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α-Synuclein interactions with phospholipid model membranes: Key roles for electrostatic interactions and lipid-bilayer structure

Katja Pirc, Nataša Poklar Ulrih *

Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, 1000 Ljubljana, Slovenia

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ABSTRACT

α-Synuclein is a small presynaptic protein that is critically implicated in the onset of Parkinson's disease and other neurodegenerative disorders. It has been assumed that the pathogenesis of α-synuclein is associated with its aggregation, while for its physiological function, binding of α-synuclein to the synaptic vesicle membrane appears to be most important. The present study investigated the mechanism of α-synuclein binding to the lipid membrane. Upon binding to negatively charged small unilamellar vesicles consisting of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol in the liquid-crystalline state, α-synuclein undergoes conformational transition from its native unfolded form to an α-helical structure. The positively charged N-terminal part of α-synuclein is likely to be involved in interactions with the negatively charged lipid surface. α-Synuclein did not associate with vesicles consisting of the zwitterionic (neutral) lipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine or 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine. The data obtained by circular dichroism spectroscopy, fluorescence anisotropy measurements, differential scanning calorimetry, and calcein efflux assays indicate that in addition to electrostatic interactions, hydrophobic interactions are important in the association of α-synuclein with membranes. The mechanism of α-synuclein binding to lipid membranes is primarily dependent on the surface charge density of the lipid bilayer and the phase state of the lipids. We propose that α-synuclein has a lipid ordering effect and thermally stabilises vesicles.

1. Introduction

α-Synuclein is a small abundant presynaptic protein of 140 amino-acids (14.5 kDa). In its β-sheet-rich fibrillar aggregated form, α-synuclein is present in the intracellular neuronal inclusions of brains affected by Parkinson's disease and other neurodegenerative conditions. Most cases of Parkinson's disease are ageing-dependent and sporadic; however, certain mutations and multiplications of the α-synuclein gene locus are associated with early-onset Parkinson's disease [1–3].

The cause of the α-synuclein-induced Parkinson's disease pathogenesis remains unknown, although it has been hypothesised that cytotoxicity arises from protein aggregation and/or fibrillation processes [4,5]. Specifically, low-molecular-weight oligomeric α-synuclein species have been proposed to show toxicity through disruption of lipid membranes [6–8]. Loss of the native function of α-synuclein might also harm neurons [4].

Although the exact physiological function(s) of α-synuclein remains unclear, strong evidence has indicated roles in synaptic transmission and dopaminergic neuron homeostasis [9]. Indeed, knock-out or over-expression of α-synuclein in animal Parkinson's disease models and neuronal cultures have resulted in impaired synaptic transmission, such as modifications to dopamine release and uptake, and changes in the presynaptic vesicle pool size and vesicle recycling [10–13]. α-Synuclein has been shown to associate with synaptic vesicles [14], and some studies have suggested that membrane binding of α-synuclein might prevent too early vesicle fusion with presynaptic membranes [15,16].

α-Synuclein is intrinsically disordered under physiological conditions *in vitro*, as it exists as a mixture of rapidly equilibrating extended conformers [17,18]. Recently, several studies have reported on a plausible native tetrameric state of α-synuclein [19,20], but this finding is still hotly debated [21]. The primary structure of α-synuclein can be divided into three distinct regions: a positively charged N-terminus (amino-acids 1–95), which includes an amyloidogenic hydrophobic NAC region (amino-acids 60–95), and a highly negatively charged C-terminus (amino-acids 96–140). The N-terminus contains seven highly conserved Lys-rich imperfect repeats with the consensus sequence of KTKEGV. These motifs have been proposed to enable membrane-induced amphipathic α-helix formation, which is a classical membrane-binding motif [22,23].

As both physiological and pathological roles of α-synuclein appear to involve binding to lipid membranes [6,24–26], understanding the molecular basis of these α-synuclein–membrane interactions is of

* Corresponding author.
E-mail addresses: katja.pirc@bf.uni-lj.si (K. Pirc), natasa.poklar@bf.uni-lj.si (N.P. Ulrih).

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great importance. However, despite intensive investigations, there remains controversy regarding the preferred membrane physico-chemical properties for α-synuclein binding, and the membrane-associated α-synuclein conformation also remains unclear [25,26].

The α-synuclein–membrane interactions affect the properties of both the α-synuclein and the membranes [27]. The driving forces for their association might involve electrostatic and/or hydrophobic interactions [24,26]. Key roles in the modulation of α-synuclein membrane binding have been assigned to several factors, which include membrane curvature, charge and phase state, solution conditions (e.g., salt concentrations, pH), and lipid–protein stoichiometry [26]. It is generally accepted that α-synuclein preferentially interacts with small unilamellar vesicles (SUVs) that contain negatively charged polar headgroups [28] or structural imperfections [15,29–31]. This protein–lipid association is accompanied by a structural transition of α-synuclein from unfolded into a partial α-helical structure [15,28]. Upon interaction with anionic lipids, the N-terminus of α-synuclein folds into an amphipathic helix, while its C-terminus remains unstructured [32,33]. α-Synuclein was suggested to reside on the membrane surface, with some regions penetrating into the hydrophobic core of the membrane [33–37]. The membrane topology was shown to dictate the α-synuclein conformation, as it folds into two antiparallel helices when bound to micelles [32], while with larger, and physiologically more relevant, SUVs, the α-synuclein structure has been proposed to include two separate helices or an extended single helix [33,38]. It is also possible that α-synuclein switches between two such conformations [39].

Based on the current literature, it is not clear whether α-synuclein interacts with neutral membranes, with such binding only described in some studies [15,27,29–31,40–42]. However, the association of α-synuclein with anionic lipids is much stronger [15,29,31,41,42], while the binding of α-synuclein to neutral and anionic membranes also appears to occur by different mechanisms [31,42].

An additional unresolved factor in the modulation of α-synuclein–membrane interactions is the phase state of the lipids. There have been reports on the formation of an α-helical structure by α-synuclein in the presence of SUVs composed of the neutral lipid 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) in the gel phase, but not in the liquid-crystalline state [15,29,30]. Also, binding of α-synuclein to vesicles of the zwitterionic lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) has been suggested [40]. There are also divided reports on whether α-synuclein binds better to anionic lipids in the gel or liquid-crystalline phase [27,28,41,43–45], while it has also been suggested that α-synuclein binding requires membrane with two distinct phases [46].

In the present study, we aimed to determine how the different physico-chemical parameters of membrane composition and lipid phase state affect interactions between monomeric α-synuclein and membranes, and how α-synuclein binding, in turn, affects the properties of the lipid bilayer. The interactions of α-synuclein with vesicles composed of phospholipids with neutral headgroups (DPPC, POPC) and with anionic polar headgroups (1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol [DPPG], 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol [POPG]) were examined, along with the DPPC:DPPG lipid mixture. These investigations included analyses by circular dichroism (CD) spectroscopy, fluorescence anisotropy of two membrane-bound probes, differential scanning calorimetry (DSC), and calcein release assays.

