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Membrane interactions and fibrillization of α-synuclein play an essential role in membrane disruption

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ABSTRACT

We studied α-synuclein (αS) aggregation in giant vesicles, and observed dramatic membrane disintegration, as well as lipid incorporation into micrometer-sized suprafibrillar aggregates. In the presence of dye-filled vesicles, dye leakage and fibrillization happen concurrently. However, growing fibrils do not impair the integrity of phospholipid vesicles that have a low affinity for αS. Seeding αS aggregation accelerates dye leakage, indicating that oligomeric species are not required to explain the observed effect. The evolving picture suggests that fibrils that appear in solution bind membranes and recruit membrane-bound monomers, resulting in lipid extraction, membrane destabilization and the formation of lipid-containing suprafibrillar aggregates.

1. Introduction

Misfolding and aggregation of proteins is associated with several diseases, including Alzheimer's, Parkinson's disease and type II diabetes mellitus [1]. A characteristic feature of these diseases is the aggregation of proteins into cross β-sheet rich structures [2]. Interaction between aggregated proteins and membranes appears to play a significant role in the pathogenesis of these diseases [3]. The prevailing hypothesis regarding toxicity in amyloid diseases invokes oligomeric protein species as the toxic agent to cells [4–6]. Oligomers that appear in solution or grow on the membrane are suspected to disrupt membranes [7–10]. In Alzheimer's disease, oligomeric protein species have been accepted as the cause of cell death [11,12]. However, it was recently shown that membrane disruption by Aβ is a two-step process, which not only includes pore formation by oligomers, but also membrane fragmentation by fibril elongation [13]. Moreover, when β2-microglobulin amyloid fibrils were incubated with large unilamellar vesicles (LUVs), membrane binding of the fibrils resulted in large membrane deformations [14]. Besides causing mechanical damage to membranes, fibrils were hypothesized to extract lipids from the outer membrane at the points of distortion [14]. In type II diabetes mellitus aggregation or fibrillization of the human islet amyloid polypeptide (IAPP) on the membrane has been observed to damage membranes [15–17]. Although different transient or stable oligomeric species that appear during aggregation are possibly toxic in many protein aggregation diseases, damage induced by fibrils or fibril growth cannot be ruled out.

In Parkinson's disease, aggregation of α-synuclein (αS) leads to the death of dopaminergic neurons. Oligomeric αS species are hypothesized to play an important role in membrane damage [18] and cell death [7]. Cell membranes consist of bilayers containing a mixture of anionic and zwitterionic lipids. The affinity of αS for lipid bilayers increases with the density of negatively charged lipids. Although different in vitro produced αS oligomers can permeabilize highly negatively charged model membranes [19–21], the effect of protein aggregation and aggregate species on cell membranes and model membranes with more physiologically relevant compositions and charge densities is still debated [20,22,23]. Here we have studied the aggregation of αS inside cell-sized giant unilamellar vesicles (GUVs). This model system provides a cell-sized environment with physiological salt

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concentration and pH. Confocal microscopy allowed us to validate the encapsulation of αS, and the disintegration of vesicles upon αS aggregation, leading to the appearance of mixed μm-sized amyloid-lipid aggregates, reminiscent of Lewy bodies that are the pathological hallmark of Parkinson's disease. In experiments with calcein filled LUVs we show that at membrane charge densities where the isolated, in vitro produced, oligomers that we have extensively studied [20,24,25] do not induce damage, aggregation of membrane-bound protein disrupts the vesicles, possibly by extraction of lipids from the bilayer. Colocalization of fluorescently labeled lipids with preformed αS amyloid fibrils supports this idea.

2. Materials and methods

2.1. Expression and purification of αS

The expression and purification of human wild type alpha synuclein (αS) and 140 cysteine mutant alpha synuclein (140C αS) was performed as previously described [26]. The protein concentration was estimated by measuring absorbance at 276 nm, using molar extinction coefficients of 5600 M^{-1} cm^{-1} for αS and 5745 M^{-1} cm^{-1} for 140C αS respectively, on a Shimadzu spectrophotometer [25,27].

For more details refer to Supplementary Information (SI).

