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Membranes as modulators of amyloid protein misfolding and target of toxicity

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Abstract

Abnormal protein aggregation is a hallmark of various human diseases. α -Synuclein, a protein implicated in Parkinson's disease, is found in aggregated form within Lewy bodies that are characteristically observed in the brains of PD patients. Similarly, deposits of aggregated human islet amyloid polypeptide (IAPP) are found in the pancreatic islets in individuals with type 2 diabetes mellitus. Significant number of studies have focused on how monomeric, disaggregated proteins transition into various amyloid structures leading to identification of a vast number of aggregation promoting molecules and processes over the years. Inasmuch as these factors likely enhance the formation of toxic, misfolded species, they might act as risk factors in disease. Cellular membranes, and particularly certain lipids, are considered to be among the major players for aggregation of α -synuclein and IAPP, and membranes might also be the target of toxicity. Past studies have utilized an array of biophysical tools, both *in vitro* and *in vivo*, to expound the membrane-mediated aggregation. Here, we focus on membrane interaction of α -synuclein and IAPP, and how various kinds of membranes catalyze or modulate the aggregation of these proteins and how, in turn, these proteins disrupt membrane integrity, both *in vitro* and *in vivo*. The membrane interaction and subsequent aggregation has been briefly contrasted to aggregation of α -synuclein and IAPP in solution.

Graphical Abstract

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Keywords

membrane; amyloid; helical; α -synuclein; IAPP; aggregation

1. INTRODUCTION

The ability of a protein to function properly is highly correlated with its ability to maintain the native structure(s). Protein homeostasis pathways within cells control and maintain the synthesis, structure and degradation of proteins [1]. Any deleterious effect on these pathways may culminate in improper conformational changes in a protein or lead to the accumulation of misfolded proteins within a cell. Protein aggregation due to misfolding is the basis of a multitude of diseases [2]. Misfolding of a protein can result in a loss of function, and/or a toxic gain of function. The toxicity of misfolded/aggregated proteins could be due to, but not limited to, erroneous interaction with other macromolecules, mislocalization or damage to intracellular membranes. Due to the apparent nature of aggregated proteins to inflict cellular toxicity, numerous attempts have been made to unravel the underlying mechanism(s) of protein aggregation. A generalized pathway of protein aggregation is displayed in Fig. 1. Native proteins that have typically monomeric structure form oligomers, and these 'on-pathway' oligomers eventually form fibrils. Some 'off-pathway' oligomers are not converted into fibrils. The obvious and imperative question is what triggers the monomeric form to translate into oligomers and fibrils. The factors that trigger or promote aggregation can be classified as either intrinsic or extrinsic [3]. The intrinsic factors include mutations in the protein, truncations and overexpression of proteins. The extrinsic factors that have been commonly incriminated are variation in pH, lipid membranes, posttranslational modifications, metal ions, and other small molecules including polyamines and pesticides [3]. In this review, we focus on the role of lipid membranes in modulating the aggregation of α -synuclein and IAPP. Here we describe the structure, membrane interaction and interaction with lipid-like molecules of α -synuclein and islet amyloid polypeptide (IAPP), and their implications in disease. For comparative purpose, we also discuss aggregation of α -synuclein and IAPP in solution. Finally, we examine modulation of the membrane binding of these proteins as a possible therapeutic strategy in Parkinson's disease and type II diabetes.

2. PHYSIOLOGICAL AND PATHOLOGICAL RELEVANCE

α -Synuclein has been implicated in development of Parkinson's disease (PD) and IAPP in type 2 diabetes mellitus. Although a complete understanding of the physiological and pathological roles of these proteins is still lacking, many studies have provided insightful information into the biological significance of these proteins.

2.1 α -Synuclein

α -Synuclein is a presynaptic protein that is highly expressed in the brain and implicated in PD. PD is the most prevalent age-related movement disorder that is characterized by the presence of Lewy bodies in the neurons of PD patients [4, 5]. In PD, the dopaminergic neurons degenerate, leading to lack of dopamine that is required for proper neuronal

functioning [4]. The underlying mechanism of neuronal degeneration in PD is unclear. Apart from the observation of aggregated α -synuclein in Lewy bodies, several genetic studies have implicated α -synuclein in PD [5]. Different α -synuclein missense mutations, A30P, E46Q, H50Q, G51D, A53E and A53T, are linked to the autosomal dominant form of PD [6–10]. Further, α -synuclein gene duplication and triplication is also linked to early-onset PD [11, 12]. Transgenic animals overexpressing α -synuclein display motor deficit that further corroborate the role of this protein in PD [13–15].

Although this protein was discovered almost two decades ago, the exact function of α -synuclein is still not fully understood. The initial discovery linked the protein to regulation of neural plasticity [16]. Subsequent studies have associated α -synuclein with functions such as regulation of synaptic vesicle recycling, dopamine release, exo- and endocytosis, vesicular trafficking, interaction with SNARE proteins, and roles in lipid/fatty acid metabolism and transport among many others [17–25]. Apart from these functions, α -synuclein is reported to interact with a wide range of intracellular membranes, which is suggested to be relevant for its normal physiological functioning. A misregulated or abnormal membrane interaction, on the other hand, is suggested to result in neuronal malfunctioning and eventual cell death.

2.2 Islet amyloid polypeptide (IAPP)

IAPP, also called amylin, is a peptide hormone consisting of 37 amino acid residues. Initially produced as an 89-residue preproprotein in β -cells of the pancreas, it undergoes a series of proteolytic and post-translational modifications leading to its final mature form [26, 27]. Mainly produced by β -cells of the islets of Langerhans, IAPP is co-packaged and co-secreted with insulin in the secretory granules of the β -cells [28, 29]. Although not all tissue targets and physiological functions of IAPP are completely known, it is thought to mainly affect the liver, gut, and brain, where it might play a role in the control of glucose homeostasis and satiety [30, 31].

