NAC: Non-amyloid β-component.

Fibrillar conformations of αSyn are perhaps the most widely recognized among the broader scientific community, in part owing to analogies that can be drawn with the notorious deposits of fibrillar amyloid-β found in Alzheimer's disease patients. Recent applications of advanced structural characterization techniques such as electron microscopy (EM) and solid-state nuclear magnetic resonance (NMR), along with support from theoretical experiments, have shed light on the atomic details of polymorphic fibrillar structures (**Figure 3** top right) (Lemkau et al., 2013; Gath et al., 2014; Rodriguez et al., 2015; Tuttle et al., 2016; Guerrero-Ferreira et al., 2018; Li et al., 2018a, b; Chakraborty and Chattopadhyay, 2019; Hwang et al., 2019). Cryo-EM studies have demonstrated that fibrillization of αSyn is promoted by intermolecular interactions whereby the NACore ($_{68}$GAVVTGVTAVA$_{78}$) self-associates in β-strands to form a steric zipper, as does the preNAC segment ($_{47}$GVVHGVTTVA$_{56}$), which can then stack into in-register β-sheets to form elongated fibrils (Rodriguez et al., 2015). Interfilament packing around these distinct zipper interfaces derived from preNAC and NACore segments give rise to two different classes of polymorphic fibrils, termed rods and twisters, respectively (Li et al., 2018a). Fibrillar αSyn can act as seed material for aggregation, with variable seeding potency and cytotoxicity for different fibril polymorphs (Bousset et al., 2013; Oueslati et al., 2014; Peelaerts et al., 2015; Okuzumi et al., 2018; Peng et al., 2018; Candelise et al., 2019).

Similarly, αSyn oligomerization is the consequence of β-sheet stacking varying in size, interaction, and hydrophobicity exposure, resulting in a vast assortment of structurally

diverse oligomers (**Figure 3** bottom right) (van Diggelen et al., 2017). Oligomers can be on- or off-pathway with respect to fibrillization, and many species of oligomeric αSyn demonstrate toxicity linked to membrane disruption (Tsigelny et al., 2012). For example, αSyn oligomers that accumulate during fibrillization share a hollow core as part of their architecture (Chen et al., 2015). In another example, oligomerization is induced by iron(II) under aerobic conditions, resulting in αSyn oligomers that are both rich in right-twisted anti-parallel β-sheet character, reminiscent of β-II proteins (Sreerama and Woody, 2003), and resistant to fibrillization (Abeyawardhane et al., 2018b).

Disordered monomeric αSyn can also be encouraged to adopt discrete folded states by interaction with other biomolecules. For example, upon interaction with membranes, the N-terminal region of αSyn gains α-helical character, and the curvature of the membrane has a direct impact on global structure (**Figure 3** bottom left) (Trexler and Rhoades, 2009; Jiang et al., 2013). Modifications at the N-terminus such as Cu^{2+} binding have been demonstrated to increase the α-helical propensity of monomeric αSyn (Lucas and Lee, 2011).

Tetrameric αSyn represents one of the newest and most controversial conformational states of αSyn (Bartels et al., 2011a; Burré et al., 2013). This α-helical conformer is aggregation-resistant, prompting much interest in its biological role(s) and therapeutic potential. A clear understanding of the functional role the tetramer plays in neuronal health and neurodegeneration has been hampered due to its lability, and challenges in isolating this elusive conformer have impeded systematic biochemical and biophysical studies. Solution NMR studies on a recombinant variant of tetrameric αSyn provided the basis for a structural model (Wang et al., 2011; Kara et al., 2013), in which the familial PD-associated point mutations are grouped together near a putative hairpin turn in the α helical N-terminal regions of tetrameric αSyn (**Figure 3** top left). A library of αSyn structural multimers generated using NMR chemical shifts and NH residual dipolar coupling suggest the tetramer to be a storing conformation upon exposure to high protein concentration (Gurry et al., 2013). The NACore, also referred to as NAC(8–18) (el-Agnaf and Irvine, 2002), was observed to serve as an initiator of toxic oligomerization; however, the tetrameric helical species buried this region, supporting the concept of the helical tetramer as a nontoxic multimer. Indeed, experiments in transfected M17D neural cells have confirmed that tetramer formation is hampered by familial missense mutations, shifting the tetramer:monomer ratio by 10–40%, depending on the mutant (Dettmer et al., 2015b). Furthermore, tetramer abrogation disrupts normal vesicle trafficking mediated by αSyn in live neurons (Wang et al., 2014) and induces a PD-like motor syndrome in mice (Nuber et al., 2018), suggesting that tetrameric αSyn plays a key role in normal neuronal health and neurotransmission.