2. Materials and methods

2.1. Materials

The phospholipids DPPC, DPPG, POPC and POPG were from Avanti Polar Lipids (Alabaster, AL, USA) and were used without further purification. 4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 1,6-diphenyl-1,3,5-hexatriene (DPH), N,N,N-trimethyl-4-(6-phenyl-1,3,5-hexatrien-1-yl)phenylammonium p-toluenesulfonate (TMA-DPH), and calcein (fluorescein-methylene-iminodiacetic acid) were from Sigma-Aldrich Co. (St. Louis, MO, USA). Organic solvents were from Merck KGaA (Darmstadt, Germany) and were of the purest grades available. All of the other chemicals and reagents were from Sigma-Aldrich Co. (St. Louis, MO, USA) and Merck KGaA (Darmstadt, Germany). Aqueous solutions were prepared in bidistilled water (Millipore, Billerica, MA, USA) and filtered through 0.22-μm filters (Sartorius AG, Goettingen, Germany). All of the experiments were performed in 150 mM NaCl, 20 mM HEPES, pH 7.0 (HEPES buffer, pH 7.0).

2.2. α-Synuclein expression and purification

Expression and purification of recombinant human wild-type α-synuclein was performed as described previously [47], with some modifications [48]. Final confirmation of the molecular mass and homogeneity of the α-synuclein was achieved with matrix-assisted laser-desorption/ionisation-mass spectrometry and sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

2.3. α-Synuclein preparation

The α-synuclein stock solutions were prepared as described previously [48]. The α-synuclein concentrations were determined spectrophotometrically using a Cary Eclipse spectrophotometer (Varian, Mulgrave, Australia), as described previously [47]. Crosslinking with 2.5% glutaraldehyde confirmed the predominant monomeric conformation of the α-synuclein (Supplementary Data, Fig. S1).

2.4. Vesicle preparation

Lipid vesicles were prepared fresh for each set of experiments. Appropriate amounts of the lipids were dissolved in chloroform or chloroform:methanol (7:3, v/v), with mixing at the appropriate ratios for DPPC:DPPG. Following their transfer into round-bottomed flasks, the solvent was removed under reduced pressure (17 mbar) overnight. The thin films obtained were hydrated in warm HEPES buffer, pH 7.0. Then multilamellar vesicles (MLVs) with lipid concentrations of 2 mg/mL to 4 mg/mL were prepared by 10 min vortexing of the lipid suspensions in the presence of glass beads. The MLVs were further transformed to SUVs by sonication on ice for a total of 30 min, with 10 s on–off cycles at 40% amplitude using a Vibracell Ultrasonic Disintegrator VCX 750 (Sonics and Materials, Newtown, USA). To separate the debris from the SUVs after sonication, the samples were centrifuged at 6000 $\times g$ for 5 min. The SUVs were used for CD and fluorescence spectroscopy measurements, while the MLVs were used for the DSC measurements.

SUVs filled with calcein were prepared by hydrating the dried lipids with 80 mM calcein in HEPES buffer, pH 7.0, from which SUVs were prepared as described above. Unencapsulated dye was separated from the vesicles by gel filtration through a column packed with Sephadex G-50 (Pharmacia Fine Chemicals AB, Uppsala, Sweden).

2.5. Circular dichroism spectroscopy

Circular dichroism spectroscopy was performed using an AVIV model 62A DS spectropolarimeter (Aviv Associates, Lakewood, NJ, USA) equipped with a thermoelectrically controlled cell holder. Spectra of 35 μM α-synuclein in the absence and presence of SUVs (R [lipid/α-synuclein molar ratio] = 10) were recorded in the temperature range from 25 °C to 70 °C. As the pure lipids do not have a CD signal in the far-UV, the CD spectra reflect the structural/conformational characteristics of the α-synuclein [49]. Spectra were recorded using a 1-mm path length quartz cuvette, with a scan range from 200 nm to 260 nm at 0.5 nm intervals, a bandwidth of 1 nm, and an averaging time of 4 s.

The scans were buffer subtracted, 'baseline-corrected', smoothed using a polynomial fitting function (Aviv Associates, Inc.), and converted to mean residue ellipticity, $[\theta]_{(\lambda)}$, according to Eq. (1):

$$[\theta]_{(\lambda)} = (\theta_{(\lambda)} M_0)/(100cl) \quad (1)$$

where M_0 is the mean residue molar mass (103.3 g mol^{-1} for α-synuclein), $\theta_{(\lambda)}$ is the measured ellipticity in degrees, c is the protein concentration in g/mL, and l is the path length in decimetres. The secondary structure content was calculated from the far-UV CD spectra using the Contin software package [50].

The temperature in the cuvette of the CD spectropolarimeter and fluorescence spectrophotometer (for fluorescence anisotropy and calcein release measurements, see below) was additionally monitored using a thermistor (YSI 44004 Thermistor Precision, YSI Inc., USA).

2.6. Fluorescence anisotropy measurements

To determine the impact of α-synuclein binding on the lipid ordering, fluorescence anisotropy measurements of two fluorophores were performed: DPH and TMA-DPH. A 10-mm path length quartz cuvette was used with a Cary Eclipse fluorescence spectrophotometer (Varian, Mulgrave, Australia). Varian Auto Polarisers were used, with slit widths with a nominal band-pass of 5 nm for both excitation and emission. The SUVs were labelled with DPH or TMA-DPH (1 mM or 2 mM stock solutions, respectively, in dimethyl sulfoxide). The final lipid concentration in the cuvette was 0.1 mg/mL (~130 μM), and the final DPH and TMA-DPH concentrations were 1 μM and 2 μM, respectively. The α-synuclein stock solution was titrated into suspensions of fluorophore-labelled SUVs to an R (lipid/protein molar ratio) of 500 (0.26 μM α-synuclein), 100 (1.3 μM α-synuclein) and 10 (13 μM α-synuclein). For the control, SUVs were titrated with HEPES buffer, pH 7.0.

DPH and TMA-DPH fluorescence anisotropy was measured after 10 min incubations with constant stirring, at 25 °C, 40 °C and 50 °C. The excitation wavelength was 358 nm, with the excitation polariser oriented in the vertical position, while the vertical and horizontal components of the polarised emission light were recorded through a monochromator at 410 nm. The emission fluorescences of both of the fluorophores in aqueous solution were negligible. The anisotropy (r) was calculated using the built-in software of the instrument, according to Eq. (2):

$$r = I_{HH} - GI_{HV}/(I_{HH} + 2GI_{HV}), \quad (2)$$

where I_{HH} and I_{HV} are the parallel and perpendicular emission intensities, respectively. The value of the G-factor, as the ratio of the sensitivities of the detection system for the vertically (I_{HV}) and horizontally (I_{HH}) polarised light, was determined for each sample separately. From the anisotropy value, the lipid order parameter, S, was calculated using Eq. (3) [51]:

$$S = \left[(1-2r/r_0) + 5(r/r_0)^2\right)^{1/2} - 1 + r/r_0\Big]/(2r/r_0) \quad (3)$$

where r_0 is the fluorescence anisotropy of DPH in the absence of any rotational motion of the probe. The theoretical value of r_0 is 0.4, while the experimental values of r_0 were between 0.362 and 0.394 [51]. In our calculations, the experimental values used were $r_0 = 0.370$ and $r_0 = 0.369$ for DPH and TMA-DPH, respectively, in DPPC at 5 °C.

2.7. Differential scanning calorimetry

The phase transition measurements of MLVs and SUVs prepared from DPPC, DPPG and DPPC:DPPG (1:1) were performed using a Nano DS series III calorimeter (Calorimetry Science, Provo, UT, USA). Before the measurements, the samples were degassed under vacuum and then loaded into the calorimetric cell. The lipid concentration was 0.5 mg/mL (~680 μM). The DSC melting profiles for the lipid vesicles in HEPES buffer, pH 7.0, and in the presence of α-synuclein (R [lipid/α-synuclein molar ratio] = 10) were recorded over the temperature range from 10 °C to 70 °C at a constant heating/cooling rate of 1 °C/min. The corresponding baselines were obtained using cells filled with the same amount of the HEPES buffer, pH 7.0, and were subsequently subtracted from the lipid/α-synuclein thermograms. The first DSC scan was used to obtain the phase transition temperature, T_m, and the transition enthalpy, ΔH_{cal}, as described previously [49], while the subsequent scans were used to determine the reversibility of the phase transition. The data were analysed using the OriginPro8.1 software (OriginLab Corporation, Northampton, USA).