3. Results

To investigate the effect of αS aggregation on membrane integrity in a simple model system we chose a mixture of zwitterionic and anionic phospholipids that is known to bind αS well, and prepared GUVs from POPC and POPG in a 1:1 ratio. Incorporation of fluorescently labeled Liss Rhod-PE in the membrane and encapsulation of fluorescently-labeled monomeric αS allowed us to concurrently monitor the fate of both proteins and lipids as a function of time. In the absence of protein, POPC/POPG GUVs remained stable for at least 95 h (Fig. 1A and B). During this time the vesicles did not change morphology. When 100 μM αS was encapsulated in the GUVs upon vesicle formation, the protein was initially homogenously distributed over the vesicle volume (Fig. 1C). We did not observe accumulation of protein at the bilayer surface. In time, GUV-encapsulated αS aggregated and the vesicles disappeared from solution. Instead of GUVs, micrometer-sized structures that contained both proteins and lipids were observed 95 h after vesicle formation (Fig. 1D). To verify that these structures consisted of β sheet-rich amyloid fibrils, the amyloid-specific dye ThT was added to the solution. Confocal microscopy images show that the protein–lipid aggregates are ThT positive and we therefore conclude that they consist of characteristic amyloid structures, most likely of fibrillar form. The colocalization of amyloid and lipid fluorescence suggests that lipids are associated with the amyloid fibrils. At t = 160 h some of the micrometer sized amyloid aggregates observed in solution have a distinct suprafibrillar morphology and are decorated with lipid structures (Fig. 1E and F). Similar suprafibrillar aggregates were observed to form in the absence of phospholipid membranes [28]. The size and heterogeneous distribution of lipids in these aggregates suggests that protein aggregation additionally caused bilayer remodeling resulting in small vesicle-like structures. Monomeric αS adopts α-helical structures on negatively charged membranes [29,30]. The conformational transition into β-sheet fibrils may take place at the membrane surface. This aggregation at the membrane may result in sequestering of lipids and cause the lipids to deposit in amyloid-rich aggregates.

To determine if the aggregation process or specific aggregate species are involved in membrane disintegration we simultaneously followed the kinetics of αS aggregation and the leakage of dye from LUVs. When αS was incubated with calcein-filled

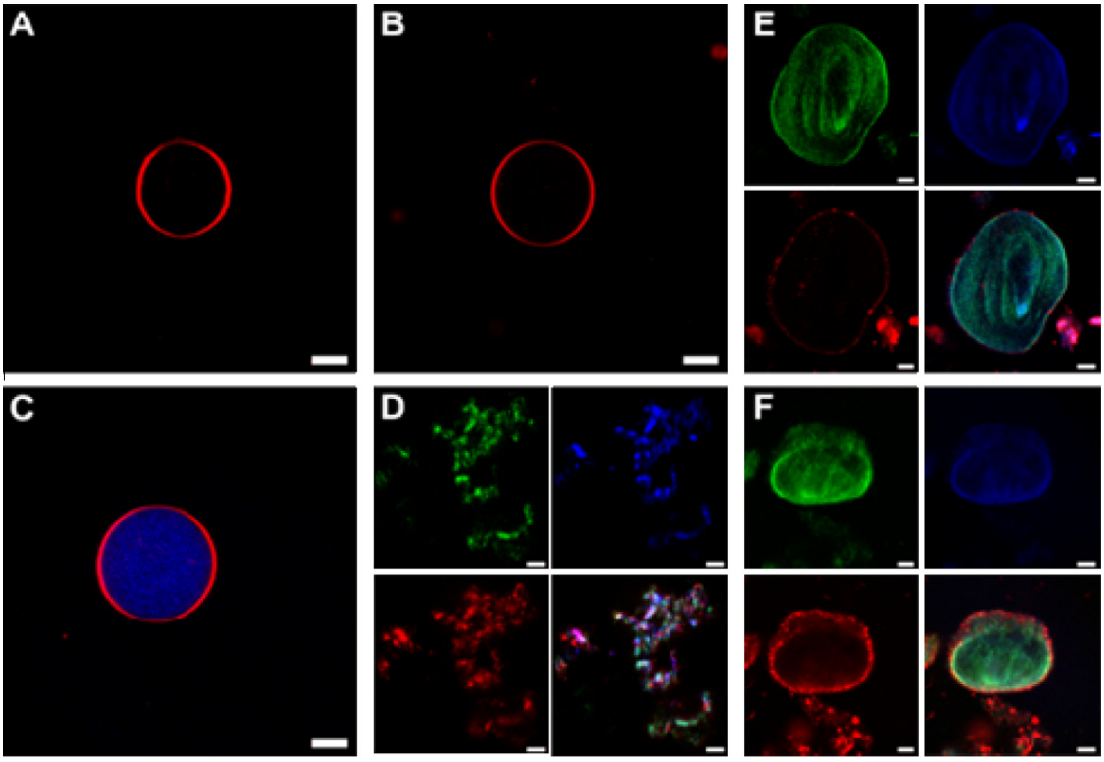

Fig. 1. αS encapsulation and aggregation at physiological salt conditions. In the absence of protein, POPC/POPG (1:1) Liss Rhod PE labeled GUVs (t = 0 h, A) remained intact for at least 95 h (t = 95 h, B). Vesicles encapsulating αS 140C-Alexa 647 (C) disintegrate into amyloid rich lipid–protein aggregates. After 95 h these amyloid aggregates were visualized using ThT fluorescence (D). After 160 h the solution additionally contains suprafibrillar aggregates coated with lipids solution (E and F). In panels D, E and F lipids are represented in red, αS 140C-Alexa 647 in blue and amyloid (ThT-stained) in green; in the bottom right images of panels E and F, all three channels are merged. Scale bars 10 μm.