

# Alpha-Synuclein Interactions with Membranes

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## 1. Introduction

Synucleinopathies are a group of neurodegenerative disorders that share common pathological intracellular deposits that contain aggregates of the protein  $\alpha$ -synuclein. Substantial evidence suggests that fibril formation by  $\alpha$ -synuclein is a critical step in the development of Parkinson's disease (PD). Indeed, *in vitro*,  $\alpha$ -synuclein forms fibrils with morphologies and staining characteristics similar to those extracted from disease-affected brains. Also, three single-point mutations and duplication or triplication of the  $\alpha$ -synuclein locus correlate with early onset of PD.

However, the function of  $\alpha$ -synuclein remains unknown. A significant portion of  $\alpha$ -synuclein is localized within membrane fractions, and especially synaptic vesicles associated with vesicular transport processes. These observations suggest that  $\alpha$ -synuclein has a role in vesicular trafficking. Although  $\alpha$ -synuclein belongs to a group of natively unfolded proteins, there is strong evidence that the membrane affinity of the protein induces an  $\alpha$ -helical conformation. A large number of studies have investigated  $\alpha$ -synuclein-lipid interactions in the search for a physiological function, as well as to understand this potential membrane involvement in the pathogenesis of  $\alpha$ -synuclein. In this review, we will predominantly focus on current opinion about the native wild-type  $\alpha$ -synuclein-lipid interactions and the structure of  $\alpha$ -synuclein that is established at the membrane surface. However, it should be noted that membranes have been reported to both accelerate and inhibit the fibril formation of  $\alpha$ -synuclein, although this will not be the focus of the present review.

## 2. Intrinsically disordered proteins

A significant number of proteins involved in protein deposition diseases have been seen to be intrinsically disordered proteins. Well-known examples include amyloid  $\beta$ -protein and tau protein in Alzheimer's disease, prion protein (PrP) in prion diseases, exon 1 region of huntingtin in Huntington's disease, and  $\alpha$ -synuclein in PD (Fink, 2005).

It has been estimated that more than 30% of eukaryotic proteins have disordered regions that are greater than 50 consecutive residues (Dunker et al., 2001). This term "disordered protein" refers to proteins that in their purified state at neutral pH, have been either shown experimentally or predicted to lack an ordered structure; such proteins are also known as natively unfolded, or intrinsically unstructured. Disordered proteins, or disordered regions

of a protein, lack specific tertiary structure and can be composed of an ensemble of conformations (Fink, 2005). Intrinsically unstructured proteins are frequently involved in important regulatory functions in the cell, and the lack of intrinsic structure is in many cases removed when the protein binds to its target molecule. Some functional advantages of these proteins might be an ability to bind to several different targets, the precise control of their binding thermodynamics, and their involvement in cell-cycle control and both transcriptional and translational regulation (Wright & Dyson, 1999). Gunasekaran et al. proposed that disordered proteins provide a simple solution to the need for large intermolecular interfaces while maintaining smaller proteins, and hence a smaller genome and a smaller cell size. For monomeric proteins with extensive intermolecular interfaces, such proteins would need to be 2-3-fold larger, and this would either increase intracellular crowding or enlarge the size of the cell by some 15% to 30%, owing to the increase in the sequence length (Gunasekaran et al., 2003).

### 3. Synucleinopathies

Protein-conformation-dependent toxicity is an emerging theme in neurodegenerative disorders, including the synucleinopathies (Ulrich et al., 2008). The group of synucleinopathies comprises many neurodegenerative diseases, among which the best known and most common is PD, but it also includes Lewy body dementia, multiple system atrophy, neurodegeneration with brain iron accumulation type I, diffuse Lewy body disease, and Lewy body variant of Alzheimer's disease (Arawaka et al., 1998; Gai et al., 1998; Spillantini et al., 1997; Wakabayashi et al., 1997). These are all brain amyloidoses with the common characteristic of pathological intracellular inclusions of aggregates that have  $\alpha$ -synuclein as the key component (Spillantini et al., 1997; Wakabayashi et al., 1997).

PD is characterized by the death of neurons that produce dopamine and are located in the *substantia nigra pars compacta* brain region (see below). This is accompanied by the appearance of Lewy bodies and Lewy neurites (Galvin et al., 1999). Lewy bodies are spherical protein inclusions that are found in the cytoplasm of surviving *substantia nigra* neurons, and they consist of a dense core surrounded by a halo of radiating fibrils of  $\alpha$ -synuclein; they also contain a variety of other proteins. The fibrils seen in PD are structurally similar to those in the amyloid diseases, and they appear as linear rods of 5 nm to 10 nm diameter (Fink, 2006). PD affects more than 1% of the population over 65 years of age (Goedert, 2001), and typical symptoms include tremor, slow movements, fine motor difficulties, and loss of postural reflexes (Jankovic, 2008). The cause of PD remains unknown, but considerable evidence suggests a multifactorial etiology that involves genetic susceptibility and environmental factors (Fink, 2006). However, substantial evidence indicates that aggregation of  $\alpha$ -synuclein is a critical step in the etiology of PD (Trojanowski & Lee, 2003).

Most cases of PD are of the late onset idiopathic type (Beyer, 2007). Evidence for an important role for  $\alpha$ -synuclein in triggering PD also emerged when certain mutations were discovered that are associated with rare inherited autosomal dominant cases of PD. While, as indicated, familial early onset PD is caused by overexpression of  $\alpha$ -synuclein due to duplication (Chartier-Harlin et al., 2004) or triplication (Singleton et al., 2003) of the  $\alpha$ -synuclein gene locus, three specific point mutations have also been identified: A53T in a large kindred of Italian and Greek origin (Polymeropoulos et al., 1997); A30P in a German family (Kruger et al., 1998); and E46K in a Spanish family (Zarranz et al., 2004).

## 4. Alpha-synuclein

In brain homogenates,  $\alpha$ -synuclein represents 0.5% to 1% of the total protein (Iwai et al., 1995). Northern blotting and *in-situ* hybridization in human and mice have shown relatively high expression of  $\alpha$ -synuclein in a restricted number of brain regions, one of which is the *substantia nigra* (Abeliovich et al., 2000; Lavedan, 1998). Here,  $\alpha$ -synuclein is localized in the presynaptic terminals (George et al., 1995; Iwai et al., 1995), with about 15% found in the membrane fraction (Lee et al., 2002); after synaptosomal lysis,  $\alpha$ -synuclein is in the soluble fraction (Iwai et al., 1995).

Although the normal physiological function of  $\alpha$ -synuclein remains unknown, it appears to be involved in maintenance of the synaptic vesicle reserve pool of the brain (Davidson et al., 1998; Fortin et al., 2004; Iwai et al., 1995; Nuscher et al., 2004). However, other roles for  $\alpha$ -synuclein have been considered: roles in lipid metabolism and transport (Scherzer et al., 2003; Sharon et al., 2001; Willingham et al., 2003), vesicle docking at the membrane (Larsen et al., 2006), exocytosis (Srivastava et al., 2007), lipid organisation (Madine et al., 2006) and prevention of oxidation of unsaturated lipids (Zhu et al., 2006). To date, no conclusive evidence showing the precise role of  $\alpha$ -synuclein in cell physiology has been provided.