IAPP derives much of its notoriety from its gain-of-toxic function that arises from its ability to misfold and ultimately form pancreatic amyloid aggregates in over 90% of patients with type 2 diabetes (T2DM) [31–35]. Although the overall etiology of T2DM is complex and not fully understood, multiple lines of evidence suggest that at least some species formed during the misfolding process are toxic agents [31–40]. IAPP toxicity has been observed in a number of cell and animal systems [37, 39, 41–45]. While there is no complete consensus regarding the toxicity of larger aggregates, which are sometimes considered either toxic or protective, smaller misfolded species that form early during misfolding are typically thought to be toxic [33–39, 46–52]. The link between IAPP misfolding and T2DM pathophysiology is further supported by comparison of IAPP sequences from different animals. Several mammals (and some non-mammals) express IAPP homologs, but not all of them are prone to develop T2DM [31]. Interestingly, species which do not spontaneously develop T2DM, for example mouse and rat, lack amyloidogenicity in their IAPP owing to species-specific sequence variations [53, 54]. These animals, however, could be made diabetic by transgenic expression of human IAPP in their β -cells [42, 44, 55, 56].

How IAPP, seemingly benign in the secretory granules, misfolds, aggregates, and acquires a toxic function has been a focus of extensive research [31, 40, 57, 58]. Evidence suggests a potential mechanism for IAPP toxicity where membranes catalyze IAPP misfolding and where IAPP, in turn, promotes membrane damage [57–59]. A review of conformational, structural, and functional consequences of this interplay between IAPP and membranes follows.

3. STRUCTURES IN SOLUTION

Both α -synuclein and IAPP in their monomeric forms are intrinsically disordered (Fig. 2). However, both, due to their structural plasticity, can adopt different conformations and structural assemblies. Many studies carried out on these proteins reveal several interesting structural features, which are summarized below. IAPP and α -synuclein can exist in different monomeric, oligomeric and fibrillar states. All of these states possess their own distinct structural features that have been studied using different biophysical tools. Depending on the conditions used, some variations in the structure have been reported.

3.1 Structure of native α -synuclein

For a long time, α -synuclein was known as an intrinsically disordered, monomeric protein with residual α -helical propensity [60–62]. In the recent past, however, reports have suggested that it could also exist in multimeric forms such as dimers, trimers, tetramers, and even octamers in its native form [63–66]. The native multimeric forms in these studies are structurally distinct from misfolded oligomers, as they are mainly helical rather than β -sheet in nature [64, 67]. Still, questions abound regarding whether the monomeric or multimeric forms represent the physiological state. It may well be possible that α -synuclein exists in a dynamic equilibrium between monomeric and different multimeric forms. Alteration of this equilibrium could have adverse effects on neuronal functioning [68, 69].

3.2 Structure of α -synuclein oligomers

It is often suggested that α -synuclein oligomers are more toxic than monomers or fibrils. α -Synuclein can form a number of different oligomers that are often transiently assembled during aggregation into fibrils, in addition to the ‘off-pathway’ oligomers. Structural studies have been difficult due to the transient, unstable and often heterogeneous nature of oligomeric species. A few studies, however, have used different methods to isolate or trap the oligomers, enabling structural analysis of some oligomeric forms (For review see [70]).

α -Synuclein oligomers have been categorized using criteria such as size, shape, extent and nature of β -sheet and proteinase K digestion [71, 72]. Oligomers with morphologies including spherical, annular, tubular, circular and elliptical have been observed [70, 73–75]. In a recent study by Fusco et. al., two distinct species of stabilized α -synuclein oligomers were structurally characterized using solid-state nuclear magnetic resonance (NMR) [67]. One of them, named type A, was non-toxic and lacked secondary structure in the rigid regions of the protein, while the toxic type B was found to have considerable amount of β -sheet content in its rigid regions. The N-terminal region of the non-toxic oligomer was less dynamic and inaccessible compared to the toxic form [67]. These structural differences

enabled the toxic form to insert more deeply into lipid bilayers of vesicles mimicking the synaptic vesicle phospholipid composition, causing membrane disruption [67].

Overall, reports suggest that α -synuclein oligomers adopt an antiparallel β -sheet structure [70, 76], whereas fibrils form a parallel arrangement [77, 78]. The oligomeric core seems to include the same amino acids that form the fibril core, however, with a different β -sheet arrangement in the latter. The number of monomeric units within different oligomeric species mostly range from ~10–90. Although the N- and C-terminal regions of α -synuclein are not part of the fibrillar core, they could have many intermolecular interactions within the oligomer and, hence, considerable structural remodeling of early oligomeric interactions is expected for fibril elongation. Various oligomeric species reported by different research groups display significant differences in their morphology, structure, physicochemical properties and cellular toxicity that requires further detailed analysis to know which structure could be pathologically more relevant.

3.3 Structure of α -synuclein fibrils

Many oligomers eventually transform into fibrillar structures. The *in vitro* fibrils formed by α -synuclein are morphologically very similar to those isolated from postmortem brains of PD patients [79, 80]. Like oligomers, α -synuclein fibrils also display large polymorphism that has hampered the identification of a consensus fibril structure. Structural studies have been carried out on full-length fibrils generated using varied methodologies and by techniques like solid-state NMR, electron paramagnetic resonance, hydrogen/deuterium (H/D) exchange mass spectrometry, and electron microscopy [77, 78, 81–86]. Both twisted and straight fibrils with varying diameters have been observed [70, 86–88]. Early mapping of the α -synuclein fibril core region has come from electron paramagnetic resonance (EPR) experiments, which also revealed this region takes up a parallel, in-register structure [77, 78]. These findings were later confirmed by a number of techniques, including solid-state NMR [81, 85, 86]. A recent study by Rodriguez et. al. used micro-electron diffraction to determine 1.4 Å structure of microcrystals formed by a segment of α -synuclein containing residues 68–78, called NACore, which has important role in α -synuclein aggregation and cytotoxicity [89]. The structural data revealed protofibrils formed of pairs of face-to-face β -sheets and a structure that had similarity to toxic fibrils of full-length α -synuclein [89]. The core region and the participation of particular amino acids in the cross- β structure of α -synuclein fibrils depends on the fibril polymorph but, in general, it is between the positions ~30–100. Experimental data also suggest the role of N-terminal region and to some lesser extent, the C-terminal region, in the formation of fibrils, especially in the interaction between protofilaments [77, 78, 85, 87, 90].

