Liquid-liquid phase separation: new insights into proteins aggregation and fibrillation

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Abstract

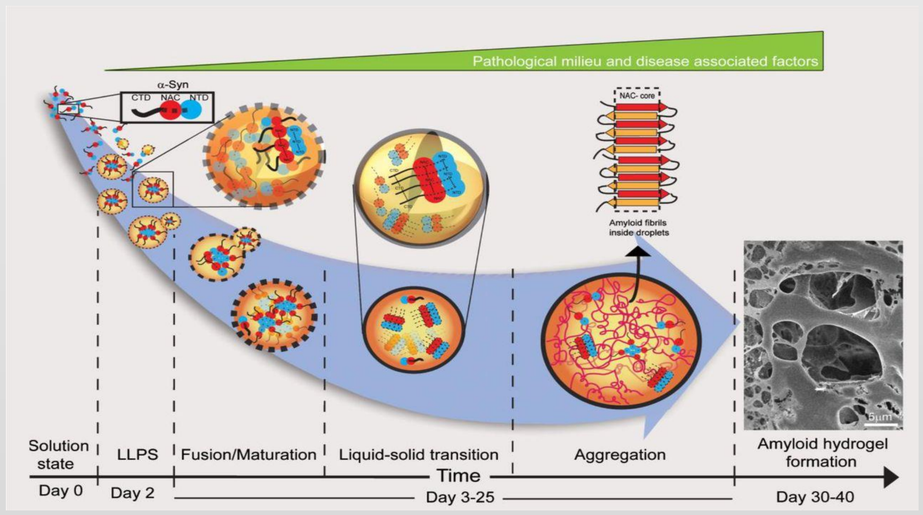
Cells are compartmentalized by numerous membrane-enclosed organelles and membraneless compartments to ensure that a wide variety of cellular activities occur in a spatially and temporally controlled manner. There have been known many evidences showing that; Membraneless compartments, called biomacromolecular condensates, have been formed via liquid-liquid phase separation (LLPS) and also have been observed in some neurodegenerative diseases. Phase-separated condensates participate in various biological activities, in the frame of this study, the presence of these structures in the proteins misfolding process can be considered. For an accurate understanding of biomacromolecular condensates that can transit into different states such as gel-like structures, solid aggregations and fibrils biophysical methods such as; Transmission Electron Microscopy (TEM), Fluorescence Recovery After Photo Bleaching(FRAP), fluorescence microscopy and atomic force microscopy measurements have been used. The existence of an association between phase separation with various human diseases have been shown involved in neurological diseases. By examining the kinetics of this process under the influence of various factors including: salts, protein concentration and physical parameters such as; temperature and pH, the mechanism of liquid-liquid phase separation is somewhat descriptive. However, the goal of primitive LLPS research was not simply triggered curiosity or any attempt to understand one of life's greatest unanswered questions, but made it possible to discover functions or structures useful to new applications. The phase separation provides a new and useful framework for understanding the mechanism of some sever human diseases, such as Parkinson and Alzheimer.

Keywords: Biophysics, Liquid-liquid phase separation (LLPS), Protein Aggregations, Fibrillation

Introduction

Membraneless compartments, called biomacromolecular condensates, have been formed via(LLPS) and also have been observed in some neurodegenerative diseases. Phase-separated condensates participate in various biological activities, in the frame of this study, the presence of these structures in the proteins misfolding process can be considered.

fig. 1. Liquid-liquid phase separation and liquid-to-solid transition mediate α-synuclein amyloid fibril containing hydrogel formation.



Schematic representation of the proposed mechanism for α-Syn LLPS and aggregation Monomeric α-Syn can undergo a phase transition event in the pathologically relevant milieu and in the presence of disease-associated factors.

