The Role of Neuronal Chaperone ProSAAS in Blocking α-synuclein Cytotoxicity

Nevin Varghese, Timothy S. Jarvela, Iris Lindberg
Department of Anatomy and Neurobiology, University of Maryland-Baltimore, Baltimore MD

Research Question

Is the neuronal chaperone proSAAS capable of decreasing α-synuclein-mediated cytotoxicity?

Abstract

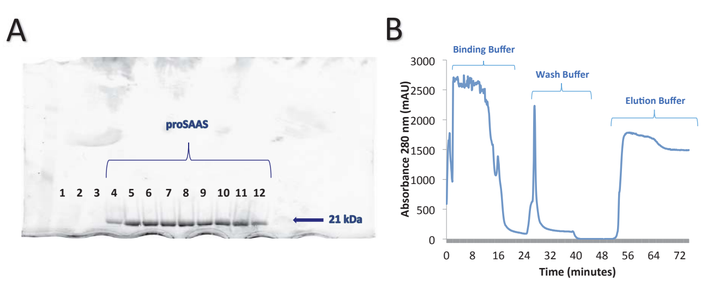
In Parkinson's Disease, abnormal aggregation of α-synuclein is toxic to dopaminergic neurons and results in serious neuronal dysfunction, impairing motor skills. Recent research suggests that molecular chaperone proteins can be cytoprotective against neurodegenerative biochemical processes including protein aggregation. ProSAAS is a widely expressed secretory chaperone in the brain. We hypothesize that proSAAS is cytoprotective against the aggregation and fibrillation of α-synuclein in Parkinson's Disease. The purpose of this research is to determine the ability of proSAAS to decrease α-synuclein cytotoxicity. Full length and truncation constructs of proSAAS were affinity purified and tested for cytoprotective effects. Addition of recombinant proSAAS to SH-SY5Y neuroblastoma cells overexpressing α-synuclein rescued the synuclein-mediated cytotoxic effects. To see if protection was specific for α-synuclein cytotoxicity, proSAAS was tested against other forms of cytotoxicity. ProSAAS had no effect on cytotoxicity due to oxidative stress (H_2O_2) or to endoplasmic reticulum stress (Tunicamycin). We conclude that proSAAS cytoprotection is specific for synuclein-mediated cytotoxicity. Ongoing research aims to determine the specific protein sequence responsible for cytoprotection, using purified truncated constructs of proSAAS, as highly conserved amino acid residues 160-180 have been shown to be critical in blocking *in vitro* fibrillation.

Background

Parkinson's Disease is the second most common neurodegenerative disease in the United States. It primarily affects the motor system, and symptoms result from dopamine loss and neuronal cell death in the substantia nigra. A key characteristic of Parkinson's Disease is abnormal α-synuclein protein aggregation into Lewy Bodies in neurons. α-synuclein is a 140 amino acid natively unfolded protein believed to have a functional role in synaptic transmission regulation. Proteostasis is a molecular process by which chaperones prepare or fix unfolded and misfolded proteins for translation or degradation. However, through environment stressors, accumulation of errors, and mutations, proteostatic mechanisms begin to fail and proteins like α-synuclein are more prone to misfolding, aggregating, and fibrillating. When the protein forms fibrils, it adapts toxic behavior which can cause proteasome disruption, mitochondrial dysfunction, and ER-Golgi traffic blockage. Probable therapeutic targets are secretory chaperones that block α-synuclein aggregation. The focus of this project is on the widely expressed neuronal secretory chaperone proSAAS. Preliminary data shows that proSAAS colocalizes with α-synuclein in Lewy Bodies in the substantia nigra. Further, proSAAS can block α-synuclein fibrillation *in vitro.* In this project, we produced proSAAS deletion mutants to test for efficacy at blocking α-synuclein toxicity in cell culture.