Most of the experimental data obtained agree with the presence of α -synuclein monomeric units stacked in a parallel in-register arrangement within a protofilament. The β -sheets are arranged anti-parallel to each other within a single monomeric subunit. The number of β -strands in each monomeric unit vary depending on the fibril polymorph. A recent study by Tuttle et. al., using solid-state NMR spectroscopy, EM and X-ray fiber diffraction, presented a new orthogonal Greek-key topology consisting of the common amyloid features including

parallel, in-register β -sheets and hydrophobic-core residues [86]. Further structural studies would determine which fibril polymorphs has structure like the *in vivo* form.

3.4 Structure of IAPP Monomer and oligomers

Early evidence from circular dichroism (CD) suggested that IAPP monomers are mainly in random coil conformation with some α -helical and β -sheet content [91–95]. This structural organization has been confirmed by a number of studies [96, 97], some including solution NMR, which revealed residual α -helical conformations [98, 99]. This structural feature of IAPP also extends to the physiologically relevant acidic conditions in the secretory vesicles from which it is released [100] (Fig. 3). Under these conditions, IAPP monomers exhibit an α -helical N-terminal region and disordered C-terminal region [100]. As described later in more detail for membrane-mediated aggregation, the presence of this helical structure also appears to promote IAPP aggregation in solution [98] (Fig. 2).

Despite being an important amyloid species, high-resolution structural studies of oligomers have been difficult, owing to the heterogeneity and limited stability of such species. A two-dimensional infrared spectroscopy (2D-IR) study observed a parallel β -sheet structure in the region within an oligomeric intermediate [101] previously shown to be a disordered loop in mature fibrils [102]. This data suggests that conversion from β -sheet in oligomer to loop in fibrils poses free energy barrier that in turn generates a lag phase in IAPP aggregation. A recent Fourier transform infrared (FTIR) and Raman spectroscopy study showed considerable α -helical content in early IAPP oligomers [103]. This study also suggested that such oligomers could have enhanced membrane affinity.

3.5 Structure of IAPP fibrils

IAPP fibrils, like those of α -synuclein and many other fibrils, can be highly polymorphic [104, 105], yet most or all of these polymorphs are largely arranged in parallel, in-register structures, as first determined by EPR and site-directed spin labeling [106] (Fig. 3). Structural models of such parallel, in-register fibrils have been generated by solid-state NMR, EPR, X-ray diffraction of microcrystals from IAPP fragments and computational techniques [102, 107–109]. Among these models, the EPR and solid-state NMR models are most similar, both having a horseshoe type arrangement with two β -strands separated by a bend region. A two-strand model is further supported by data from H/D exchange and 2D-IR spectroscopy [110, 111]. Despite their similarities, the EPR and NMR models also have some differences. First, there is a slight variation in the exact lengths of the strands, and the relative positions of the strands with respect to each other are different. While the solid-state NMR model has both strands in the same plane (Fig. 3D), the EPR model is staggered by about 15 Å (Fig 3E–G). This stagger, as per distance measurements, indicates that the two strands in the same monomer are spaced apart from each other. Interestingly, this model is analogous to a recent fibril model of Alzheimer's amyloid beta peptide obtained from solid-state NMR and cryo EM [112]. The staggered structure could provide stability and expose large hydrophobic surfaces at the fibril ends. It has been suggested that such “sticky ends” could promote capture of incoming monomers and promote fibril extension [107].

4. MEMBRANE INTERACTION

Membrane interaction appears to be an essential feature of α -synuclein in fulfilling its proposed physiological role. An abnormal membrane interaction, on the other hand, is associated with pathological consequences for both α -synuclein and IAPP. The pathological consequences in the form of membrane damage have been observed for both α -synuclein and IAPP in numerous *in vitro* assays and confirmed *in vivo*. Various studies to understand the underlying mechanism of these interactions and to tease out the role of specific membrane components and protein features are discussed below.

4.1 Membrane interaction of α -synuclein

α -Synuclein contains seven imperfect 11-amino acid repeats that have some similarities to those present in apolipoproteins (Fig. 2). The N-terminal ~100 amino acids that encompass these repeats are the primary mediators for membrane binding. α -Synuclein preferentially binds to phospholipids with negatively charged headgroups like phosphatidic acid (PA), phosphatidylserine (PS), phosphatidylinositol (PI) and phosphatidylglycerol (PG) [113, 114]. It has negligible affinity towards zwitterionic phospholipids like phosphatidylcholine (PC) and phosphatidylethanolamine (PE) headgroups. The affinity towards anionic phospholipids is due to their interaction with multiple lysine residues in the N-terminal membrane binding region of α -synuclein [115]. The binding preference can further extend for a particular negatively charged lipid headgroup over another negatively charged headgroup with same acyl chains [116], or for a particular acyl chain over another acyl chain with same phospholipid headgroup [117]. Thus, the membrane affinity of α -synuclein is modulated by both the headgroup and the acyl chain of a phospholipid molecule.

