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Trimethylamine *N*-Oxide Stabilizes RNA Tertiary Structure and Attenuates the Denaturating Effects of Urea

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RNA folding is governed by certain rules of engagement between the sequence and the solvent. Favorable electrostatic interactions involving specific or nonspecific cation binding and a set of sequence-dependent interactions, such as hydrogen bonding, base stacking, and hydrophobic bonding, drive RNA secondary and tertiary structure formation. Since the strength of these interactions is solvent-dependent, changes in the solvent would affect stability. The global stability of RNA decreases in urea-containing solution since urea destabilizes both secondary and tertiary structure.[1] Changing the solvent from water to an organic solvent−water solution decreases secondary structure stability.[2] Surprisingly, it is reported that methanol stabilizes RNA tertiary structure and trimethylamine *N*-oxide (TMAO) helps drive reconstitution of ribosomes.[3] These results raise the issue of the important role the solvent plays in stabilizing different types of RNA structure.

We are exploring how altering the solvent affects RNA stability, by measuring how TMAO perturbs RNA stability. TMAO is an osmolyte, a class of naturally found compounds used by cells to maintain viability in harsh environments, and is known to stabilize proteins. Exclusion of TMAO from the protein surface due to repulsive interactions between TMAO and the amide backbone increases protein stability.[4] Several marine organisms use a 1:2 ratio of TMAO/urea to counteract the osmotic stress of the ocean.[5] Several investigations show TMAO counteracting urea-induced protein destabilization at this and other ratios.[6] RNA, of course, must be functional in this environment. We are the first to show TMAO stabilizing $tRNA^{fmet}$ tertiary structure and counteracting the denaturing effects of urea. This is surprising, considering the differences in chemical composition of proteins and RNA. Whether both share a common mechanism of stabilization is unknown, but this result accentuates the importance of solvent effects on RNA stability. Furthermore we venture that these results are characteristic of RNA tertiary structure.

The system chosen for study is *Escherichia coli* initiator $tRNA^{fmet}$, since it is representative of a predominant type of RNA present in all species and is convenient because the thermodynamics of its folding is well documented and only monovalent ions are required for tertiary structure stabilization.[7] We measure changes in $tRNA^{fmet}$ tertiary and secondary stability as a function of osmolyte concentration by fitting the derivatives of melting curves to a three-state model shown in Scheme 1.[8] The first equilibrium corresponds to the unfolding of tertiary structure and the second equilibrium to the unfolding of secondary structure. Our analysis yields transition melting temperatures and enthalpies from which free energies are calculated by using the Gibbs−Helmholz equation and assuming transition enthalpies are temperature-independent.

In Figure 1, derivatives of the melting curves of $tRNA^{fmet}$ dissolved in 10 mM sodium cacodylate, 200 mM NaCl and 0.1 mM EDTA with and without TMAO show two fairly well resolved peaks indicating at minimum two transitions between folded and

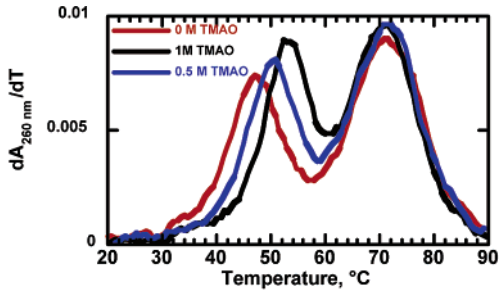
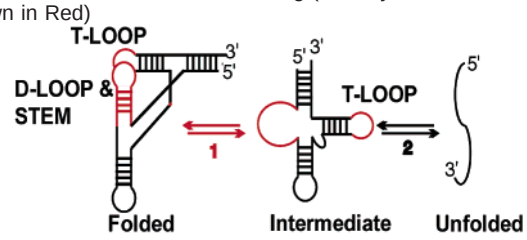


Figure 1. Derivative of melting curves obtained at constant 200 mM [NaCl].

Scheme 1. Model of tRNA Unfolding (Tertiary Interactions Are Shown in Red)



unfolded states. Tertiary structure which consists of several stacked base triples, stacking interactions, and unusual base pairing, along with the D-stem, a six nucleotide basepaired helix unfold simultaneously under the first peak and the remaining secondary structure unravels in discrete steps to the unfolded state under the second peak.[7]

These are modeled by assigning the low-temperature peak to a single transition corresponding tertiary/D-stem unfolding (equilibrium 1 in Scheme 1) and the higher-temperature transitions accounting for the remaining structure (equilibria 2 and 3). The analysis of the data according to this model reasonably recapitulates salt dependence of tertiary structure as seen in more extensive descriptions of $tRNA^{fmet}$ unfolding.[7] For 200 mM NaCl and 0 M TMAO, the T_m of the first transition is 48.1 °C, which is in reasonable agreement with reported values. The T_m of the first transition increases and eventually merges with the second transition with increasing [TMAO]. The salt activity in each solution as measured by an ion-selective electrode, was adjusted to the same value.