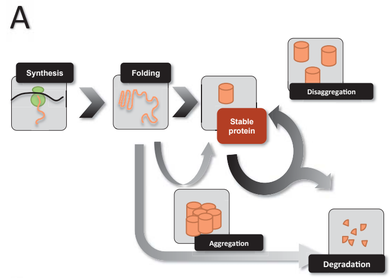
Methods

In order to prepare purified protein, recombinant proSAAS expression vectors were grown in bacteria. After inducing for protein expression with IPTG, the bacteria was incubated for 18 hours at 27°C and then centrifuged. Cell pellets were disrupted by addition of Bug Buster and lysozyme. Benzonase nuclease and PMSF were then added to break down DNA and inhibit proteases, respectively. The lysate was incubated while rocking for 15 minutes, and then centrifuged at 12.5k RPM for 20 minutes. The new sample supernatant was then affinity purified using fast protein liquid chromatography (FPLC), and collected fractions were analyzed by SDS-PAGE. Fractions with bands matching our desired proSAAS construct molecular weight were then dialyzed into 0.1 M acetic acid, concentrated by 10 kDa cutoff centrifugal filters, and diluted in 5 mM acetic acid for use in experiments.

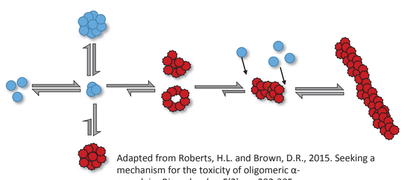


ProSAAS protein purification. A: ProSAAS protein fractions were collected and run on a SDS polyacrylamide gel. Fractions 4-12 show intense bands at 21 kDa, the molecular weight of "full length" proSAAS. **B:** Chromatogram of time versus absorbance following FPLC. Binding, wash, and elution buffer regions are shown for 21 kDa proSAAS sample.

Proteostasis Network and Fibrillation Pathway



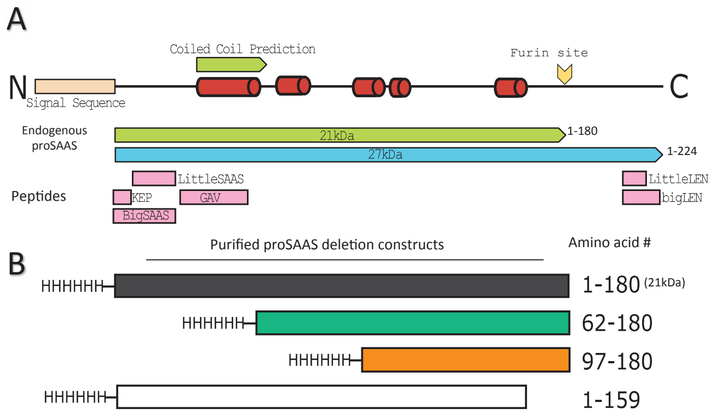
A: Proteostasis network. Proteins are synthesized in the endoplasmic reticulum. Molecular chaperones fold proteins to form stable proteins. When proteins are misfolded or destabilized from aggregation, they are sent to the ubiquitin-proteasome system for degradation.



Adapted from Roberts, H.L. and Brown, D.R., 2015. Seeking a mechanism for the toxicity of oligomeric α-synuclein. *Biomolecules*, *5*(2), pp.282-305.

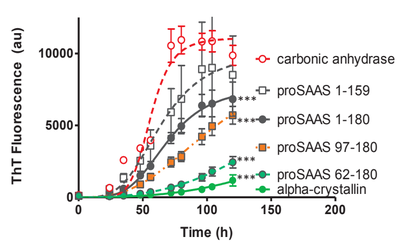
B: Fibrillation pathway. Native form monomers nucleate, forming potentially toxic annular or linear protofibrils. These protofibrils then elongate to form toxic fibrils. α-synuclein is thought to follow this unidirectional fibrillation pathway.

ProSAAS Secondary Structure



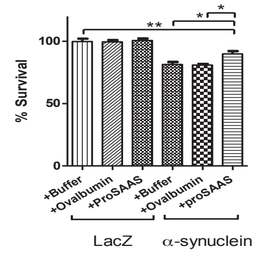
A: Structural representation of endogenous proSAAS (21 kDa and 27 kDa). Alpha helices are red, furin cleavage site is yellow, and known peptides are pink. **B:** N- or C- terminal deletion constructs of proSAAS were purified and used in cytotoxicity and fibrillation assays.