Furthermore, α -synuclein binding to membranes is affected by membrane curvature [113, 118]. Studies of the binding affinity to lipid vesicles of different sizes revealed that the protein binds preferentially to highly curved membranes [113, 118]. The more effective binding to smaller vesicles was attributed to the more prevalent membrane packing defects present in those vesicles. This property correlates well with the ability of α -synuclein to bind the relatively small synaptic vesicles (~30 nm diameter).

In addition to the influence of lipid properties on α -synuclein membrane binding, some of the familial PD mutations also affect the membrane binding of α -synuclein [119–123]. It should be noted that all the familial mutants discovered so far lie between residues 30 and 53, which is right in the middle of the membrane binding region of α -synuclein and within the core region of α -synuclein fibrils. The A30P and A53E mutations seem to reduce the membrane interaction while E46K seems to enhance the membrane binding [119, 121, 123–125]. The A53T mutation, on the other hand, does not seem to influence membrane interaction in any significant way [119].

Aside from lipids, α -synuclein binds to fatty acids and is proposed to transport fatty acids inside cells [126]. α -Synuclein shares sequence homology with fatty acid binding proteins. The binding affinity, however, does not seem to be as high as reported for classical fatty acid binding proteins [127]. It was observed that α -synuclein accumulated on phospholipid monolayers surrounding triglyceride-rich lipid droplets in cells treated with fatty acids [65].

However, the disease mutants of PD were not as efficient in surrounding the lipid droplets and protecting triglyceride turnover [65]. It has been further demonstrated that α -synuclein forms soluble oligomers in cultured mesencephalic cells upon exposing those cells to polyunsaturated fatty acids (PUFAs) [128, 129]. Since the brains of patients with PD and dementia with Lewy bodies have increased amounts of soluble, lipid-associated α S oligomers, the binding of α -synuclein to PUFAs could be important for pathology [128, 129]. In fact, neuronal cells overexpressing α -synuclein, when exposed to physiological levels of PUFA, result in the formation of proteinaceous Lewy-like inclusions that are preceded by PUFA-induced soluble oligomers. [130]. Further, the N-terminal region of α -synuclein was found, in a study, to be essential for binding to PUFA and formation of oligomers [131]. In the same report, a C-terminal truncation resulted in the acceleration of PUFA-induced α -synuclein oligomerization [131]. Further investigation is required to probe any link between PUFA-induced soluble oligomers and α -synuclein fibrillization.

4.2 Structure of membrane-bound α -synuclein

α -Synuclein readily transforms into a predominantly α -helical conformation in the presence of phospholipid membranes [113, 115, 132–134]. The conversion into a helical form also occurs in the presence of other molecules such as fatty acids or SDS micelles [117, 135, 136]. Overall, the N-terminal ~100 amino acids attain a helical conformation while the remaining ~40 amino acids in the C-terminal region remain unordered [115, 133, 134, 136–138] (Figs. 2 and 4). However, different groups have reported structures that largely differ in whether α -synuclein forms a single extended-helix or a broken-helix around a central bend. The location of this bend varies from structure to structure but is typically around position 40. The solution NMR structure of α -synuclein bound to SDS micelle showed a bent-helical structure with two antiparallel helices between 3–37 and 45–92 [136] (Fig. 4B). EPR experiments also revealed the presence of a broken-helical structure on lysophospholipid and sodium lauroyl sarcosinate (SLAS) micelles [133, 135] (Fig. 4C). However, in comprehensive studies using continuous wave EPR and DEER based distance measurements, in combination with computational refinement, it was shown that α -synuclein forms an extended amphipathic helix on phospholipid vesicles [115, 132] (Fig. 4A). The formation of an extended-helix was further validated in another EPR study that probed the structures of α -synuclein on bicelles, vesicles and rod-like micelles [134]. The observation of a broken-helical structure on detergent micelles using solution NMR could be explained by geometrical consideration. A detergent micelle (~5 nm diameter) cannot accommodate the fully-extended helical α -synuclein (~14 nm long) on its surface, and thus, a kink is introduced in the middle to accommodate α -synuclein, resulting in a broken-helical form (Fig. 4).

Solid-state NMR has recently been used to study the structural transition from a predominantly α -helical, membrane-bound conformation to β -sheet fibrils [139]. The fibrils formed in the absence and presence of lipids displayed similar morphology. Further analyses, however, revealed major structural differences in their N-termini and a minor structural variation in the central NAC domain [139]. During the fibril formation in presence of lipids, four major states of α -synuclein were observed. The first state had an α -helical secondary structure that converts to a mostly β -sheet secondary structure with a strong

association with lipid acyl chains. This was followed by a third state with key chemical shift signatures that correlates with the mature fibrils and a fourth state with further changes in chemical shifts indicated quaternary assembly of protofilaments [139].

Another study focused on understanding the role of different regions of α -synuclein towards the membrane interaction by exploring the membrane bound structure using solid-state NMR [140]. It was found that α -synuclein can be divided into three distinct regions based on structural and dynamical properties [140]. First, an N-terminal helical segment that acts as a membrane anchor, followed by a central region that determines the affinity for membranes and acts as a sensor of the lipid properties, and finally, an unstructured C-terminal region [140]. The ability of α -synuclein to adopt different binding modes and the observation of either a broken or an extended-helix conformation is attributed to a small difference in free energy between the two conformations [115, 138, 141–143].

