CHAPTER THREE

Osmolyte-IDP interactions during desiccation

Vincent Nicholson, Emma Meese, and Thomas C. Boothby*
Department of Molecular Biology, University of Wyoming, Laramie, WY, United States
*Corresponding author. e-mail address: tboothby@uwyo.edu

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Abstract

Desiccation, the extreme loss of water, poses a significant challenge to living organisms. Desiccation-tolerant organisms combat this in part by accumulating desiccation tolerance intrinsically disordered proteins (DT-IDPs) and osmolytes within their cells. While both osmolytes and DT-IDPs help maintain cellular viability on their own, combinations of the two can work synergistically to provide enhanced protection and survival. This review summarises our understanding of the interactions between DT-IDPs and osmolytes during desiccation, and explores possible molecular mechanisms underlying them. Using recent literature on DT-IDPs and on the broader study of IDP-osmolyte interactions, we propose several hypotheses that explain interactions between DT-IDPs and osmolytes. Finally, we highlight several techniques from literature on DT-IDPs that we feel are useful to the study of IDPs in other contexts.

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1. The role of IDPs and osmolytes in desiccation tolerance

Water is required for all metabolism and active life. Thus, one of organismal physiology's most captivating mysteries is the ability of certain organisms to survive prolonged periods in a desiccated state. Desiccation tolerance is observed across the tree of life and has evolved independently many times.[1,2] In order to achieve desiccation tolerance, desiccation-tolerant organisms enter into an anhydrobiotic (from Greek, meaning "life without water") state. In this state, metabolism drops to non-detectable levels, but often resumes quickly upon rehydration.[3,4]

The physiological and biochemical changes that occur in living organisms and cells during desiccation are myriad.[3–8] As the extracellular environment becomes increasingly hyperosmotic, water rapidly effluxes from the cell, concentrating and crowding the cytoplasm.[9,10] This is detrimental to the cell in several ways. At the ultrastructural level, the cell shrinking causes a disorganisation of the cytoplasm, triggering changes in organellar form and function.[11,12] At the molecular level, water loss disrupts the network of hydrogen bonds that helps maintain the fold of globular proteins, causing them to unfold and aggregate.[9,13,14] Water loss also affects membranes by destabilising them and promoting their phase transition from a liquid crystalline to a gel state.[3,8] It can further cause the accumulation of reactive oxygen species and the degradation of nucleic acids.[3,8,15,16] Combined, these changes make desiccation catastrophic for cells, and thus it is to be expected that anhydrobiotic organisms have evolved robust mechanisms for coping with these stresses.[17]

The exact mechanisms through which cells survive desiccation have been the subject of intense study.[4,18–23] Most, if not all, desiccation-tolerant organisms enrich osmolytes, especially disaccharides and polyols, during the drying process (Table 1).[24–28] In many organisms, this enrichment reaches extremely high levels, such that single osmolytes can account for up to 15% of an organism's dry mass after desiccation.[4,29,30] Several studies have concluded that osmolytes are essential for desiccation tolerance in a variety of different organisms.[27,31] Furthermore, enriched osmolytes are sufficient to increase the survival of desiccation-intolerant cells, and to stabilise labile macromolecules in vitro.[14,28,32–35]

In addition to osmolytes, several different families of intrinsically disordered proteins have been identified as mediators of desiccation tolerance, which we will refer to collectively as desiccation tolerance IDPs (DT-IDPs).[6]

Table 1 A non-exhaustive list of different desiccation tolerant organisms, their DT-IDPs, and some of their co-enriched osmolytes.

Organism	Osmolytes	Proteins	References
Arabidopsis thaliana (Mustard plant)	Proline, trehalose, sucrose, raffinose, glycerol, glycine betaine	LEA proteins, FLOE1, sHSPs	22,23,36,37,38
Hypsibius exemplaris (Tardigrade)	Trehalose	LEA proteins, TDPs, sHSPs	28,35,39
Caenorhabditis elegans (Nematode)	Trehalose, glycerol	LEA proteins, sHSPs	30,40,41
Saccharomyces cerevisiae (Budding yeast)	Trehalose, glycerol	sHSPs	8,42,43
Adineta vaga (Bdelloid rotifer)	Unknown, lack trehalose	LEA proteins, sHSPs	44–46
Artemia franciscana (Brine shrimp)	Trehalose, glycerol	LEA proteins, sHSPs	4,47–49
Physcomitrella patens (Land moss)	Mannitol, proline	LEA proteins, sHSPs, FLOE homologs	38,50–52