1. Residues 160-180 are Necessary for Optimal Anti-Aggregant Behavior



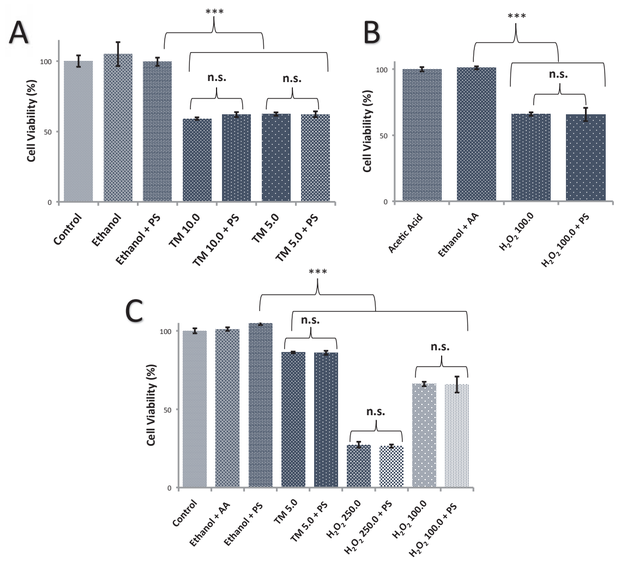
ProSAAS residues 160-180 are critical to preventing α-synuclein aggregation. Experiment of α-synuclein fibrillation inhibition by proSAAS 21 kDa, deletion constructs, carbonic anhydrase (control), and alpha-crystallin (positive control). Each point is represented by the mean ± SEM, n=6. (*p<0.05, **p<0.01, ****p<0.001, two-way ANOVA, vs.* carbonic anhydrase control).

2. ProSAAS Blocks α-synuclein Cytotoxicity



ProSAAS blocks α-synuclein mediated cytotoxicity. SH-SY5Y neuroblastoma cells were transfected with α-synuclein expression and LacZ control vectors. After 24 hours, they were split into a 96 well plate and treated with proSAAS, acetic acid buffer, and ovalbumin. After 48 hours, the percentage of surviving cells was measured through the WST-1 assay. Cell survival increased from 75% to 85% after proSAAS addition. Each point is represented by the mean ± SEM (*p<0.05, **p<0.01, *two-way t-test*).

3. ProSAAS Protection Against α-synuclein Cytotoxicity



ProSAAS cytoprotection is specific for α-synuclein toxicity. A: SH-SY5Y neuroblastoma cells were seeded into a 96 well plate. ProSAAS (PS) and two concentrations (5.0 µg/mL and 10.0 µg/mL) of ER stressor Tunicamycin (TM) were added. After 48 hours, cells were measured for percentage survival using the WST-1 assay. **B:** Cells were treated with proSAAS and oxidative stressor 100.0 µg/mL hydrogen peroxide (H_2O_2). **C:** Cells were treated with proSAAS, two concentrations (100.0 µg/mL and 250.0 µg/mL) of hydrogen peroxide, and 5.0 µg/mL Tunicamycin. Variability in toxicity between experiments is due to concentration of cells per well, but not in the relative effects of (+/-) proSAAS. Each point is represented by the mean ± SEM (n.s. p>0.05, ****p<0.001, two-way t-test*).

Conclusions

- **ProSAAS residues 160-180 are essential to its anti-aggregant function.**
- **ProSAAS is effective at reducing α-synuclein cytotoxicity in SH-SY5Y cells.**
- **ProSAAS cytoprotection is specific for α-synuclein toxicity in SH-SY5Y cells, as it has no effect on toxicity from ER or oxidative stress.**

Future Studies

- **Does proSAAS interact with α-synuclein in the extracellular space to prevent cytotoxicity?**
- **Does proSAAS block α-synuclein oligomerization intra- and/or extracellularly?**
- **Does proSAAS have an effect on an increased fibrillation α-synuclein A53T mutant?**
- **Create a finer map of proSAAS functional domains.**

Acknowledgements

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