2.8. Calcein efflux assay

Calcein-loaded SUVs were used to determine the α-synuclein-induced vesicle permeability, and changes in the fluorescence intensity were followed on a Cary Eclipse fluorescence spectrophotometer (Varian, Mulgrave, Australia). Calcein is a small fluorophore that can be released from vesicles after pore formation or other lipid bilayer destruction [52]. The concentration of calcein in the intact SUVs was 80 mM, a concentration at which calcein self-quenches. After being released from the SUVs, the calcein was diluted and its fluorescence intensity was greatly increased. The calcein-loaded SUVs in HEPES buffer, pH 7.0, were stirred in a 10-mm path length quartz cuvette at a lipid concentration of 0.1 mg/mL (~130 μM). Aliquots of the α-synuclein solution were added to achieve lipid/α-synuclein molar ratio (R) of 10. The time-dependent changes in the fluorescence intensity were followed for 20 min at constant temperature (25, 40, 45, 50 °C), with an excitation wavelength of 495 nm and the intrinsic emission fluorescence of calcein at 515 nm. The permeabilisation induced by α-synuclein was expressed in terms of the percentage of released calcein, as compared to the maximal permeabilisation obtained at the end of the assay with 2 mM Triton X-100 detergent. Calculations were performed according to Eq. (4):

$$\text{Calcein release}(\%) = (F - F_{min})/(F_{max} - F_{min}) \times 100 \quad (4)$$

where F is the fluorescence emission intensity at the end of the incubation of the vesicles with α-synuclein, F_{min} is the fluorescence of free calcein for the same emission intensity as the vesicles filled with calcein at the start of each experiment, and F_{max} is the maximal fluorescence of calcein obtained after the detergent addition.

3. Results

3.1. SUVs as the model lipid system

In this study of α-synuclein–membrane interactions, SUVs obtained by sonication were used as the model lipid membranes. The size of these SUVs resembles ~40 nm synaptic vesicles [53], as α-synuclein has been shown to have a higher affinity for curved membrane surfaces provided by such SUVs, as compared to that for 'flatter' membranes [28,41]. Additionally, due to the light scattering, the spectroscopic techniques used (i.e., CD spectroscopy and fluorimetry) limit the use of larger sized liposomes.

A lipid bilayer composed of one type of phospholipid is characterised by a phase transition between the relatively non-mobile (gel phase) and highly mobile (liquid-crystalline phase) states of the lipid. At room temperature, the monounsaturated POPC and POPG are in their liquid-crystalline state, as their T_m is at <0 °C. However, the saturated DPPC and DPPG are in their gel phase at room temperature. MLVs composed of DPPC, DPPG and DPPC:DPPG (1:1) have a very narrow main phase transition, between 41 °C and 42 °C (see Section 3.4.). In contrast to MLVs, the phase transitions of SUVs are broader and less-cooperative [15]. This has been attributed to the highly curved membrane of these

smaller SUVs, which causes abnormalities in the lipid packing. DSC analysis of these DPPC, DPPG and DPPC:DPPG (1:1) SUVs showed the phase transitions in the temperature range from ~33 °C to 45 °C (Supplementary Data, Fig. S2). Therefore, for further experiments, three temperatures were selected for these measurements: 25 °C (corresponding to the gel phase of saturated lipids); 40 °C (corresponding to the phase transition temperature, T_m); and 50 °C (corresponding to the liquid-crystalline phase). Fluorescence anisotropy measurements and the calculated order parameters of the lipid bilayers confirmed these three different phases at these three different temperatures (see Section 3.3).

3.2. Anionic lipids induce α-helical secondary structure in α-synuclein

α-Synuclein binding to lipid membranes induces its structural transition from an unfolded into an α-helical conformation [26]. We used CD spectroscopy to follow the changes in the far-UV CD range of α-synuclein, in the presence of the different lipid SUVs. The minimum of the average mean residue ellipticity, [θ], at 220 nm ($[\theta]_{220}$) is characteristic for proteins with an α-helical secondary structure [54]. Lower $[\theta]_{220}$ values indicate higher α-helical content in a protein structure. Representative far-UV CD spectra over a temperature range from 25 °C to 70 °C at a lipid/α-synuclein molar ratio, R, of 10 are shown in Fig. 1.

Regardless of the temperature during the measurements, α-synuclein in buffer solution had a CD spectrum that was characteristic of a random coil conformation (Fig. 1A). Analysis of these far UV-CD spectra with the Contin programme [50] demonstrated that the α-helical content did not exceed 10% (Supplementary Data, Fig. S3). Addition of SUVs prepared from DPPC and POPC (Fig. 1B, C) had no significant impact on this α-synuclein secondary structure, as the CD spectra in the presence of these neutral lipid SUVs did not differ significantly from the spectra of the lipid-free α-synuclein (Fig. 1A). This thus indicated an absence of α-synuclein–membrane interactions.

On the contrary, the negatively charged SUVs had a great impact on the far-UV CD spectra of α-synuclein (Fig. 1D, E). The temperature also had a profound influence on the α-synuclein CD spectra in the presence of DPPG vesicles (Fig. 1D). Here, $[\theta]_{220}$ started to decrease at 40 °C, and reached a minimum at around 50 °C to 60 °C, after which $[\theta]_{220}$ increased again. In the presence of POPG, the far-UV spectra of α-synuclein became more negative, which is typical for a predominantly α-helical structure with low $[\theta]_{220}$. In the presence of POPG, $[\theta]_{220}$ of α-synuclein has already reached the minimum at 25 °C, the further increase in temperature to 45 °C and 70 °C had no significant impact on the far-UV CD spectra of α-synuclein (Fig. 1E). These data indicate that negatively charged phospholipids (i.e., DPPG, POPG) in the liquid-crystalline phase induce the formation of the maximum α-helicity in α-synuclein. As determined by the Contin programme, the α-helical content of α-synuclein did not exceed 30% (Supplementary Data, Fig. S3).

Next, we examined the importance of the anionic lipids for α-synuclein α-helix formation. Here, an equimolar mixture of DPPC:DPPG SUVs was used (Fig. 1F), and at temperatures above 40 °C, only a slight decrease in the $[\theta]_{220}$ was observed. An increase in the amount of the anionic lipid in these SUVs (i.e., DPPC:DPPG, 1:2) did not induce any significant further reduction in $[\theta]_{220}$ (Fig. 1G).

