Structural Effects of Spermine Binding to WT $\alpha$-Synuclein and its A30P & A53T Mutants

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Introduction

- Parkinson’s disease is one of the most common age-related neurodegenerative disorders, affecting more than one million people in the United States.

- $\alpha$-Synuclein is a 140 residue protein which aggregates into amyloid fibrils that comprise a major component of the plaques (Lewy Bodies) found in the brains of Parkinson’s disease victims.

- Spermine is a naturally occurring polyamine in neuronal cells that increases the rate of aggregation and fibrillation of $\alpha$-synuclein, without inducing secondary structure.

- Spermine binds to the highly acidic tail, but also affects glycine and threonine residues in the N-terminal region.

- Two familial mutants associated with early onset PD exist with substitutions in this region: A30P and A53T. These mutants were investigated to determine whether or not these substitutions would have an effect on the [$\alpha$-synuclein + spermine] complexes.

Methods

- Ion Source
- Ion Funnel
- Drift Cell
- MS
- Detector

\[ F_{friction} - F_{el} \]

\[ \text{p(He)} \]

\[ \text{Arrival Time Distribution} \]

Time is measured starting from when the ions are pulsed into the drift cell, until they reach a detector. Inside the drift cell they encounter -5 Torr of helium gas. Larger structures will experience more collisions with the He, increasing the frictional drag, and lengthening the amount of time they spend in the cell, as compared to a more compact structure. This enables us to temporarily separate structures of different collisional cross-sections.

\[ v = \text{const.} \]
\[ \ddot{v} = K \cdot E \]
\[ K = \text{ion mobility} \]

Get shape information from ion mobility

Conclusions

- The A30P mutant only exists in a highly compact form with net charge -6.

- All three proteins behave the same in the presence of spermine.

- Spermine binds to an extended conformation, inducing a more compact form by lowering the net charge.

- Mass spectral results suggest spermine complexation may induce oligomerization of all three forms of $\alpha$-synuclein.

- Future plans include:
  - Investigating $\alpha$-synuclein complexes with other natural polyamines such as spermidine and putrescine.
  - Studying possible complexes formed between $\alpha$-synuclein and various pesticides.
  - Exploring whether the mitigating effects of nicotine and caffeine consumption are related to the folding of $\alpha$-synuclein.

References

Results

Mass Spectra

The mass spectrum of A30P α-synuclein shows a broad distribution at pH 7.5 which is shifted and significantly narrowed at pH 2.5. This behavior is consistent with a protein that is natively unfolded at pH 7.5 and partially folded at pH 2.5.

Cross section vs. charge state for WT α-synuclein and its A53T and A30P mutants. Two families of structures are clearly evident, one dominant at low injection energies, circles, and one dominant at high injection energies, diamonds.

Spermine

- Naturally occurring polyamine found in neuronal cells
- Known to be involved in neurodegenerative processes
- Chemical shifts show spermine binds to negatively charged C-terminus
- Significant intensity changes observed in region x22-93
- Intensities of G and T residues in this region 2-4 times higher than same residues in spectrum of free protein

Spermine Addition

ATDs

ATDs of several charge states taken at an injection energy of 50eV at pH 2.5. For charge states -7 and -8 the A30P mutant ATDs are much narrower than the WT and A53T mutant ATDs.

-6 Charge State

ATDs of the A53T α-synuclein mutant showing the presence of similar conformational families in the free protein in solution (a), the unbound protein in the complex solution (b) and the protein-spermine complex (c). A greater percentage of the population is in the compact form in the complex as compared to the unbound protein.