Experimental study of protein phase separation, impact in Parkinson's disease.

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Abstract

Recent studies have shown that numerous proteins involved in neurodegenerative disorders have a high propensity to undergo liquid-liquid phase separation (LLPS). It's been suggested that, like amyloid formation, the ability to undergo LLPS may be a universal property of proteins and nucleic acids under specific conditions. Here, we demonstrate that α-synuclein LLPS might be driven by the interaction with biologically relevant cofactors such as spermine (Sp) or other neurodegenerative related protein as the Tau protein.

Motivation

Neuronal death

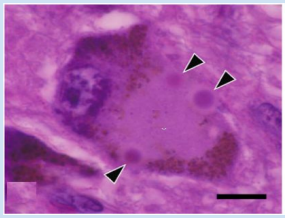
Mal-functioning of neuronal connections

Neurodegenerative disorders

Parkinson's disease **(PD)**, Parkinson's disease with dementia **(PDD),** and dementia with Lewy bodies **(DLB)** are known as Lewy bodies **(Lb)** diseases.

Lb inside of the neuronal cells of patients with this **NMD**

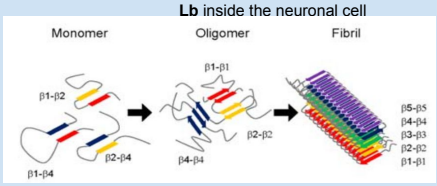
Amyloid fibrils of **α-synuclein (AS)** are the main component of Lewy bodies.



Lb inside the neuronal cell

Typically the protein in the cell is in the monomeric state.

Under certain conditions, the protein forms aggregates.



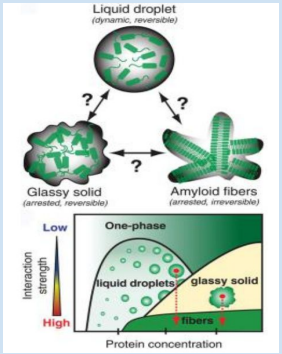
Hypothesis:

In the pathway to amyloid fibril formation do we observe liquid-liquid phase separation (formation of liquid droplets)?

Do we only have the formation of liquid droplets without reaching the fibril state?

Could this lead to a way of preventing Parkinson's disease?

Objective :
Evaluate the formation of the liquid droplets of α-synuclein under different experimental conditions

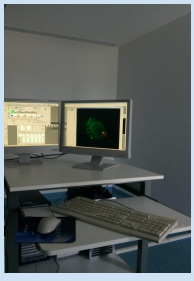


Experiments

The experimental technique used is fluorescence confocal microscopy.

The size of the droplets is typically 1-10 μm

Two types of images are taken: differential interference contrast (DIC) and fluorescence images.



Results

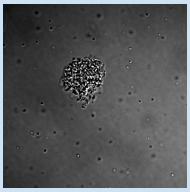
In all experiments the As is incubated at 37 °C for a short time (1 h), using 25 mM Tris-HCl buffer at pH 7.4.

We use the crowding agent, polyethylene glycol of molecular weight 6000 Da (PEG-6000), to simulate the highly-dense cell environment.

Set 1: Only AS

AS/PEG-6000	10 - 250(μM)	100 (μM)
0%- 20% (w/v)	noise	
10% (w/v)		noise
		NaCl/KCl 70/150(mM)

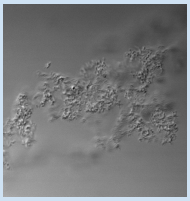
AS at 150 μM



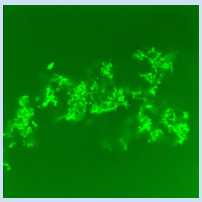
Set 2: (AS + ATP)/ AS-synuclein + Polyamines (AS 100 μM and PEG-6000 10% (w/v))

Polyamine (μM)	0-50	100	250-1000	ATP(1 and 5mM)
Spermidine		Noise	Aggregates	Aggregates
Spermine	Noise	Droplets		Aggregates

ATP at 5 mM, AS forms aggregates

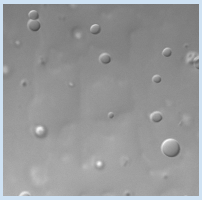


DIC image



Fluorescence image

Spermine at 100 μM
AS forms droplets

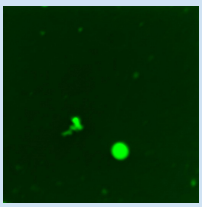


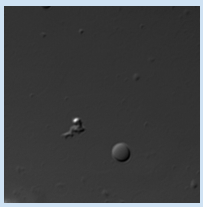
DIC image

Set 3: (α-synuclein + TAU)

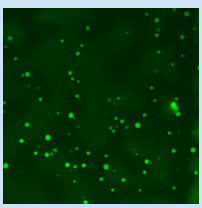
TAU (μM)	AS 0 μM	AS 100 μM
1	noise	Droplets
5	Droplets (TAU)	x
10	Droplets (TAU)	x

TAU 1 uM + AS 100 μM





TAU 5 μM



Conclusions

We have observed that under physiologic temperature and pH conditions, AS doesn't form liquid droplets for short periods of incubation time. The three biomolecules included in the experiments impact the phase behavior of AS. ATP and Spermidine promote a rapid aggregation of the protein, while spermine fosters the formation of liquid droplets. More extended experiments are required to determine if this will lead to the formation of AS amyloid fibrils under these conditions. Tau and AS can synergistically form liquid droplets implying a co-recruitment phenomenon.