Considering the relatively small energy differences between various membrane or lipid-bound forms of α -synuclein, one might expect that the structural plasticity allows α -synuclein to take up different conformations on membranes that vary in shape and curvature, for example tubules and nanoparticles [115, 135, 144]. This is extremely important to keep in mind when carrying out structural studies, as α -synuclein can remodel membranes into nanoparticles and membrane tubes (micellar tubes and bilayer tubes) by induction of membrane curvature [117, 144–150]. Tubulation and formation of lipoprotein nanoparticles are a common property of synucleins and apolipoproteins [144, 145, 151]. The structures of the nanoparticle and tubule-bound forms of synuclein are distinct. While a broken-helical structure is present on nanoparticles, an extended-helical structure is formed on tubes [117, 144]. Similar data were obtained for α -synuclein's structure on tubes and nanoparticles formed in the presence of oleic acid [144]. Overall, the available structural data from the different membrane or detergent-bound forms of α -synuclein reveal the common theme that the conformation is strongly correlated with the size of the binding template. α -Synuclein adopts a broken-helical structure on surfaces that have smaller dimensions than the length of the extended α -synuclein helix (~14 nm) (Fig. 4). Such surfaces include detergent micelles (~5 nm diameter) or nanoparticles (~10 nm diameter). On the other hand, an extended-helix is strongly favored on surfaces that can accommodate the helix in its extended form, such as tubes or intact membranes or vesicles.

Although the complete significance of these studies to α -synuclein biology remains to be determined, there are several implications. First, some conformations of α -synuclein may represent functionally active structures. These structures could be formed in response to the changes in lipid/fatty acid metabolism. For example, considering that α -synuclein might play some role in lipid transport and metabolism *in vivo*, its apolipoprotein-like binding to lipoprotein nanoparticles in a broken-helical conformation could be of functional relevance. Mutations that affect the conformation of protein on these lipoprotein nanoparticles or the formation of these nanoparticles could have pathological implications. Other structures like that observed on cylindrical micelles (membrane tubes) may represent binding of α -synuclein to lipid assemblies, often referred to as the “hemifusion” or hemifission” states. These states are thought to form during membrane fusion and fission during processes such as endocytosis or exocytosis. Since α -synuclein is linked to synaptic vesicle recycling, the

α -synuclein conformation on cylindrical micelles might represent a functionally active form during synaptic vesicle recycling. Regarding pathological implications, any imbalance between the monomeric and multimeric forms could trigger non-physiological binding or protein aggregation. α -Synuclein in its helical conformation induces membrane remodeling, and excessive membrane curvature induction can lead to membrane disruption. Some lipids, particularly negatively charged lipids or lipids with different intrinsic curvature could shift the equilibrium towards more α -synuclein binding to the membrane surface, and thereby cause membrane disruption. Indeed, this has been observed in case of interaction with many cellular membranes [152–156].

4.3 Membrane interaction of IAPP

Several studies have indicated that membrane interaction of IAPP can have deleterious effects on membranes [37, 157–160]. IAPP is normally secreted but it has also been observed cytosolically, where it could promote toxicity [55, 161–163]. IAPP has been shown to be toxic to islet β -cells and insulinoma cell lines, regardless of whether it is exogenously applied or endogenously overexpressed. Moreover, there is evidence that exogenously applied IAPP can be taken up into the cell in a process that can be further modulated by cholesterol [164]. Once in the cell, IAPP can target and disrupt mitochondrial, lysosomal and other intracellular membranes, and cause toxicity [161, 165–171]. Interestingly, synthetic molecules impairing membrane binding of IAPP can protect cells against toxicity, further supporting the notion that membranes are a target of IAPP cytotoxicity [172].

4.4 Structure of membrane-bound IAPP

The potential role of IAPP membrane interactions in disease [37, 48, 58, 59, 158, 165, 173] has fostered interest in this process on a mechanistic and structural level. IAPP aggregation can be significantly enhanced by the presence of membranes. The first evidence for a membrane-bound intermediate that is generated prior to β -sheet fibril formation came from CD studies, which revealed transient formation of α -helical structure in the presence of anionic lipids [166]. The transient nature of this helical structure has complicated some structural studies, but it was ultimately possible to stabilize it long enough for analyses using site-directed spin labeling and EPR spectroscopy [174]. This study found that the IAPP forms a single α -helix encompassing residues 9–22, with flanking N- and C-terminal regions being largely disordered (Figs. 2 and 3A) see more detailed discussion below).

NMR studies have used membrane-mimetic detergent micelles to study IAPP structure [175, 176]. These studies focused on human IAPP in detergent micelles [175, 176] where it takes on a more extensive two-helical structure in the region between residues 5–28 [176] and residues 7–28 [175] (Fig. 3B). While the structural models of membrane-bound IAPP [174, 177] have substantial similarities, they nonetheless differ significantly from those obtained for micelles. This is especially the case with respect to the one helical structure on membranes versus the two-helical structure on detergents (Fig. 3A–B). This scenario is reminiscent of what was observed for α -synuclein (discussed above, Fig. 4) and illustrates that structures of proteins can be different on micelles and on membranes.

The structure of membrane-bound IAPP oligomers had long been inaccessible because of rapid aggregation in presence of membranes. A recent solution NMR study, however, managed to stabilize IAPP oligomers in the presence of lipid nanodiscs [177]. This structure shows three antiparallel β -strands formed by residues A8-L12, F15-H18, and I26-S29 connected via flexible loops in each monomeric unit [177]. This structure is remarkably different from the fibril structure underscoring the structural plasticity of this amyloidogenic peptide.

5. MEMBRANE-MEDIATED AGGREGATION

Lipids can modulate the aggregation behavior of α -synuclein and IAPP (Fig. 5). Both increases and decreases in the rate of aggregation have been reported and these effects are highly dependent on the lipid compositions and protein-to-lipid ratios.

