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Lipid Dynamics and Phase Transition within α-Synuclein Amyloid Fibrils

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Supporting Information

ABSTRACT: The deposition of coassemblies made of the small presynaptic protein, α-synuclein, and lipids in the brains of patients is the hallmark of Parkinson’s disease. In this study, we used natural abundance 13C and 31P magic-angle spinning nuclear magnetic resonance spectroscopy together with cryo-electron microscopy and differential scanning calorimetry to characterize the fibrils formed by α-synuclein in the presence of vesicles made of 1,2-dimyristoyl-sn-glycero-3-phospho-L-serine or 1,2-dilauroyl-sn-glycero-3-phospho-L-serine. Our results show that these lipids coassemble with α-synuclein molecules to give thin and curly amyloid fibrils. The coassembly leads to slower and more isotropic reorientation of lipid molecular segments and a decrease in both the temperature and enthalpy of the lipid chain-melting transition in amyloid fibrils formed by α-synuclein in the absence of lipids. Moreover, these proto-fibrils were found to be able to convert into mature fibrils through an increase in temperature. Finally, the concentration of α-synuclein lipid-induced proto-fibrils formed in these experiments was found to be proportional to the concentration of the lipids (DMPS and DLPS) ([fibrils] ~ 0.1[lipids]), suggesting that lipids may not only be involved in the initial steps of the reaction resulting in amyloid formation but also act as reactants in this process.

In this study, we used 13C and 31P MAS NMR, differential scanning calorimetry (DSC), and cryo-electron microscopy (cryo-EM) to characterize the proto-fibrils formed by α-synuclein in the presence of DMPS and DLPS vesicles under quiescent conditions at 30 °C for 4 d (see Methods in the Supporting Information and refs 11 and 12 for more details). Cryo-EM images show that α-synuclein forms thin and curly proto-fibrils. The term proto-fibrils has been used to describe these structures as they are observed to be thinner (~5 nm in thickness) and curlier than the mature fibrils formed by α-synuclein in the absence of lipids. Moreover, these proto-fibrils were found to be able to convert into mature fibrils through an increase in temperature. Finally, the concentration of α-synuclein lipid-induced proto-fibrils formed in these experiments was found to be proportional to the concentration of the lipids (DMPS and DLPS) ([fibrils] ~ 0.1[lipids]), suggesting that lipids may not only be involved in the initial steps of the reaction resulting in amyloid formation but also act as reactants in this process.

In this study, we used 13C and 31P MAS NMR, differential scanning calorimetry (DSC), and cryo-electron microscopy (cryo-EM) to characterize the proto-fibrils formed by α-synuclein in the presence of DMPS and DLPS vesicles under quiescent conditions at 30 °C for 4 d (see Methods in the Supporting Information and refs 11 and 12 for more details). Cryo-EM images show that α-synuclein forms thin and curly proto-fibrils.
fibrils in bulk solution under these conditions (Figures 1A,B and S1), an observation in agreement with images of these fibrils acquired using atomic force microscopy and electron microscopy on dried samples.\textsuperscript{11,12,16}
The morphology of these proto-fibrils is different from that of mature proto-fibrils, as indicated by differences in the cryo-EM images of the DMPS-induced α-synuclein proto-brils. We attributed these structures to the DMPS or DLPS bilayers in the pure lipid system and within α-synuclein proto-fibrils at varying temperatures. Left y-axis: Change in the δ13C of the pure DMPS (blue circles) and DMPS-induced α-synuclein proto-fibrils (purple squares) with increasing temperatures. Right y-axis: Change in the molecular heat capacity (Cp) of DMPS solubilized as vesicles (blue dashed line) or of DMPS-induced α-synuclein proto-fibrils untreated (purple dashed line) or treated with proteinase-K (black dashed line) with increasing temperatures. The chain melting enthalpies were found to be ca. 25 (pure lipid system, blue dashed line), ca. 6 kJ mol⁻¹ (DMPS-induced α-synuclein proto-fibrils, purple dashed line) and ca. 17 kJ mol⁻¹ (proteinase-K treated DMPS-induced α-synuclein proto-fibrils, black dashed line).