First identified in plants, DT-IDPs have now been identified in all kingdoms of life and appear to be ubiquitous among desiccation-tolerant organisms (Table 1).[4,6,19] They have been reported to perform a large number of diverse functions in vitro and in vivo. These include, but are not limited to, maintaining proteostasis, forming vitreous solids, stabilising membranes, and slowing metabolism.[53–57]

Far from being a uniform set of proteins, DT-IDPs can be divided into several evolutionarily distant groups, which can be broken down further into distinct families. One prominent group of DT-IDP is late embryogenesis abundant (LEA) proteins. LEA proteins have been known in the stress tolerance field for nearly 40 years, but many of the mechanisms underlying their function remain elusive.[18,36,58] As is implied by their

name, LEA proteins are heavily enriched in the late embryonic phases of plant seed development, during which many seeds become desiccation tolerant concomitantly as they dry. LEA proteins are thought to help maintain the seeds viability until imbibition and germination occurs.[18,22,59] While LEA proteins were first identified in plants, they have subsequently been identified in a variety of organisms across many kingdoms of life.[21,60,61]

Several other families of DT-IDPs are unique to the phylum *tardigrada*. These include the secreted-, mitochondrial-, and cytoplasmic-abundant heat soluble (SAHS, MAHS, and CAHS, respectively) proteins.[62,63] Together, these families are categorised as tardigrade disordered proteins (TDPs) and are essential for desiccation tolerance in tardigrades. The best studied TDPs, CAHS proteins, are capable of protecting labile molecules both in vitro and in vivo.[6,14,28,34,35,64–66] They also readily self-assemble into fibrous hydrogels, which while dispensable for in vivo osmotic stress tolerance and in vitro proteostasis, have been found to enhance the proteins' protective functions.[35,57,64,67–69]

In addition to DT-IDPs, there are many desiccation tolerance mediators that are only partially disordered. Small heat shock proteins (sHSPs) are well-folded proteins that contain intrinsically disordered regions (IDRs).[70,71] They are thought to play a role as active chaperones in desiccating cells.[72–74]

The exact mechanism through which DT-IDPs protect cells and cellular constituents from the rigours of drying is an area with many outstanding questions. One important facet of IDP biology in this regard is the fact that the ensemble and function of IDPs is heavily influenced by changes in their chemical environment. Due to the susceptibility of an IDP's ensemble to environmental change, it may be surprising that IDPs play important roles in desiccation, during which there are massive changes to the intracellular chemistry. However, accumulating evidence indicates that these changes to the intracellular environment may enhance the protective function of DT-IDPs. A prominent example is the presence of osmolytes, which several research groups have documented as working synergistically with DT-IDPs.[28,60,75,76]

There are several functional outcomes that could occur upon the interaction of an IDP with an osmolyte. A synergistic interaction is one in which the function of the resulting mixture is greater than the sum of its parts. Conversely, an osmolyte-IDP mixture may function additively (equal to the sum of its parts) or antagonistically (less than the sum of its parts).

Specifically, synergistic interactions between DT-IDPs and osmolytes have been described in desiccation tolerance literature for nearly 20 years.[60] However, past research on this subject has described this phenomenon without thoroughly investigating its nature, leaving several unanswered questions. For example, even though a wide variety of osmolytes have been implicated in desiccation tolerance, there is a large amount of literature that explores synergy with just a single osmolyte: trehalose. Additionally, the exact molecular mechanisms underlying osmolyte-IDP synergy are not well understood.

In this chapter, we explore interactions between IDPs and osmolytes in the context of the extreme chemistry of desiccation. We will summarise the empirical evidence that supports functional cooperativity between osmolytes and IDPs in desiccating systems. We will highlight what is known not only about trehalose-induced synergy, but also the potential of other small molecules to induce synergy. Using a simple model for the effect of solution chemistry on protein folding, we outline several hypotheses surrounding the underlying molecular mechanism behind osmolyte-IDP interactions in the context of desiccation.

2. Empirical evidence for osmolyte-IDP synergy during desiccation

Each group of desiccation tolerance mediators described above contains members that have been observed to work synergistically with at least one osmolyte.[60] This section will explore existing literature about osmolyte-IDP synergy during desiccation, highlight recent progress, and identify key gaps in the literature that beg further study.