Fig. 2A shows representative CD spectra of α-synuclein in the absence and presence of SUVs at 55 °C, a temperature that generally induced the largest reductions in $[\theta]_{220}$ with the saturated lipid SUVs (for POPG and POPC, 45 °C was used). For clarity, the $[\theta]_{220}$ obtained from these far-UV CD spectra of α-synuclein at the different temperatures are also shown in Fig. 2B. As can be seen, the temperature only had a minor effect on $[\theta]_{220}$ of α-synuclein in the absence of lipid SUVs and in the presence of the neutral lipid SUVs. However, the presence of the DPPG and POPG SUVs decreased $[\theta]_{220}$ at all of the measurement temperatures. In the presence of the DPPG SUVs, $[\theta]_{220}$ sharply decreased from 40 °C to 50 °C, and reached its lowest value around 50 °C to 60 °C, at which point it was equivalent to the $[\theta]_{220}$ in the presence of POPG SUVs. With equimolar amounts of DPPC and DPPG in the SUVs, only a small decrease in $[\theta]_{220}$ was observed. Surprisingly, an increase in the anionic lipid content in the DPPC:DPPG SUVs (i.e., to the 1:2 molar ratio) only slightly decreased $[\theta]_{220}$, which indicated that there was only a small increase in the α-helical content of α-synuclein within this temperature range.

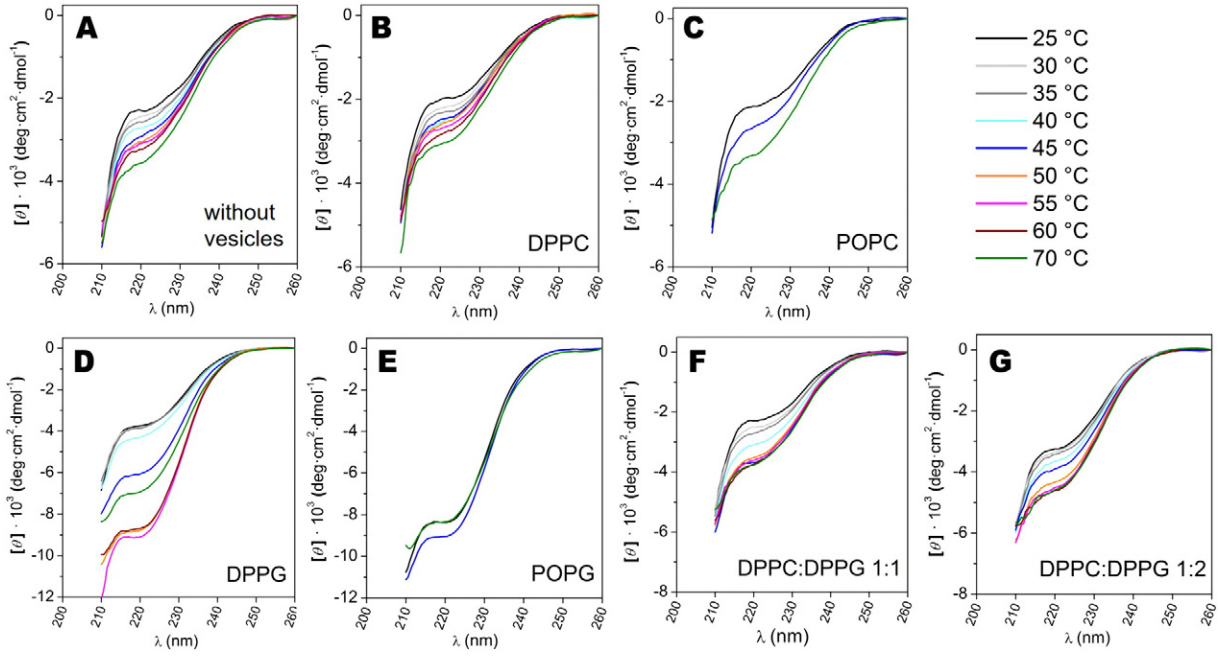

Fig. 1. Representative far-UV CD spectra of α-synuclein in the absence of SUVs (A) and in the presence of different SUVs (B–G; as indicated). The spectra were recorded in 150 mM NaCl, 20 mM HEPES, pH 7.0, for a temperature range from 25 °C to 70 °C, every 5 °C (as indicated). Note the different scales for the average mean residue ellipticity, [θ].

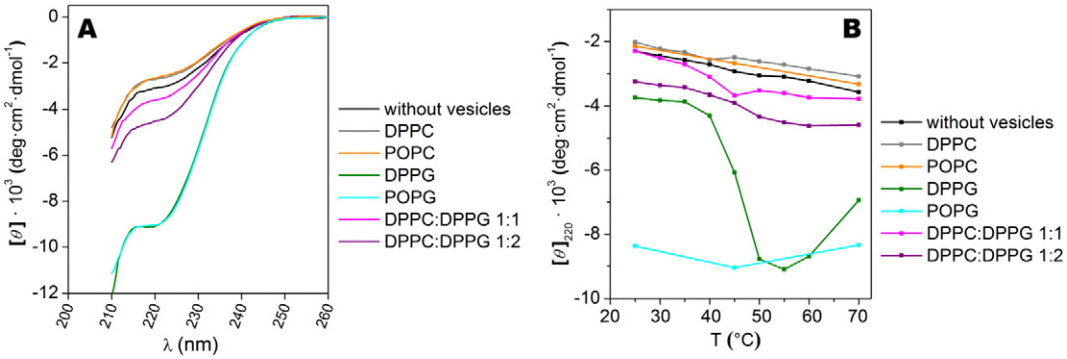

Fig. 2. (A) Representative CD spectra of α-synuclein in the absence and presence of SUVs of the different lipid compositions (as indicated), as recorded at 55 °C (or 45 °C for POPC, POPG SUVs). (B) Average mean residue ellipticity of α-synuclein at 220 nm ($[\theta]_{220}$) at the different measurement temperatures (T) in the absence and presence of SUVs of the different lipid compositions (as indicated). All of the spectra were recorded in 150 mM NaCl, 20 mM HEPES, pH 7.0.

3.3. Effects of α-synuclein on the lipid ordering parameter

To determine the impact of α-synuclein on the degree of order of the lipid bilayer, α-synuclein was titrated into suspensions of these SUVs and the fluorescence anisotropy of the fluorophores DPH and its cationic derivative TMA-DPH were measured. Anisotropy measurements reveal the degree of reorientation of the fluorophore that occurs between the polarised excitation and emission planes. As the degree of reorientation of fluorescence emission depends on the rotational diffusion of the fluorophore, information on the viscosity of the environment can be obtained [55]. DPH is hydrophobic and can be used to monitor changes in the middle of the bilayer, whereas TMA-DPH is amphiphilic, such that its polar region remains anchored at the membrane–water interface, which allows the study of the membrane properties more towards the lipid headgroup region [55]. As the anisotropy of DPH and TMA-DPH depends on the degree of packing of the lipid acyl chains in membranes, they can be associated with an order parameter, S, as described in Section 2.6 [56].

The dependence of the lipid order parameter S of these SUVs on the molar ratio of lipid/α-synuclein R at three different temperatures is shown in Fig. 3. For the control, the SUV suspensions were titrated with HEPES buffer, pH 7.0, which did not affect the fluorescence anisotropy of the fluorophores.