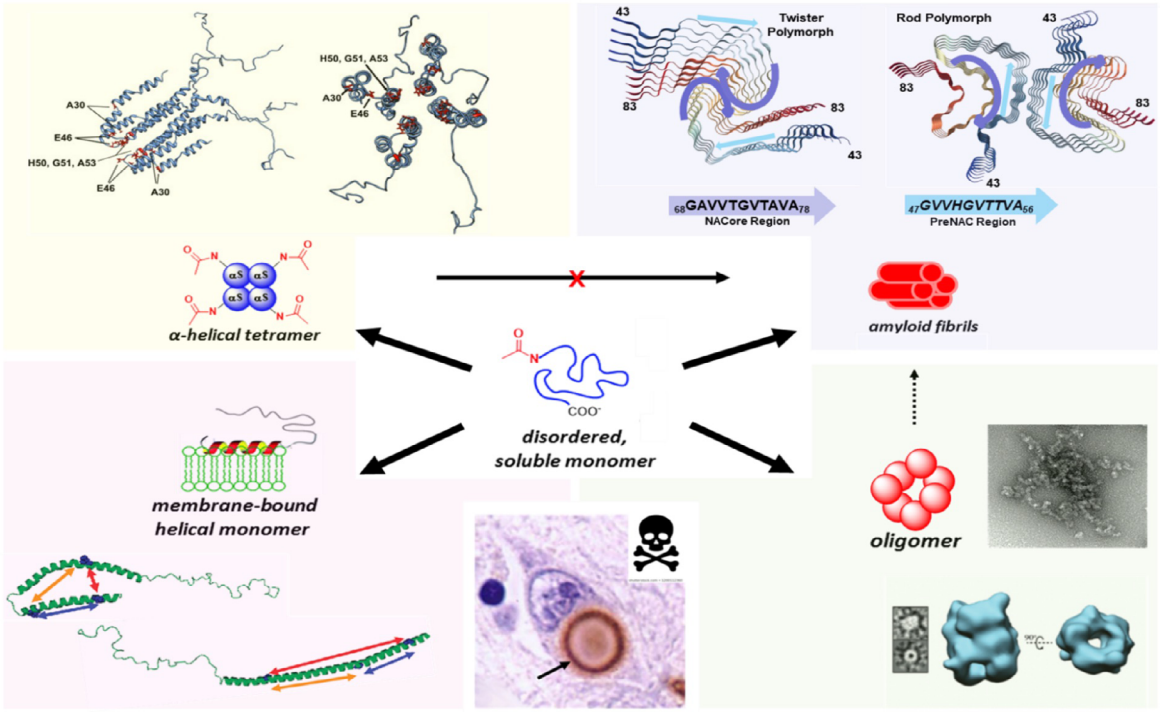

Figure 3 Schematic representation of α-synuclein (αSyn) multimeric conformations.
Upper left: Cartoon representation and ribbon model of tetrameric structure derived from solution NMR data and modified from Kara et al. (2013). Missense mutations (red) are found in proximity to loops connecting two helices of the tetramer. Upper right: Atomic modeling of amyloid fibril αSyn derived from cryo-electron microscopy (EM) for both the twister (PDB: 6CU8) and rod polymorph (PDB: 6CU7). Lower left: Cartoon representation and ribbon model of monomeric αSyn bound to membrane mimics based on modifications of Trexler and Rhoades (2009) and Lucas and Lee (2011). Lower right: Cartoon representation, transmission electron microscope image, and 3-dimension reconstruction from cryo-EM modeling of αSyn oligomers adapted from Abeyawardhane et al. (2018b) and Chen et al. (2015).

Controversy and Consensus Surrounding Tetrameric α-Synuclein

The discovery of tetrameric αSyn in 2011 by Bartels and coworkers generated both excitement and skepticism. In their report, *in vivo* crosslinking experiments revealed the presence of endogenous αSyn tetramers in several human cell lines, red blood cells, and mammalian brain tissue, supported by native and denaturing polyacrylamide gel electrophoresis. Biophysical techniques including EM imaging and sedimentation-equilibrium analytical ultracentrifugation indicated a molecular weight of about 58 kDa for tetrameric αSyn, and circular dichroism spectroscopy described the structural conformation of red blood cell-purified tetrameric αSyn to be helical. Aggregation conditions did not induce fibrillization or β-sheet formation of αSyn tetramers, indicating an ability to resist neurotoxic assembly that was immediately recognized for its therapeutic potential. Until this point, αSyn had been universally considered a natively disordered monomer with a strong affinity towards negatively charged phospholipids and a propensity for aggregation, so the original report sparked widespread debate.