2.1 Late embryogenesis abundant proteins

Of all DT-IDPs, LEA proteins have by far the most documented examples of synergy with osmolytes. The first observation of synergy between an LEA protein and osmolyte was with AavLEA1, a LEA protein from the nematode *Aphelenchus avenae* and trehalose.[60] Synergy was assessed using lactate dehydrogenase (LDH), a desiccation-sensitive enzyme, which can be protected during drying by the addition of excipients.[60] While both trehalose and AavLEA1 were found to protect LDH independently, a mixture of the two protected significantly better than the sum of their parts. In this study, BSA, a well-folded protein with a similar ability to AavLEA1

to preserve LDH on its own, was unable to work synergistically with osmolytes.[60] This suggests that the synergistic effect was dependent on the disordered nature of AavLEA1.

Synergy with trehalose has also been demonstrated in AfrLEA6, a LEA protein from brine shrimp.[75] Synergy was assessed using several in vitro enzyme functional assays, including LDH.[75] Intriguingly, trehalose appeared to induce this synergistic effect despite not noticeably affecting the secondary structure of AfrLEA6.[61] The mechanism(s) underlying synergy between AavLEA1 or AfrLEA6 and trehalose have remained elusive.

In addition, the synergy of LEA proteins has been assessed comparatively.[76] Using the LDH assay, 5 LEA proteins were assessed for synergistic protection when paired with two osmolytes: trehalose and sucrose.[76] Synergy was shown in 4 of the 5 LEAs. Importantly, LEA proteins were most synergistic when paired with an osmolyte that is enriched in the same organism during drying. One LEA protein, AvLEA1C, was not synergistic with either osmolyte. This protein was derived from a bdelloid rotifer which enriches neither trehalose nor sucrose during desiccation. On the whole, this suggests two key points. First, that synergy between LEA proteins and osmolytes is relatively widespread, and second, synergy is enhanced by endogenous osmolytes, suggesting a potential coevolution of DT-IDP sequence and the chemical environment.[76] Like in the AfrLEA6 study,[61] no changes in secondary structure were observed here.[76]

2.2 Tardigrade disordered proteins

Like LEA proteins, synergy has been observed between osmolytes and TDPs.[28] Trehalose, which is enriched in tardigrades during desiccation, increased the protective capacity of a representative TDP, CAHS D, by several fold.[28] Performing the same experiment with sucrose, which is not enriched by tardigrades, produced a significantly weaker synergistic effect. Interestingly, synergy with trehalose only manifests at or above the biological ratio of trehalose to CAHS protein found in dry tardigrades. In addition, synergy between trehalose and several different CAHS proteins was shown to be enhanced in vivo relative to the in vitro LDH assay.[28]

Also using CAHS D, synergy has been studied in the context of promoting certain structural changes.[76] Trehalose and sucrose were probed for their impact on CAHS D's local ensemble (secondary structure), global ensemble (expansion/compaction), and quaternary structure. Ultimately, the results from this study suggest that synergistic osmolytes do not heavily influence the local or global ensemble but instead induce

the oligomerization of CAHS D. While CAHS D polymerises into a fibrous hydrogel without the influence of any osmolytes, it does so at a lower concentration when either trehalose or sucrose is present, with trehalose being more effective than sucrose. Another osmolyte, glycine betaine, inhibits oligomerization of CAHS D and is antagonistic to CAHS D's protective function.

This work further demonstrates that this phenomenon may be explained from a physical chemistry perspective using transfer free energies (TFEs), a set of experimentally derived values that can be used to approximate the effect of an osmolyte on a protein's fold.[77] These values are specific to each combination of one amino acid and one osmolyte, and are additive such that they can be summed to approximate an omsolyte's effect on the conformational change of a whole protein. The following equation can be applied to calculate the ΔG_{tr}, which measures the change in free energy a protein undergoes upon transfer from water to a solution containing 1 M of some osmolyte, assuming no change in the protein's conformation.

$$\Delta G_{tr} = \sum_{N} \sum_{i=1} \alpha_i g_N$$

Here, α is the surface area of an amino acid residue in square angstroms, and g is the transfer free energy for that amino acid per square angstrom of exposed surface area.[77–79] Finally, N represents a particular amino acid, and i is the numerical index for all instances of that amino acid.[77–80] Negative ΔG_{tr} represent "attractive" solution environments, in which the osmolyte acts as a denaturant, and positive ΔG_{tr} values represent "repulsive" solution environments, in which the osmolyte promotes structure. By simply comparing the ΔG_{tr} of two protein conformations, one can mathematically approximate which conformational changes are favourable or unfavourable in the presence of some osmolyte.[77]