### 4.1 Primary sequence

Alpha-synuclein is a small (140 amino acid; 14 kDa) highly acidic protein (Figure 1), and it is intrinsically disordered under physiological conditions *in vitro* (Bisaglia et al., 2009; Fink, 2006). The first 89 residues are composed almost entirely of seven 11-amino-acid imperfect repeats, with a consensus sequence of KTKEGV (George et al., 1995). This strongly resembles sequence motifs found in exchangeable apolipoproteins, which are believed to constitute amphipathic helical lipid-binding domains (Segrest et al., 1992). This 11-residue periodicity is broken in one point by the insertion of four uncharged amino acids, separating units 4 and 5. There are no Cys or Trp residues in the  $\alpha$ -synuclein sequence (George et al., 1995).

The structure of  $\alpha$ -synuclein can be divided into three regions (Figure 1). The N-terminal domain (residues 1-60) is positively charged and contains five of the imperfect repeats (Fink, 2006; George et al., 1995). The sequence 61-95 is the most hydrophobic portion of the protein, and this was originally described as the "non-amyloid-beta component" (NAC) of Alzheimer's disease plaques (Takeda et al., 1998). Several studies have defined this region as responsible for  $\alpha$ -synuclein aggregation and  $\beta$ -sheet formation (Bodles et al., 2001; Giasson et al., 2001). The homologous  $\beta$ -synuclein, which is distinct from  $\alpha$ -synuclein by the absence of the central hydrophobic sequence, is much less prone to self-aggregation. The interaction between  $\beta$ -synuclein and  $\alpha$ -synuclein has been argued to inhibit aggregation (Park & Lansbury, 2003). The highly acidic C-terminal domain of  $\alpha$ -synuclein is rich in Pro and acidic residues, with a predominance of Glu residues over Asp (George et al., 1995). This domain contains three of the four Tyr residues, at positions 125, 133 and 136; the fourth Tyr residue is at position 39. It has been shown that monomeric  $\alpha$ -synuclein has a more compact structure than expected for a completely unfolded polypeptide, and this compactness has been linked to its inhibition of fibril formation due to burial of the hydrophobic NAC domain (Bertoncini et al., 2005; Dedmon et al., 2005). In addition, it has been shown that the 1-102 and 1-110 C-terminal-truncated  $\alpha$ -synuclein fragments, but not that of 1-120, are efficient in the promotion of  $\alpha$ -synuclein aggregation. The negatively charged 104, 105, 114 and 115 residues in the C-terminus have been suggested to be responsible for reduced  $\alpha$ -synuclein aggregation and a lack of seeding of wild-type  $\alpha$ -synuclein (Murray et al., 2003).

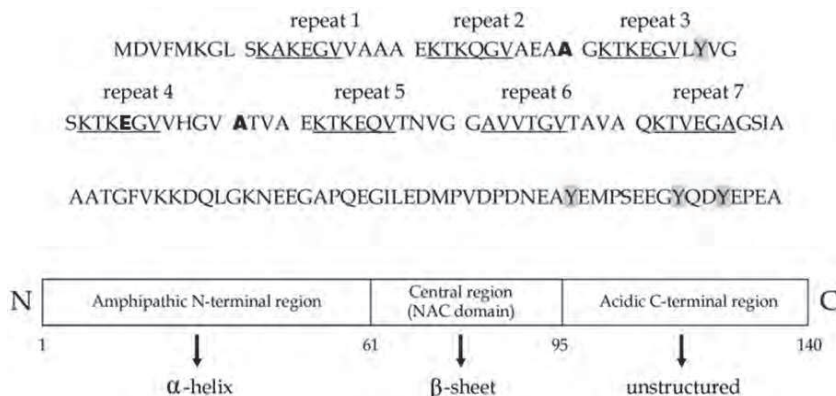


Fig. 1. Top: Amino-acid sequence of human  $\alpha$ -synuclein. The imperfect 11-mer repeats are as indicated, with the predominant KTKEGV consensus sequences underlined. The locations of the three point mutations that have been linked to early-onset PD (A30T, E46K, A53T) are shown in bold type, and the four Tyr residues are shaded. Bottom: The  $\alpha$ -synuclein sequence can be divided into three regions: the N-terminus adopts an  $\alpha$ -helix upon binding to lipids, the hydrophobic NAC domain can form  $\beta$ -sheet structure, and the negatively charged C-terminus is unstructured.

#### 4.2 Alpha-synuclein structure under physiological conditions

Weinreb et al. were the first to attempt to define the secondary structure of  $\alpha$ -synuclein. Sedimentation of  $\alpha$ -synuclein under physiological conditions is slower than for globular proteins of a similar molecular weight, suggesting an elongated structure of the native protein. Circular dichroism has demonstrated the lack of  $\alpha$ -synuclein secondary structure in solution: 68% as random coils and less than 2% as helical content. The remainder of the protein is  $\beta$ -sheet, although it is difficult to quantify the  $\beta$ -sheet structure by circular dichroism. Fourier-transform infrared spectroscopy has confirmed that native  $\alpha$ -synuclein is unstructured. The conformational properties of  $\alpha$ -synuclein were not changed by heat denaturation and were independent of  $\alpha$ -synuclein concentration, salt concentration, chemical denaturants and pH. These features prompted the conclusion that under physiological conditions,  $\alpha$ -synuclein exists as a mixture of rapidly equilibrating extended conformers, and that it is representative of a class of natively unfolded proteins (Weinreb et al., 1996). With a slightly different isolation protocol, circular dichroism showed 9%  $\alpha$ -helix, 35%  $\beta$ -sheet, and 56% random coil structure in solution (Narayanan & Scarlata, 2001). Dedmon et al. (2005) used paramagnetic relaxation enhancement and nuclear magnetic resonance (NMR) to show interactions between different parts of the  $\alpha$ -synuclein molecule. Some  $\alpha$ -synuclein mutants were prepared, with the insertion of nitroxide-labeled cysteine residues, which allowed the observation of short-life-time interactions. Partial condensation of  $\alpha$ -synuclein is driven by long-range contacts between residues 30-100 in the center of the molecule, and residues 120-140 in the C-terminal tail. It appears that this interaction can shield the NAC region (residues 61-95) from aggregation, which is the most hydrophobic part of  $\alpha$ -synuclein (Dedmon et al., 2005). Bertoncini et al. used a similar methodology to show that the most important interaction is a hydrophobic cluster that comprises the C-terminal part of the NAC region (residues 85-95) and the C-terminus (residues 110-130),

which is probably mediated by Met<sup>116</sup>, Val<sup>118</sup>, Tyr<sup>125</sup> and Met<sup>127</sup>. Within the C-terminal domain, residues 120-130 contact residues 105-115, and the region around residue 120 also interacts with the N-terminus around residue 20. These long-range interactions that stabilize the monomeric conformations of  $\alpha$ -synuclein also inhibit its oligomerization and aggregation. The autoinhibitory conformations fluctuate in the range of nanoseconds to microseconds (Bertoncini et al., 2005). Consistent with this, small-angle X-ray scattering analysis has shown that the radius of gyration, which is used to describe the dimensions of polypeptide chain, is  $\sim 40$  Å with native  $\alpha$ -synuclein, which is much larger than that predicted for a folded globular protein of 140 residues (15 Å), although it is significantly smaller than that of a fully unfolded random coil (52 Å) (Uversky et al., 2001).

Using an atomic-force-microscopy-based single-molecule mechanical unfolding methodology, Sandal et al. (2008) studied the  $\alpha$ -synuclein conformation equilibrium under various conditions. Their method allowed the measuring of the force required for unfolding a single protein molecule. It was thus possible to detect conformers with a lifetime that was longer than  $10^{-3}$  s, which due to their longevity, might be the most biologically relevant structures. In 10 mM TRIS/HCl buffer solution at pH 7.5, the  $\alpha$ -synuclein secondary structure contains a random coil (38.2%) and  $\beta$ -structure (7.3%) (Sandal et al., 2008).