As can be seen from Fig. 3, the fluorescence anisotropy, and consequently the order parameter, of these SUVs formed from the saturated lipids DPPC and DPPG, or from an equimolar mixture of DPPC:DPPG,

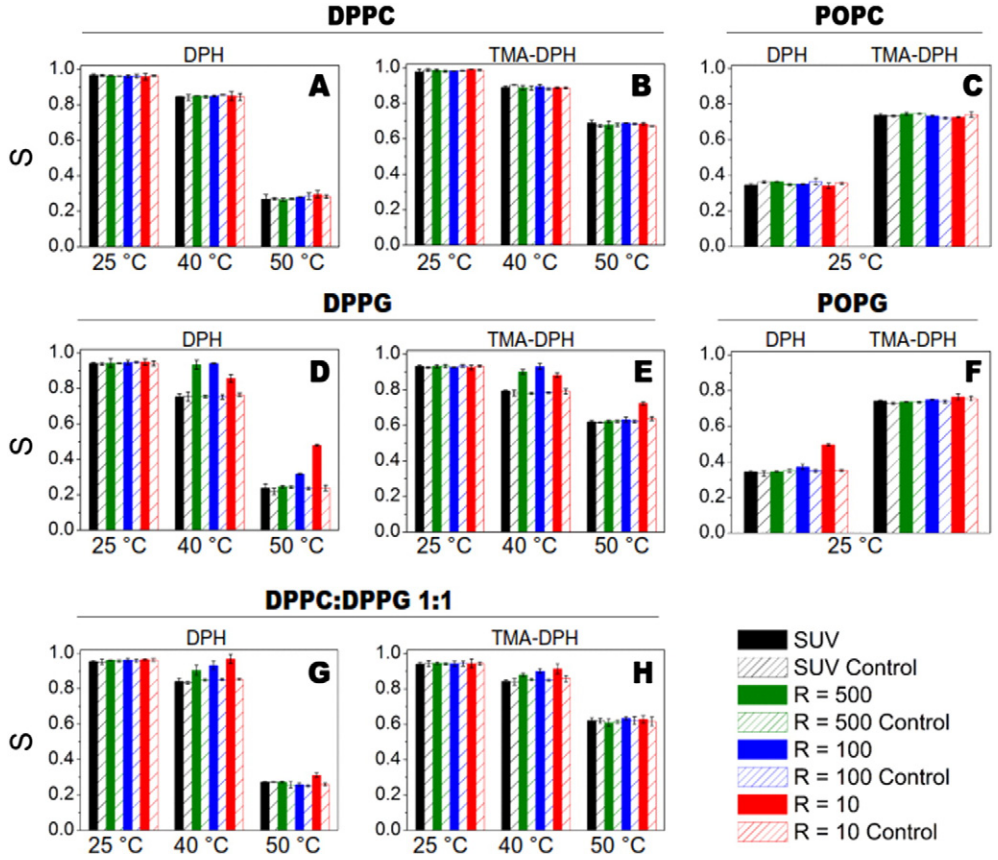

Fig. 3. The lipid order parameter, S, of SUVs formed from DPPC (A, B), POPC (C), DPPG (D, E), POPG (F) and DPPC:DPPG 1:1 (G, H) in the absence and presence of α-synuclein. R (lipid/α-synuclein molar ratio) was 500, 100 and 10 (as indicated). The measurements were performed at 25 °C, 40 °C and 50 °C (as indicated). Data are means ± standard error from at least two independent experiments.

was highest in the gel phase (i.e., at 25 °C), intermediate at the temperature of the lipid phase transition (i.e., at 40 °C) and lowest in the liquid-crystalline state (i.e., at 50 °C) (Fig. 3A, B, D, E, G, H). This was the case for both the inner (as detected by DPH) and the outer (as detected by TMA-DPH) parts of the SUV membranes. At 50 °C the lipid order parameters S for the saturated lipid SUVs were comparable to those for the POPC and POPG SUVs at 25 °C (Fig. 3C, F), which confirms their liquid-crystalline phase.

α-Synuclein did not impact on the lipid order parameter S of the DPPC SUV bilayer at any of the measurement temperatures (Fig. 3A, B). Also, there were no changes in the lipid order parameter when the POPC SUVs were titrated with α-synuclein (Fig. 3C). On the basis of these data, it can be concluded that α-synuclein does not affect the order of the acyl chains in the bilayer of SUVs made from neutral lipids, regardless of the lipid phase state.

α-Synuclein also caused no significant changes in S for the DPPG SUV bilayer at 25 °C, when DPPG was in the gel phase (Fig. 3D, E). However, there were changes seen at and above the phase-transition temperature of DPPG (i.e., at 40 °C, 50 °C). At both of these higher temperatures, α-synuclein addition caused increases in S in the inner part of the SUV membrane (as detected by DPH; Fig. 3D) and at the water–lipid interface (as detected by TMA-DPH; Fig. 3E). These increases in the order parameter S indicated higher order in the microenvironment of these fluorescent probes. However, the effects of α-synuclein on the lipid order of these DPPG SUVs were different at 40 °C and 50 °C. At 40 °C, the lipid order parameter S in the regions of the acyl chains and the lipid headgroups was increased already at the low α-synuclein concentration (R = 500). These data indicate lipid ordering by α-synuclein when the lipid bilayer of the DPPG SUVs was in the state of the phase transition. Above the phase transition temperature of the DPPG, when the SUV membrane is in the liquid-crystalline state, the lipid ordering occurred at the higher α-synuclein concentrations; namely, at R = 100 in the interior of the membranes, and R = 10 in the vicinity of the lipid headgroups. These results are comparable with the effects of α-synuclein on the order parameter of POPG SUVs (Fig. 3F), where there was a slight increase in the lipid order parameter S in the inner part of the SUV membrane at R = 100, while the order of the SUV membrane increased at R = 10. Changes in the lipid order parameter S with TMA-DPH, which detects changes more towards the SUV membrane surface (i.e., the interfacial area), were minimal in the case of these POPG SUVs. The S for the acyl chain region at R = 10 was about 0.5 (Fig. 3F), which was comparable with the S for DPPG at 50 °C (Fig. 3D).

As for the DPPG SUVs, addition of α-synuclein did not affect the lipid order parameter S of SUVs formed from the DPPC:DPPG (1:1) mixture at 25 °C (Fig. 3G, H). The greatest impact on the lipid ordering occurred at the phase transition temperature (40 °C), where the addition of α-synuclein to this mixed lipid SUV suspension led to a gradual increase in the ordering of the lipid acyl chains (Fig. 3G) and the water–lipid interface region (Fig. 3H). This increase in the SUV bilayer order was most pronounced at R = 10.

From these data presented in Fig. 3, it can be concluded that α-synuclein does not cause changes in the lipid ordering parameter S when the SUV bilayer is in the gel phase, even if the lipids are negatively charged. The lipid ordering occurs only during the phase transition (i.e., gel state to liquid-crystalline state) or above the T_m, and therefore only in at least partially liquid-crystalline phase (i.e., 40 °C, 50 °C for the saturated lipids). The impact of α-synuclein on the lipid order parameter S was concentration dependent.

3.4. Impact of α-synuclein on the gel to liquid-crystalline phase transitions of the lipids

Differential scanning calorimetry can be used to measure changes in enthalpy and temperature of lipid phase transitions from gel to liquid-crystalline state. Sharp phase transitions in a narrow temperature range are characteristic only for lipid systems that include saturated fatty acids [57]. As the DSC method in the temperature range from 0 °C to 100 °C fails to detect phase transitions of unsaturated lipids, only vesicles prepared from the saturated lipids DPPC, DPPG and DPPC:DPPG (1:1) were used. As presented above, SUVs have less-cooperative phase transitions across a wide temperature range (see Supplementary Data, Fig. S2), which would make it difficult to objectively evaluate the impact of α-synuclein on the lipid structure. Therefore, instead of SUVs, MLVs were used. We recorded the DSC thermograms of these MLVs in the absence and presence of α-synuclein at a lipid/α-synuclein molar ratio R of 10, as shown in Fig. 4. The thermodynamic values of the phase transitions in the presence of α-synuclein are given in Table 1.