Methods

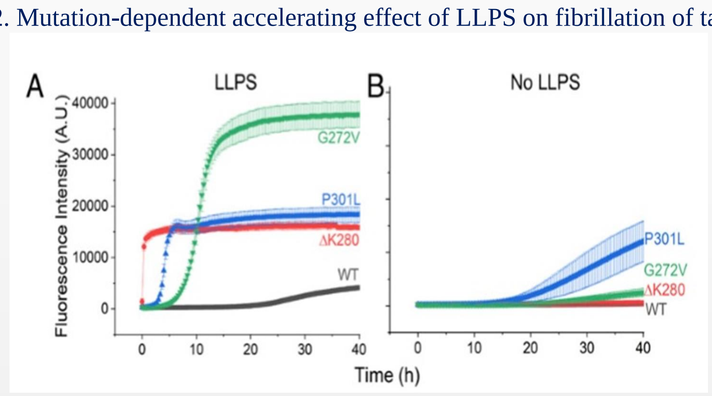
Biophysical methods:

- Microscopy → To provide detailed information on biomolecular condensates (To visualize the structure and composition)
- Bioinformatic tools or databases
- Fluorescence Recovery After Photo bleaching(FRAP) → To detect the material properties and dynamics of biomolecular condensates

Results & Discussion

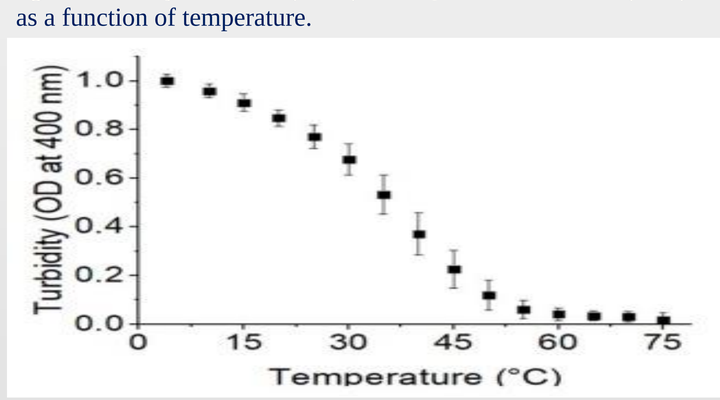
The existence of an association between phase separation with human diseases have been shown involved in neurological diseases. By examining the kinetics of this process under the influence of various factors including: salts, protein concentration and physical parameters such as; temperature, the mechanism of LLPS is somewhat descriptive. However, the goal of primitive LLPS research was not simply triggered curiosity or any attempt to understand one of life's greatest unanswered questions, but made it possible to discover functions or structures useful to new applications.

fig. 2. Mutation-dependent accelerating effect of LLPS on fibrillation of tau441.



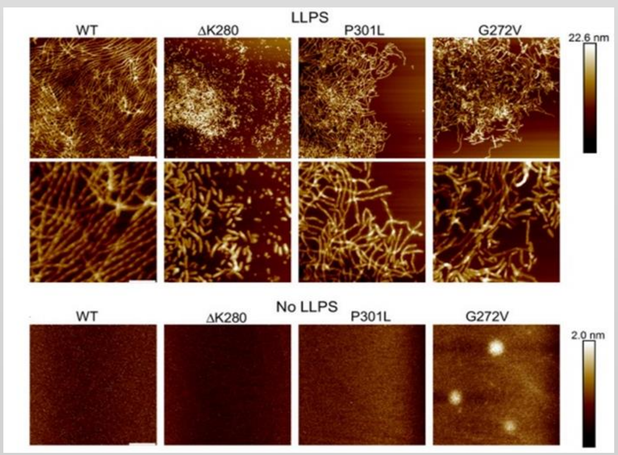
A.ThT fluorescence traces for wild-type tau441 and disease-related tau441 mutants (5 μM protein, 10 μM heparin in each case) under the conditions of LLPS (A) or in the absence of LLPS (B).

fig. 3. Turbidity of tau411 (10 M) in the presence of PEG (10%) as a function of temperature.



Samples were prepared by rapidly mixing protein and PEG solutions preheated to 75 °C, and turbidity was monitored in a cooling cycle by decreasing temperature at a rate of 2.5 degrees/min.

fig. 4. Mutation-dependent accelerating effect of LLPS on fibrillation of tau441.



Representative atomic force microscopy images obtained at the end of the growth phase of fibrillation reaction for each protein under LLPS conditions & absence of LLPS at the same time.

Conclusion

The phase separation provides a new and useful framework for understanding the mechanism of some sever human diseases, such as Parkinson and Alzheimer diseases.

References

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