Following the initial disclosure of tetrameric αSyn, a vigorous discourse played out in the literature (Bartels et al., 2011a; Fauvet et al., 2012b; Burré et al., 2013; Selkoe et al., 2014). Monomeric αSyn exists in an extended state due to its lack of secondary structure. As a result, it presents with decreased mobility in gel electrophoresis. Moreover, hydrodynamic radius measurements by dynamic light scattering have not been able to discriminate between the compact helically folded tetramer and the unfolded monomer. The unusual behavior of monomeric αSyn complicates data interpretation, contributing to early reluctance regarding tetrameric αSyn and an assertion by some groups that monomeric, disordered αSyn is the predominant form *in vivo*. It is now well established that the tetramer is highly labile outside of the cellular environment, requiring chemical crosslinking techniques to isolate it from native sources. The realization that tetrameric αSyn dissociates to its monomeric units during traditional cell lysis protocols has reconciled some of the original controversy.

The existence of tetrameric αSyn has since been corroborated by repeated experiments from the original lab and by many others. A consensus is beginning to form that tetrameric αSyn exists in a dynamic equilibrium with disordered monomeric αSyn, and that the distribution of the ensemble depends on the cell type and other biochemical factors. For example, glucocerebrosidase 1 deficiency has been shown to reduce the tetrameric αSyn content and increase the monomeric αSyn content in SH-SY5Y cells (Kim et al., 2018). In mammalian cells expressing αSyn, the tetrameric population has been shown most prevalent in human erythroid leukemia cells (Dettmer et al., 2013). In gastrointestinal neuronal cells from rats, however, the population of tetrameric αSyn is absent, suggesting that cells of the enteric nervous system generate predominantly monomeric αSyn (Corbillé et al., 2016). A dynamic equilibrium between monomer and tetramer has been observed following purification of recombinant αSyn under mild purification conditions from *Escherichia coli* (Wang et al., 2011; Trexler and Rhoades, 2012; Fernández and Lucas, 2018b). The conditions that govern the dynamic equilibrium between monomeric and tetrameric αSyn remain unexplained; however, misfolding of αSyn to toxic aggregates is well established to proceed from monomers and is thus connected to tetramer disassembly.

Destabilization of α-Synuclein Tetramers

It has been suggested that a shift from metastable tetramers to the monomeric form could serve as a mechanism for disease initiation (Dettmer et al., 2015b); yet, the underlying molecular pathology causing this type of shift remains unknown. Given that tetrameric αSyn may serve as a benign storage form of this otherwise amyloidogenic protein, current and future research is expected to place an emphasis on understanding the factors that control αSyn conformational homeostasis and on stabilization of tetrameric αSyn as a therapeutic strategy for PD and other synucleinopathies.

The N-terminal regions of αSyn contain a series of imperfect KTKEGV motifs (**Figure 4** in green font) embedded within 11-residue repeats that are predicted to form an amphipathic 11/3 helix (Bendor et al., 2013). Given the helical character of tetrameric αSyn, the KTKEGV consensus sequences were targeted for mutational studies aimed at understanding the contribution of this conserved motif to tetramer formation (Dettmer et al., 2015a). Indeed, altered

