The Dual Dependency of Varying Liposomes and Peptides on Available Conformers of αS

Kathryn Peterson, Christian Coffman, Katie Dunleavy, Stephanie Kobany, Jonathan Sachs, and Anne Hinderliter

Introduction

Parkinson's disease (PD) affects more than half a million Americans and it's estimated that the U.S. spends about 25 billion dollars a year treating and caring for patients with PD.[1] As a result, research needs to be done on this currently incurable disease. Through investigation of patient's brains with PD via autopsy, there is a high concentration of amyloid proteins present with the largest component being the protein α-Synuclein (αS). Amyloids are an aggregation of transiently stable proteins, a common sign of PD.[2] αS is an 140-amino acid long protein that binds to the membrane and has a dual effect. The protein undergoes a structural change from being intrinsically disordered to a largely α-helical structure upon membrane binding. It also plays a role in lipid annealing within the membrane.

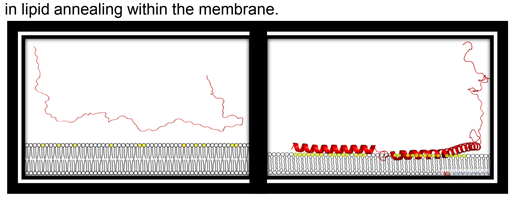

Figure 1. αS unbound and bound to PS and PE containing membrane
αS is intrinsically disordered in solution and the membrane shows no lipid domains when αS is unbound (left). Upon αS binding αS shows a change in secondary structure and promotes domain formation.

This dual dependency makes the study of this protein very complex. αS is found in the presynaptic terminal of most neurons and is thought to play a role in synaptic vesicle (SV) trafficking and neurotransmitter release. The sensitivity of αS to membranes of high curvature and high cholesterol content was studied using Differential Scanning Calorimetry (DSC) by monitoring the lipid transition. Proteins role in lipid rearrangement was studied using CF release assay. We propose that SV membranes dictate αS binding affinity based on the curvature and/or cholesterol content of SVs and upon binding, αS decreases the SV membrane fusion potential by relieving the rigidity of SV membranes. On the other hand we want to track the membranes effects on available protein conformers, this can be done using CD. To further investigate available αS conformers in response to changing compositions of membrane DSC can be used to monitor the protein transition in the presence of membrane. Finally, using all of the information about αS's complex relationship with SV membrane Isothermal Titration Calorimetry can be employed to propose a possible binding mechanism of αS. We propose that full-length αS in the presence of SV mimic will be able to undergo all of the possible conformers needed to bind membrane with high affinity, while a shortened αS fragment won't be able to find all possible conformations and will bind with lower affinity. A possible binding mechanism would give insight into PD pathogenesis and the cause of protein aggregation in the cells.

Materials/Methods

Materials:
POPE, SOPE, POPS, and cholesterol from Avanti Polar Lipids were used directly from the stocks. Purity was assessed before each use using thin layer chromatography. Truncated αS was from Yale University and was used without further purification.
Vesicle Preparation:
No Cholesterol: Lipid samples were aliquoted in appropriate volumes and put under nitrogen to evaporate the chloroform solvent off. Samples were placed under pressure for 4 hours, lyophilized, and placed under pressure for an additional 4 hours.
Cholesterol: Lipid samples and cholesterol were aliquoted in appropriate volumes into a Rotovap tube. Sample was ideally mixed in Rotovap tube. The tube was then placed under pressure for 8 hours.
Lipids were hydrated in 20mM MOPS and 100mM KCl pH7.5 buffer and extruded to create unilamellar vesicles..
Carboxyfluorescein Release Preparation:
LUVs containing CF were put through a transition from liquid to gel phase while monitoring for fluorescence using a Fluorolog 3 double excitation and double emission monochromator (Horiba Jobin Yvon).
Circular Dichroism Spectroscopy:
αS was prepared for CD data collection. Data points were collected in a range of 200-260 nm in 1 nm increments from -2°C to 60°C.
Differential Scanning Calorimetry:
The sample underwent heating phases as well as isothermal phases to establish equilibrium. This method was used to monitor a lipid transition and a protein transition.

Results

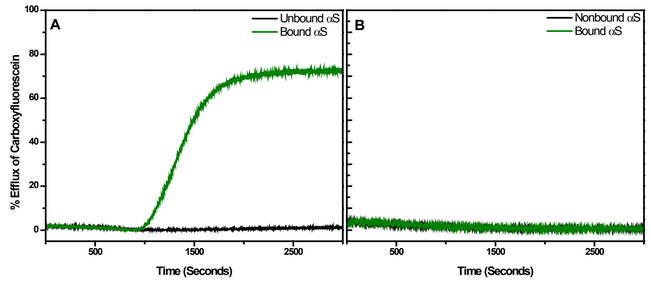

Figure 2. Carboxyfluorescein (CF) Release Assay
Monitoring CF release through a liposome fluid to gel transition in the absence of cholesterol (left) and presence of cholesterol (right) to probe for lipid rearrangement. In the absence of αS no CF was released, but in the presence of αS CF was released showing αS's annealing ability upon binding. In the presence of cholesterol no CF efflux was seen, not implying rearrangement wasn't occurring, but rather that cholesterol may be acting like a plug preventing CF release.