This method is well established for measuring the effect of osmolytes on well folded proteins, but is not commonly applied to IDP conformational changes.[76,77] It is, however, particularly powerful in the case of DT-IDPs. Not only do DT-IDPs (like all IDPs) have a high solvent-accessible surface area, which increases the strength of their interactions with osmolytes, but DT-IDPs function in the context of extremely high osmolyte concentrations, which further exaggerates this effect.[3]

In the case of CAHS D, transfer free energy predicts that trehalose and sucrose promote the dimeric state of the protein (which would promote

gelation), and that glycine betaine promotes the monomeric state (inhibiting gelation). This prediction generally agrees with structural and functional experiments on CAHS D-osmolyte interactions.

2.3 Small heat-shock proteins

Many sHSPs contain IDRs. While they are not fully disordered and thus cannot be considered DT-IDPs, several studies have investigated the effect of osmolytes on sHSPs. Literature dating back to the mid 90's has characterised synergistic interactions between sHSPs and trehalose.[81] As it stands, only two studies have documented synergy between sHSPs and osmolytes during drying, both using trehalose,[82,83] however, synergy has been demonstrated in other contexts such as thermal stress and oxidative stress.[81–85] For example, Kim et al. demonstrated that while HSP12 and trehalose can promote yeast survival, when mixed together they provide protection that is significantly greater than the sum of their individual parts.[83] Existing literature does not make an attempt to characterise the exact mechanism through which trehalose induces synergy in sHSPs.[82,83] However, the effect appears in a range of sHSPs from different species, indicating that synergy between IDPs/IDRs and osmolytes might be a common mechanism for surviving various types of stress.[82,83] The relevance of sHSPs to biology extends well beyond just desiccation, and further investigation of their synergy with osmolytes further will likely continue to be fruitful.[86]

2.4 Key gaps in knowledge

The existing knowledge summarised above informs us about the nature of DT-IDP osmolyte interactions, but they leave several unanswered questions and major gaps in our understanding.

- All but two of the studies limit themselves to a single osmolyte: trehalose. While trehalose is certainly important to desiccation tolerance in many organisms, it is certainly not the only relevant osmolyte. Notably, some desiccation-tolerant organisms make no trehalose at all during desiccation.[44,45,87]
- While there appears to be a relationship between osmolyte-induced oligomerization and synergy in TDPs, no such relationship has been identified in LEA proteins or in sHSPs.[76] Lastly, it is currently unclear what differentiates synergistic interactions from antagonistic ones, but TFE has been proposed as a possible explanation.

3. Deciphering the molecular basis of osmolyte-IDP synergy

A long-term goal in the protein biochemistry field has been to predict the impact of solution chemistry on a protein's fold. As such, there is nearly 60 years of literature dedicated to the subject. While this chapter cannot begin to explore protein-solution interactions from a theoretical or mathematical perspective, we direct readers to the dozens of articles on the subject, especially the works of Martin Gruebele, Wayne Bolen, and Charles Tanford.[77,88,89]

For the purposes of this review, we summarise this theoretical work with a simple but effective model in which "repulsive" osmolytes preferentially promote the burial of specific protein residues and "attractive" osmolytes preferentially promote the exposure of specific protein residues (Fig. 1). This model is relevant to the regulation of both intra-chain and inter-chain interactions, leading to diverse effects on proteins at all structural levels. Using this model, we can conceptualise hypotheses that potentially explain the structural basis for osmolyte-IDP synergy during desiccation.

Given the diversity of DT-IDPs, there are a large number of possible mechanisms through which synergy with osmolytes could arise. The below hypotheses are supported by evidence from the broader study of osmolyte-IDP interactions, and from biophysical studies of DT-IDPs. However, we

