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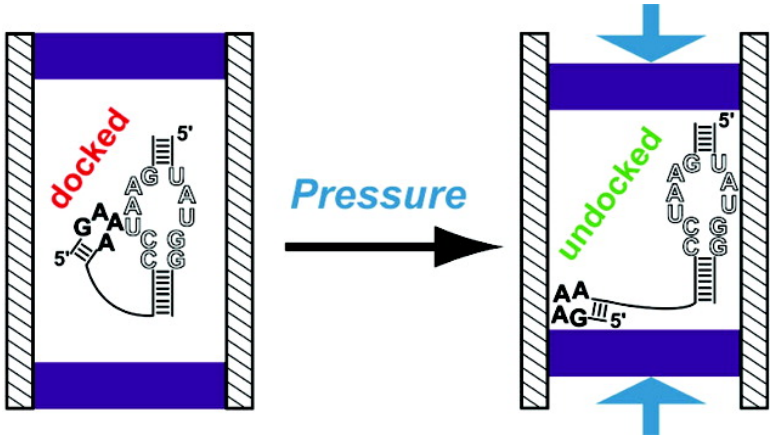

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Influence of Hydrostatic Pressure and Cosolutes on RNA Tertiary Structure

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RNAs fold into complex three-dimensional structures that are critical to their various biological functions. These 3D structures generally form independently of the RNA secondary structure and are stabilized by tertiary interactions between specific motifs. However, the detailed molecular mechanisms that drive formation of RNA tertiary interactions are not well understood. Temperature, metal ion concentration, and chemical denaturant concentration are the most commonly used variables when probing the thermodynamics or kinetics for forming RNA−RNA interactions. Here, we investigate the effects of hydrostatic pressure and nondenaturing cosolutes on formation of the commonly occurring GAAA tetraloop−receptor motif (Figure 1a).[1] Formation of this RNA tertiary interaction was probed by fluorescence resonance energy transfer (FRET). The results show that hydrostatic pressure (P) slightly destabilizes the GAAA tetraloop−receptor interaction. Analysis of these data showed that the change in partial molar volume (ΔV) for forming this RNA−RNA interaction was small compared to that of typical protein−protein or protein−nucleic acid interactions.[2,3] The effects of cosolutes on GAAA tetraloop−receptor stability were also studied and showed that increasing concentrations of polyethylene glycol (PEG) or dextran favor tertiary structure formation, whereas sucrose and glycerol had little effect on the RNA structure.

The effects of P on protein systems have been studied extensively.[2,3] Intermolecular protein−protein and protein−nucleic acid interactions are usually disrupted by P ranging from 1 to 2 kbar, and protein monomers are denatured by 4−15 kbar. Previous studies have demonstrated that P has a very small effect on the double- to single-strand transition for DNA or RNA duplexes.[4] However, little is known about how P affects formation of tertiary interactions or other higher order RNA structures.

To test the influence of P on an RNA tertiary interaction, the well-characterized GAAA tetraloop−receptor interaction motif was employed as a model system.[1,5−10] We utilized a construct with the GAAA tetraloop and receptor connected by a flexible A_7 linker. Docking of the tetraloop to the receptor was probed by FRET (Figure 1a). The FRET efficiency (E_{FRET}) for this RNA decreases with increasing P, which is consistent with a decrease in the docked population. The same trend was observed for a range of [Mg^{2+}], and it is clear from these data that P has a much smaller influence on docking than [Mg^{2+}] (Figure 1b). Little effect was observed for an RNA with the GAAA tetraloop replaced by a nondocking UUCG tetraloop, ($\Delta E_{FRET} < 0.01$, Supporting Information), confirming that the results in Figure 1b represent a decrease in GAAA tetraloop−receptor docking. The equilibrium constant for GAAA tetraloop−receptor docking, K_{dock}, was calculated from the FRET data as described in the Supporting Information. Values for K_{dock} at 1 bar and 2.5 kbar at each Mg^{2+} concentration are shown in Table 1.

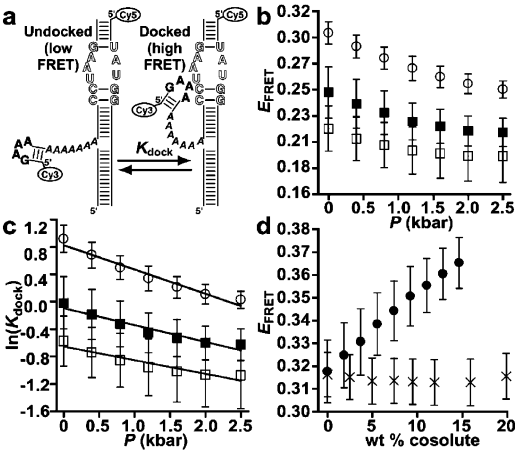

Figure 1. Effects of hydrostatic pressure and nondenaturing cosolutes on GAAA tetraloop−receptor docking. (a) GAAA tetraloop−receptor RNA construct with Cy3 (donor) and Cy5 (acceptor) FRET probes. (b) FRET efficiency vs P at 0 (□), 0.20 (■), and 1.0 (○) mM $MgCl_2$. (c) P dependence of ln(K_{dock}) calculated from FRET data in (b). The data are fit to lines with slopes equal to $-\Delta V/RT$. (d) FRET efficiency vs [PEG 400] (●) and [sucrose] (×) at 1 bar, 1.0 mM Mg^{2+}. All experiments are at 25 °C, with 50 mM HEPES pH 7.5, 100 mM NaCl, and 0.10 mM EDTA.