## 5. Fibril formation

*In-vitro* studies of recombinant  $\alpha$ -synuclein have demonstrated that purified  $\alpha$ -synuclein forms fibril aggregates that resemble those found in Lewy bodies (Serpell et al., 2000). In contrast to its helical secondary structure in the presence of lipids,  $\alpha$ -synuclein monomers form soluble oligomers (sometimes referred to as protofibrils) that can undergo a conformational change from disordered to predominantly beta secondary structure. These oligomers can assemble and form insoluble fibrils, which are found in inclusion bodies, together with other proteins (Conway et al., 2000; Fink, 2006; Wood et al., 1999).

Extensive data suggest that the first step of fibrillogenesis is the formation of a partially folded intermediate that promotes self-association of  $\alpha$ -synuclein and formation of various oligomeric species (Uversky et al., 2001). Factors that increase the concentrations of these intermediates will favor aggregation (Fink, 2006). Protein aggregation and the kinetics of fibril formation typically appear sigmoidal, and they are usually attributed to a nucleated polymerization process in which the initial lag phase corresponds to the requirement for the formation of critical nuclei; the subsequent exponential growth phase corresponds to fibril elongation, and the final plateau is ascribed to the exhaustion of the soluble monomers and intermediates (Ulrich et al., 2008).

All three of the above-mentioned PD-related point mutations have been shown to accelerate  $\alpha$ -synuclein aggregation *in vitro* (Uversky, 2007). The A53T and A30P point mutations both accelerate oligomer formation, although only A53T readily forms large amyloid fibrils (Conway et al., 2000). E46K appears to be even more effective in the promotion of aggregate formation in cultured cells than these other two mutations (Pandey et al., 2006).

As fibril formation of native  $\alpha$ -synuclein occurs in most cases of synucleinopathies, most studies have dealt with the mechanisms that trigger this process. Both physical and chemical factors have been demonstrated to affect this aggregation process (Lundvig et al., 2008).

As mentioned above, it is believed that interactions between the C-terminus and the central portion of  $\alpha$ -synuclein can prevent or minimize its aggregation/fibril formation. As the majority of hydrophobic interactions in the C-terminal of  $\alpha$ -synuclein arise through its three

Tyr, we created Tyr to Ala mutants to examine the importance of these Tyr residues in fibril formation of  $\alpha$ -synuclein *in vitro*. This was completely inhibited in the timescale over which measurements were made (70 hours) when the three C-terminal Tyr were replaced with Ala. In addition, substitution of Tyr<sup>133</sup> by Ala also inhibited fibril formation, whereas the individual Y125A and Y136A mutants showed limited inhibition. Replacement of Tyr<sup>39</sup> by Ala also resulted in substantial inhibition of fibril formation. Structural analysis showed that the Y133A  $\alpha$ -synuclein mutant has a substantially different conformation, as it is rich in  $\alpha$ -helical secondary structure, as compared with wild-type  $\alpha$ -synuclein and its other mutants. However, no formation of any tertiary structure was seen, as judged from the near-UV circular-dichroism spectra. These observations suggest that the long-range intramolecular interactions between the N-terminal and C-terminal of  $\alpha$ -synuclein are crucial for the process of fibril formation (Ulrich et al., 2008).

## 6. Alpha-synuclein and membranes

### 6.1 Lipid-binding domains

A characteristic feature of the  $\alpha$ -synuclein amino-acid sequence is the set of seven degenerate 11-residue repeating motifs. These are reminiscent of the amphipathic  $\alpha$ -helical domains of the exchangeable apolipoproteins, which mediate a variety of lipid and protein interactions (Davidson et al., 1998, George et al., 1995). Amphipathicity corresponds to the segregation of polar and nonpolar residues to the two opposite faces of the  $\alpha$ -helix, a distribution that is well suited for membrane binding (Drin & Antony, 2009).

Depending upon the distribution of residues to the polar and nonpolar faces of the helices, Segrest et al. divided the apolipoprotein  $\alpha$ -helices into different classes: class A helices bind lipids and are characterized by a clustering of basic residues at the polar/nonpolar interface and acidic residues at the center of the polar face, while class G helices are implicated in protein interactions and are characterized by a random radial distribution of charged residues to the polar face of the helix (Segrest et al., 1992). Davidson et al. subjected the entire  $\alpha$ -synuclein sequence to helical wheel analysis and identified five potential amphipathic  $\alpha$ -helices that encompass all of the 11-mer repeats and some of the adjacent amino acids. The first four of these five theoretical helices share the defining properties of class A<sub>2</sub> lipid-binding helices, and they are distinguished by clustered basic residues at the polar-apolar interface and positioned  $\pm 100^\circ$  from the center of the nonpolar face, with a preponderance of Lys over Arg, and the presence of Glu residues on the polar face. The  $\alpha$ -helix on the fifth 11-mer repeat resembles a class G helix, and it is thus a candidate for protein-protein interactions (Davidson et al., 1998).

There is a notable feature that can be used to distinguish between putative amphipathic  $\alpha$ -synuclein helices and those in the exchangeable apolipoproteins: the Thr residues at the center of the nonpolar faces of helices 2-4 (Davidson et al., 1998). Although polar, these can reside on the nonpolar face of the helix due to its relatively long aliphatic side chain (Segrest et al., 1992). Thr are conserved among the  $\alpha$ -synuclein sequences from canary, human and rat, suggesting that they indeed have an important function. Another unique aspect of the  $\alpha$ -synuclein 11-mer repeat sequences is the absence of Pro, which in exchangeable apolipoproteins introduces helix-breaking hairpin turns. In contrast,  $\alpha$ -synuclein helices 1-4 appear to be punctuated by nonpolar residues that are predicted to disrupt the amphipathicity of a helix (Davidson et al., 1998).

## 6.2 Lipid and membrane selectivity

Data that have documented the tendency of  $\alpha$ -synuclein to colocalize with synaptic vesicles *in vivo* (Maroteaux et al., 1988) and the presence of the 11-residue repeated domains in a pattern similar to that found in the apolipoproteins (George et al., 1995) sparked a series of studies to determine the  $\alpha$ -synuclein lipid-binding ability. Alpha-synuclein interactions with membranes have been found to be one of the most contentious areas regarding this protein (Fink, 2006), as there have been numerous reports on sometimes completely contradicting results, and as there might be major differences between the situation *in vivo* and *in vitro*. Also, membranes have been reported to both accelerate (Lee et al., 2002) and inhibit (Narayanan & Scarlata, 2001; Zhu & Fink, 2003)  $\alpha$ -synuclein fibril formation, so this probably reflects the varying conditions used in the different studies (Zhu & Fink, 2003).

All three of these  $\alpha$ -synuclein mutations occur within the N-terminus, which is responsible for its membrane binding, hence suggesting an effect on membrane interactions (Fortin et al., 2010). The A30P  $\alpha$ -synuclein mutation, and to a lesser extent that of A53T, disrupts the helical structure of  $\alpha$ -synuclein (Bussell & Eliezer, 2001), although it does not significantly affect the structure of membrane-associated  $\alpha$ -synuclein (Bussell & Eliezer, 2004). The E46K  $\alpha$ -synuclein mutant binds to negatively charged vesicles with a higher protein/lipid ratio than does wild-type  $\alpha$ -synuclein (Choi et al., 2004), while A30P affects the localization, and presumably the membrane binding, of  $\alpha$ -synuclein *in vivo* (Fortin et al., 2010).