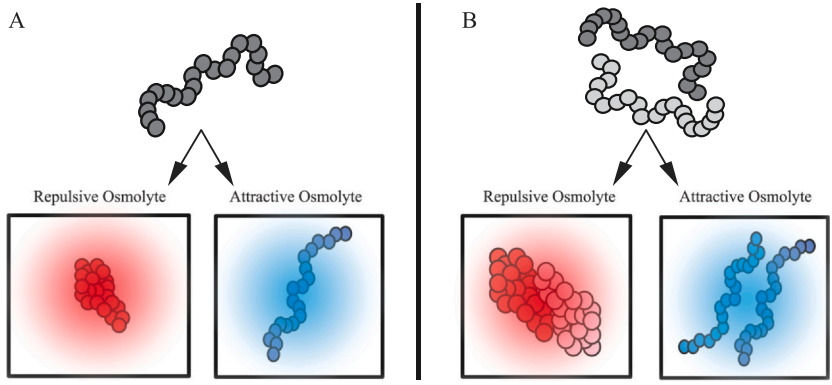

Fig. 1 Overview of how IDP ensembles could be influenced by the repulsiveness or attractiveness of their solution environment. (A) In a single protein chain, repulsive osmolytes induce global compaction and structuralization (red), while attractive osmolytes induce global expansion and disorder (blue). (B) Repulsive osmolytes stabilise protein-protein interactions (red) while attractive osmolytes stabilise dispersity (blue).

wish to stress that these hypotheses are not mutually exclusive. In addition, the mechanism(s) for synergy in one family of DT-IDPs may be, and likely are, different from mechanism(s) of synergy in other DT-IDP families.

3.1 Alpha helicity

A prominent hypothesis explaining synergy in DT-IDPs is that osmolytes induce the formation of alpha-helical secondary structure (Fig. 2A). Many DT-IDPs undergo a disorder-to-helix transition during the drying process.[68,90–92] Many research articles have suggested a link between this transition and a DT-IDP's ultimate function,[90,92] but only recently has this been demonstrated empirically.[66] Outside of desiccation tolerance, it is well documented that osmolytes can induce particular secondary structures, including helicity, in IDPs.[93–98] This generally involves an attraction interaction between the osmolyte with the protein's R groups, and a repulsive interaction between the osmolyte and the protein's backbone.[99–101]

LeBlanc and Hand (2021), which investigated synergy between the LEA protein AfrLEA6 and trehalose, implies this exact relationship.[75] By incubating solutions of AfrLEA6 at different relative humidities, they

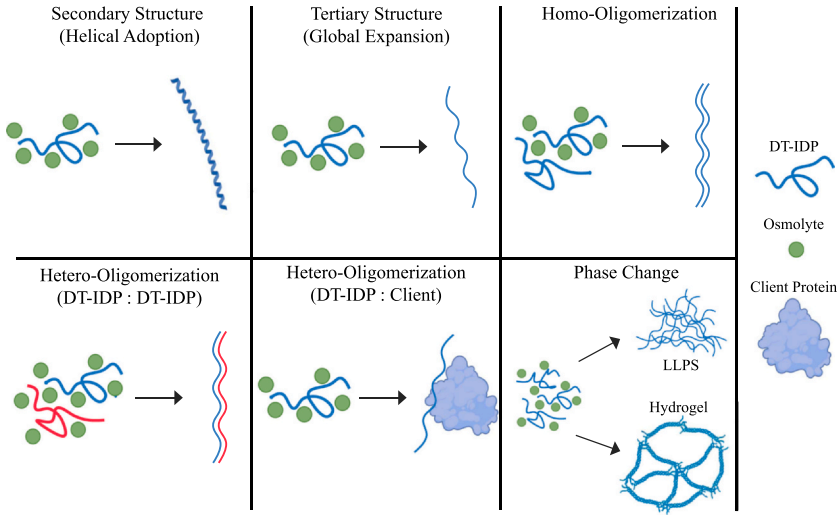

Fig. 2 Possible structural bases for osmolyte-induced synergy. In each panel, osmolytes (green circles) induce a change in the structure of a DT-IDP (blue). (A) Local secondary structure (helical adoption). (B) Global dimensions (expansion). (C) Homo-oligomerization. (D) Hetero-oligomierzation (DT-IDP: DT-IDP). (E) Hetero-oligomerization (DT-IDP: Client). (F) Phase change into an LLPS droplet (top) or hydrogel (bottom).

observe an increase in helicity, as water content decreases, that correlates with an increase in the function of the protein. They do not demonstrate, however, that this is the mechanism by which AfrLEA6 behaves synergistically with trehalose. KC et al. measured the secondary structure of six different DT-IDPs at varying concentrations of trehalose and sucrose. Strikingly, while these mixtures result in synergistic protection, neither osmolyte induces changes in secondary structure of the proteins in the hydrated state. Similarly, while each of these proteins became more helical upon drying, the presence of trehalose or sucrose did not affect this structural adoption.[76] Finally, it has been shown that the LEA protein AtLEA4–5 does not suffer reduced function when its helicity is broken through the insertion of prolines, although this is measured through freezing stress instead of desiccation stress.[102] Nonetheless, the diversity of DT-IDPs leaves the possibility that helicity is important for synergy in some, but not all systems.