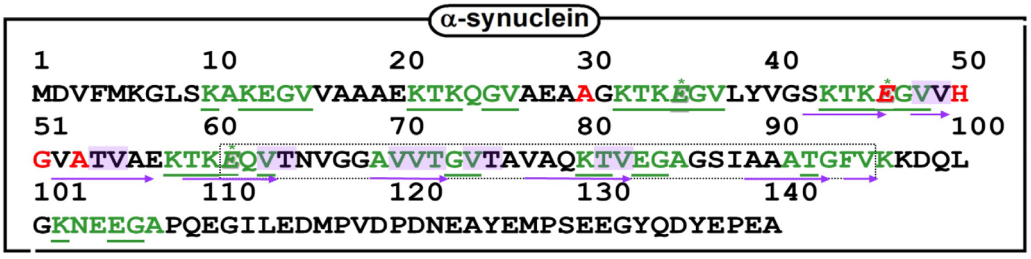

Figure 4 Primary sequence features of α-synuclein.
The primary amino acid sequence of human α-synuclein is shown, with key sequence features of this 140-amino acid protein highlighted. The core sequence of the 9 imperfect repeats that predict α-helicity are shown in green font, and the amino acids consistent with the KTKEGV consensus sequence are underlined. Locations of known familial Parkinson's disease mutations are shown in red font. Pairs of β-branched amino acids that promote β-sheet formation are highlighted in purple, with the β-strand regions observed inα-synuclein fibrils notated by purple arrows below the sequence.

repeat sequences KLKEGV, KTKKGV, KTKEIV, and KTKEGW abolished tetramer formation in neural cells, whereas other consensus motif alterations (GTKEGV, KTEEGV, and KTKEGR) preserved the tetrameric αSyn population. Moreover, the motif alterations that eliminated tetrameric αSyn also conferred neural cytotoxicity and generated cytoplasmic αSyn inclusions in neurites. Notably, the E46K familial PD point mutation falls within a KTKEGV core consensus sequence, garnering it special attention. The E46K mutation was demonstrated to induce a decrease in the tetrameric αSyn population in human M17D neural cells (Dettmer et al., 2015b). Moreover, this shift in the tetramer:monomer ratio could be progressively amplified by introducing additional E-to-K mutations in the nearby consensus sequences, which was mirrored by a stepwise reduction in cell viability. When expressed in mice, the tetramer-abrogating αSyn triple mutant E35K/E46K/E61K induced a PD-like motor syndrome phenotype, highlighting a thread that links the KTKEGV consensus motif with α-helix stabilization and tetrameric αSyn stabilization with neuronal health (Nuber et al., 2018).

Another notable conserved sequence feature of αSyn is the presence of sequential pairs of the β-branched amino acids valine and threonine (**Figure 4**, highlighted in purple). These amino acid pairs have an opposing effect on the helical propensity of αSyn, as they are embedded throughout the same region as the imperfect KTKEGV consensus motifs; thus, αSyn is nudged towards β sheet formation in order to alleviate steric side-chain interactions due to adjacent β-branched VT residues by adopting an extended conformation as is common among cross-β-sheet fibrils (Pochapsky, 2015). This dichotomy emphasizes a delicate balance that underpins one aspect of the conformational variability of αSyn, a balance that cells must be poised to preserve in order to maintain neuronal health.

Recombinant Expression Systems

Mounting evidence implicates degradation of tetrameric αSyn in neuronal cytotoxicity and in the onset of pathological phenotypes associated with PD and other synucleinopathies. Thus, a therapeutic strategy aimed at stabilization of αSyn tetramers is an obviously compelling target. These efforts would be greatly facilitated by a more complete picture of the structure and dynamics of tetrameric αSyn, which in turn would be enabled by access to purified tetrameric αSyn for biophysical and biochemical studies. Problematically, isolation of tetrameric αSyn from human cell lines and erythrocytes requires chemical cross-linking, thus undermining efforts to use this material to probe factors that may influence the tetramer:monomer ratio. Recent advances in the recombinant production and isolation of tetrameric αSyn, described herein, have shown promise in increasing access to the elusive αSyn tetramer for rigorous biophysical studies.

The earliest successful production of recombinant tetrameric αSyn was through expression in *E. coli* as a GST fusion protein, described by Wang et al. (2011), just after the initial report of tetrameric αSyn being identified in mammalian tissue and human cell lines. Following cleavage of the GST tag, the soluble recombinant αSyn construct adopted a helical tetrameric conformation even in the absence of micelles, as confirmed by circular dichroism spectroscopy. Further support for a tetrameric αSyn conformer was obtained through native gel electrophoresis, chemical crosslinking, and mass spectrometry. A set of NMR studies corroborated that the helical structure of αSyn tetramers resides within the N-terminal regions, specifically within the first 100 residues. These same NMR studies have provided the basis for structural models of tetrameric αSyn that have been used for various applications, including mapping disease-related mutations and engineering theoretical αSyn variants that are expected to favor the tetrameric conformation (Gurry et al., 2013; Kara et al., 2013; Xu et al., 2018). This recombinant construct also supported the first preliminary investigations into factors impacting tetramer stability, as both heat and dilute conditions were shown to disrupt the tetrameric conformation (Wang et al., 2011). It should be noted that this construct maintains a 10-residue N-terminal extension originating from the fusion protein linker, so data generated from this construct should be interpreted with that awareness. For example, the influence of biometals on αSyn structure and dynamics is of interest, in part because diminished copper pools have been observed in PD brain tissue (Davies et al., 2014), and αSyn is known to bind reduced copper at the N-terminus with potential downstream effects on global structural dynamics (Binolfi et al., 2011; Abeyawardhane et al., 2018a). Accordingly, the impact of the residual 10-amino acid linker region which resides at the N-terminus must be taken into consideration in downstream studies.