### 6.2.1 Membrane interactions *in vitro*

It is generally accepted that  $\alpha$ -synuclein preferentially interacts with small unilamellar vesicles (SUVs) containing negatively charged head groups (Davidson et al., 1998; Jo et al., 2000) or interfacial packing defects (Kamp & Beyer, 2006; Nuscher et al., 2004), and that upon SUV binding,  $\alpha$ -synuclein undergoes a conformational transition from an intrinsically disordered state to an  $\alpha$ -helical structure (Davidson et al., 1998; Jo et al., 2000; Nuscher et al., 2004). Various combinations of charged and uncharged lipids have been used in these studies. These negatively charged acidic phospholipids include phosphatidylglycerol (PG), phosphatidic acid (PA), phosphatidylserine (PS) and phosphatidylinositol (PI), while the uncharged, neutral lipids commonly used include phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (Valenzuela, 2007).

The interactions of  $\alpha$ -synuclein with membranes have been shown to affect the properties of both the protein and the membranes, and both electrostatic and hydrophobic interactions are important in the protein-bilayer association (Zhu et al., 2003). There are several factors that are believed to have central roles in modulation of the binding equilibrium of  $\alpha$ -synuclein to membranes, including chemical properties of the membranes (Davidson et al., 1998; Jo et al., 2000), ionic strength of the solution (Davidson et al., 1998; Zhu et al., 2003), vesicle size, or more precisely, the curvature of the phospholipid surface (Davidson et al., 1998; Narayanan & Scarlata, 2001; Rhoades et al., 2006), and mass ratio of  $\alpha$ -synuclein to the lipids (Zhu & Fink, 2003). Here, an overview of some of the more important findings regarding the lipid specificities of  $\alpha$ -synuclein are given.

Davidson et al. were the first to demonstrate that  $\alpha$ -synuclein binds only to acidic phospholipids and preferentially to vesicles with smaller diameters. Circular dichroism spectroscopy was used to determine the effects of this lipid binding on the secondary structure of  $\alpha$ -synuclein. In buffer solution,  $\alpha$ -synuclein is mainly unstructured, with less than 3% of the structure as  $\alpha$ -helix. Incubation of  $\alpha$ -synuclein with vesicles made of a

mixture of acidic and neutral phospholipids is accompanied by a large increase in its  $\alpha$ -helical content. Alpha-synuclein does not bind to SUVs or multilamellar vesicles composed of PC only, or of a mixture of PC and PE. Here,  $\alpha$ -synuclein binds to SUVs containing at least 30% to 50% acidic lipids, per vesicle weight. Comparisons of the ability of  $\alpha$ -synuclein to bind to vesicles of different sizes, as SUVs with 20 nm to 25 nm diameters and large unilamellar vesicles (LUV) with  $125 \pm 30$  nm diameter, have shown that  $\alpha$ -synuclein does not bind to vesicles that contained neutral 1-palmitoyl 2-oleoyl PC (POPC) alone. Also, binding to LUVs composed of POPC/1-palmitoyl 2-oleoyl PA (POPA) was less than to SUVs of the same composition (Davidson et al., 1998).

Binding to negatively charged SUVs was confirmed in another study where they incubated  $\alpha$ -synuclein together with vesicles and then fractionated the solution with gel filtration chromatography. Alpha-synuclein eluted together with the SUV fraction when incubated with either POPC/POPA or POPC/1-palmitoyl 2-oleoyl PS (POPS) phospholipids, while its binding to vesicles composed of POPC alone was not detected (Perrin et al., 2000). Although in nervous tissue PA comprises approximately 1% to 3% of the total phospholipids, while PS is more abundant (12% to 22% of the total phospholipids) (Sastry et al., 1985), it is difficult to relate these values directly to the local composition of specific membranes inside the cell, since these lipids are not distributed evenly in the cell and are generated and metabolized rapidly (Perrin et al., 2000).

Using thin-layer chromatography overlay, it has been shown that  $\alpha$ -synuclein binds to the brain or commercially available lipids PE, PI and lyso-PE. These interactions were much weaker with POPS and brain PS, and absent with POPC, POPA, sphingomyelin and cholesterol (Jo et al., 2000). Surprisingly, in contrast with a previous report (Davidson et al., 1998),  $\alpha$ -synuclein does not bind to PA, which was attributed to the properties of the thin-layer chromatography overlay method. Replacing PC with PE in acidic lipid vesicles greatly increased the binding of  $\alpha$ -synuclein (Jo et al., 2000). Although both PE and PC are neutral phospholipids that have similar electrostatic properties, they differ in their head-group orientation, lipid bilayer packing, and hydrogen-bonding capacity (Hauser et al., 1981, as cited in Jo et al., 2000). When the neutral head-groups are tightly packed, PE forms a lipid monolayer with high negative curvature (Bazzi et al., 1992). It is believed that  $\alpha$ -synuclein can relieve this negative curvature strain, and hence stabilize the PE/acidic lipid vesicles. This study also showed that both SUVs and multilamellar vesicles composed of POPC/POPS induce  $\alpha$ -helical secondary structures, which suggests that the vesicle size does not impact on the  $\alpha$ -synuclein secondary structure. With the neutral charged PE in the presence of acidic phospholipids (PA and PI), this significant increases the  $\alpha$ -synuclein  $\alpha$ -helicity. However, it should be emphasized that the changes in  $\alpha$ -synuclein secondary structure are much lower in the presence of neutral PC in combination with negatively charged lipids (Jo et al., 2000).

In contrast to previous studies that used prolonged incubation times and mechanical separation of the products, and which found that  $\alpha$ -synuclein only bound to SUVs composed of acidic phospholipids (Davidson et al., 1998; Jo et al., 2000; Perrin et al., 2000), in another study, the association between  $\alpha$ -synuclein and lipids was viewed immediately after the addition of lipids to  $\alpha$ -synuclein. Data were then obtained either by monitoring the change in intrinsic fluorescence emanating from four  $\alpha$ -synuclein Tyr residues, or by adding the Laurdan fluorescent probe to the vesicles. Surprisingly,  $\alpha$ -synuclein bound with similar affinities to LUVs composed of the negatively charged POPS and the electrically neutral



POPC. This initial binding did not induce changes in the secondary structure of  $\alpha$ -synuclein. This study also supported the role of the  $\alpha$ -synuclein C-terminus in membrane binding, by showing that lowering the pH of folded  $\alpha$ -synuclein, which reduces the negative charge of  $\alpha$ -synuclein, greatly increases the binding affinity without altering the secondary structure (Narayanan & Scarlata, 2001).

Fluorescence correlation spectroscopy was used as a tool for rapid and quantitative analysis of the lipid binding of  $\alpha$ -synuclein. Some studies have confirmed the importance of the negatively charged lipids (PA and PS) for  $\alpha$ -synuclein binding to LUVs with 120 nm diameter, when no pre-incubation of  $\alpha$ -synuclein and the vesicles was used. Alpha-synuclein has a significantly higher affinity for vesicles that contain some POPA, over those that contain an equivalent amount of POPS. The reason for this could be the polar POPA head-group, which is smaller in size compared to that of POPS and might therefore be able to pack more closely together in a lipid bilayer, producing a higher charge density. Alpha-synuclein shows slightly higher binding affinity to POPE compared to POPC (Rhoades et al., 2006). Combined with other data in the literature (Davidson et al., 1998; Jo et al., 2000; Nuscher et al., 2004), these suggest that each molecule of  $\alpha$ -synuclein can bind to a lipid bilayer patch composed of  $\leq 85$  acidic lipid molecules, corresponding in the case of POPS to a weight ratio of bound protein to total lipid of approximately 1:5. Interestingly, at higher  $\alpha$ -synuclein concentrations, the amount of bound  $\alpha$ -synuclein decreases, suggesting a destabilization of the membrane. This study also confirmed the importance of electrostatic interactions for the binding between  $\alpha$ -synuclein and the lipids (Rhoades et al., 2006).

