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Regulation and aggregation of intrinsically disordered peptides

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Intrinsically disordered proteins (IDPs) are a unique class of proteins that have no stable native structure, a feature that allows them to adopt a wide variety of extended and compact conformations that facilitate a large number of vital physiological functions. One of the most well-known IDPs is the microtubule-associated tau protein, which regulates microtubule growth in the nervous system. However, dysfunctions in tau can lead to tau oligomerization, fibril formation, and neurodegenerative disease, including Alzheimer's disease. Using a combination of simulations and experiments, we explore the role of osmolytes in regulating the conformation and aggregation propensities of the R2/wt peptide, a fragment of tau containing the aggregating paired helical filament (PHF6*). We show that the osmolytes urea and trimethylamine N-oxide (TMAO) shift the population of IDP monomer structures, but that no new conformational ensembles emerge. Although urea halts aggregation, TMAO promotes the formation of compact oligomers (including helical oligomers) through a newly proposed mechanism of redistribution of water around the perimeter of the peptide. We put forth a "superposition of ensembles" hypothesis to rationalize the mechanism by which IDP structure and aggregation is regulated in the cell.

protein folding | intrinsically disordered proteins | molecular dynamics simulations | osmolytes | tau protein

Most proteins in the human body have a well-defined, stable three-dimensional structure that is intimately tied to their physiological function. In the past few decades however, researchers have also identified a class of proteins that are natively unstructured. The latter, often referred to as intrinsically disordered proteins (IDPs) (1), are widespread and have the ability to quickly change their conformations to participate in a variety of biological processes. IDPs typically contain multiple charged side chains and few hydrophobic residues. Despite these characteristics, IDPs are not typically found in extended states but rather populate compact states due to hydrogen bonds and salt bridges (2, 3). IDP structures are highly regulated in the cell, and aberrant regulation often results in protein aggregation.

In this paper we consider the effect of external agents, specifically osmolytes, in regulating IDP structure and aggregation properties. To carry out this study, we focused on the microtubule-associated protein tau, a soluble (4), archetypical IDP found in the nervous system that helps regulate and stabilize microtubules (5, 6). When the regulation of tau structure and activity is compromised, tau loses its ability to bind to microtubules, and disassociated tau proteins can aggregate into supramolecular assemblies with a cross-β structure (7–9) typical of amyloid fibers. This aggregation process is a pathological hallmark of Alzheimer's disease and other forms of dementia known as tauopathies (10, 11). We consider here a segment of tau, the R2/wt fragment $_{273}$GKVQIINKKLDL$_{284}$, which contains the highly aggregation prone paired helical filament (PHF6*) (VQIINK) region. R2/wt aggregates in vitro in a manner qualitatively similar to full-length tau, therefore we expect that the effects of osmolytes on R2/wt are similar to those on full-length tau.

Our earlier work on this peptide showed that R2/wt is unstructured in solution, populating an ensemble of interconverting conformations (12). Two primary structural families emerged: a family consisting of extended conformations and a family consisting of compact conformations. The latter included structures that were stabilized by hydrogen bonding (primarily hairpins and, to a lesser extent, helices) or by salt bridges between aspartic acid (D) and lysine (K) groups, with salt bridges between K274 and D283 (located at opposite ends of the peptide) more prevalent than between adjacent residue pairs K280 and D283 or K281 and D283. Hydrogen bonded conformations are stabilized for enthalpic reasons, however, salt bridge ensembles are stabilized by both energetic and entropic contributions, as multiple conformations of the backbone are possible for any given salt bridge. The competition between salt bridge formation and hydrogen bonding is the reason that this peptide is intrinsically disordered, unable to find a unique structure. Conformations stabilized by hydrogen bonds are continually disrupted by the formation of salt bridges, and vice versa.

Here, we propose a new hypothesis that we term "superposition of ensembles." We propose that external regulating agents (osmolytes, crowders, etc.) or internal regulating mechanisms (mutations, posttranslational modifications, etc.) can shift the population of protein conformations, but do not necessarily introduce fundamentally new structures, and can thus define the basis of regulation. The subtle conformational shifts caused by these regulatory effects can then either enhance or suppress particular states, such as those possessing different levels of normal activity, or states with propensities to aggregate into abnormal pathological conformations. To explore this idea further, we consider the effect of the following external agents on the R2/wt tau conformation: (*i*) urea, an osmolyte used to

Significance

The microtubule-regulating protein tau is a prototypical intrinsically disordered protein (IDP) that plays an important physiological role in the human body; however, aggregates of tau are a pathological hallmark of Alzheimer's disease. Here we demonstrate through simulations and experiments with an aggregating tau fragment that cosolvent interactions can significantly affect the balance between hydrogen bonds and salt bridge formation in IDPs, subsequently determining their preferred conformations. These subtle perturbations can dramatically shift IDPs from compact ensembles to extended ones, thereby influencing aggregate formation. These results lend considerable insight into the biophysics of the regulation and aggregation of IDPs.

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The authors declare no conflict of interest.