5.1. Effect of lipids on α -synuclein aggregation

At physiological pH, α -synuclein aggregates in the test tube into fibrils that are morphologically indistinguishable from those found in Lewy bodies [73, 87]. However, the fibrillization reaction requires high protein concentrations and incubation times of several days [73, 74, 178]. The speculation that membranes might modulate aggregation of α -synuclein originated as lipids were found in the amyloid deposits in different human diseases [179, 180]. In Parkinson's disease, Lewy bodies (LB) contain aggregated α -synuclein along with other protein components and a significant proportion of lipids that are thought to be derived from degraded membrane organelles [181, 182].

Membranes influence the aggregation of α -synuclein in two different ways: one, by affecting the rate of aggregation, whether it is inhibition or acceleration, and two, by becoming incorporated into lipid-protein co-aggregates/fibrils. The co-aggregation occurs by the 'pulling-out' of the lipids from the membrane during the aggregation of α -synuclein on the membrane surface. A recent *in vitro* study found that this is particularly prevalent in case of membranes containing negatively charged lipids [183]. A close association between lipids and α -synuclein was observed at high protein/lipid ratios while at low ratios, lipid vesicles adsorbed along the fibrils. The process of co-aggregation generated lipid-protein assemblies that had distinct structure, dynamics and morphology as compared to structures formed by either lipid or protein alone [183].

Regarding the effect of membranes on the rate of α -synuclein aggregation, there was some discussion initially whether membranes enhanced or inhibited α -synuclein aggregation [184–186]. It now appears that there is a strong dependence on the exact conditions and results differ due to the use of different lipids and different protein/lipid ratios in the respective studies. Inhibition of fibrillization has been observed under conditions favoring stabilization of the helical form of α -synuclein. These conditions typically require a low protein/lipid ratio and membranes to which α -synuclein has a high affinity. Such membranes contain a significant fraction of negatively charged lipids, like PS, PG and GM1 [184–187]. In contrast, the use of net neutral lipids, to which synuclein only has a marginal affinity, does not affect aggregation kinetics [188].

At high protein/lipid ratios, an acceleration in the rate of fibrillization was observed in the presence of negatively charged phospholipids [184, 187], including physiological membranes isolated from the brain, synaptosomal membranes and synaptic vesicles mimicking membranes [188–190]. Apart from the acceleration of aggregation of α -synuclein in the intracellular space, a recent work demonstrated acceleration of α -synuclein aggregation by exosomes, and particularly by ganglioside lipids GM1 or GM3 present in exosomes [191]. Galvagnion et al. recently found that high protein/lipid ratios result in a primary nucleation rate that is at least three orders of magnitude greater than the nucleation in bulk solution [187]. The enhanced nucleation rate on the membrane surface was attributed to the higher local concentration of protein molecules due to the reduced dimensionality [187] (Fig. 5). This hypothesis is supported by aggregation of α -synuclein at a nanomolar or even lower bulk concentration in the presence of surfaces like hydrophilic glass and cell membrane-mimicking supported lipid bilayers [192, 193]. Another aspect to favor nucleation on the membrane surface is the possibility of membranes directing α -synuclein into conformations that may favor primary nucleation. The conformations that favor nucleation would also be present in solution albeit rarer than at the membrane surface. Many groups have suggested that such nucleation-promoting conformations could be further favored by a reduction in the membrane interaction of the region around the hydrophobic core (~60–90 position) of α -synuclein, for example, by familial mutations like A30P and G51D [143, 194–196]. In these mutations, the membrane interaction through their N-terminal region remains unaffected as that region still works as a membrane anchor. On the other hand, the membrane interaction of the central hydrophobic region is affected, and this region then becomes available for lateral protein-protein interaction on the membrane surface. Such interactions in turn would augment membrane-mediated aggregation of α -synuclein.

Apart from point mutations, truncation of the C-terminal region encompassing several negative charges enhances the aggregation propensity of α -synuclein [90, 188, 197]. Moreover, C-terminal truncation mutants have been observed in Lewy bodies, thereby signifying the biological relevance of this α -synuclein fragment [90, 197–201].

Another membrane factor that can influence α -synuclein aggregation is membrane curvature. Smaller vesicles were more efficient in increasing the rate of fibrillization, presumably due to the aforementioned enhanced binding [184]. This could indicate that highly curved membranes or membrane regions could be ‘hot spots’ for aggregation. As described previously, α -synuclein can bend membranes and form lipoprotein nanoparticles and membrane tubes. The lipoprotein particles have multiple α -synuclein molecules coming together in close proximity [144]. Similarly, the membrane tubes are formed at high protein/lipid ratios and α -synuclein molecules are likely to be locally concentrated on tubes. These close contacts on lipoprotein nanoparticles or membrane tubes could aid nucleation of α -synuclein molecules and progress them towards the aggregation pathway. Concentration of α -synuclein on highly curved membrane regions within the cells could, thus, be a mechanism of aggregation. In this case, membrane remodeling would precede protein aggregation. Lipids or fatty acids with intrinsic curvatures that affect the highly curved regions in the cellular membranes could assist α -synuclein molecules to concentrate in the highly curved regions. Whether an alteration in the manner these proteins come together on

the lipoprotein nanoparticles or on highly curved regions on cellular membranes leads to protein aggregation remains to be tested.

5.2 Effect of lipids on IAPP aggregation

A number of studies have shown that membranes can significantly promote the aggregation of IAPP into β -sheet rich species [94, 166, 202, 203]. This aggregation is most pronounced in the presence of negatively charged membranes [166, 204–207], for which the positively charged IAPP has a high affinity. Interestingly, cholesterol and gangliosides, which are often found enriched in raft-like membranes, significantly modulate IAPP aggregation [208–212]. Presence of metal ions such as Ca^{2+} may further modulate interaction of IAPP with cholesterol containing vesicles by interfering with the pre-fibrillar phase [211]. The helical structure of membrane-bound IAPP provided some first insight into potential aggregation mechanisms [174]. This helical structure anchors the peptide to the membrane while leaving the most amyloidogenic region (~20–29) exposed for aggregation (Fig. 3A). Once on the membrane, IAPP molecules are highly concentrated and can collide with each other in a two-dimensional manner (Fig. 5). Thus, the highly amyloidogenic regions of IAPP are likely to come into frequent contact, allowing them to take up β -sheet containing oligomeric form that ultimately proceeds toward fibril formation. Does such transition happen in cellular environment too? This is difficult to answer since measuring secondary structure of a peptide in cellular environment, within cellular time-frame, without disrupting normal physiology, has been beyond the reach of current tools and techniques.