The binding of  $\alpha$ -synuclein to SUVs has been monitored by measuring the changes in intrinsic fluorescence emanating from the four Tyr residues in  $\alpha$ -synuclein. These data have suggested that  $\alpha$ -synuclein binds to both negatively charged and electrically neutral SUVs, although slightly weaker for the latter. This binding to electrically neutral vesicles is presumably due to electrostatic interactions between the negatively charged C-terminal region of  $\alpha$ -synuclein and the positively charged choline. Binding to different types of vesicles was also detected in high ionic strength solutions. These data indicate that for  $\alpha$ -synuclein binding to lipids, not only are electrostatic interactions important, but also hydrophobic interactions. The influence of  $\alpha$ -synuclein binding to the membrane has also been examined. Due to the differences in the excitation spectra and polarisation of the Laurdan dye after incubation of dipalmitoyl PA (DPPA)/dipalmitoyl PC (DPPC) and dipalmitoyl PG (DPPG)/DPPC SUVs with  $\alpha$ -synuclein, this study concluded that  $\alpha$ -synuclein is inserted deep into the membrane and does not remain bound only on the surface. A lack of significant penetration of  $\alpha$ -synuclein into the DPPC vesicle bilayer was observed. Random coil-helix structure transition was most notable when SUVs composed of DPPG or dipalmitoyl PS (DPPS) or their mixtures with DPPC or dipalmitoyl PE (DPPE) were used. The amount of helix induced was smaller for DPPA/DPPC. SUVs made of DPPC only do not trigger the formation of the  $\alpha$ -synuclein  $\alpha$ -helix structure; presumably  $\alpha$ -synuclein binds to the surface of these vesicles due to electrostatic interactions, but does not induce the helical structure (Zhu et al., 2003).

The small diameter of the SUVs leads to curvature stress in the bilayer, which results in a rather broad phase transition that is centered at  $\sim 4$ -5 °C below the chain-melting phase-transition temperature ( $T_m$ ), and thus vesicles made of DPPC undergo melting transition at 36 °C rather than at 41 °C (Gaber & Sheridan, 1982). Using isothermal titration calorimetry, differential scanning calorimetry (Nuscher et al., 2004), spin label electron paramagnetic

resonance (EPR), and fluorescence spectroscopy (Kamp & Beyer, 2006), it has been shown that  $\alpha$ -synuclein affects the lipid packing in neutral SUVs. Here  $\alpha$ -synuclein induces chain ordering below the  $T_m$ , but not in the liquid crystalline state of zwitterionic vesicle membranes. Binding of  $\alpha$ -synuclein leads to an increase in the temperature and cooperativity of the phase transition, which was attributed to defect healing in the curved vesicle membranes. Binding to the vesicles also induces coil-helix transitions of  $\alpha$ -synuclein (Kamp & Beyer, 2006; Nuscher et al., 2004). SUVs made of POPC/POPG at a molar ratio of 1:1 and 2:1 cause  $\alpha$ -helix formation in the structure of  $\alpha$ -synuclein, and this is more pronounced at the 1:1 ratio. A helix structure is not observed in LUVs of the same composition. This again highlights the importance of the negative charge and size of lipid vesicles for  $\alpha$ -synuclein  $\alpha$ -helix formation. A more important finding is the formation of the helical structure by binding to SUVs of neutrally charged DPPC under the  $T_m$  and not above that temperature (Nuscher et al., 2004).

Recently, Bartels et al. used circular dichroism spectroscopy and isothermal titration calorimetry to investigate peptide fragments from different domains of the full-length  $\alpha$ -synuclein protein. They showed that membrane recognition of the N-terminus is essential for the cooperative formation of helical domains in the protein. This suggests that the membrane-induced helical folding of the first 25 residues of  $\alpha$ -synuclein might be driven simultaneously by electrostatic attraction and by changes in lipid ordering (Bartels et al., 2010).

### 6.2.2 Membrane interactions *in vivo*

Compared with the  $\alpha$ -synuclein-lipid interaction *in vitro*, the interaction of  $\alpha$ -synuclein with membranes in cells is less well understood. Cole et al. investigated  $\alpha$ -synuclein interactions with intracellular lipid stores in cultured cells treated with high concentrations of fatty acids. Here,  $\alpha$ -synuclein accumulated on phospholipid monolayers surrounding triglyceride-rich lipid droplets and protected the stored triglycerides from hydrolysis. Chemical cross-linking experiments led to the suggestion that dimers or trimers of  $\alpha$ -synuclein were associated with the droplet surface (Cole et al., 2002).

Alpha-synuclein can be imported into cells (Sung et al., 2001) and can be secreted from cells, although it lacks a conventional signal sequence for secretion (Lee et al., 2005). Lee et al. reported that a portion of  $\alpha$ -synuclein is stored in the lumen of vesicles in the cytoplasm, and that the  $\alpha$ -synuclein in vesicles might be secreted through an unconventional exocytosis pathway. This study also demonstrated that intravesicular  $\alpha$ -synuclein is more prone to aggregation than cytosolic  $\alpha$ -synuclein, and that aggregated forms of  $\alpha$ -synuclein are also secreted from cells (Lee et al., 2005). They thus used a series of deletion mutants and recombinant peptides to determine the amino-acid sequence motifs of  $\alpha$ -synuclein that were required for its membrane translocation. The N-terminal region and the NAC peptide were shown to be necessary for translocation, although the NAC was less effective than the N-terminal region. This thus suggested that the 11-amino acid repeat sequences bind to the lipid bilayer and that this binding interaction is crucial for  $\alpha$ -synuclein translocation. Cellular uptake of  $\alpha$ -synuclein was not significantly affected by treatment with inhibitors of endocytosis, suggesting that this occurs via a mechanism distinct from normal endocytosis (Ahn et al., 2006).

Sharon et al. showed that free fatty acids have specific roles in the formation and maintenance of the soluble  $\alpha$ -synuclein oligomers, and they suggested that  $\alpha$ -synuclein

might be a fatty-acid-binding protein (Sharon et al., 2001). In contrast, a later NMR study excluded high-affinity binding of fatty-acid molecules to specific  $\alpha$ -synuclein sites (Lucke et al., 2006). Exposure of living mesencephalic neurons to polyunsaturated fatty acids (PUFAs) increased the  $\alpha$ -synuclein oligomer levels, whereas saturated fatty acids decreased these. Here,  $\alpha$ -synuclein interacts with the free PUFAs to form the first soluble oligomers, which then aggregate into insoluble high-molecular-weight complexes (Sharon et al., 2003a). Indeed, elevated PUFA levels have been detected in the soluble fractions of PD and Lewy bodies dementia brains. The levels of saturated and monounsaturated fatty acids did not change in these PD brains or in cells overexpressing  $\alpha$ -synuclein, which indicated that  $\alpha$ -synuclein is involved specifically in the maintenance of PUFA levels (Sharon et al., 2003b).