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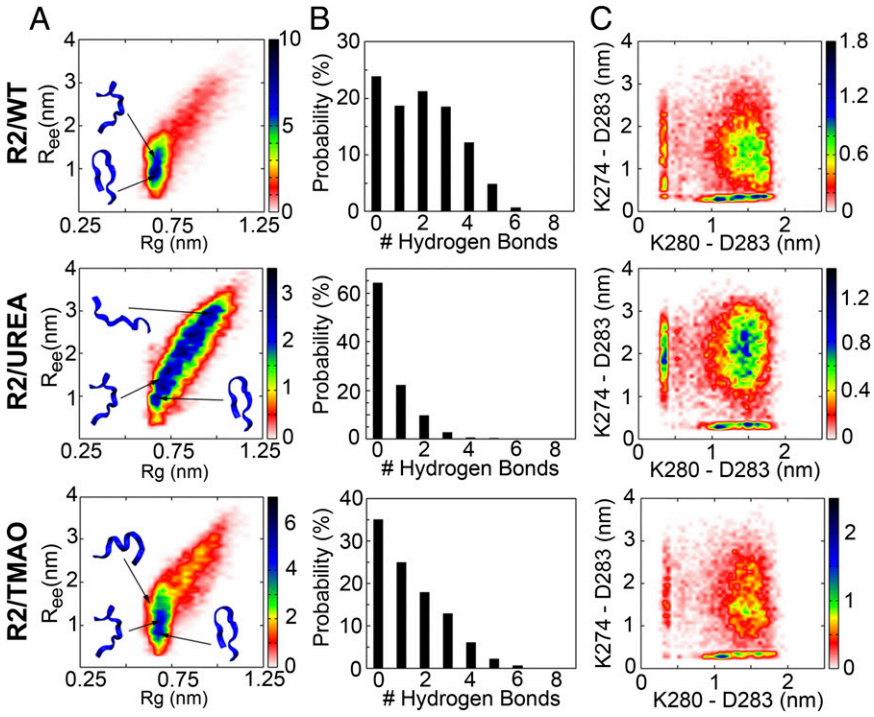

Fig. 1. (*A*) Population densities (in normalized arbitrary units) of peptide conformations at room temperature over 150 ns (R2/wt, R2/urea) or 100 ns (R2/TMAO). R_{ee} is a measure of the peptide end-to-end distance (C terminus to N terminus), and R_g is the peptide radius of gyration about its center of mass. Highly populated conformations are indicated in blue, whereas less populated conformations are indicated in red and yellow. (*B*) Probabilities of forming intramolecular hydrogen bonds (C=O to *N*–H) over the final 100 ns of simulations are shown. (*C*) Population densities of monomeric salt bridges are indicated by the relative proximities between lysine and aspartic acid.

denature globular proteins through presumed perturbations in peptide hydrogen bonding (13), and (*ii*) trimethylamine N-oxide (TMAO), an osmolyte that is known to stabilize globular proteins and act as a crowding agent (13). The effect of these agents on the monomeric state of R2/wt is studied using replica exchange molecular dynamics simulations, a methodology that enables efficient sampling of the diverse conformations populated by this peptide. The ability of R2/wt to aggregate in the presence of these external factors, in turn, is assayed experimentally using Thioflavin T (ThT) fluorescence spectroscopy to quantify aggregation rates and transmission electron microscopy (TEM) to determine aggregate morphologies. By combining statistics from molecular simulations with experimental observations, it becomes possible to study, at an atomic level, the intrinsic origins of protein disorder and regulation that play a leading role in most physiological processes.

Results and Discussion

Replica exchange molecular dynamics simulations were performed, as described in *Materials and Methods*, on R2/wt in the presence of urea (hereby referred to as R2/Urea) and in the presence of TMAO (R2/TMAO). Population densities are plotted for each case in Fig. 1 as a function of the peptide end-to-end distance and radius of gyration, along with the probabilities of forming hydrogen bonds or salt bridges.

Urea Promotes the Formation of Extended R2/wt Structures. It is apparent from Fig. 1 that the addition of urea leads to a dramatic decrease in the number of peptide hydrogen bonds, with a smaller effect on salt bridge formation. The overall result is a population shift for R2/wt into more extended structures (coexisting with semicompact ones, as shown in Fig. S1). Because the peptide becomes more extended in the presence of urea, salt bridges between distant residues (e.g., K274–D283) become less likely, favoring, if at all, salt bridges between nearby residues (in particular K280–D283). Similar to what we observe in pure water systems, R2/wt monomers in urea appear to favor K280–D283 salt bridges more than K281–D283 salt bridges, even though the two systems (pure water and urea) produce radically different monomer morphologies. As a whole however, the total number of R2/wt salt bridges in urea is less than in pure water, as reflected in Fig. 1 by the amount of R2/wt states in urea containing no salt bridges at all (i.e., states where the lysine-to-aspartic acid distance is more than 1 nm). Additionally, Table 1 shows significant R2/wt dehydration within 5 Å of the peptide surface (about two hydration shells) when urea is introduced, compared with pure water systems. Monomers in urea are only hydrated by 153 water molecules, compared to 200 water molecules in pure systems (a 25% reduction). The mechanism for this reduction is bound urea molecules that interact with the peptide side chains and backbone (Fig. S2), subsequently excluding water from the surface (Fig. 2). A more complete, per-residue osmolyte profile can be found in Fig. S3, where urea tends to aggregate near positively charged side chains such as K274, K280, and K281. As we will see in subsequent sections, the magnitude of the osmolyte's affinity for water plays a large role in determining how and where R2/wt is hydrated. For R2/wt monomers, we observed about 23 bound urea molecules for every 153 water molecules on the peptide surface, a ratio of only 6.7 water molecules per urea in the vicinity of R2/wt. Using a similar force field for urea, Pettit and coworkers (14) observed a ratio of 4.5 water molecules per urea in the vicinity of a denatured deca-alanine peptide, consistent with our observations.