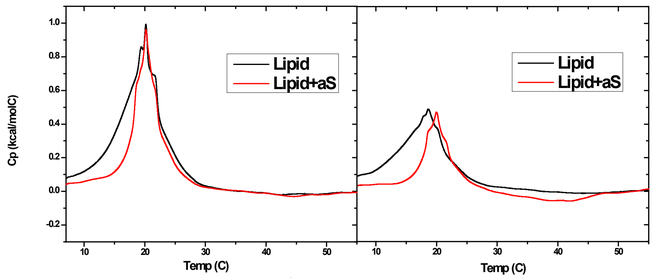

Figure 3. DSC Thermogram-Lipid Transition in the presence of 3-lipid mimic
DSC thermal denaturation of αS in absence (black) and presence of (red) LUV's (left) and SUV's (right). In the presence of αS in both LUV and SUV sized liposomes there is an increase in Tm and narrowing of the thermograms further showing αS annealing ability to create favorable van der Waals interactions between the acyl chains making the membrane more stable. The enthalpy and entropy decrease upon αS binding suggesting αS orders the membrane and makes it more rigid. The three phospholipids present in the mimic are shown below in Fig. 4.

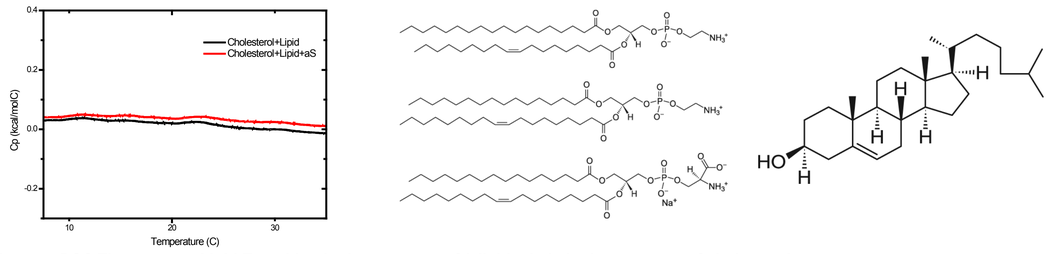

Figure 4. DSC Thermogram-Lipid Transition in the presence of 3-lipid mimic in the presence of cholesterol
DSC thermal denaturation of cholesterol containing LUV's in absence (black) and presence (red) of αS. No obvious phase transition was seen, resulting from cholesterol's ability to form domains and local Tms. DSC was done on liposomes containing the lipids shown above in a 38:38:24 POPE:SOPE:POPS mixture (pictured from top to bottom) in the presence (Fig.4) and absence (Fig.3) of 45% cholesterol (pictured to the right).

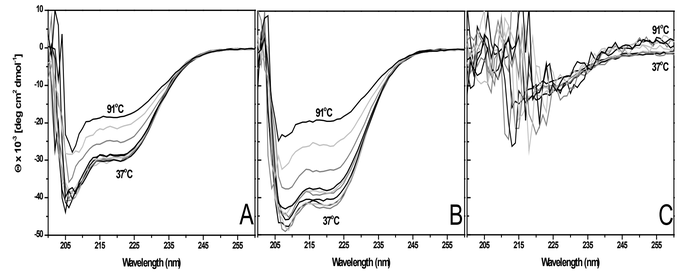

Figure 5. Circular Dichroism (CD)
The thermal denaturation of αS in the presence of (A) LUV's monitoring change in secondary structure, (B) SUV's, and (C) synaptic vesicle mimic SUV's. Enhanced helical character is seen in the membrane with the highest curvature showing αS specificity for curvature. The secondary structure of αS in the presence of complex mimic needs to be studied using a different method.

Figure 6. DSC Thermogram-Protein Transition
αS in the presence of complex synaptic vesicle mimic with physiologically relevant cholesterol composition and high curvature. A protein transition from folded to unfolded only took place in the presence of complex SV mimic. This suggests there are specific αS conformers only available in the presence of this complex composition and high curvature membrane.

Table 1. Thermodynamic parameters of 38:38:24 POPE:SOPE:POPS liposome LUV's or SUV's in the presence and absence of αS and cholesterol. Values determined through analysis of DSC thermograms.

	ΔH (kcal/mol)	Tm (°C)	ΔS (kcal/molK)
LUVs	7.76	19.3	0.0265
LUVs in the presence of cholesterol	0.47	15	0.0016
LUVs in the presence of αS	6.35	20.1	0.0217
LUVs in the presence of cholesterol and αS	0.85	17	0.0029
SUVs	3.31	17.6	0.0114
SUVs in the presence of αS	4.38	19.7	0.0149

Discussion

- I hypothesize that αS has varying available conformers due to changing membrane composition and curvature. The available conformers will then impact αS's binding and functionality.
- αS and membranes have a complex and intricate relationship.
- αS has the ability to anneal lipids leading to a more stressed membrane, possibly being a mechanism for neurotransmitter release.
- Membrane plays a role in available αS conformers, a change in available conformers could allow for the regulation of SV fusion.
- All of this information put together can lead to a binding mechanism that will shed light on the cause of protein aggregation in patients with PD.

References

1. Bartels, T.; Ahlstrom, L. S.; Leftin, A.; Kamp, F.; Haass, C.; Brown, M. F.; Beyer, K. The N-Terminus Of the Intrinsically Disordered Protein α-Synuclein Triggers Membrane Binding and Helix Folding. *Biophysical Journal*. **2010**, *99*, 2116–2124.
2. Fajardo, V. A.; Mcmeekin, L.; Basic, A.; Lamb, G. D.; Murphy, R. M.; Leblanc, P. J. Isolation Of Sarcolemmal Plasma Membranes by Mechanically Skinning Rat Skeletal Muscle Fibers for Phospholipid Analysis. *Lipids*. **2013**, *48*, 421–430.
3. Rhoades, E.; Ramlall, T. F.; Webb, W. W.; Eliezer, D. Quantification Of α-Synuclein Binding to Lipid Vesicles Using Fluorescence Correlation Spectroscopy. *Biophysical Journal*. **2006**, *90*, 4692–4700.