A recombinant construct for human αSyn was subsequently reported by Trexler and Rhoades that maintained the native sequence of αSyn (Trexler and Rhoades, 2012). Bacterial co-expression of αSyn with an acetylation complex from fission yeast provided the acetyl group capping the N-terminal free amine, as is found natively in human αSyn. When purified in the presence of octyl β-D-glucopyranoside, a non-ionic detergent, this construct yielded a multimeric α-helical species consistent with tetrameric αSyn based on size exclusion chromatography and sedimentation-equilibrium analytical ultracentrifugation. Both the acetyl PTM and octyl β-D-glucopyranoside were required to attain the α-helical conformer.

Two recent reports by Fernández and Lucas describes a platform for the isolation of intact tetrameric N-terminally acetylated αSyn from *E. coli* that does not require crosslinking agents or detergents (Fernández and Lucas, 2018a, b). In this work, a mild isolation protocol that avoids harsh or abrupt environmental conditions throughout the process and maintains minimal pressure during chromatography steps enables access to tetrameric αSyn without the need for potentially structure-modifying additives. This tetrameric αSyn construct, which has only the native human sequence and carries the acetyl PTM, displays the characteristic α-helical circular dichroism signature, and its assignment is further supported by size exclusion chromatography, im-

munoblotting, and native gel electrophoresis. Concurrent purification of the non-acetylated form resulted in reduced structural rigidity and led to unfolding and accelerated β-sheet formation. As observed for tetrameric αSyn from mammalian sources, the recombinant N-terminally acetylated αSyn tetramer reported by the Lucas group is resistant to aggregation, indicating that it can serve as a reliable source material for biophysical studies and downstream drug development efforts.

Conclusion

The aggregation-prone neuronal protein αSyn is universally recognized as being a key player in PD, however the biochemical events linking αSyn misfolding and the disease state remain to be fully elucidated. Likewise, αSyn is thought to have an important role in maintaining neuronal health and facilitating normal neurotransmission, yet the molecular details of this process are also still largely unresolved. The dynamic nature of this conformational chameleon is at the heart of its impact on human health and neuropathology.

Widely considered an IDP, αSyn can access an overwhelming variety of ordered states as well, including beautifully crystalline β-sheet fibrils with several polymorph variations, a multitude of higher-order oligomers that are often linked to toxicity, structured monomers that are typically membrane-associated, and the controversial α-helical tetrameric αSyn that is the focus of this review. Each species may have unique implications on disease onset and progression. Thus, teasing apart the molecular function of crucial αSyn conformers and understanding the factors that control and influence the dynamic equilibria among them is of utmost importance.

Despite initial uncertainty surrounding the elusive tetrameric αSyn, it has now been established as one of the native states of αSyn, present in variable amounts depending on the cell type and the local biochemical environment. Moreover, compelling studies from multiple groups have documented pathological downstream events that result from systematically disrupting αSyn tetramers in human cell lines, live neurons, and mouse models. Based on these observations, a therapeutic strategy targeting αSyn tetramer stabilization could have far-reaching effects, particularly given the range of neurodegenerative disorders linked to αSyn misbehavior, including PD, Alzheimer's disease, dementia with Lewy bodies, and multiple system atrophy. A deeper understanding of the structure and dynamics of tetrameric αSyn, along with a systematic account of factors that can influence the tetramer:monomer ratio, are needed as a foundation for drug discovery and development efforts in this area. These efforts have been hampered by technical challenges associated with harvesting tetrameric αSyn from mammalian sources, but recent advances in the recombinant production and successful isolation of native tetrameric αSyn show promise in providing a reliable source material for the development of therapies for PD and other synucleinopathies targeting tetramer stabilization.

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P-Reviewers: Bernstein HG, Adachi K; C-Editors: Zhao M, Yu J; T-Editor: Jia Y