TMAO Stabilizes Compact R2/wt Monomers. When TMAO is introduced to R2/wt monomers, there is only a modest change in the probability density plots of Fig. 1, with the peptide populating similar compact states as it did in water. This is perhaps not surprising, as TMAO is known to promote folding of globular proteins. Hence it is reasonable that an R2/wt peptide in an already-compact conformation remains compact with the addition of TMAO. TMAO reduces the formation of strand and turn secondary structures in R2/wt by 5–10%, however it does not affect the formation of helical monomers, which persist in TMAO to the same extent as in pure water, roughly 2.3% (the fourth most prevalent peptide conformation in each system). We observe preferential TMAO interactions with the peptide backbone (Fig. S3), although interactions with side chains were also observed to a lesser extent. This is in agreement with Cho et al.

Table 1. Average number of waters and osmolytes found within 0.5 nm of the surface of R2 in its most dominant conformations

Peptide	Solvent + cosolvent	No. waters ≤ 0.5 nm	No. osmolytes ≤ 0.5 nm	Water/ osmolytes
Monomer	Water	201	0	N/A
	Water + TMAO	174	9	19.3
	Water + urea	153	23	6.7
Dimer	Water	330	0	N/A
	Water + TMAO	258	13	19.8

(15), who modeled the effects of TMAO on dipeptide monomers and concluded that TMAO-mediated volume exclusion was responsible for peptide stabilization.

Although urea significantly reduces intramolecular hydrogen bonding in R2/wt, TMAO has a smaller effect on hydrogen bonding (Fig. 1*B*), with a 65% chance of observing one or more intramolecular hydrogen bonds (compared to 76% in water and 36% in urea). TMAO also reduces the probability of forming K280–D283 salt bridges, but increases the probability of forming K274–D283 salt bridges (which are located at opposite ends of the peptide). As a result, the slight reduction in hydrogen bonding is compensated by a moderate increase in salt bridging; therefore, R2/wt monomers in TMAO do not significantly shift their conformations from those found in pure water. TMAO also dehydrates the peptide surface, but to a lesser extent than urea. In Table 1 we show a reduction of water (within 5 Å of the monomer surface) from 201 waters to 174 waters (a 15% reduction), however there were significantly less TMAO molecules bound to R2/wt compared with urea (Fig. S2). We observed only nine bound TMAO molecules to 174 waters at the peptide surface, resulting in a water-to-TMAO ratio of 19.3. As with urea, a representative snapshot can be found in Fig. 2, which shows the relative amounts of water and TMAO present in the first two hydration shells. Although urea heavily crowds the peptide through electrostatic binding to its side chains, TMAO is much more diffuse at the surface and does not cluster as much.

Osmolyte Hydration Affects Peptide Stabilization. To explain the divergent behaviors of R2/wt in urea and TMAO, we examined the (radial) distribution of water and osmolyte relative to the entire peptide surface (Fig. S4). In pure water there are two hydration peaks on the surface of R2/wt at about 1.9 Å and 2.8 Å. Although the first hydration shell is unaffected by the presence of urea or TMAO, the second hydration shell is significantly reduced by both osmolytes. Urea, which has a larger dipole moment compared to TMAO, binds close to the surface of R2/wt at a distance of 3.2 Å. TMAO binds to R2/wt in much smaller quantities at a distance of 4 Å. The resulting changes in surface water density (Fig. S4) are consistent with the measurements in Table 1. However, our analysis also reveals interactions between the bound urea molecules themselves. There were multiple instances (some of which are visible in Fig. S2) where the negatively charged carbonyl oxygen on bound urea was oriented toward a positively charged amino hydrogen on a neighboring urea. This preferential attraction between bound urea helps explain why there is significant urea clustering near the surface of R2/wt. Another reason for enhanced urea clustering is that the osmolyte chemically mimics the C=O and NH peptide backbone. Therefore, it is often more energetically favorable for the peptide backbone to bind to a nearby urea molecule rather than wait for the peptide to rearrange itself to find a complementary amino acid residue (13). As a consequence we obtain the trends in Fig. 1*B*, where there is a strong urea-facilitated reduction in intramolecular hydrogen bonds. This reduction shifts the peptide into more extended conformational ensembles, which it was restricted from sampling when a large number of intramolecular hydrogen bonds were present.