These thin and curly assemblies may therefore be called proto-fibrils, consistently with the nomenclature used for these assemblies described in our previous studies.1,11,12,15,16 The cryo-EM images of the DMPS-induced α-synuclein proto-fibrils are characterized by the presence of spherical structures that appear to either be opened or closed (indicated with star symbols on Figure 1A). We attributed these structures to either loops in the proto-fibrils or remaining vesicles. Finally, the morphology of these proto-fibrils is very different from that of mature fibrils formed by α-synuclein alone; the latter were found to be thicker and straighter than the proto-fibrils and to be organized as parallel bundles using cryo-EM.6 We then used natural abundance 13C MAS NMR to investigate whether lipid molecules are incorporated within the proto-fibrils. We first acquired 13C MAS NMR spectra of the protein-free lipid lamellar phase, where lipid molecules, DMPS or DLPS, are organized as multilayer stacks of bilayers (Figure 2A (DMPS) and B (DLPS), gray spectra). These reference samples will be referred to as "pure lipid system" throughout the rest of the Letter. The 13C MAS NMR spectra of the pure lipid systems were measured at temperatures above the melting temperature (Tm) of the lipids, at 60 °C for DMPS (Tm ≈ 39 °C) and 30 °C for DLPS (Tm ≈ 20 °C), and were used as references for the DMPS or DLPS bilayers in the fluid phase. We then acquired 13C MAS NMR spectra of DMPS- and DLPS-induced α-synuclein proto-fibrils at these temperatures, and we observed the presence of 13C resonances from the different lipid carbons (Figure 2A (DMPS) and B (DLPS), gray spectra), implying that DMPS and DLPS coassemble with α-synuclein into proto-fibrils.

To get insight into the molecular dynamics of DMPS and DLPS within α-synuclein proto-fibrils, we then used polarization transfer 13C MAS NMR.10 Indeed, it is possible to obtain qualitative site-specific information about the molecular mobility of each lipid carbon from the experimental data by comparing the intensities of each of the cross-polarization (CP), direct polarization (DP), and insensitive nuclei enhanced by polarization transfer (INEPT) signals. The signal intensity of a given carbon in an INEPT and a CP spectrum, ICP and INEPT, respectively, depends on the correlation time (τC) and the order parameter (SCH) for the C–H bond vector in the molecular segment.10 In the CP experiment, polarization is transferred from 1H to 13C via through-space dipolar couplings, which are averaged to zero by fast isotropic reorientation. Therefore, CP spectra are expected to yield maximal signals for rigid segments, with τC > 10 μs and/or |SCH| < 0.05.10 In an INEPT experiment, polarization is transferred from 1H to 13C through covalent bonds and will show a signal as long as the 1H and 13C transverse relaxation times are longer than the time required for 1H−13C polarization transfer.10 INEPT spectra will yield intensity signals for mobile segments with τC < 0.01 μs and |SCH| < 0.05.10 We acquired sets of 13C CP-DP-INEPT MAS spectra of the DMPS- and DLPS-induced α-synuclein proto-fibrils that we compared to those of the pure lipid system samples (Figure 2A,B) at 60 and 30 °C, respectively. At these temperatures, the DMPS and DLPS bilayers in the pure lipid system are in the fluid phase, as illustrated by the presence of all the resonances of the different 13C on their respective INEPT spectra (red spectra Figure 2A (DMPS) and B (DLPS)). In the case of DMPS- and DLPS-induced α-synuclein proto-fibrils, we observed only the resonances corresponding to the carbons Cα, Cβ of the polar head, Cγ, of the glycerol group, and the carbons of the end methyl (C14 for DMPS (Figure 2A) and C12 of DLPS (Figure 2B)) of the lipids on their respective INEPT spectrum. We compared the relative intensities (IDP, INEPT, and ICP) for the different carbons of DMPS and DLPS in the proto-fibrils to those in the pure lipid system, and we found that all carbons, except the end methyls, have a lower reorientation rate (increase of τC) and/or a more anisotropic reorientation (increase of SCH) after coassembly with α-synuclein into amyloid proto-fibrils (Figure 2C,D). Interestingly, the relative intensities (IDP, INEPT, and ICP) of the end methyl C12 DLPS or C14 DMPS suggested that τC < 0.01 μs and SCH < 0.05 and that these carbon atoms have a high reorientation rate and a highly isotropic reorientation in both the pure lipid system and in the proto-fibrils (Figure 2C,D).

