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Effects of impaired membrane interactions on α-synuclein aggregation and neurotoxicity

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ABSTRACT

The post-mortem brains of individuals with Parkinson's disease (PD) and other synucleinopathy disorders are characterized by the presence of aggregated forms of the presynaptic protein α-synuclein (aSyn). Understanding the molecular mechanism of aSyn aggregation is essential for the development of neuroprotective strategies to treat these diseases. In this study, we examined how interactions between aSyn and phospholipid vesicles influence the protein's aggregation and toxicity to dopaminergic neurons. Two-dimensional NMR data revealed that two familial aSyn mutants, A30P and G51D, populated an exposed, membrane-bound conformer in which the central hydrophobic region was dissociated from the bilayer to a greater extent than in the case of wild-type aSyn. A30P and G51D had a greater propensity to undergo membrane-induced aggregation and elicited greater toxicity to primary dopaminergic neurons compared to the wild-type protein. In contrast, the non-familial aSyn mutant A29E exhibited a weak propensity to aggregate in the presence of phospholipid vesicles or to elicit neurotoxicity, despite adopting a relatively exposed membrane-bound conformation. Our findings suggest that the aggregation of exposed, membrane-bound aSyn conformers plays a key role in the protein's neurotoxicity in PD and other synucleinopathy disorders.

Introduction

Parkinson's disease (PD) is an age-related neurodegenerative disorder defined by the presence of cytoplasmic inclusions named Lewy bodies, which contain aggregated forms of the protein α-synuclein (aSyn) (Rochet et al., 2012; Shulman et al., 2011; Spillantini et al., 1997). aSyn is a highly expressed protein that localizes to presynaptic nerve terminals. Several autosomal dominant mutations in the SNCA gene encoding aSyn have been linked to familial forms of PD, including substitutions (A30P, E46K, H50Q, G51D, A53E, and A53T) (Kiely et al., 2013; Kruger et al., 1998; Lesage et al., 2013; Pasanen et al., 2014; Polymeropoulos et al., 1997; Proukakis et al., 2013; Shulman et al., 2011; Zarranz et al., 2004) and gene multiplications (Chartier-Harlin et al., 2004; Singleton et al., 2003). Some of the substitution mutants have been shown to have an increased propensity to form high molecular weight oligomers (Conway et al., 2000; Greenbaum et al., 2005; Li et al., 2001), and gene multiplications are predicted to promote aSyn aggregation via mass action (Rochet et al., 2012). These observations suggest that aSyn oligomerization is a key event in PD pathogenesis. Additionally, aSyn accumulation has been observed in other neurodegenerative diseases referred to as 'synucleinopathies', including dementia with Lewy bodies and multiple system atrophy (Spillantini et al., 1997; Wakabayashi et al., 1998). A better understanding of the events that initiate aSyn aggregation is critical for designing neuroprotective strategies to treat PD and other synucleinopathy disorders.

aSyn is most commonly expressed as a 14 kDa (140-residue) protein. Biophysical studies have revealed that the protein is natively unfolded in solution (Weinreb et al., 1996). The protein consists of 3 regions: an N-terminal region, a central hydrophobic region, and a C-terminal region (Fig. 1A). The N-terminal region spans the first 67 amino acid residues and contains 5 conserved lysine-rich repeats. The

Abbreviations: aSyn, α-synuclein; CD, circular dichroism; DOPC, 1,2-dioleoyl-*sn*-glycero-3-phosphocholine; DOPE, 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine; DOPS, 1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine; EGFP, enhanced green fluorescent protein; HSQC, heteronuclear single quantum coherence; MAP2, microtubule associated protein 2; MOI, multiplicity of infection; PBS-T, PBS + Tween 20; PC, L-α-phosphatidylcholine; PD, Parkinson's disease; PG, L-α-phosphatidylglycerol; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol; PUFA, polyunsaturated fatty acid; SUV, small unilamellar vesicle; TH, tyrosine hydroxylase.

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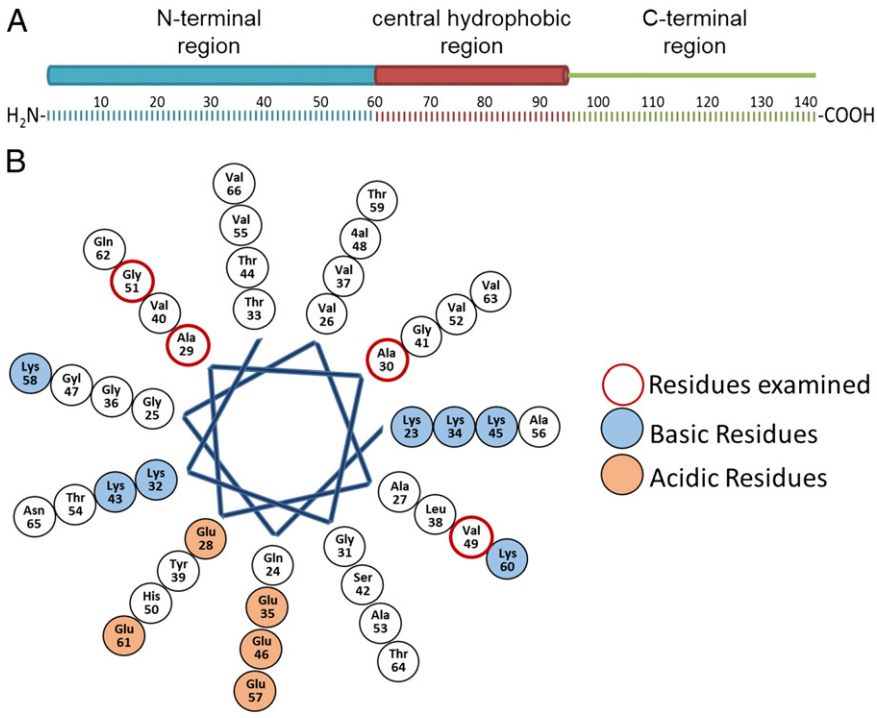