In contrast to urea, the mechanism of action for TMAO stabilization appears to be much more subtle. If we examine the radial distribution function between osmolyte and water relative to the osmolyte's center of mass (Fig. S5), we see very different hydration profiles when comparing TMAO to urea. Urea is hydrated more tightly than TMAO (because it is more polarized). However, TMAO is more likely to interact with adjacent water molecules rather than with neighboring TMAO. This is consistent with our earlier simulations that showed that TMAO forms a complex with 2–3 water molecules, in agreement with experimental observations (16). We also show in Fig. S5 that the hydration shell around TMAO is wider than for urea. Urea has a hydration shell spanning 1.3 Å [at g(r) = 1], whereas TMAO has a hydration shell spanning 1.9 Å. Moreover, TMAO has been reported to stabilize native peptide states by acting as a molecular crowder (15, 17), as well as by virtue of being depleted from the surface as the protein adopts its native state (18, 19). Our earlier computational studies on bulk TMAO showed that the osmolyte's methyl groups did not act as conventional hydrophobic moieties, in agreement with the experiments of Sagle (18), which demonstrated that the methyl groups of TMAO oriented away from hydrophobic surfaces. In the presence of proteins, however, this effect (and related depletion of TMAO) may be muted, as protein surfaces do not necessarily present extended hydrophobic patches (20). In addition to the effect of TMAO crowding, a minor source of stabilization of the protein may arise from an entropic gain resulting from the release of TMAO/water from the vicinity of the protein, as suggested by van der Vegt and coworker (21) in the context of polymer compaction in the presence of urea.

The Superposition of Ensembles and Its Implication for Regulation. As highlighted in the work of Weinkam et al. on modeling the denatured state of the protein cytochrome *c* (22), peptides that are intrinsically unstructured necessitate a different theoretical framework than the one used for natively folded proteins. Polyampholyte models, such as those studied by Müller-Späth et al. (23), although attractive, are not well-suited to describe the tau protein, as these models treat the peptide as an ideal

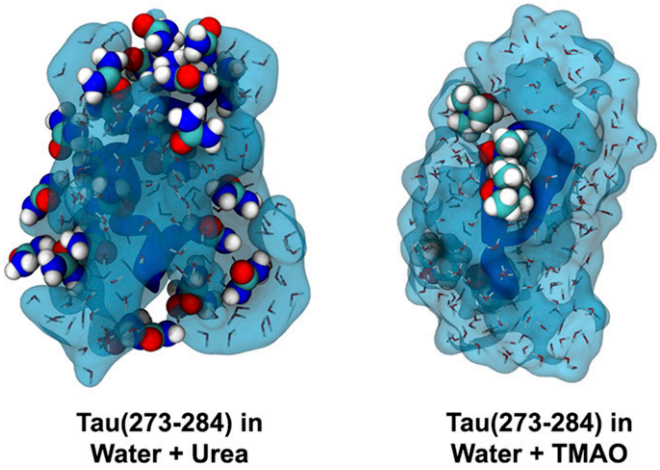

Fig. 2. Typical solvent and cosolvent surfaces around tau in the presence of urea and TMAO, out to 0.5 nm from the peptide surface. We observed significant peptide dehydration for systems containing urea and moderate peptide dehydration for systems containing TMAO. Urea also binds more closely and numerously to the peptide surface compared to TMAO.

polymer chain (i.e., a peptide that has no preference for any specific secondary structures). The tau peptide, on the other hand, although capable of exploring a large ensemble of conformations, is statistically more likely to populate a few subsets of such conformations. Taken together, the data presented here paints a picture in which external factors (e.g., osmolytes) can regulate R2/wt by shifting the conformational ensemble. An important observation is that no new structures emerged at the monomeric level. This highlights the fact that each ensemble is complementary, together sampling the entire conformational space accessible to R2/wt.

These simulations also indicate that extended conformations are more likely to appear when stable hydrogen bonds are not permitted, rather than when salt bridges are not allowed. This can be explained by the fact that multiple backbone conformations are possible for a given salt bridge connection. Therefore, peptides can change their conformations without necessarily breaking existing salt bridges, because such transitions are entropically favorable and do not incur energetic penalties when hydrogen bonds are absent. As a result, compact peptide conformations containing salt bridges can be viewed as transitional structures between fully hydrogen bonded structures and fully extended structures, with no hydrogen bonds present. Conformations containing salt bridges can also transition into a hydrogen bond-stabilized structure, with the associated loss of entropy compensated by a gain in enthalpy. Conversely, the salt bridge can detach and encourage extended peptide conformations, with the loss of backbone entropy (associated with multiple backbone conformations for a given salt bridge) and energy (due to the breaking of ionic bonds) compensated by an overall increase in the conformational entropy of the system. In the case of urea, extended peptide conformations are further stabilized through interactions with the osmolyte. TMAO has a more modest effect on R2/wt monomers. Despite the superposition of reduced hydrogen bond ensembles mixed with increased K274–D283 salt bridge ensembles, R2/wt maintains its compactness in the presence of TMAO primarily due to crowding effects (15). Other mechanisms for TMAO-facilitated stabilization in the context of larger oligomer systems (i.e., dimers) are discussed below.