Using binding assays, it has been demonstrated that  $\alpha$ -synuclein binds saturably and with high affinity to detergent-resistant membranes, to lipid rafts, in permeabilized HeLa cells, and in the presence of synaptosomal membranes from transgenic mice expressing human  $\alpha$ -synuclein. The A53T  $\alpha$ -synuclein mutation has no detectable effects on this binding, while the A30P mutation disrupts the association, which supports the specificity of the interaction (Fortin et al., 2004). It should also be mentioned that both of these mutations do not generally affect the interactions of  $\alpha$ -synuclein with artificial membranes (Perrin et al., 2000), probably because these membranes fail to reproduce the full characteristics of lipid rafts (Fortin et al., 2004). In contrast, the A30P mutation disrupts  $\alpha$ -synuclein association with native membranes, such as those of axonal transport vesicles, lipid droplets produced in HeLa cells by the administration of oleic acid, and yeast (Cole et al., 2002; Jensen et al., 1998; Outeiro et al., 2003). Alterations in the electrophoretic mobility of  $\alpha$ -synuclein upon membrane binding have confirmed its binding to lipid rafts, with this interaction resistant to digestion of the rafts with proteinase K, which suggests that the lipids, rather than proteins, are required. This assumption is also supported by high affinity binding of  $\alpha$ -synuclein to artificial membranes that do indeed mimic lipid rafts. Cholesterol does not appear to be required for the binding, but rather for maintenance of raft integrity; sphingolipid also appears not to be crucial for these interactions (Kubo et al., 2005).

Similar to previous reports (Davidson et al., 1998; Perrin et al., 2000), Kubo et al. reported that  $\alpha$ -synuclein binding requires acidic phospholipids, with a preference for PS. Synthetic PS with defined acyl chains did not support this binding when used individually, with the combination of 18:1 PS and PS with polyunsaturated acyl chains required both to bind to and to shift the electrophoretic mobility of  $\alpha$ -synuclein. The addition of 18:1 PC to 20:4 PS, or conversely, the addition of 20:4 PC to 18:1 PS, also promoted  $\alpha$ -synuclein binding. The requirement for both monounsaturated and polyunsaturated acyl chains suggests that the interaction of  $\alpha$ -synuclein requires membranes with two distinct phases: lipid rafts in a liquid-ordered phase, and the rest of the cell membrane in a liquid-disordered phase. Alpha-synuclein binds with higher affinity to artificial membranes with the PS head-group on the polyunsaturated fatty acyl chain rather than on the oleoyl side chain, apparently reflecting an interaction of  $\alpha$ -synuclein with both the acyl chain and the head-group (Kubo et al., 2005).

In contrast to artificial membranes, the interactions of  $\alpha$ -synuclein with biological membranes are highly dynamic and they show rapid dissociation. Thus, rather than electrostatic interactions, Kim et al. suggested the involvement of hydrophobic interactions. Furthermore, the interactions of  $\alpha$ -synuclein with cellular membranes occurred only in the presence of nonprotein and nonlipid cytosolic components, which distinguished it from the

spontaneous interaction with artificial membranes. Here, addition of a cytosolic preparation to the artificial membranes resulted in similar binding of  $\alpha$ -synuclein as for biological membranes (Kim et al., 2006).

Lipid rafts contain a lot of the ganglioside GM1, and it has been suggested that the gangliosides mediate or facilitate the association of  $\alpha$ -synuclein with neuronal membranes (Martinez et al., 2007). However, recently Di Pasquale et al. identified a ganglioside-binding domain in  $\alpha$ -synuclein that showed a marked preference for interactions with GM3, which is a minor brain ganglioside for which the expression increases with age; the Lys<sup>34</sup> and Tyr<sup>39</sup> residues were shown to have critical roles in the GM3 recognition by  $\alpha$ -synuclein (Di Pasquale et al., 2010).

## 7. Structural properties of membrane-bound $\alpha$ -synuclein

High resolution structural and dynamics information of  $\alpha$ -synuclein in its lipid-bound state appear to be sufficient for the development of a better understanding of the physiological role of  $\alpha$ -synuclein, as well as to identify the structural features that appear to be relevant to  $\alpha$ -synuclein misfolding (Ulmer et al., 2005). However, despite the abundance of structural information for soluble proteins, relatively little is known about the structures of membrane-associated proteins in the physiologically important lipid bilayer environment (Jao et al., 2008). Consistent with this, the conformation of membrane-bound  $\alpha$ -synuclein still remains unclear and somewhat contradictory.

Several biophysical methods have provided valuable insights into the structural features of the disordered and folded  $\alpha$ -synuclein, including circular dichroism spectroscopy (Davidson et al., 1998; Chandra et al., 2003; Perrin et al., 2000), fluorescence spectroscopy (Rhoades et al., 2006), NMR (Bisaglia et al., 2005; Bussel & Eliezer, 2001, 2003; Bussel et al., 2005; Chandra et al., 2003; Dedmon et al., 2005; Eliezer et al., 2001; Ulmer et al., 2005), and EPR (Bortolus et al., 2008; Drescher et al., 2008; Georgieva et al., 2008; Jao et al., 2004, 2008). Binding of  $\alpha$ -synuclein to anionic membranes induces folding of its N-terminal part into an amphipathic helix, whereas the C-terminus (residues ~98-140) remains unstructured (Bisaglia et al., 2005; Bussel & Eliezer, 2003; Chandra et al., 2003; Davidson et al., 1998; Eliezer et al., 2001; Ulmer et al., 2005). The helical content of  $\alpha$ -synuclein is much lower in buffer and in the presence of zwitterionic membranes (Davidson et al., 1998; Zhu & Fink, 2003).

It has generally been proposed that the natural binding target of  $\alpha$ -synuclein *in vivo* is the synaptic vesicles, the surface topology of which is most closely approximated *in vitro* by synthetic lipid vesicles (Bisaglia et al., 2005; Bussel & Eliezer, 2003; Bussell et al., 2005; Chandra et al., 2003; Jao et al., 2004; Ulmer et al., 2005). The slow tumbling rate of intact phospholipid vesicles has hindered direct studies of the vesicle-bound conformation of  $\alpha$ -synuclein using solution NMR methods (Georgieva et al., 2008). Consequently, most of the structural information available concerns studies where detergent micelles were used as membrane-mimetic environments, because their small size facilitates high-resolution structural analysis by NMR. The conformation of micelle-bound  $\alpha$ -synuclein has thus been thoroughly investigated, and there is a general consensus on the presence of two curved helices, with a break in the  $\alpha$ -synuclein 38–44 region (Bisaglia et al., 2005; Bussel & Eliezer, 2003; Chandra et al., 2003; Ulmer et al., 2005). On the contrary, the structure of  $\alpha$ -synuclein bound to lipid vesicles, which would be more relevant physiologically, remains a matter of debate. EPR analyses of  $\alpha$ -synuclein derivatives bound to SUVs have provided evidence for

an elongated helical structure that is devoid of any significant tertiary packing (Jao et al., 2004), or they have suggested a broken helical structure (Bortolus et al., 2008; Drescher et al., 2008). A number of recent studies have highlighted the ongoing debate regarding the physiologically relevant form, as the bent or extended membrane-bound helix (Figure 2).