3.2 Global dimensions

Another potential mechanism underlying osmolyte-IDP synergy during desiccation relates to the global dimensions of DT-IDPs. The molecular shielding hypothesis argues that DT-IDPs protect aggregation-prone proteins during desiccation by physically blocking the interaction of proteins that would otherwise aggregate.[19,60,103] Under this hypothesis, one might assume that DT-IDPs that are generally more expanded would occupy more space and thus prevent the association of aggregation-prone proteins. Such conformations could be stabilised by the presence of osmolytes (Fig. 2B). Indeed, several reports from non-desiccation-related research demonstrate that the global dimensions of IDPs are highly sensitive to the presence of osmolytes, and that osmolytes can induce global expansion through an attractive interaction.[94,97,104,105]

Despite this, a screen of DT-IDPs' structural plasticity in vivo found that their global dimensions decrease drastically when overexpressed in cells undergoing hyperosmotic shock.[98] In addition, KC et al. (2024) suggests that the global dimensions of LEAs and TDPs are insensitive to the presence of common desiccation-enriched osmolytes, even at the same molar concentrations that result in synergy.[76]

3.3 Homo-oligomerization

In addition to local and global changes to the DT-IDP ensemble, there are several promising hypotheses related to quaternary structure. Oligomerization

is commonly observed in DT-IDPs.[57,64,67–69,106] There is a large body of evidence indicating that LEA proteins form low-level oligomers, and TDPs are known to self-assemble into cytoskeleton-like networks.[18,57,64,67–69] Both TDP and LEA researchers have suggested that oligomerization may be important to the function of their respective proteins.[18,64,67–69] If one looks beyond desiccation tolerance literature, one finds many examples of osmolytes inducing or stabilising oligomerization of IDPs.[107–112] This points to a model in which osmolytes may modulate the function of DT-IDPs by inducing or stabilising oligomerized states through repulsive interactions (Fig. 2C).

There is already some research to support the idea that small molecules can modulate the formation of homo-oligomers in DT-IDPs. High concentrations of glycerol (>5 osM) induce the dimerisation of the LEA protein COR15A.[109] However, the authors do not directly attribute this effect to osmolyte-IDP interactions, but rather to non-specific effects arising from high solution osmolarity.[109] Another study demonstrated the ability of metal ions to induce the formation of "fuzzy complexes" in the LEA protein AtLEA4–5.[110] While both of these papers demonstrate oligomerization induced by the presence of osmolytes, neither of them show a specific structure-function relationship. As discussed above, some evidence suggests osmolyte-inducible oligomerization of the TDP, CAHS D, which correlates with the protein's protective function.[76] It also applies a general computational approach that may predict osmolyte-inducible oligomerization in other DT-IDPs. Together, these studies make osmolyte-induced oligomerization an especially strong hypothesis for the molecular basis of synergy.

3.4 Hetero-oligomerization

An alternative hypothesis for synergy looks at protein-protein interactions in a different light. In the same way that osmolytes can stabilise homotypic protein interactions, they could also stabilise heterotypic protein interactions (Fig. 2D). Importantly, many desiccation-tolerant organisms make more than one type of DT-IDP.[6,18,58] For example, there are 51 LEA proteins in *Arabidopsis thaliana* alone.[36] Several studies which investigated in vivo heterotypic interactions between LEA proteins using bimolecular fluorescence complementation found strong evidence that some combinations of LEA proteins associate with one another, while others do not.[106,113] The exact nature of these interactions are unclear, but the authors of these studies speculate that heterotypic interactions could be important for the protective function of LEA proteins.

