



Dehydration under Cellular Conditions: A Study of Multi Charged Metabolites

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DESCRIPTION

In the presence of charge-balancing anionic counterparts, intrinsically disordered proteins rich in cationic amino acid groups can undergo Liquid-Liquid Phase Separation (LLPS). Arginine and Lysine are the two most common cationic amino acids in LLPS proteins, with arginine-rich proteins undergoing LLPS more readily than lysine-rich proteins, a feature commonly attributed to arginine's ability to form stronger cation interactions with aromatic groups. They show that arginine's ability to promote LLPS is independent of the presence of aromatic partners, and that at high salt concentrations, arginine-rich peptides, but not lysine-rich peptides, exhibit re-entrant phase behavior. They also show that the hydrophobicity of arginine is the determining factor in the dense LLPS phase's reentrant phase behavior and tunable viscoelastic properties.

Controlling the behavior of arginine-induced reentrant LLPS using temperature and salt concentration opens up possibilities for bioengineering stress triggered biological phenomena and drug delivery systems. Liquid-liquid phase separation is a key organizing principle for bimolecular condensates that compartmentalize specific metabolites in the absence of a biological membrane. This phenomenon involves macromolecules separating into two immiscible liquid phases: a dense phase rich in macromolecules (the coacervate phase) and a dilute phase depleted of macromolecules (the supernatant phase). Simple or complex coacervation driven by electrostatic interactions is the most basic form of LLPS. In polyelectrolyte and polyampholyte systems, this LLPS is easily realized.

However, naturally occurring proteins have more complex sequences, and LLPS is driven not only by electrostatic cation/anion interactions, but also by a slew of other enticing interactions such as cation interactions, hydrophobic interactions, interactions, hydrogen bonding, and van der Waals interactions. Both globular and Intrinsically Disordered Proteins (IDPs) can form LLPS, but IDPs account for the majority of biological LLPS. IDPs are frequently enriched in charged, aromatic, and polar amino acids, and their structural flexibility increases the likelihood of multivalent interactions. The amino

acid composition of IDPs influences the physical properties of the dense coacervate phase, such as diffusivity, interfacial tension, density, viscoelasticity, and exchange dynamics.

These LLPS properties are critical for bioengineering applications such as delivery agents for protein based drugs and mRNA vaccines. Thus, it is critical to comprehend the role of amino acid composition in tuning the relevant physical properties of the coacervate phase. Because of their cationic nature, nucleic acid binding ability, cell-penetrating properties, and biocompatibility, arginine-rich peptides or proteins is an important class of biomolecules with applications ranging from gene carriers to drug and even adhesive molecules. These proteins can undergo LLPS, and the resulting protein droplets have controllable cargo recruitment and release properties in response to external cues, making them one of the most promising delivery platform development options.

Arginine is a much more potent inducer of LLPS than other cationic amino acids with the same charge (such as lysine), indicating that electrostatic interactions do not fully account for cationic polymers' LLPS proclivity. They looked into two cationic-rich systems that could be subjected to LLPS: poly-lysine and protamine, the latter being an arginine-rich, clinically relevant, highly biocompatible, and injectable biomolecule used in cardiac surgery to counteract the anticoagulant effects of heparin. At low salt concentrations, both protamine and poly-lysine undergo LLPS, but only protamine exhibits salt and temperature-dependent reentrant phase behavior. They also demonstrate that the viscoelastic physical properties of the protamine and poly-lysine coacervate phases differ.

High concentrations of multi-charged metabolites found under cellular conditions or molecular chowders can be used to simulate similar dehydration conditions as those modeled in this study. The high salt concentration and the reentrant phase behavior in this study are expected to account for cellular protein phase separation under cellular conditions with enhanced hydrophobic interactions given the abundance of multi-charged metabolites and the fact that their levels are altered by cellular conditions, such as stress and cell division.

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