Fig. 1. aSyn adopts an α-helical structure upon binding phospholipid membranes. (A) Schematic representation of the secondary structure of membrane-bound aSyn. The amphipathic α-helix, shown here spanning the N-terminal region (blue) and the central hydrophobic region (red), and the unfolded C-terminal region (green) are depicted above a map of the polypeptide chain with identical color coding. (B) Helical wheel plot portraying residues 23–66 in the membrane-binding region of aSyn as an amphipathic α11/3 helix. Because A29 and G51 are located on the hydrophobic face of the helix embedded in the membrane, replacement of either residue by an acidic residue is predicted to disfavor membrane binding and helix formation. In contrast, V49 is located on the hydrophilic face of the helix exposed to solvent. Accordingly, the replacement of this residue with an acidic residue should have a less pronounced effect on membrane binding and helix formation, though the proximity of residue 49 to the predicted membrane interface suggests that a negative charge at this position could be repelled by anionic lipid head groups. Residues examined in this study are outlined in red. Basic and acidic residues are highlighted with blue and orange shading, respectively.

central region (spanning residues 61–95) is highly hydrophobic and contains a 6th lysine-rich repeat. Within the central region, residues 71–82 are required for aggregation of the protein (Giasson et al., 2001). The C-terminal region spanning residues 96–140 contains many proline residues and acidic residues and is thought to be involved in long-range interactions that stabilize the protein and prevent aggregation (Bertoncini et al., 2005; Dedmon et al., 2005).

The ability of aSyn to bind phospholipid membranes has been well documented and is presumably necessary for the protein's function in regulating synaptic vesicle trafficking (Davidson et al., 1998; Jensen et al., 1998; Venda et al., 2010). A number of groups have shown that aSyn interacts with anionic phospholipid vesicles or detergent micelles by forming an amphipathic α-helix (most likely an α11/3 helix) with various lengths, ranging from a short helix spanning residues ~1–25 to a long helix spanning residues ~1–97 and including the central hydrophobic region (Bartels et al., 2010; Bodner et al., 2009, 2010; Bussell and Eliezer, 2003; Davidson et al., 1998; Jao et al., 2004; Jo et al., 2000) (Fig. 1A). The protein adopts a predominantly broken or continuous α-helical structure upon binding high- or low-curvature membranes, respectively (Chandra et al., 2003; Ferreon et al., 2009; Georgieva et al., 2010; Trexler and Rhoades, 2009; Ulmer et al., 2005). Interactions involving an N-terminal segment spanning residues 1–25 are critical for membrane binding and for the adoption of an α-helical structure (Bartels et al., 2010).

aSyn has been shown to undergo accelerated aggregation at the membrane surface when incubated with isolated rat membrane homogenates (Lee et al., 2002), synaptosomal membranes (Jo et al., 2004), exosomes (Grey et al., 2015), detergent micelles (Giehm et al., 2010; Necula et al., 2003), phospholipid vesicles (Galvagnion et al., 2015), and supported phospholipid bilayers (Haque et al., 2010; Pandey et al., 2009) at relatively high protein:lipid ratios. The increased propensity of aSyn to undergo aggregation in the presence of membranes versus in solution could be because the two dimensional surface of the lipid bilayer increases the probability of molecular interactions needed for oligomerization (Abedini and Raleigh, 2009; Pandey et al., 2009). It has also been observed that aSyn exhibits an enhanced propensity to form high molecular weight aggregates in the presence of polyunsaturated fatty acids (PUFAs) (Perrin et al., 2001; Sharon et al., 2003). PUFAs are thought to stimulate aSyn self-assembly because they are susceptible to oxidation to 4-hydroxy-2-nonenal and 4-oxo-2-nonenal, and the incubation of aSyn with these oxidation products results in the formation of large, covalently crosslinked β-sheet-rich oligomers (Nasstrom et al., 2011; Qin et al., 2007). Membrane-induced aSyn aggregation may play a role in neurodegeneration by triggering membrane thinning, a process that could result in increased ion permeability (Comellas et al., 2012; Lee et al., 2012; Ouberai et al., 2013; Pfefferkorn et al., 2012; Reynolds et al., 2011).

Although evidence suggests that phospholipid membranes can stimulate aSyn aggregation, conformational properties of membrane-bound aSyn favoring its self-assembly are poorly understood. We hypothesize that structural perturbations which disrupt interactions between the central hydrophobic region and the phospholipid bilayer promote the formation of neurotoxic aSyn aggregates at the membrane surface. To address this hypothesis, we characterized a set of aSyn mutants (A29E, A30P, V49E, G51D) in terms of their membrane affinity, membrane-bound conformation, propensity to undergo membrane-induced aggregation, and neurotoxicity as compared to WT aSyn. The rationale for examining these mutants was that their amino acid substitutions were predicted to disrupt aSyn-phospholipid interactions at various sites on the amphipathic α11/3 helix formed by the membrane-bound protein (Fig. 1B). Our findings suggest a molecular basis for the enhanced neurotoxicity of the familial mutants A30P and G51D, and they yield insight into the molecular features of aSyn that