Another hypothesis relating oligomerization and synergy has to do with IDP-client interactions. For example, if a LEA protein protects a sensitive enzyme by directly associating with it, osmolytes may promote or stabilise this interaction (Fig. 2E). However, Crilly et al. presented evidence implying that CAHS D (a TDP) and PvLEA4 (a LEA protein) interact with their clients through mainly non-specific interactions.[114] It is unclear whether or not osmolytes are able to stabilise such interactions. In this light, it is important to note that sHSPs, acting as chaperones, are confirmed to undergo specific interactions with their clients.[115] Thus, the stabilisation of protein-client interactions remains a promising explanation for osmolyte induced synergy during desiccation, at least in some systems. More research is needed to elucidate the relevance of protein-client interactions to osmolyte-induced synergy. This can likely be accomplished using LOVE NMR, molecular dynamics simulations, microscale thermophoresis, or several other techniques.[114,116–118]

3.5 Phase behaviour and localisation

A final mechanism through which osmolytes may modulate DT-IDP function is through inducing changes in phase behaviour or localisation (Fig. 2F). Many IDPs are capable of forming biomolecular condensates—large networks of macromolecules whose stable interactions overcome the entropic drive to disperse.[119,120] A commonly studied example is liquid-liquid phase separation (LLPS), which is the tendency for biomolecules to sequester themselves into membraneless condensates.[121,122] Research outside of desiccation tolerance has indicated that osmolytes can stabilise LLPS droplets.[123,124] The missing link is a direct correlation between LLPS and the function of DT-IDPs, which has yet to be demonstrated.[18,125] Another example of biomolecular condensation in DT-IDPs is the formation of hydrogels, which is observed in some TDPs and at least one LEA protein.[19,57,64,67,126] Hydrogel formation is not required, but promotes the protective function of TDPs, both in vitro and in vivo.[57,64,68] KC et al. (2024) suggests that the presence of synergistic osmolytes promotes the formation of hydrogels in CAHS D, whereas antagonistic osmolytes directly inhibit it.[76] It is unclear, however, whether or not this is merely a consequence of osmolytes promoting or inhibiting lower-level oligomerization.[76]

3.6 Overview

Having explored a wide variety of potential hypotheses explaining synergy in DT-IDPs (Fig. 2), it should be clear that there are many unanswered

questions in the field of IDP-osmolyte interactions during desiccation. DT-IDPs are highly diverse in their function and structure and have evolved to function in different organisms under different conditions. Osmolytes too are diverse in the nature of their interactions with IDPs. The hypotheses above are not an exhaustive list of all possibilities, but rather a partial collection of ideas supported by existing literature. We feel it is important to note that synergy may arise in a desiccated osmolyte-IDP mixture without any change in the IDP ensemble at all. One study found that the presence of LEA proteins was sufficient to change the physical properties of the vitreous solids formed by dried sucrose.[127] Vitreous solids are known to have a protective function inside desiccated cells, meaning that a change in the structure of vitreous solids could increase their efficacy and explain osmolyte-IDP synergy.[12,35,65,128–133] The list of possible explanations for synergy is vast, but we hope that the hypotheses above may guide future research into osmolyte-IDP interactions during desiccation.

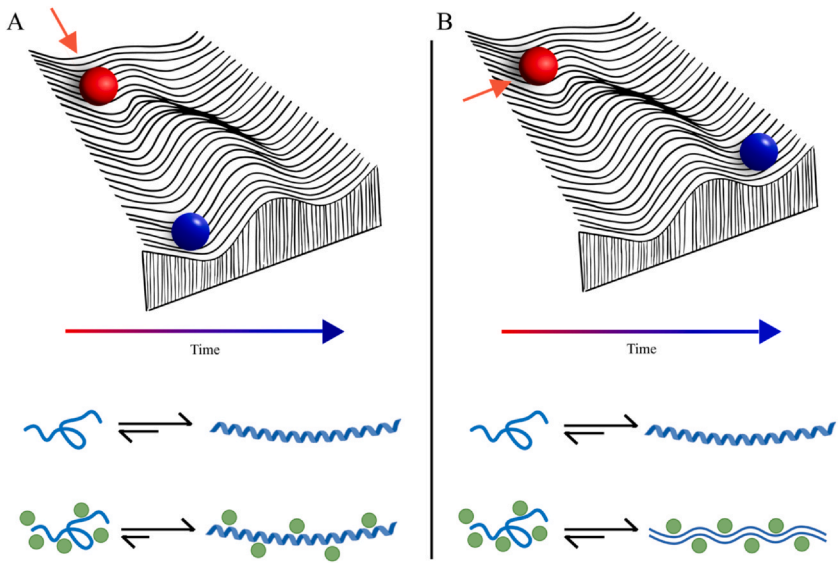

Fig. 3 Two hypotheses depicting the effect of osmolytes on the ensemble of DT-IDPs. Waddington's landscape is used to depict the conformational outcomes of DT-IDPs before and after drying. Gravity represents the thermodynamic forces imposed on DT-IDPs during drying without osmolytes present. The red arrow represents the thermodynamic forces imposed on DT-IDPs by osmolytes. (A) Osmolytes act cooperatively with other influences to reach the same end state. This enhances the stability of the end-state structure (bottom). (B) Osmolytes act perpendicularly with other influences, resulting in the formation of emergent structures.

