

# INTERACTIONS OF HELIX 1 IN ALPHA SYNUCLEIN WITH SDS MICELLES

Alpha-synuclein is a pre-synaptic terminal protein that is the primary protein component of Lewy body deposits. These deposits are considered the hallmarks of the neurodegenerative disease, Parkinson's, one that results from dopamine deficiency. Alpha-synuclein has also been shown to interact with vesicles containing neurotransmitters. Previous studies have determined that alpha-synuclein, although a member of the intrinsically unstructured family of proteins, adopts a helical structure in the presence of SDS micelles and lipid membranes. Further structural study of the protein has identified that the N-terminal tail contains two helical structures that have a propensity for reversibly binding to membranes, while the C-terminal tail is acidic, remains highly unstructured and is free of binding. The protein's structure is speculated to be ideal in securing synaptic vesicles to plasma membranes and regulating fusion. To further investigate the relationship between the first helix in the N-terminal tail and vesicle and plasma membranes, a truncated form of alpha-synuclein, only forty residues, was harvested. Experimental observation using NMR of helix 1 and its interactions with SDS micelles showed how tightly the protein binds with the membranes and whether or not the helices interact differently with micelles when independent of one another. Future studies comparing our results with the results of the interactions between the truncated protein and plasma membrane mimetic liposomes could reveal whether helix 1 has a specific inclination for a certain type of membrane, either vesicle or plasma. Determining whether this helical part of the protein will always bind to the same type of membrane can help us further elucidate the function of alpha-synuclein and its role in the pathogenesis of Parkinson's disease.

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## INTRODUCTION

The precise function of the  $\alpha$ -Synuclein pre-synaptic terminal protein still remains unknown, but a great amount of headway has been made to lead us to more insight on the topic. The small amyloid fibril-forming protein, Lewy body deposits in the *substantia nigra* part of the brain, was discovered to be a major component of the distinctive characteristics of Parkinson's disease. Two mutations of the  $\alpha$ -Synuclein protein have also been proven to lead to the early onset of the disease. The protein's interaction with hDAT, the human dopamine transporter, has further garnered interest in  $\alpha$ -Synuclein and its potential role in the disease's pathogenesis.

Structural study of the protein using, among other tools, circular dichroism, nuclear magnetic resonance (NMR), and fluorescence correlation spectroscopy have designated  $\alpha$ -Synuclein as a member of the intrinsically unstructured family of proteins. In the presence of lipid membranes and SDS micelles, however,  $\alpha$ -Synuclein adopts a helical structure in the N-terminal tail. It contains a series of 11-mer repeats there which substantiates that it can reversibly bind to lipid membranes, just like apolipoproteins with similar repeating series can. NMR studies have proven that the N-terminal tail of  $\alpha$ -Synuclein reversibly binds to lipid membranes and SDS micelles, while the C-terminal tail of the protein remains unbound and without a helical conformation. Due to its association with dopamine synthesis, it is postulated that the unbound C-terminal tail of  $\alpha$ -Synuclein is there to interact with other proteins while the N-terminal tail binds to lipids. Further study of the binding N-terminal tail has identified two helices,

one extending from residues 3 to 35, while the other helix encompasses residues 45 to 92. The break in the helices provides for a hairpin structure that is apparent when the protein is bound to micelles.

$\alpha$ -Synuclein has also been associated with PLD, an enzyme linked to vesicle formation and fusion.  $\alpha$ -Synuclein's structure seems to be ideal for oppositely oriented membranes, which is the case during vesicle fusion. Eliezer's model hypothesizes that both helices of  $\alpha$ -Synuclein can straddle the space between synaptic vesicles and plasma membranes when they are ready to fuse and stabilize the interaction. The C-terminal tail remains unbound and ready to interact with other proteins like PLD to further the interaction.  $\alpha$ -Synuclein's apparent role in the pathogenesis of Parkinson's disease could be its involvement in the vesicle fusion of dopamine-related vesicles. Study of each of the helices independent of one another can confirm whether each helix interacts differently when independent from the other and whether each helix will always bind to the same membrane in the same way.

## METHODS/MATERIALS

### Protein Growths

Using a plasmid PT-7 construct containing the DNA for WT  $\alpha$ -synuclein, provided by Dr. Peter Lansbury, and a QuikChange Site-Directed Mutagenesis Kit, a stop codon, TGA, was inserted at position 40, normally GTA. The truncated protein was grown in BL21(de3) cells using LB broth then spun down at 65000 rpm in a centrifuge at 24°C for fifteen minutes. The pellets were re-suspended in a wash media containing 200 ml 5×M9 salts, 700 ml

distilled H<sub>2</sub>O, 100  $\mu$ L CaCl<sub>2</sub>, 1 ml MgSO<sub>4</sub> and 1 ml ampicillin. This solution was spun down again and the pellets were then re-suspended in N<sup>15</sup> wash media containing all of the above and a combination of 1 g labeled Ammonium Chloride, 4 g unlabelled dextrose, 10 ml 100 $\times$  vitamins, and 50 ml distilled H<sub>2</sub>O. The solution was then aerated for three hours. The O.D. was checked at 30 min and one hour and induced with 500  $\mu$ L of IPTG at one hour when O.D. had reached 0.3. The solution was then transferred to centrifuge jars and spun at 65000 rpm at 4°C for fifteen minutes. The supernatant was discarded and the pellet stored in -20°C freezer overnight.