These studies also suggest that the forming and breaking of hydrogen bonds and salt bridges can induce dramatic effects on the balance between different conformational ensembles available to intrinsically disordered peptides. Consequentially, changes in the local environment shift the equilibrium in one direction or another. The latter can be considered a regulatory effect, in which a set of conformations [including, presumably, the active form(s) of the protein] can be selected, as depicted in Fig. 3. An example can be found in full-length tau, where its affinity for microtubules, its ability to regulate microtubule growth, and its aggregation potential are all regulated through phosphorylation of serine and threonine side chains (24).

Regulation and Aggregation. When tau is misregulated, it can detach from microtubules and self-assemble into the amyloid fibrils that make up neurofibrillary tangles. Here, we investigate the effects of our regulating agents on R2/wt aggregation using both ThT assays and TEM studies, and interpret the experimental results using simulations. The ThT and TEM data are shown in Fig. 4. Large ThT signals are indicative of high β-sheet content, and are thus useful for quantifying the amount of aggregation as well as for following aggregation kinetics. TEM images reveal macroscopic fibril structures and are useful for gaining qualitative insight into the dominant aggregate morphologies. Together these descriptions offer a complementary picture that can be deconstructed and understood in terms of the mechanisms observed in simulations.

Urea hinders the aggregation process, as evidenced by the near-background ThT signals and empty TEM grids (Fig. 4). To

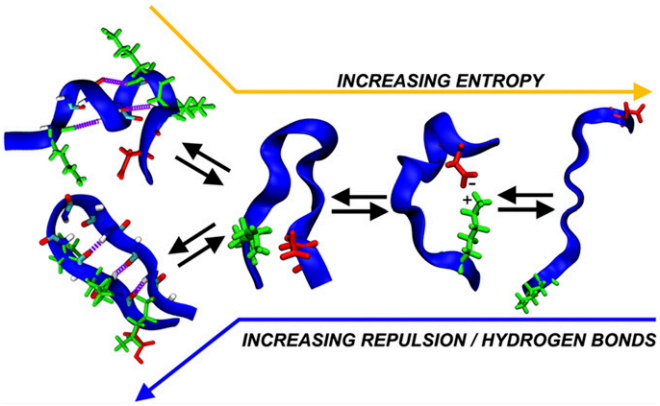

Fig. 3. The most likely conformations depend on environmental conditions. Adding urea breaks hydrogen bonds, which makes compact conformations unfavorable. Adding TMAO, on the other hand, redistributes water around the peptide exterior, which makes intramolecular hydrogen bonding more favorable.

explain this observation, it is helpful to revisit the effects of urea at the monomer level. Our simulations indicated that urea favors extension of the peptide backbone at the expense of stable hydrogen bonds. However, because hydrogen bonds are the main source of stabilization of small oligomers (and larger fibrils), urea may prevent aggregation by destabilizing or hindering the formation of the earliest aggregates. This result mirrors the work of Klimov et al. (25) and Cai et al. (26), which observed destabilization of amyloid-beta and NFGAIL oligomers in the presence of urea. There is also significant crowding of urea around the exterior of the peptide, as seen in Fig. S2, which subsequently reduces the number of intramolecular hydrogen bonds. Similar studies with urea have also looked at how changes in intermolecular hydrogen bonds affect dimerization. In the case of lysozyme (27) and NFGAIL (26), there is evidence that urea-facilitated decreases in intramolecular hydrogen bonds also contributes to decreases in intermolecular hydrogen bonds, thus reducing dimerization. When we attempted to simulate R2/wt dimers in the presence of urea, we also did not observe significant dimerization, in agreement with observations from other peptide systems (23–25). Thus, our simulations confirm that a lack of intermolecular hydrogen bonds, as a result of competitive urea binding, results in the formation of few or no peptide dimers. This in turn explains the small ThT signals and lack of aggregates on TEM grids.

TMAO Promotes the Formation of Helical Oligomers. Unlike urea, TMAO dramatically increases the rate of R2/wt aggregation, as evidenced by the rapid rise in the ThT signal (Fig. 4*A*). TEM demonstrates that these aggregates have morphologies typical of tau fibers (Fig. 4*B*). Aggregation on experimental time scales remained heparin-dependent. These results are in agreement with previous work on a longer tau segment containing the entire microtubule binding repeat region, and the carboxyl-terminal tail of the protein (Tau187) (28). To further investigate the effects of TMAO on R2/wt aggregation, we also performed simulations of R2/wt dimerization in the presence of TMAO. In contrast to urea, R2/wt dimers form quite effortlessly in TMAO (R2/TMAO). The probability densities for R2/TMAO are shown in Fig. 5. TMAO stabilizes compact dimer geometries, and we observe multiple conformations where one peptide chain becomes more compact as a dimer than as a monomer. A representative snapshot of the R2/wt dimer with nearby TMAO is presented in Fig. 5.