4. Conclusions and future perspectives

Beyond stress tolerance, desiccation is a potentially fruitful model for studying osmolyte-IDP interactions. Studying IDPs is difficult both in vivo and in vitro thanks to their lack of a stable three-dimensional structure and their propensity for transient, non-specific binding. This means that osmolyte-IDP interactions can be difficult to characterise, often manifesting as subtle changes in an IDP ensemble or in some binding equilibrium. Desiccation makes osmolyte-IDP interactions far easier to study by acting as an exaggerated version of normal cellular conditions. Desiccation increases the concentration of both IDPs and osmolytes in any system, which increases the magnitude of osmolyte-IDP interactions relative to aqueous conditions. In addition, the function readouts for in vitro and in vivo assays used in the desiccation field are straightforward and quantitative. As such, desiccation tolerance provides researchers with a situation where the enrichment of IDPs and changing osmolyte levels are extreme as well as easily characterised in terms of function.

Importantly, there are several unanswered questions and gaps in our knowledge that must be addressed by future research. The existing literature on osmolyte-IDP synergy during desiccation is focused overwhelmingly on trehalose, despite the fact that it is not the only osmolyte produced by desiccation-tolerant organisms and is in fact absent from some desiccation-tolerant organisms altogether.[28,44,45] Several other desiccation-enriched osmolytes, such as glycerol and TMAO, would be excellent candidates for future research.[28,134] Additionally, more work is needed to understand the molecular mechanisms behind synergy. While there is evidence linking oligomerization and synergy in CAHS proteins, no such evidence currently exists for LEA proteins or sHSPs. Given the fact that the ensemble of LEA proteins seem to be unaffected by the presence of osmolytes in both the fully aqueous and fully dehydrated state, it is likely necessary to probe intermediate stages of drying.

Another major unanswered question relates to the exact role of osmolyte during desiccation. As discussed in this chapter, DT-IDPs are capable of disorder-to-order transitions during desiccation even when no osmolytes are present. This leaves two possibilities for the potential role of osmolytes. Osmolytes may influence DT-IDPs in a manner that is cooperative with the thermodynamic influences created during desiccation, meaning that they simply stabilise structure that would otherwise form anyway (Fig. 3). On the other hand, they may act perpendicularly with the

thermodynamic influences of desiccation to promote the formation of emergent structures (Fig. 3). There are examples of osmolytes showing both emergent and cooperative effects in non-desiccation-related IDPs.[94,135,136] When it comes to DT-IDPs specifically, KC et al. (2024) suggests a cooperative role for osmolytes with CAHS D, but this finding might not apply to every DT-IDP.[76]

An important implication of the study of synergy relates to organismal cross-tolerance. We feel it is likely that observations related to synergistic interactions during desiccation may also inform us about synergy during other stresses, and especially during freezing. Many organisms exhibit cross-tolerance between freezing and desiccation stress, and many DT-IDPs are enriched during both freezing and desiccation.[18,35,137] Likewise, osmolytes are enriched to high levels in organisms during freezing stress.[37,138]

We expect that several of the techniques and approaches highlighted in this review will be instrumental to answering these questions going forward. Two that we expect will be particularly important are the use of TFE to quantify the effects of osmolytes on an IDPs ensemble, and the use of LOVE NMR to probe the function of DT-IDPs at the residue level.[76,114] Both of these techniques are relatively new to the desiccation tolerance field and to the study of IDPs as a whole. Importantly, both of these techniques are not limited to the study of desiccating systems, and will likely be useful towards the study of osmolyte-IDP interactions in other contexts.

In conclusion, desiccation provides the ideal conditions for synergy between IDPs and osmolytes to occur. Within the desiccation tolerance field, these interactions have become increasingly important to understanding the desiccation response in different species. Knowledge of these processes has applications relevant to guarding the global food supply by increasing the drought resilience of crops, and to expanding access to medicine by protecting sensitive pharmaceuticals in the dry state. Looking beyond just desiccation tolerance, we see that the study of osmolyte-IDP interactions in desiccating systems can increase our understanding of these interactions in the hydrated state.

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