Of critical importance for the enhancement of aggregation are negatively charged lipids [166]. Thus, in principle, exposure to such lipids in a cellular environment could be highly toxic. Under normal circumstances, negatively charged lipids are mainly located on the cytosolic leaflets of membranes. IAPP has been found in the cytosol, and might, therefore, have access to such lipids. The vast majority of IAPP, however, is secreted to the extracellular surface, where it would only encounter the external leaflet of the plasma membrane, which may not promote aggregation as potently. This scenario could be altered by plasticizers and fatty acids, two risk factors implicated in T2DM [203]. Both of these molecules are amphiphilic, containing an extensive hydrophobic region as well as a negatively charged group. A recent study found that both molecules strongly partition into uncharged membranes where they can enhance the charge density. Intriguingly, the enhanced charge density also strongly promotes IAPP aggregation just as negatively charged lipids do. The implications of this study are that these risk factors may facilitate IAPP aggregation even on cellular membranes that would otherwise be uncharged [203].

6. Membrane disruption by α -synuclein and IAPP

Many amyloidogenic proteins, including α -synuclein and IAPP, can disrupt the integrity of cellular membranes [153–156, 213, 214]. Several mechanisms of membrane disruption have been proposed and it is important to keep in mind that they are not mutually exclusive. This is perhaps best illustrated by the different temporal phases described for IAPP membrane disruption [173, 215–217]. The early phase of membrane disruption (leakage) precedes the formation of β -sheet structure, while later stages occur around the time of aggregation. Thus,

different structures and properties of IAPP appear to be responsible for compromising membrane integrity.

Some commonly suggested mechanisms for membrane damage include pore (or channel) formation, detergent-like membrane dissolution, lipid extraction during co-aggregation, and membrane remodeling. Aside from the more recently discovered membrane remodeling mechanism, many of the above mentioned mechanisms have been described in several reviews before, and will, therefore, be only briefly mentioned here (for review see [218]). Pores (or channels) have been reported to be formed by many amyloidogenic proteins, including α -synuclein and IAPP [157, 158, 219, 220]. Typically, they involve oligomeric structures that span the bilayer and allow passage of certain solutes through the membrane. It is easy to see how such a disruption of bilayer integrity could adversely affect local concentrations of ions and other molecules and, ultimately, lead to toxicity. Although the outcome of disruption of membrane integrity is similar, a detergent-like membrane dissolution and incorporation into growing fibrils present an entirely different mechanism as they directly attack the bilayer integrity rather than by providing a proteinaceous pore in an otherwise unperturbed membrane.

Membrane disruption is not unique to the misfolded β -sheet forms, but it is also a property of the α -helical forms of α -synuclein and IAPP. Peptides/proteins that do not typically aggregate like rIAPP [202], truncated IAPP [221], and β -synuclein [145], as well as IAPP and α -synuclein in helical form (prior to the formation of β -sheet) are capable of causing membrane leakage. Recent studies have indicated that the membrane damage from the α -helical forms of these proteins can arise from membrane remodeling [145, 222]. In this mechanism, the helical form acts as a 'wedge' on the membrane surface inducing curvature effects and causing tubulation, vesiculation and formation of lipoprotein particles/lipid-protein complexes. Like α -synuclein, IAPP can also remodel vesicles into tubes, smaller vesicles and non-vesicular protein-lipid complexes [222]. The membrane remodeling effect by both α -synuclein and IAPP was observed only above a certain protein/lipid ratio implying the requirement of a threshold local protein concentration on the membrane surface for curvature induction. Thus, the concerted action of multiple wedges is required to provide enough driving force to trigger membrane remodeling. This also means that membrane remodeling should be more pronounced in cases where the local protein concentration is very high. In this context, one might speculate that oligomers could act like 'delivery vehicles' to furnish a high local protein concentration on the membrane surface for curvature induction. An overexpression of α -synuclein or IAPP could also provide high protein concentration for membrane remodeling effects. The pathological implication of this mechanism was shown by Boassa et. al. in an *in vivo* study using transgenic mice, where overexpressed α -synuclein remodeled membranous organelle system into highly curved structures at presynaptic termini [223]. The membrane remodeling by both α -synuclein and IAPP was observed in the presence of negatively charged lipids. Any change in the cellular membrane composition that increases negative charge density due to either altered lipid or fatty acid metabolism or due to plasticizers could be a risk factor for membrane disruption. In summary, the presence of different structural forms (monomers, oligomers, fibrils) that can interact with cellular membranes differing widely in their lipid compositions suggest concerted effect of multiple mechanisms for membrane disruption in disease pathology.