In simulations of R2/wt dimers in the presence of TMAO, we also observed dimer morphologies that were more spherical, or

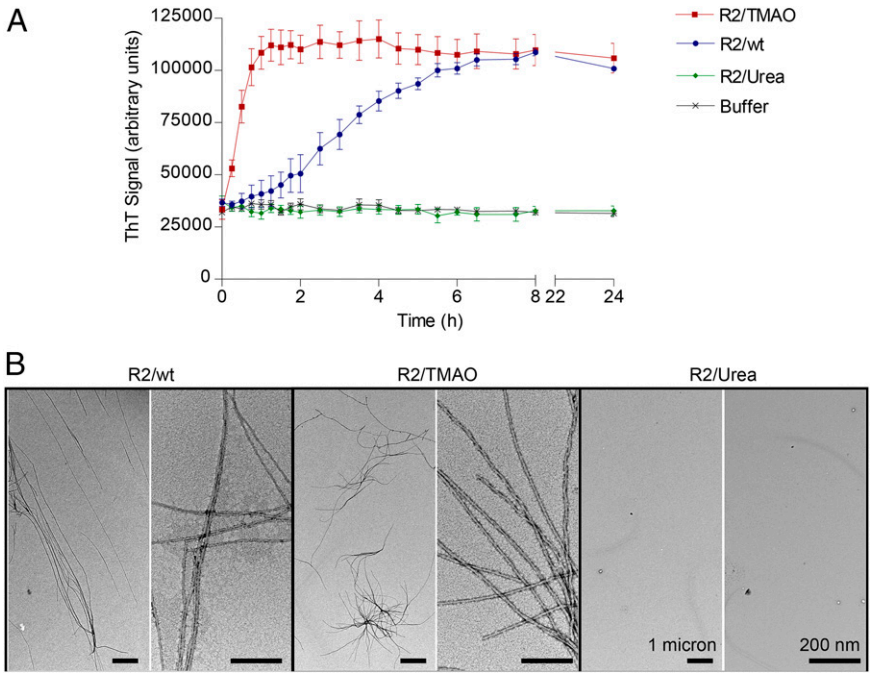

Fig. 4. (*A*) ThT and (*B*) TEM experiments reveal that urea suppresses R2/wt aggregation, whereas TMAO decreases the lag phase of tau aggregation. Experiments were performed in the presence of heparin, which greatly speeds up the time course of aggregation.

clumped together, compared with the R2/wt dimers in water. The two major structural families included dimers without clear relative orientation to one another (Fig. S6) stabilized by salt bridges, as well as a family of helical dimers. The emergence of a helical dimer structure (shown in Fig. S1, *Right*) occurs as the sixth most prominent conformation (3.5%) in R2/TMAO. A similar structure exists for R2/wt, but only appears as the 21st most prominent conformation (0.8%). This suggests that although TMAO has little effect on the population of helical peptide monomers, it can significantly increase the population of helical peptides in dimers or oligomers. In addition, Pazos and Gai (29) used IR spectroscopy to study the hydrogen bonding of water to a nitrile probe. Their experiments showed that the strength of the water–nitrile hydrogen bond was reduced in the presence of TMAO. This observation would imply a strengthening of intraprotein hydrogen bonds, which could explain the increase in helical structure of tau oligomers in the presence of TMAO. Although the mechanism of R2/wt aggregation is not known, there is experimental evidence (for several aggregating peptides) that fibril formation is preceded by the formation of helical intermediates. Our results suggest that TMAO may accelerate fibril formation by increasing the likelihood of forming such helical oligomers (30–32).

TMAO Maintains Compact R2/wt Dimers Through Water Redistribution. Looking further, we extracted per-residue osmolyte and hydration profiles for R2/wt dimers in the presence of TMAO (Fig. S7). We found that backbone hydration is much more uniformly distributed with TMAO present compared with dimers in pure water. Recall from Fig. S5 that TMAO preferentially interacts with nearby water molecules rather than with neighboring TMAO. In addition, we also observed that each TMAO that binds to an R2/wt dimer also redistributes its hydrating water molecules around the peptide surface, so that the overall hydration profile across each residue becomes more uniform. In contrast, urea forces the peptide hydration shell to become more asymmetric, where some residues lose water while others gain it. The uniformity in peptide hydration in the presence of TMAO encourages R2/wt dimers to take on more compact conformations, where hydrophobic residues seek refuge within the peptide interior (where water is typically excluded). Even though net dehydration occurs, the redistribution of water by TMAO subsequently stabilizes existing intra- and intermolecular hydrogen bonds, thus promoting the formation of clumped or spherical dimers. This effect is augmented by the effect of the TMAO–water complexes that act as microcrowding agents, favoring protein–protein association and priming R2/wt for further aggregation.