Figure 2 shows that the T_m's of tertiary and secondary structure transitions vary linearly with log[NaCl]. The salt dependence is a measure of the entropic contribution salt makes to RNA stability. The tertiary structure salt dependence indicated by the slope of 25 °C is in agreement with previous reports.[7] This slope increases to 29 °C when 1M TMAO is present. The increased slope may reflect a change in TMAO activity, or the affinity salt has for tertiary or

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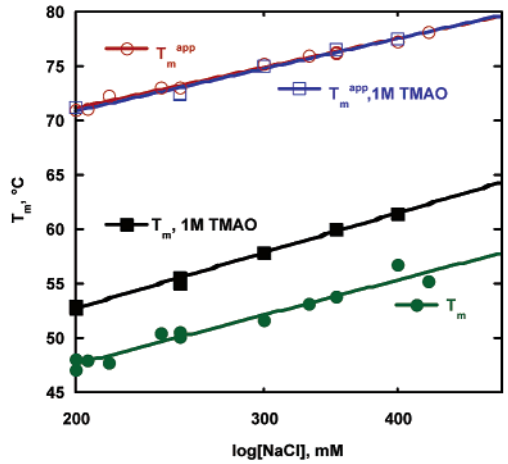

Figure 2. Comparison of T_m's or T_m^{app} for tertiary structure and secondary transitions in the presence or absence of TMAO.

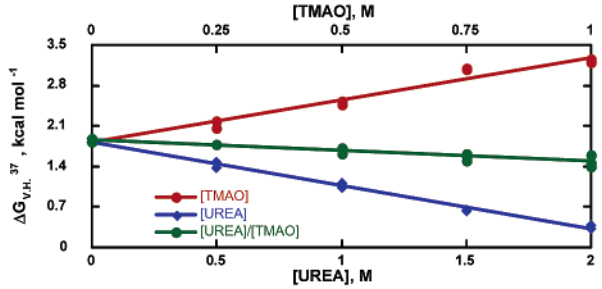

Figure 3. Comparison of $\Delta G_{v.H.}^{37}$ (van't Hoff free energy of unfolding extrapolated to 37 °C) of $tRNA^{fmet}$ tertiary structure in urea(bottom scale) and 2:1 urea/TMAO (bottom scale) and TMAO (top scale.)

intermediate structure. The apparent T_m (T_m^{app}) taken as the maximum of the second transition is essentially independent of [TMAO].

We found that TMAO significantly counteracts urea induced destabilization of tertiary structure without affecting secondary structure. Figure 3 shows that at constant 200 mM NaCl tertiary structure is destabilized by increasing [urea], stabilized by raising [TMAO], and TMAO compensates for urea at ratios of 1 mol TMAO to 2 mol urea, an effect that is comparable to that seen for proteins.[9]

In proteins, preferential exclusion of TMAO from amide backbone, which is less exposed in the folded than unfolded state, drives protein folding and counteracts the effect of urea.[4] We are tempted to say that TMAO is preferentially excluded or repulsed from the RNA phosphodiester backbone. Secondary structure stability remains unchanged in TMAO since exposure of phosphates to solvent are more or less the same in both helical and unfolded states.[10] Tertiary structure becomes more stable in TMAO because phosphodiester backbone is engaged in tertiary interactions leaving them less exposed to TMAO. Urea interactions with RNA might be fundamentally different. Perhaps, urea drives RNA unfolding by binding more urea to the more surface exposed unfolded, than the less exposed folded state as is the case with proteins.[2,4] Whether RNA tertiary structure is stabilized then depends on the relative concentrations of TMAO and urea. This is certainly not unreasonable because urea denaturation can be counteracted with salt.[11]

Alternatively, TMAO may counteract urea denaturation of tertiary structure by binding to a hydrophobic pocket in tRNA; such pockets are apparently found in isoleucine and valine RNA aptamers.[12] Similar pockets might only exist in tRNA tertiary structure and explain why TMAO counteracts tertiary structure denaturation and not secondary structure. Further experimentation is required to resolve some of these points.

These results are significant for several reasons. First its surprising that both proteins and RNA, two very chemically distinct macromolecules behave similarly in TMAO, urea and TMAO:urea solutions. Second, these results suggest that tertiary structure stability is not only sensitive to cations, but also to the aqueous composition and properties of the solvent, a result consistent with other studies.[2,3] Third, TMAO can possibly become a thermodynamic yardstick to distinguish between RNA secondary and tertiary structure. Fourth, these results may be applied to study RNA:protein interactions, especially when the protein is unstable in aqueous solutions.

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Supporting Information Available: Examples of fits to derivatives and the derivatives of melting curves obtained in the presence of urea and TMAO (PDF). This material is available free of charge via the Internet at http:// pubs.acs.org.

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