The phase transitions of these MLVs without α-synuclein were very cooperative and narrow, with the temperatures of the main transitions in the range of 41 °C to 42 °C. Pre-transition is seen for the DPPC and DPPC:DPPG (1:1) MLVs, but not for the DPPG MLVs (Fig. 4). The cooperativity of the transition decreased slightly for the MLVs formed from DPPC to DPPC:DPPG (1:1) to DPPG. The changes in the enthalpy values of the transitions were comparable for DPPC and DPPC:DPPG (1:1), while for DPPG, the enthalpy was slightly lower (Table 1).

In the presence of α-synuclein at R = 10, the temperature and enthalpy of the pre-transition and main phase transition of the DPPC MLVs did not change, which indicated that α-synuclein did not significantly affect the phase transition of the zwitterionic lipid MLVs (Fig. 4A, Table 1). Changes in the thermodynamic parameters of the transition occurred when the anionic lipids were present in the MLVs (Table 1). α-Synuclein caused thermal stabilisation of both DPPG:DPPC (1:1) and DPPG MLVs, and reduced the cooperativity of transition (i.e., a wider temperature range for the transition) (Fig. 4B, C). Thermal stabilisation of the lipids (i.e., an increase in T_m) means that α-synuclein stabilises

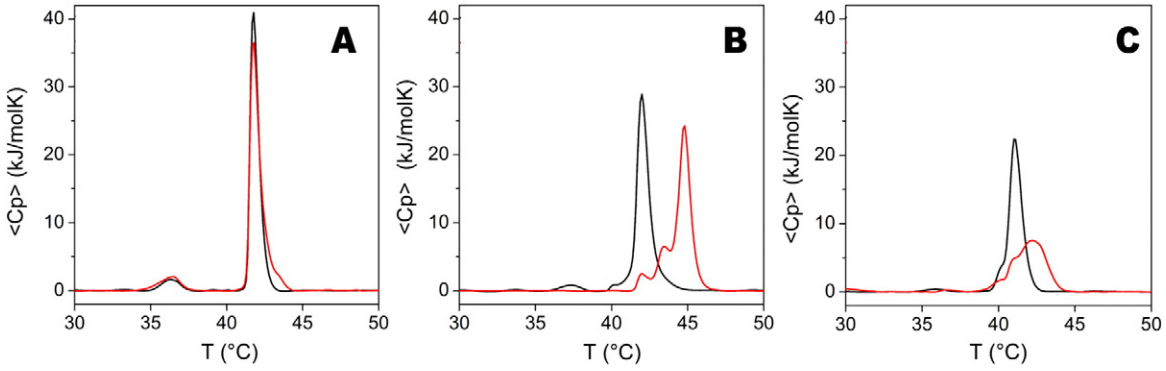

Fig. 4. Representative DSC thermograms of the MLVs formed from DPPC (A), DPPC:DPPG (1:1) (B) and DPPG (C), in the absence (black line) and presence of α-synuclein at R = 10 (red line), in 150 mM NaCl, 20 mM HEPES, pH 7.0.

Table 1
Thermodynamic profiles of the MLVs formed from DPPC, DPPC:DPPG (1:1) or DPPG in the absence and presence of α-synuclein, at R = 10. T_m, temperature of the gel-to-liquid crystalline phase transition; ΔH_{cal}, enthalpy of the gel-to-liquid crystalline phase transition.

Sample	T_m (°C)	ΔH_{cal} (kJ/mol)
DPPC	41.8 ± 0.5	29.7 ± 3.0
DPPC + α-synuclein	41.8 ± 0.5	30.3 ± 3.0
DPPC:DPPG (1:1)	41.9 ± 0.5	31.2 ± 3.0
DPPC:DPPG (1:1) + α-synuclein	44.6 ± 0.5	32.8 ± 3.0
DPPG	41.0 ± 0.5	24.6 ± 3.0
DPPG + α-synuclein	42.4 ± 0.5	21.0 ± 3.0

the gel state. For the DPPC:DPPG (1:1) MLVs, there was no pre-transition and the T_m increased by ~3 °C (Table 1), while the effect on the enthalpy of the main transition was small. We noted the appearance of new peaks in the DSC thermograms, of which the smallest corresponded to the T_m of the pure lipid MLVs without α-synuclein. On the contrary, for the DPPG MLVs, there was a reduction in the enthalpy of the phase transition, the transition became less cooperative, and the T_m increased by ~1.5 °C (Table 1).

3.5. Detection of membrane permeabilisation

The disruption of the model-lipid bilayer structural integrity in the presence of α-synuclein was investigated using the calcein dye-leakage assay. The SUVs were loaded with the fluorescent dye calcein and then incubated with α-synuclein. Any weakening of the lipid packing of the SUVs as a result of α-synuclein binding to the SUV bilayer allows the calcein to leak from the SUV interior into the external buffer medium, where its fluorescence emission intensity increases [52]. Fig. 5 shows the effects of α-synuclein on calcein release from the SUVs formed from the different lipids. The inset of Fig. 5, shows that α-synuclein induced relatively fast calcein release from the POPG SUVs. This process was complete within 20 min. The kinetics profile of the calcein release showed an initial rapid phase and a subsequent slower phase. The calcein release was also dependent on the α-synuclein concentration, with the greatest release at R = 10 (when α-synuclein was at 13 μM).

The SUV permeabilities for calcein after the 20 min incubations with α-synuclein at different temperatures are shown in Fig. 5. SUVs made from the neutral DPPC and POPC were resistant to α-synuclein and did not leak calcein at any of the incubation temperatures (i.e., 25 °C, 40 °C, 50 °C), therefore remaining independent of the lipid phase of neutral lipids. Also, there was no significant permeabilisation of the negatively charged DPPG or the DPPC:DPPG (1:1) SUVs at 25 °C, and thus when the lipids were in the gel phase. At the phase-transition temperature of the saturated lipids (i.e., 40 °C) and in the liquid-crystalline phase (i.e., 50 °C), the incubations with α-synuclein caused calcein release from the DPPC:DPPG (1:1) and DPPG SUVs. This release was greater for the DPPG SUVs (~95%), as compared with the DPPC:DPPG (1:1) mixture (~70%). However, the permeabilities of the SUVs at these two temperatures were comparable for both of these lipid compositions. The permeabilities of the DPPG SUVs at 40 °C and 50 °C were also the same as the permeability of the POPG SUVs at 45 °C, while the permeability of the POPG SUVs at 25 °C was slightly lower than at 45 °C. It can thus be concluded that α-synuclein permeabilises only the negatively charged lipid SUVs at the phase transition state or the liquid-crystalline state.

4. Discussion

The precise function(s) of α-synuclein is not yet known, but strong evidence indicates a role in relation to lipid membranes. In neurons, α-synuclein localises in the vicinity of, and/or is associated with, synaptic vesicles [1,14,58]. α-Synuclein might have an important role in the regulation of synaptic vesicle transport and fusion, and therefore in neurotransmitter release [6,9]. In vitro, SUVs represent a good alternative to mimic the size of synaptic vesicles [26,53]. It has been shown previously that different α-synuclein domains exhibit various lipid affinities, with first 25 N-terminal residues being essential for helix folding [30]. To further determine which forces (i.e., electrostatic and/or hydrophobic) contribute to the full-length α-synuclein–membrane interactions and how α-synuclein affects the thermotropic properties and structure of the lipid bilayer, we systematically studied the protein–lipid association at neutral pH and physiological ionic strength.

4.1. Effects of the lipid bilayer on α-synuclein structure

It has been shown before that membrane binding of α-synuclein is accompanied by a conformational transition of α-synuclein from a native disordered form into a partially α-helical structure [26,28]. By measuring the CD spectra of α-synuclein in the far-UV wavelength range, a characteristic minimum at 220 nm can be used to determine its α-helical content.