Conclusions

The human tau protein exemplifies the dynamics and characteristics of an IDP and is physiologically relevant for the study of Alzheimer's disease and other degenerative amyloid disorders. By atomically characterizing the conformational changes and aggregation propensities of IDPs using a computationally tractable, aggregating fragment of tau, critical interactions with the surrounding environment can be identified. Such interactions

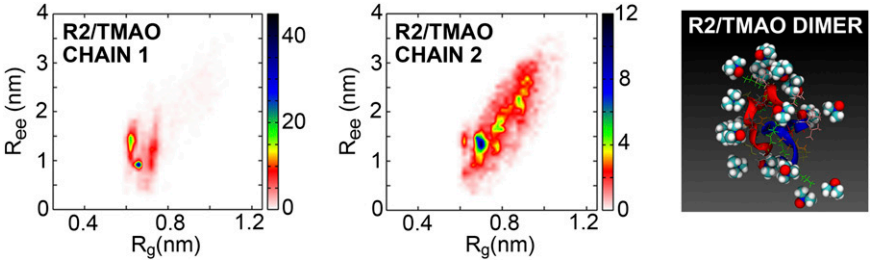

Fig. 5. Probability distribution of R2/TMAO dimers as a function of end-to-end distance and radius of gyration. Compared with R2/wt dimers, which are extended and anti-parallel, R2/TMAO dimers are spherical and remain compact.

can dramatically affect the folding and subsequent fibrilization of amyloid proteins. Here we performed replica exchange molecular dynamics simulations on a fully atomistic R2/wt tau peptide in the presence of osmolytes and studied the aggregation of these peptides using ThT fluorescence spectroscopy and TEM. These studies showed that both urea and TMAO affect the hydration of the peptide. Once urea is bound to both the peptide backbone and side chains, it interacts favorably with itself and tau in the vicinity of the peptide surface, thus repelling water. In contrast, TMAO interacts weakly with itself, and instead promotes water redistribution from hydrophilic residues to hydrophobic ones around the peptide exterior, encouraging the formation of stable (yet compact) tau conformations. Although TMAO is primarily attracted to the peptide backbone, interactions also exist between TMAO and R2/wt side chains. Our observations with urea are consistent with previous studies, however our observation of TMAO water redistribution and its effect on protein aggregation has not been reported previously. We chose to study a small system so that we could perform a comprehensive sampling of conformational space. However, we anticipate that our results would translate to longer IDPs as well. An important outcome of our simulations is that the addition of osmolytes merely shifts the conformational ensemble of tau but does not generate new monomer conformations. The resulting superposition of ensembles hypothesis provides a useful tool for decomposing complex IDP conformations into simplified ensembles for further study, allowing a better understanding of IDP regulation and aggregation in the context of protein-related diseases.

Materials and Methods

Simulation Conditions. As in previous publications (12), we used a short tau protein fragment $_{273}$GKVQIINKKLDL$_{284}$, which was studied by Mandelkow and coworkers (33). This fragment of tau (known as R2/wt) encompasses the PHF6* fragment VQIINK, which contributes significantly to the high aggregation propensity of full-length tau (34). A leap-frog algorithm was used to integrate Newton's equations of motion using an integration time step of 2 fs. Short-range electrostatics and Van der Waals interactions were truncated at 1.2 nm, and long-range electrostatics were calculated using the Particle Mesh Ewald algorithm (35), which uses Fast Fourier Transforms to reduce the computational complexity of molecular dynamic simulations. Periodic boundary conditions were also used to mitigate system size effects. Additional information can be found in *SI Materials and Methods*.

Experimental Conditions. The R2/wt peptide (Ac-GKVQIINKKLDL-NH2, 98.7% pure) was synthesized by GenScript. A 2-mM stock solution was prepared in deionized water and then flash frozen in liquid nitrogen and stored at –80 °C. For aggregation studies, R2/wt peptide was diluted to 50 μM in 20 mM ammonium acetate buffer, pH 7, with or without TMAO (2 M final) or urea (4.4 M final). When present, heparin (Sigma, product H5515) was added to 12.5 μM final to initiate aggregation. Additional information can be found in *SI Materials and Methods*.