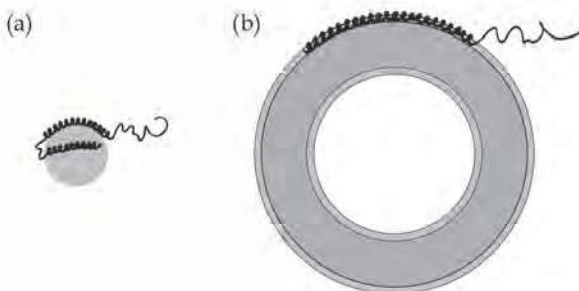


Fig. 2. Illustrations of the generally proposed  $\alpha$ -synuclein structures on (a) micelles (bent helix model) and (b) SUVs (elongated helix model) (Jao et al., 2008; Trexler & Rhoades, 2009).

### 7.1 Helix periodicity

Several studies have raised the question of the periodicity of the helix that is formed upon binding of  $\alpha$ -synuclein to membranes. As indicated above, the N-terminus of  $\alpha$ -synuclein contains seven imperfect 11-residue repeats. Using site-directed spin-labeling, it has been shown that repeats 5–7 of  $\alpha$ -synuclein are bound to SUVs with an 11/3 periodicity (11 residues to complete three full turns) (Jao et al., 2004). Sodium dodecyl sulphate (SDS) micelle-bound  $\alpha$ -synuclein shows the same periodicity, as opposed to the 18/5 periodicity of an ideal  $\alpha$ -helix (Bussell et al., 2005). In this ideal 18/5 periodicity, there are 3.6 residues per turn and the rotation per residue is  $100^\circ$ . In the  $\alpha$ -synuclein 11/3 periodicity, the number of residues per turn is 3.67 and the rotation per residue is  $98.18^\circ$ . Using theoretical methods, it has indeed been concluded that the periodicity of  $\alpha$ -synuclein is 11/3, and that through the positioning of the charged residues, this has implications for  $\alpha$ -synuclein membrane binding. These calculations show that the energy penalty for a change in periodicity from the 18/5 to 11/3 on anionic membranes is overcome by the favorable solvation energy (Mihajlovic & Lazaridis, 2008).

### 7.2 Analysis of $\alpha$ -synuclein structure by nuclear magnetic resonance

Eliezer et al. were the first to use NMR spectroscopy to characterize the conformational properties of  $\alpha$ -synuclein when bound to lipid vesicles and lipid-mimetic detergent micelles. They demonstrated that only the first 100 residues of the N-terminal region of  $\alpha$ -synuclein bind to both SDS micelles and PA/PC vesicles and fold into an amphipathic helix, while the acidic C-terminal region of  $\alpha$ -synuclein remains unstructured (Eliezer et al., 2001).

Ulmer et al. have described the structure and dynamics of  $\alpha$ -synuclein in the micelle-bound form according to solution NMR spectroscopy. In binding to SDS micelles or SDS micelles with dodecylphosphocholine (DPC),  $\alpha$ -synuclein forms two curved  $\alpha$ -helices (Figure 3), helix-N (Val<sup>3</sup>-Val<sup>37</sup>) and helix-C (Lys<sup>45</sup>-Thr<sup>92</sup>). These helices are connected by a well-ordered, extended linker in an unexpected anti-parallel arrangement, which is followed by another short extended region (Gly<sup>93</sup>-Lys<sup>97</sup>) that overlaps with a chaperone-mediated autophagy recognition motif and a predominantly unstructured highly mobile tail (Asp<sup>98</sup>-Ala<sup>140</sup>) (Ulmer et al., 2005).



Fig. 3. Structure of  $\alpha$ -synuclein bound to SDS micelles. Picture represents the two curved  $\alpha$ -helices (Val<sup>3</sup>-Val<sup>37</sup> and Lys<sup>45</sup>-Thr<sup>92</sup>), connected by extended linker. The disordered C-terminus has been partially omitted. The image was generated from the PDB (accession number 1XQ8).

Although the presence of the helix break in the micelle-bound state of  $\alpha$ -synuclein has been suggested to be a consequence of the small size of the micelle (Jao et al., 2004), the well-ordered conformation of the helix-helix connector indicates a defined interaction of  $\alpha$ -synuclein with the lipid surfaces, suggesting that when it is bound to larger diameter synaptic vesicles, this can act as a switch between this broken helix structure and the uninterrupted helix structure (Ulmer et al., 2005). Therefore, the presence or absence of the helical break in  $\alpha$ -synuclein appears to be the more controversial structural feature of  $\alpha$ -synuclein when bound to lipids (Bisaglia et al., 2009). Other studies have also shown that there are two helical regions in the N-terminal sequence of  $\alpha$ -synuclein that are interrupted by a single helix break around residue 42 (Bisaglia et al., 2005; Bussel & Eliezer, 2003; Chandra et al., 2003). Data from Bisaglia et al. show that the region of residues 61-95 (the NAC region) is partially embedded in the micelle (Bisaglia et al., 2005)

Analysis of the dynamic processes of the  $\alpha$ -synuclein backbone on a fast timescale (picoseconds to nanoseconds) revealed the presence of three distinct helical regions that have greater mobility with respect to the other helical fragments: Ala<sup>30</sup>-Val<sup>37</sup>, Asn<sup>65</sup>-Val<sup>70</sup>, and Glu<sup>83</sup>-Ala<sup>89</sup> (Ulmer et al., 2005). All three of these regions have two Gly residues in close sequential proximity, which might serve to mitigate a possible effect of  $\alpha$ -synuclein binding on membrane fluidity. The helix curvature is significantly less than predicted based on the native globular micelle shape, which indicates a deformation of the micelle by  $\alpha$ -synuclein. Ulmer et al. suggested that the interactions of the positively charged Lys side chains, which emanate sideways from the helices, with the negatively charged headgroups of SDS can lead to the deformation of the globular micelle along the helix axes, to form a prolate, ellipsoid particle (Ulmer et al., 2005).

As indicated above, there are four Tyr residues in  $\alpha$ -synuclein. One of these, Tyr<sup>39</sup>, is located in the break region. Bisaglia et al. (2005) suggested that this Tyr<sup>39</sup> is buried in the SDS micelle and proposed that this insertion might protect  $\alpha$ -synuclein from aggregation (Zhou & Freed, 2004; Ulrih et al., 2008), as well as to protect Tyr<sup>39</sup> from phosphorylation by p72<sup>syk</sup> tyrosine kinase (Negro et al., 2002, as cited in Bisaglia et al., 2005). This is in contrast with other models that have predicted that this Tyr<sup>39</sup> is located either on the hydrophilic side of the helix or at the membrane-water interface (Bussell & Eliezer, 2003; Chandra et al., 2003; Jao et al., 2004; Mihajlovic & Lazaridis, 2008).

### 7.3 Analysis of $\alpha$ -synuclein structure by electron paramagnetic resonance

EPR analysis of 47 singly labeled  $\alpha$ -synuclein mutants has shown that the membrane interactions are mediated by major conformational changes within seven of the N-terminal 11-amino-acid repeats: these reorganize from highly dynamic structures into an elongated helical structure. The equivalent positions within each of these different repeats are located in structurally comparable positions with respect to the membrane proximity, which suggests a curved membrane-dependent  $\alpha$ -helical structure of  $\alpha$ -synuclein, wherein each of these 11-aminoacid repeats takes up three helical turns (Jao et al., 2004). The  $\alpha$ -synuclein helix is over 90 amino acids in length and it extends parallel to the curved membrane in a manner that allows the conserved Lys and Glu residues to interact with the zwitterionic headgroups, while the uncharged residues penetrate into the acyl-chain region (Jao et al., 2008). This structural arrangement is significantly different from that of  $\alpha$ -synuclein in the presence of the commonly used membrane-mimetic detergent, SDS (Bisaglia et al., 2005, Ulmer et al., 2005). Thus these structural analyses also show that it is important to consider the lipid composition of any given bilayer, as this can have pronounced effects on the protein and bilayer structures (Jao et al., 2008).