We then used 31P MAS NMR in order to determine the influence of protein–lipid coassembly into amyloid fibrils on the rate or the anisotropy of the reorientation of the lipid phosphate groups. In particular, we determined the value of the 31P chemical shift anisotropy (Δν) of lipid molecules in the...
pure lipid system or in the α-synuclein proto-fibrils (Figures 3 and S2) by spectral deconvolution and by fitting the spinning sideband amplitudes of the 31P MAS NMR spectra using the Herzfeld–Berger method of sideband analysis. In the case of DMPS, we observed that the 31P MAS NMR line widths of the lipids were larger in the proto-fibrils than in the pure lipid system (Figure 3A) at 60 °C, suggesting that the lipid–protein coassembly leads to a decrease in the rate of reorientation of the DMPS phosphate group. Moreover, the values of 31P Δσ were found to be ca. 8 ppm lower for DMPS in the proto-fibrils (40 ppm) compared to those in the pure lipid system (48 ppm). This observation implies that the coassembly of DMPS molecules with α-synuclein into amyloid fibrils induces a more isotropic reorientation of the phosphate group. In the case of DLPS and DLPS-induced α-synuclein proto-fibrils, we observed very broad sidebands that could not be fitted accurately (Figure S2). The broadening of these lines is likely due to the fact that the reorientation of the DLPS phosphate group is on the same time scale as the magic-angle spinning (τC ≈ 1 ms). Altogether, the 13C and 31P MAS NMR measurements show that the coassembly of DMPS and DLPS molecules with α-synuclein into amyloid proto-fibrils decreases the rate and the anisotropy of their reorientation (see illustration in Figure 4).

Finally, we investigated the dynamics of DMPS molecules in the pure lipid system and in the DMPS-induced α-synuclein proto-fibrils at temperatures ranging from 25 to 60 °C (Figures 3 and 5). This temperature range spans values below and above the melting temperature of pure DMPS, i.e., ca. 39 °C. We used the spectra of pure DMPS measured at 25 and 60 °C as references for the bilayer in the gel (solid) and liquid phases, respectively. At 25 °C, the broad peak corresponding to the unresolved resonances of the acyl C4−C11 carbons (C4−C11 peak) was found to be centered at 33 ppm for both the pure lipid system and the protein–lipid proto-fibrils. This chemical shift value is characteristic of hydrocarbon chains in an all-trans conformation. Moreover, we observed that the line width of this peak for DMPS was larger in the proto-fibrils than in the pure lipid system. This observation suggests that the C4−C11 carbon chains of DMPS sample a wider range of conformations in the proto-fibrils than in the pure lipid system. When the temperature was gradually increased to 60 °C, we observed a decrease in the intensity of the C4−C11 peak and an increase in the intensity of a new peak at 30 ppm for both the pure lipid system and the protein–lipid proto-fibrils. The chemical shift value of this new peak is characteristic of hydrocarbon chains in an all-trans conformation.