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1. Uversky VN (2013) A decade and a half of protein intrinsic disorder: Biology still waits for physics. *Protein Sci* 22(6):693–724.
2. Teufel DP, Johnson CM, Lum JK, Neuweiler H (2011) Backbone-driven collapse in unfolded protein chains. *J Mol Biol* 409(2):250–262.
3. Mao AH, Crick SL, Vitalis A, Chicoine CL, Pappu RV (2010) Net charge per residue modulates conformational ensembles of intrinsically disordered proteins. *Proc Natl Acad Sci USA* 107(18):8183–8188.
4. Schweers O, Schönbrunn-Hanebeck E, Marx A, Mandelkow E (1994) Structural studies of tau protein and Alzheimer paired helical filaments show no evidence for beta-structure. *J Biol Chem* 269(39):24290–24297.
5. Hirokawa N, Noda Y, Tanaka Y, Niwa S (2009) Kinesin superfamily motor proteins and intracellular transport. *Nat Rev Mol Cell Biol* 10(10):682–696.
6. Desai A, Mitchison TJ (1997) Microtubule polymerization dynamics. *Annu Rev Cell Dev Biol* 13:83–117.
7. Chiti F, Dobson CM (2006) Protein misfolding, functional amyloid, and human disease. *Annu Rev Biochem* 75:333–366.
8. Bucciantini M, et al. (2002) Inherent toxicity of aggregates implies a common mechanism for protein misfolding diseases. *Nature* 416(6880):507–511.
9. Maji SK, Wang L, Greenwald J, Riek R (2009) Structure-activity relationship of amyloid fibrils. *FEBS Lett* 583(16):2610–2617.
10. Avila J, Lucas JJ, Perez M, Hernandez F (2004) Role of tau protein in both physiological and pathological conditions. *Physiol Rev* 84(2):361–384.
11. Lee VMY, Goedert M, Trojanowski JQ (2001) Neurodegenerative tauopathies. *Annu Rev Neurosci* 24:1121–1159.
12. Larini L, et al. (2013) Initiation of assembly of tau(273-284) and its ΔK280 mutant: An experimental and computational study. *Phys Chem Chem Phys* 15(23):8916–8928.
13. Canchi DR, García AE (2013) Cosolvent effects on protein stability. *Annu Rev Phys Chem* 64:273–293.
14. Kokubo H, Hu CY, Pettitt BM (2011) Peptide conformational preferences in osmolyte solutions: Transfer free energies of decaalanine. *J Am Chem Soc* 133(6):1849–1858.
15. Cho SS, Reddy G, Straub JE, Thirumalai D (2011) Entropic stabilization of proteins by TMAO. *J Phys Chem B* 115(45):13401–13407.
16. Larini L, Shea JE (2013) Double resolution model for studying TMAO/water effective interactions. *J Phys Chem B* 117(42):13268–13277.
17. Ma J, Pazos IM, Gai F (2014) Microscopic insights into the protein-stabilizing effect of trimethylamine N-oxide (TMAO). *Proc Natl Acad Sci USA* 111(23):8476–8481.
18. Sagle LB, et al. (2011) Methyl groups of trimethylamine N-oxide orient away from hydrophobic interfaces. *J Am Chem Soc* 133(46):18707–18712.
19. Canchi DR, Jayasimha P, Rau DC, Makhatadze GI, Garcia AE (2012) Molecular mechanism for the preferential exclusion of TMAO from protein surfaces. *J Phys Chem B* 116(40):12095–12104.
20. Athawale MV, Dordick JS, Garde S (2005) Osmolyte trimethylamine-N-oxide does not affect the strength of hydrophobic interactions: Origin of osmolyte compatibility. *Biophys J* 89(2):858–866.
21. Rodríguez-Ropero F, van der Vegt NF (2014) Direct osmolyte-macromolecule interactions confer entropic stability to folded states. *J Phys Chem B* 118(26):7327–7334.
22. Weinkam P, Pletneva EV, Gray HB, Winkler JR, Wolynes PG (2009) Electrostatic effects on funneled landscapes and structural diversity in denatured protein ensembles. *Proc Natl Acad Sci USA* 106(6):1796–1801.
23. Müller-Späth S, et al. (2010) From the cover: Charge interactions can dominate the dimensions of intrinsically disordered proteins. *Proc Natl Acad Sci USA* 107(33):14609–14614.
24. Stoothoff WH, Johnson GV (2005) Tau phosphorylation: Physiological and pathological consequences. *Biochim Biophys Acta* 1739(2-3):280–297.
25. Klimov DK, Straub JE, Thirumalai D (2004) Aqueous urea solution destabilizes Abeta (16-22) oligomers. *Proc Natl Acad Sci USA* 101(41):14760–14765.
26. Cai Z, et al. (2014) Effect of urea concentration on aggregation of amyloidogenic hexapeptides (NFGAIL). *J Phys Chem B* 118(1):48–57.
27. Hua L, Zhou R, Thirumalai D, Berne BJ (2008) Urea denaturation by stronger dispersion interactions with proteins than water implies a 2-stage unfolding. *Proc Natl Acad Sci USA* 105(44):16928–16933.
28. Scaramozzino F, et al. (2006) TMAO promotes fibrillization and microtubule assembly activity in the C-terminal repeat region of tau. *Biochemistry* 45(11):3684–3691.
29. Pazos IM, Gai F (2012) Solute's perspective on how trimethylamine oxide, urea, and guanidine hydrochloride affect water's hydrogen bonding ability. *J Phys Chem B* 116(41):12473–12478.
30. Abedini A, Raleigh DP (2009) A role for helical intermediates in amyloid formation by natively unfolded polypeptides? *Phys Biol* 6(1):015005.
31. Kirkitadze MD, Condron MM, Teplow DB (2001) Identification and characterization of key kinetic intermediates in amyloid beta-protein fibrillogenesis. *J Mol Biol* 312(5):1103–1119.
32. Anderson VL, Ramlall TF, Rospigliosi CC, Webb WW, Eliezer D (2010) Identification of a helical intermediate in trifluoroethanol-induced alpha-synuclein aggregation. *Proc Natl Acad Sci USA* 107(44):18850–18855.
33. von Bergen M, et al. (2001) Mutations of tau protein in frontotemporal dementia promote aggregation of paired helical filaments by enhancing local beta-structure. *J Biol Chem* 276(51):48165–48174.
34. Li W, Lee VM (2006) Characterization of two VQIXXK motifs for tau fibrillization in vitro. *Biochemistry* 45(51):15692–15701.
35. Essmann U, et al. (1995) A smooth Particle Mesh Ewald method. *J Chem Phys* 103(19):8577–8593.