Our data presented here show that formation of the α-helical structure in α-synuclein occurs only in the presence of negatively charged SUVs, and not in the presence of neutral SUVs formed from DPPC or POPC. DPPC and POPC SUVs had no influence on the α-synuclein far-UV CD spectra, and therefore on the $[\theta]_{220}$, at any of the investigated temperatures (i.e., from 25 to 70 °C). From these data, it can be concluded that α-synuclein remains in an unfolded conformation and does not interact with SUV membranes with a net surface charge of 0.

For the DPPG SUVs, the reduction in $[\theta]_{220}$ was highly temperature dependent. The DPPG SUVs in the gel phase (or up to ~40 °C) did not significantly affect the α-helical content of α-synuclein, which was only slightly higher than that determined for the SUV-free protein and in the presence of the neutral lipid SUVs. However, the content of the α-helical structure of α-synuclein started to increase at and above the temperature of the lipid phase transition (T_m) of the DPPG lipid SUVs. Phase transition occurs when the alkyl chains of the lipid molecules are converted from a relatively rigid and stretched (i.e., mainly *trans*) conformation in the gel state, to an orientationally more disordered state, which is characterised by increased rates of intramolecular and intermolecular movement [59]. The α-helical content of α-synuclein reached its maximum at temperatures from 50 °C to 60 °C, when the lipids of the SUVs were in the liquid-crystalline phase. This was also confirmed by the analysis of the content of secondary structure with the Contin programme. At higher temperatures, the α-helicity decreased again, which might be attributed to the denaturing of an ordered secondary protein structure and/or to excessive acyl chain mobility.

Membrane-induced α-helix formation of α-synuclein is an exothermic process, and an increase in temperature will thus produce a melting of the α-helix [60]. Temperature dependent α-helix formation in the presence of DPPG has been reported before, but only for the larger unilamellar vesicles and not for sonicated SUVs [45]. As our data show, the formation of the α-helical structure of α-synuclein is highly dependent on both the surface charge and the phase state of the lipid membranes of the SUVs. This was also confirmed by the observation that the CD spectra of α-synuclein in the presence of DPPG at 55 °C and POPG at 45 °C are practically the same.

Some previous studies have also shown that α-synuclein binds exclusively to negatively charged vesicles in the liquid-crystalline phase [43–45]. This association was attributed to the electrostatic attraction between positively charged ε-amino groups of Lys (which are situated in the imperfect N-terminal repeats) and anionic lipids [16,28,30,33,61]. It is generally accepted that the formation of an amphipathic helix at the water–lipid membrane interface involves at least three different steps; long-range electrostatic interactions between the anionic lipids and a cationic polypeptide increase the concentration of the protein in the vicinity of the membrane surface, which is then followed by the

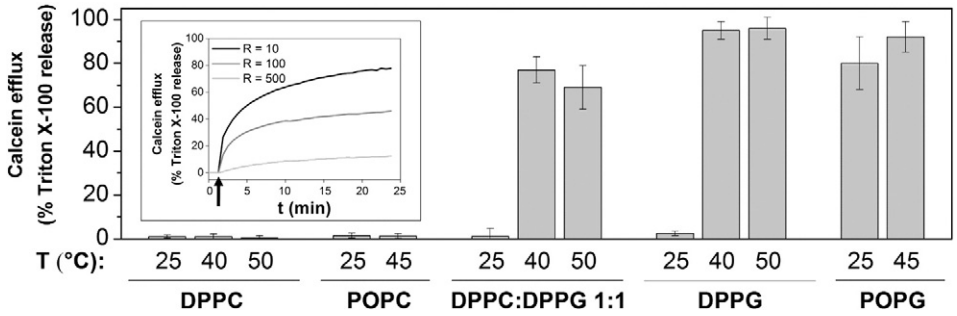

Fig. 5. Calcein efflux from SUVs formed from the different lipids induced by α-synuclein after 20 min of incubation. Measurements were performed at 25 °C, 40 °C, 45 °C and 50 °C (as indicated), at the lipid/α-synuclein molar ratio R of 10. Data were normalised, and are shown ± standard error, from at least two independent experiments. Inset: Kinetics of calcein efflux from 130 μM POPG SUVs induced by α-synuclein (R = 10, 100, 500) at 25 °C. The arrow indicates the α-synuclein addition. Each curve is representative of three separate experiments (SD < 15%). Measurements were performed in 150 mM NaCl, 20 mM HEPES, pH 7.0.

(sequentially or simultaneously) adsorption and folding of the protein [60,62].

The impact of surface charge on α-helix formation of α-synuclein was also examined here with the use of SUVs composed of a mixture of neutral (DPPC) and negatively charged (DPPG) lipids, wherein the addition of DPPC to DPPG lipids reduced the surface charge density of the membrane [63]. The electrostatic effect is complex and hard to precisely predict, as it depends on the protein charge, the membrane surface potential, or the membrane charge density, and the ionic strength of the solution [60,64]. Very simplified, the average net charge (i.e., charge per lipid molecule) of 100% DPPG vesicles is −1, for DPPC:DPPG SUVs at 1:2 it is −0.66, and for DPPC:DPPG SUVs at 1:1 it is −0.5 [31]. While for the DPPG SUVs the increase in the α-helicity of α-synuclein coincides with the lipid chain melting, for the DPPC:DPPG SUVs, the liquid-crystalline phase did not induce significant changes in $[\theta]_{220}$. CD analysis did not provide conclusions on whether with the DPPC:DPPG SUVs there is less α-synuclein association, or whether a shorter α-helix was formed with the same amount of bound protein, as in the case of DPPG SUVs. Bartels et al. also did not detect any significant differences in the α-helical content of α-synuclein between the DPPC:DPPG gel phase and the liquid-crystalline state, although they did show comparable α-helicity of α-synuclein in the presence of DPPC SUVs below the T_m [30]. Electron paramagnetic resonance analysis showed that a reduction in the surface charge density impairs the α-synuclein affinity for POPC:POPG large unilamellar vesicles, whereby, as in our case, significant binding of α-synuclein to POPC vesicles was not detected [65]. In contrast, the data obtained with fluorescence correlation spectroscopy showed greater α-synuclein affinity for an equimolar mixture of DPPC:DPPG in the gel phase than for POPC:POPG [41].

As our data show, the precise negative surface charge density is crucial for α-synuclein adsorption to these SUV membranes. This confirms the role of the N-terminal domain, which has many Lys residues for the interaction of α-synuclein with the lipid bilayer. However, as well as electrostatic interactions, formation and/or stabilisation of an amphipathic helix requires additional, hydrophobic interactions. It has been suggested that α-synuclein binds to both neutral and anionic lipids below their T_m [15,29,41]. Due to the high curvature strain, SUVs are characterised by lipid packing defects, which can expose the hydrophobic areas of the lipid bilayer [66]. Such defects are even more pronounced in the gel phase, when the membrane is more rigid [15]. The formation of low levels of α-synuclein α-helix was observed also in the presence of the DPPG SUVs in the gel phase in the present study, although the α-helicity of α-synuclein was significantly higher when the lipids were in a liquid-crystalline phase. In the liquid-crystalline state, the lipid headgroups and acyl chains are more loosely packed, which will allow easier insertion of α-synuclein into the hydrophobic core of the bilayer, and thus promote α-helix formation. In the liquid-crystalline phase, accommodation of α-synuclein within the membrane also requires less membrane reorganisation.