and occurs at a lower temperature (ca. 29 °C) (Figure 3B). The DSC thermograms of DMPS dispersed as vesicles and of DMPS-induced α-synuclein proto-fibrils were characterized by the presence of one transition centered at 39 and 29 °C (Figure 3B), respectively. This observation suggests that the changes in DMPS 31P Δσ occur at the same temperature as that of the chain-melting transition (ca. 39 °C for the pure DMPS and ca. 29 °C for DMPS in the α-synuclein proto-fibrils) (Figure 3B). Moreover, the enthalpy of DMPS chain-melting was found to be ca. four times smaller in the proto-fibrils than in the pure lipid system at low temperatures and more rigid in the proto-fibrils than in the pure lipid system at high temperatures (Figure 5). We then investigated whether the observed change in the chain-melting transition of DMPS associated with protein–lipid coassembly could be reversed by measuring the DSC thermograms of the DMPS-induced α-synuclein proto-fibrils after incubation with proteinase-K. The thermogram of the digested proto-fibrils is characterized by the presence of a broader transition centered at ca. 39 °C and with a transition

![Figure 4. Illustration of the influence of the coassembly of DMPS and α-synuclein within proto-fibrils on the lipid dynamics measured at 60 °C. Theoretical 1H−13C polarization transfer efficiency as a function of τC and ISCD for a CH2 segment at the magnetic field 11.7 T and the magic-angle spinning frequency 5 kHz (adapted from ref 20 with permission; copyright 2013 Elsevier). The map is color-coded according to the calculated CP (blue) and INEPT (red) intensities. White indicates the absence of signal for both CP and INEPT. Each circle corresponds to a carbon of the acyl chain (C1−C14) of DMPS in the pure lipid system (white open circles) or in the protein–lipid proto-fibrils (black open circles). The position of the carbons results from the estimations of τC and ISCD that are based on previous quantitative measurements of those parameters (Pure DMPS: |S|CD(C−C) ≈ 0.2, |S|CD(C6−C12): 0.2 to 0.05, |S|CD(C14) ≈ 0.01, |S|CD: C4−C11: 1−1 ns, |S|CD(C14) ≈ 0.01 ns; DMPS in α-synuclein proto-fibrils: τC increased for all carbons by approximately a factor of 100, and |S|CD decreased for all carbons by a factor of 1.3 (see Figure 3B)).](image)
enthalpy equal to ca. 70% of that of DMPS in the pure lipid system. These results suggest that most of the DMPS molecules are released from the proto-fibrils after proteinase-K treatment and confirm that lipid molecules are present within these structures. Taken together, these results show that the reduced melting temperature and melting enthalpy of DMPS is related to the lipid−protein interaction in the amyloid proto-fibrils and not to a potential loss of lipids and that this process is partly reversible.

In conclusion, the results described in this study show that DMPS and DLPS lipid molecules can coassemble with α-synuclein molecules in the formation of proto-fibrils. From the present data we cannot distinguish the location and organization of lipids in the proto-filaments, i.e. whether lipids are incorporated within the fibril structure as individual molecules or as distorted vesicles/membranes. However, our data clearly implicate strong lipid−protein interactions in the coassemblies. In particular, our combined 13C, 31P MAS NMR, and DSC data indicates that this protein−lipid coassembly was found not only to influence the lipid dynamics by decreasing the rate and the anisotropy of the orientation of their hydrocarbon chains and phosphate groups but also to affect the lipid chain melting by decreasing both its temperature and enthalpy. These results contribute to a better understanding of the properties of lipid molecules within protein−lipid assemblies such as those found in the brain of patients with Parkinson’s disease.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcl.9b03005.

Materials and Methods, cryo-EM images of proto-fibrils formed by α-synuclein in the presence of DLPS and DMPS vesicles (Figure S1), and 31P MAS NMR spectra of pure DLPS/DMPS and DLPS/DMPS-induced α-synuclein proto-fibrils measured at different temperatures (Figure S2) (PDF)

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Notes
The authors declare no competing financial interest.

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