Several other independent studies have appeared, with contradictory results. In one such study (Bortolus et al., 2008), the 35-43 region of  $\alpha$ -synuclein bound to SUVs and to SDS micelles was investigated using site-directed spin labeling and EPR spectroscopy. The distance distributions were compatible with the presence of conformational disorder in this region, rather than for the formation of a continuous helical structure. These data showed that  $\alpha$ -synuclein shows very similar behavior in micelles and in SUVs, and they ruled out an unbroken helical structure of the region around residue 40. This propensity for helix breaking was confirmed by their molecular dynamics simulations of the 31-52 fragment interacting with a lipid bilayer (Bortolus et al., 2008).

In a study by Drescher et al. (2008), four  $\alpha$ -synuclein mutants were prepared by inserting Cys residues labeled with the spin-label reagent (*S*-(2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonylthioate) (MTSL), with each containing one label in the proposed helix 1, and a second label in helix 2. Between the labeled Cys residues within the molecule the distance resulting from their binding to the membrane was measured using dual-frequency pulsing EPR (double electron-electron resonance). Consistent with a previous report (Bortolus et al., 2008), these data showed that  $\alpha$ -synuclein even adopts a two-helix, antiparallel arrangement on vesicles that are large enough to accommodate an extended helix, which suggests that this bent structure is also the preferred conformation of  $\alpha$ -synuclein on larger vesicles (Drescher et al., 2008).

Also using pulsed dipolar EPR, Georgieva et al. came to somewhat different conclusions. Here the distances measured between the pairs of nitroxide spin labels introduced were close to those expected for a single continuous helix. To circumvent problems associated with SUVs and rodlike SDS micelles, here they used lipid bicelles (providing a lipid-bilayer structure, yet having a particle size nearly as small as that of micelles), which produced very similar results to liposomes while offering a major improvement in experimentally accessible distance ranges and resolution. According to these data, they suggested that when  $\alpha$ -synuclein is bound to SUVs, it forms a single  $\alpha$ -helix, without the intermediate region of the interruption. The idea that  $\alpha$ -synuclein can interconvert between these broken and extended helical forms was also suggested, and it thus remains possible that *in vivo*  $\alpha$ -synuclein occupies one or the other form depending on conditions (Georgieva et al., 2008).

#### 7.4 Analysis of $\alpha$ -synuclein structure with other methods

Contradictory to these current models of membrane-bound  $\alpha$ -synuclein that have been deduced mostly from NMR studies, limited proteolysis experiments have indicated that the C-terminal part of membrane-bound  $\alpha$ -synuclein has a more rigid structure. The negatively charged C-terminus appears to bind  $\text{Ca}^{2+}$  in the presence of SDS micelles, and in doing so it becomes sufficiently rigid and structured to resist extensive proteolysis (de Laureto et al., 2006). In another study based on site-directed fluorescence labeling, they also examined the effects of  $\text{Ca}^{2+}$  on the acidic tail conformation of lipid-bound  $\alpha$ -synuclein (Tamamizu-Kato et al., 2006). Here, they suggested that the  $\text{Ca}^{2+}$  either bridges  $\alpha$ -synuclein to the membrane, possibly by coordinating with the negative charge on the  $\alpha$ -synuclein acidic tail and the acidic head-groups in the phospholipid bilayer, or it facilitates the traversing of the membrane bilayer by this segment of  $\alpha$ -synuclein (Tamamizu-Kato et al., 2006).

Another study highlighted the role of the physical parameters of the membrane mimetic in determining the  $\alpha$ -synuclein conformation (Trexler & Rhoades, 2009). Single molecule Förster resonance energy transfer was used to probe the helical structure of  $\alpha$ -synuclein bound to SDS micelles and LUVs. Single and double Cys  $\alpha$ -synuclein mutants were engineered to allow for site-specific labeling with maleimide fluorophores. When bound to highly curved detergent micelles,  $\alpha$ -synuclein formed a bent-helix, whereas the structure of the elongated helix was adopted when bound to the more physiological 100-nm-diameter lipid vesicles (Trexler & Rhoades, 2009).

Single-molecule Förster resonance energy transfer was also used to provide evidence for the structural interplay between the broken and extended  $\alpha$ -helix structures of  $\alpha$ -synuclein, as induced by the binding of  $\alpha$ -synuclein to SDS and phospholipid SUVs (Ferreon et al., 2009). The switch between a broken and an extended helical structure can be triggered by changing the concentrations of the binding partners or by varying the curvature of the binding surfaces presented by the micelles or bilayers composed of SDS. The use of lipid vesicles of various compositions showed that a low fraction of the negatively charged lipids, as similar to that found in biological membranes, was sufficient to drive  $\alpha$ -synuclein binding and folding that resulted in the induction of the extended helical structure (Ferreon et al., 2009).

The structure of the N-terminal domain of  $\alpha$ -synuclein has also been determined using theoretical methods (Mihajlovic & Lazaridis, 2008). This computational study of the binding of truncated  $\alpha$ -synuclein (residues 1-95) to planar bilayers showed that  $\alpha$ -synuclein forms a bent helix, with the largest bend around residue 47. This bending of the helix was not due to the protein sequence or membrane-protein interactions, but to the collective motions of the long helix (Mihajlovic & Lazaridis, 2008).

## 8. Conclusions

In this chapter, we have presented the state-of-the-art for the field of  $\alpha$ -synuclein structure, and for its fibril formation and interactions with membranes. There are still many unanswered questions regarding the correlation between  $\alpha$ -synuclein membrane affinity, and its function and its role in synucleinopathies. As the disruption of membranes by  $\alpha$ -synuclein correlates with the binding affinity of  $\alpha$ -synuclein for particular membrane compositions and with the induced helical conformation of  $\alpha$ -synuclein, this suggests that inappropriate membrane permeabilization is the cause of cell dysfunction, and even cell



death, in amyloid diseases. Protofibrillar or fibrillar  $\alpha$ -synuclein results in a much more rapid destruction of membranes than soluble monomeric  $\alpha$ -synuclein, which indicates that protofibrils or fibrils are likely to be significantly neurotoxic. Further studies of  $\alpha$ -synuclein interactions with membranes are still very important to provide us with a fuller understanding of the molecular mechanisms of its implications in Parkinson's disease.

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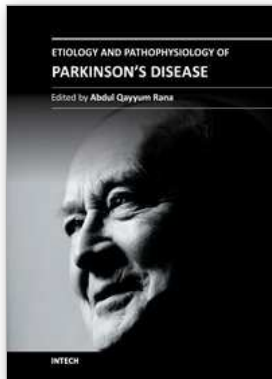
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## **Etiology and Pathophysiology of Parkinson's Disease**

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This book about Parkinson's disease provides a detailed account of etiology and pathophysiology of Parkinson's disease, a complicated neurological condition. Environmental and genetic factors involved in the causation of Parkinson's disease have been discussed in detail. This book can be used by basic scientists as well as researchers. Neuroscience fellows and life science readers can also obtain sufficient information. Beside genetic factors, other pathophysiological aspects of Parkinson's disease have been discussed in detail. Up to date information about the changes in various neurotransmitters, inflammatory responses, oxidative pathways and biomarkers has been described at length. Each section has been written by one or more faculty members of well known academic institutions. Thus, this book brings forth both clinical and basic science aspects of Parkinson's disease.

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