4.2. Effects of α-synuclein on the membrane properties

To obtain further insight into the impact of α-synuclein binding on the order of these SUV lipid bilayers, the changes in fluorescence anisotropy of two probes were measured: DPH and TMA-DPH, which localise to different positions within the bilayer [56]. α-Synuclein did not affect the lipid order parameter of these DPPC and POPC SUVs at any of the temperatures investigated. Together with the absence of the α-synuclein α-helical structure according to the $[\theta]_{220}$ in the CD spectra, this confirms the lack of binding of α-synuclein to these neutral lipids. α-Synuclein also had no effect on the lipid order of the anionic SUVs when they were in the gel phase. However, recent NMR study [67] reported that α-synuclein can influence lipid packing within raft-like domains containing POPC, sphingomyelin and cholesterol.

Conversely, α-synuclein had an ordering effect on the anionic DPPC:DPPG (1:1) and DPPG lipid bilayers of these SUVs at and above the T_m. As expected, the impact on the lipid order was smaller in the case of DPPC:DPPG (1:1). The degree of lipid ordering thus depends on the membrane charge and the phase state, and also on the α-synuclein concentration (see below). Also, the lipid-ordering effects on these anionic SUVs caused by α-synuclein and monitored with DPH and TMA-DPH indicate that α-synuclein affected both the acyl chains region and the water–lipid interface. We can thus conclude that α-synuclein has an influence on the ordering of both the acyl chains and the lipid headgroups.

The ordering of the DPPG bilayer at the T_m occurred at a lower α-synuclein concentration (i.e., already seen at R = 500, where α-synuclein was at 0.26 μM) than when the membranes were in the liquid-crystalline state, where the lipid ordering occurred at R = 100 (where α-synuclein was at 1.3 μM). Here, the effects were comparable to those with the POPG SUVs. At the T_m, the DPPG membranes of these SUVs were in the equilibrium state between the liquid-ordered and liquid-crystalline phases. From this it follows that in comparison with the liquid-crystalline phase, at the T_m, the hydrophobic regions are less accessible. This might be the reason for the lower structural conversion of α-synuclein, as there are fewer binding sites available for amphipathic α-helix formation. Consequently, lower levels of the membrane-bound α-synuclein can have greater impacts on the lipid order. In the liquid-crystalline phase, the α-helicity of α-synuclein was maximal; however, we assume that even at R = 10, the α-synuclein concentration is too low to order the highly dynamic lipid chains. We propose that an increase in the α-synuclein concentration would lead to lipid ordering.

Surprisingly, α-synuclein also had concentration-dependent ordering effects on the DPPC:DPPG (1:1) SUVs. This SUV membrane composition did not induce substantial amounts of α-helical structure of α-synuclein at any of the measurement temperatures (i.e., 25, 40, 50 °C). The formation of the α-helical structure is required for α-synuclein insertion into the membrane. The effects on the lipid order show that α-synuclein was adsorbed onto the membrane surface. The consequential shielding of the negative DPPG charges thus reduced the headgroup repulsion and led to lipid ordering.

Fluorescence anisotropy is sensitive to the molecular dynamics in a certain vicinity to the probe, while DSC can be used to measure changes in enthalpy and temperature of the phase transition of lipids, and hence determine the thermodynamic parameters associated with heat-induced phase changes. Therefore, DSC provides more direct evidence for these α-synuclein–membrane interactions. As SUVs show less-cooperative phase transitions over a wide temperature range, for the DSC analysis, MLVs were used. As expected, addition of α-synuclein to the DPPC MLVs at R = 10 had no effects on the lipid phase transition; however, α-synuclein affected the phase transition of the negatively charged MLVs. α-Synuclein thermally stabilised both the DPPG and DPPC:DPPG (1:1) MLVs (in both cases, there was an increase in T_m). The increase in T_m indicates the stabilisation of the gel phase of these MLVs after α-synuclein binding. At the same time, the cooperativity of the phase transition decreased. α-Synuclein did not affect the enthalpy of the phase transition of the DPPC:DPPG (1:1) MLVs, whereas for the DPPG MLVs, the enthalpy decreased slightly. This indicates that α-synuclein does not act only at the membrane surface, but instead it can insert into the MLV bilayer and affect the energy of the packing of the acyl chains.

These CD spectroscopy, fluorescence anisotropy and DSC measurements suggest that the interaction of α-synuclein with the membrane surface and its insertion into the bilayer depend on the vesicle surface charge and lipid phase. The calcein release assay results indicate that α-synuclein addition (at R = 10) induced calcein leakage from the SUVs composed of unsaturated POPG, saturated DPPG, and DPPC:DPPG (1:1) at and above the T_m. Although the calcein release assay cannot provide conclusions about the mechanism of vesicle disruption [52], it has been shown that vesicle permeabilisation correlates with α-helicity [68]. However, α-synuclein also permeabilised the DPPG SUVs at the T_m (i.e., at 40 °C), where the α-synuclein α-helix content was considerably lower than in the liquid-crystalline phase (i.e., at 50 °C). In addition, α-synuclein also caused calcein release from the DPPC:DPPG (1:1) SUVs, which compared to the DPPG SUVs, induced significantly lower α-synuclein α-helicity. Moreover, the SUV permeability also coincided with the α-synuclein effects on the lipid bilayer order.

It has been proposed that the oligomeric α-synuclein structures, which might also preferentially bind to anionic membranes in the liquid-crystalline phase [8], are particularly membrane destructive [69]. Although α-synuclein in solution at used concentrations is in monomeric state (Supplementary Data, Fig. S1), it is not excluded that it oligomerise on the membrane surface [70], and neutralises the negative charge on the surface and release the calcein from the interior of the liposomes. These monomers have, however, also been shown to induce some calcein release from POPG large unilamellar vesicles, although at the same time, α-synuclein did not permeabilise POPC:POPG (1:1) vesicles [8]. It is likely that α-synuclein adsorption onto the negative lipid headgroups neutralises the membrane charge, thus facilitating calcein leakage, which at pH 7.0, carries a negative charge.

It has been suggested that pore formation is unlikely for α-synuclein and that vesicle leakage might be due to lateral lipid expansion and membrane thinning [71]. α-Synuclein might induce membrane permeability by bilayer remodelling, such as membrane tubulation [72]. As in the liquid-crystalline state, the membrane is *per se* permeable to calcein, it is possible that adsorption of α-synuclein to the lipid headgroups increases the dye release. Anionic vesicles might be more prone to disruption by mechanical forces [73]. This also coincides with a lower permeability of the DPPC:DPPG (1:1) SUVs with respect to the DPPG and POPG SUVs.

5. Conclusions

We have shown here that α-synuclein binds to negatively charged surfaces of membranes in the phase-transition range and in the liquid-crystalline state. This binding to the membranes by α-synuclein induces its α-helix formation, which has an ordering effect on the packing of the acyl chains in the lipid bilayer. In the process of neurotransmitter release, synaptic vesicles fuse with the presynaptic plasma membrane, and are then re-formed by endocytosis [74]. We suggest that α-synuclein has an ordering effect and stabilises the anionic lipid bilayer of such highly curved SUVs. α-Synuclein maintains the integrity of the SUVs, which in a physiological context would mean that α-synuclein can stabilise the membranes of synaptic vesicles, as previously suggested [15,16,29,66]. This would prevent premature fusion of these vesicles with the presynaptic membrane.

Conflict of interest

The authors declare that they have no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found, in online version.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbamem.2015.06.021.

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