### Protein Purification

Cells were split open by re-suspending the pellet in 40 mL lysis buffer consisting of 200  $\mu$ L of 0.5 M EDTA, 1 mL of 1 M Tris, 0.0154 g of DTT, 1 mL of 100 mM PMSF and ~98 mL of distilled water and having the solution sonicated. The resulting solution was then put in a centrifuge with more lysis buffer to spin at 40000 rpm for one hour at 4°C. The pellet was re-suspended in 40 mL lysis buffer, sonicated again, and centrifuged as previously described. 0.5 g of Streptomycin Sulfate was added to the supernatants of both the first and second spin downs and stirred in a cold room for one hour. The resulting mixture was then put in a centrifuge again to spin at 15000 rpm for 15 minutes at 4°C. The supernatant was dialyzed overnight in a cold room in a buffer containing 25 mL 1 M Tris, 20 mL 1 M NaCl, 2 mL 0.5 M EDTA, and 953 mL distilled H<sub>2</sub>O to prepare for FPLC.

The protein was then purified using FPLC. Fractions, where there was a peak present in the chromatogram, were pooled and dialyzed overnight to be ready for HPLC. The fractions present in the HPLC peak were pooled and lyophilized.

### NMR Sample Preparation

In order to produce 700  $\mu$ L of 636  $\mu$ M sample to use in NMR studies

we dissolved 1.8 mg of our lyophilized N15 WT  $\alpha$ -Syn40stop protein in 700  $\mu$ L of SDS NMR Buffer pH 7.4. This buffer contained 40 mM SDS, 100 mM NaCl, 10 mM Na<sub>2</sub>HPO<sub>4</sub>, and 10% D<sub>2</sub>O. 2-D NMR was run using a Varian 600 MHz NMR magnet.

## RESULTS

### Protein Purification

Reviewing mass over time, we found a peak present between 30 and 40 minutes. The peak had a height of about a 100 mAU using a wavelength of 280 nm. A gel with samples of scattered fractions between 15 and 42 confirmed protein expression within the fractions we pooled to dialyze for HPLC. We also found protein elution between 30 and 46 minutes using a wavelength of 229 nm. We collected our fractions manually and were able to obtain about 75 mL of sample to lyophilize. A gel of different stages of purification and a sample of the HPLC fraction we lyophilized confirmed protein expression. The sample was lyophilized for two days and yielded about 2 mg of solid protein.

## DISCUSSION

We re-suspended our protein in an NMR buffer, already containing SDS micelles to mimic the synaptic vesicle membrane. The resulting NMR spectra plot peaked of resonant frequencies (in ppm) of the hydrogen nuclei against that of the N15 nuclei. We found that the interactions of the helix were different when it was by itself compared to when it was part of the full length construct.

We had expected the residues of our truncated protein to indicate that helix one bonded in the same way with the SDS micelles as the full length protein. However, that that was not the case. The peaks did not match up, suggesting that helix one interacts differently with micelles when independent of helix two. However being

wary of this very preliminary data, we analyzed it further and noticed that the peaks seemed to be from the C-terminal tail of the full length protein instead of the N-terminal tail. We ran the NMR experiment again at 10°C to produce a clearer spectrum. At 10°C, SDS becomes solid and anything attached to it will not show up in the NMR spectrum, but everything that is free, will. These data show the C-terminal tail very clearly implying that the protein we put in the sample is not, in fact, the truncated protein we had wanted to create. What we think happened is that the bacteria did not properly read the mutated DNA we introduced into them and instead created a new version of the wild-type protein. New primers were designed with two stop codons in succession to prevent this error from occurring again. Unfortunately we were unable to receive them before the allotted time and could therefore not run another experiment with the proper protein.

## FUTURE DIRECTIONS

The entire process will have to be redone using new primers. Hopefully, this new protein will be correct and the lab can accurately investigate the interactions of helix one with SDS micelles. The next step would be to investigate the helix's interactions with lipid mimetic liposomes. Future research will also be done using a construct of only  $\alpha$ -synuclein's helix two and its interactions with SDS micelles and lipid mimetic liposomes.

### RESOURCES

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