7. Strategies for inhibition of membrane-mediated aggregation

Membrane-mediated aggregation can be inhibited by preventing membrane interaction or by trapping the helical membrane-bound form. Inhibition of membrane interaction was used in the case of squalamine, a natural compound with a net positive charge and a high affinity for anionic phospholipids [224]. This compound can easily transport into eukaryotic cells and displace proteins bound to the cytoplasmic face of plasma membranes [224]. Squalamine was found to displace α -synuclein from lipid membranes, thereby inhibiting α -synuclein aggregation by competing for binding sites on vesicles composed of negatively charged DMPS. Further, the reduction in aggregation of α -synuclein was observed in the PD model of *C. elegans* with mitigation of PD-associated paralysis [224]. Endosulfine- α (ENSA), a protein that is down-regulated in the brains of synucleinopathy patients acts via the trapping mechanism [225]. It possesses the ability to specifically interact with the membrane-bound form of α -synuclein. ENSA was found to inhibit membrane-mediated α -synuclein aggregation, prevent α -synuclein-induced vesicle disruption and neurotoxicity [225]. A similar mechanism was used for IAPP by Miranker and colleagues, who designed and synthesized an oligopyridylamide IS5 which binds membrane-bound α -helical intermediate of IAPP and inhibits membrane-mediated aggregation and IAPP cytotoxicity in INS-1 cells [172]. In another approach, screening IAPP-derived peptides for their inhibition efficacy in the presence of anionic lipids showed that these peptides can form hetero-complexes with IAPP and inhibit IAPP fibrillation [226]. Using yet another strategy, a water soluble derivative of curcumin, CurDAc, was used to inhibit IAPP aggregation in presence of membranes at stoichiometric concentration [227].

In summary, the multitude of studies described in this review on α -synuclein and IAPP have revealed important lipid and protein determinants that influence toxic protein aggregation. It appears that membrane-mediated aggregation is a common feature of all amyloids. In a recent study, for example, using huntingtin exon-1 (Httex1), which has a central role in Huntington's disease, it was shown that membranes can potentially accelerate aggregation that finally results in significant membrane damage [228]. The membrane-mediated aggregation of Httex1 was driven by the N17 domain (N terminus containing 17 amino acids) that acts as a helical membrane anchor. Like, α -synuclein and IAPP, membrane binding leads to high local protein concentration, thereby promoting intermolecular collision via two-dimensional diffusion, which results in formation of aggregates. Membrane damage is a common theme of all amyloid diseases, and therefore, strategies focused on preventing disruption of cellular membranes could be an effective therapeutic approach in amyloidogenic diseases.

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HIGHLIGHTS

- α -Synuclein aggregates in Parkinson's disease and IAPP in type 2 diabetes mellitus
- α -Synuclein and IAPP interact with cellular membranes in health and disease
- Lipid membranes and lipid-like molecules catalyze protein aggregation
- Some risk factor molecules in diabetes can promote membrane-mediated aggregation
- Membranes are targets of toxicity
- Membrane integrity is disrupted by aggregates as well as membrane remodeling

Figure 1.

Aggregation pathway depicting conversion of native protein into oligomers and fibrils. The native protein is typically monomeric. Under the influence of various intrinsic and extrinsic factors the native protein is converted to 'on-pathway' oligomers that eventually form fibrils or 'off-pathway' oligomers.

Figure 2.

Membrane binding and main amyloidogenic regions of α -synuclein and IAPP. (A) The N-terminal ~100 amino acids of α -synuclein are involved in membrane binding while 61–95 (NAC region) is the most hydrophobic region. (B) The α -helical region of IAPP when bound to membranes is between 9–22 and the amyloidogenic region is between 20–29.

Figure 3.

Structural models of human IAPP (IAPP) monomers and fibrils. (A) EPR based model of monomeric IAPP bound to phospholipid membrane. The red-colored thicker ribbon shows the central helical region (residues 9–20) while the red-colored thinner ribbon (residues 21 and 22) indicates the C-terminal end of the helix. The N- and C-termini flanking the central helical region are unstructured and depicted with dashed lines. Gold-colored spheres indicate phosphates (Apostolidou et al, 2008) (B) NMR structure of monomeric IAPP bound to SDS micelles showing α -helical conformation of the peptide (PDB 2L86, Nanga et al, 2011). (C) The NMR structure of monomeric IAPP in acidic solution (pH 5.3) shows α -helical conformation near the N-terminus (residues C7-F15) while the C-terminal region remains unstructured (PDB 5MGQ, Rodriguez-Camargo et al, 2017). (D) Solid-state NMR based model of IAPP monomers in fibrils with striated ribbon morphology. The monomeric subunits have β -hairpin conformations and interact with each other through their C-terminal

β -stands (Luca et al, 2007). (E, F, and G) EPR based structural model of IAPP fibrils. The N- and C-terminal β -strands are separated by a long loop region (Bedrood et al, 2011). (E) Individual monomers (shown as blue, green, and orange ribbons) in fibrils have considerable stagger. The i to $i+3$ contacts between monomers are indicated for the blue monomers. (F) Rotated view of fibril in (E). (G) The structural model of fibril consisting of 101 monomers shown to emphasize a left-handed helical turn of $\sim 90^\circ$ between the center of the red boxes.

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Figure 4.

Structure of membrane-bound α -synuclein. (A) The membrane-bound structure of α -synuclein was determined using electron paramagnetic resonance. α -Synuclein mainly adopts an extended-helix on small unilamellar vesicles. (B) NMR structure of SDS micelle-bound α -synuclein (PDB ID: 1XQ8). (C) Structure of SLAS micelle-bound α -synuclein (PDB ID: 2KKW) obtained using NMR and pulsed EPR measurements. α -Synuclein adopts a broken-helical conformation on highly curved SDS and SLAS micelles.

Figure 5.

Schematic of membrane-mediated aggregation. Native IAPP or α -synuclein molecules bind to the membrane and adopt helical conformation. The reduced dimensionality on the membrane surface increases local concentration of IAPP or α -synuclein, which facilitates intermolecular interaction through their respective amyloidogenic region. This interaction results in the formation of oligomers, and finally, fibrils. The red cylinder represents helical region and the double-headed arrow shows intermolecular interaction in the amyloidogenic region of IAPP and α -synuclein. The C-terminal of α -synuclein is not depicted and the peptide/protein length is not to scale.