Table 1. Thermodynamic Values for GAAA Tetraloop−Receptor Docking from Hydrostatic Pressure Data[a]

Mg^{2+} (mM)	K_{dock} at 1 bar	K_{dock} at 2.5 kbar	$\Delta\Delta G^{\circ}_{dock}$ (kcal/mol)[b]	ΔV (kcal/mol)[c]
1.0	2.5 ± 0.2	1.0 ± 0.2	0.53 ± 0.08	9 ± 2
0.20	1.0 ± 0.5	0.55 ± 0.12	0.36 ± 0.15	6 ± 3
0	0.59 ± 0.41	0.38 ± 0.12	0.29 ± 0.15	5 ± 3

[a] Errors represent standard deviations of at least three experiments and uncertainty from calculating K_{dock} from E_{FRET} values. [b] $\Delta\Delta G^{\circ}_{dock}$ represents ΔG°_{dock}(at 2.5 kbar) − ΔG°_{dock} (at 1 bar) at 25 °C. [c] ΔV is determined from the slope of ln(K_{dock}) vs pressure (Figure 1c).

These results show that even the highest P leads to only a modest decrease in stability, with a $\Delta\Delta G^{\circ}_{dock} < 0.6$ kcal/mol (Table 1).

Analysis of ln(K_{dock}) versus P (Figure 1c) yields the ΔV for the GAAA tetraloop−receptor docking reaction at each [Mg^{2+}].[2] The ΔVs range from 5 to 9 mL/mol (Table 1). These ΔVs are smaller than typically observed for protein folding (10−160 mL/mol) or for forming intermolecular protein−protein or protein−nucleic acid complexes (50−260 mL/mol).[2−4] These large ΔVs compared to the tetraloop−receptor could reflect that P affects RNAs and proteins differently or that the ΔVs are per mol and therefore can be influenced by the sizes of the molecules.

RNA−RNA interactions are stabilized by hydrogen bonds, stacking between the aromatic bases, and electrostatic interactions between the RNA and metal cations.[11] P stabilizes aromatic stacking and has little effect on the hydrogen bonding interactions that stabilize biomolecular structures.[12] In contrast, P disrupts charge−charge interactions and favors full hydration of individual charged

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groups.[12,13] This effect arises from electrostriction of the waters around the individual ions, causing these waters to have a higher density (smaller volume) than in bulk water. Previous studies have shown that the GAAA tetraloop−receptor binds at least one Mg^{2+} ion upon docking.[6,8,10] Thus, formation of the additional RNA−Mg^{2+} interactions in the docked state makes a positive contribution to the observed ΔV. Other factors that can contribute to the observed ΔV include differences in hydration of the docked and undocked states and differences in the void volumes of these states. Void volumes arise from solvent-free cavities caused by imperfect packing of groups in the molecule.[12,14] Thus, imperfect packing at the tetraloop−receptor interface could cause additional void volume, which would make a positive contribution to the observed ΔV.

A previous study showed that hydrostatic pressure decreases the cleavage rate of the hairpin ribozyme (activation volume $\Delta V^{\ddagger} =$ 34 mL/mol), which was interpreted as pressure destabilizing formation of a catalytically critical loop−loop tertiary interaction in the ribozyme.[15] This model requires a positive ΔV for forming the loop−loop interaction, which is what was observed here for the GAAA tetraloop−receptor. However, many other factors could affect the observed $\Delta V^{\ddagger}$ for the hairpin ribozyme since the loop−loop docking equilibrium is not directly probed by kinetic data.

Another common technique for modulating protein−protein interactions is the addition of cosolutes to the system.[16,17] Cosolute and *P* experiments are often used as complementary probes.[2,18] Many cosolutes (e.g., glycerol) stabilize native protein folds and complexes, whereas others (e.g., urea) are denaturants.[16−18] Nucleic acid equilibria have also been probed with cosolutes; for example, it has been shown that various cosolutes stabilize a DNA G-quadruplex relative to a random coil.[19] These effects arise because cosolutes perturb the interactions between the surface of a biomolecule and water. For instance, by lowering the activity of water, a cosolute can shift the biomolecule toward a state with fewest bound water molecules. This example is a limiting case where the biomolecule and cosolute do not interact. In most systems, there is also a contribution from the interaction of the cosolute and biomolecule.[17] Thus, the influence of different cosolutes falls on a continuum based on how favorably they bind to the biomolecule relative to water.[17] Cosolutes that bind with less affinity than water favor the state with the lowest solvent accessible surface area (with fewest bound cosolute and water molecules), stabilizing native folds or complexes. Conversely, cosolutes that bind with greater affinity than water shift the equilibrium toward the state with the highest exposed surface area and therefore act as denaturants. Thus, stabilizing cosolutes represent a valuable alternative to denaturants for modulating RNA−RNA interactions.

To probe the effects of nondenaturing cosolutes on GAAA tetraloop−receptor docking, FRET was measured at various concentrations of PEG (400 Da), dextran (12 000 Da), sucrose, or glycerol (Figure 1d and Supporting Information). The results show that PEG 400 significantly increases docking (Figure 1d). Since ~730 Å^2 is buried at the GAAA tetraloop−receptor interface,[7] a simple interpretation of this result is water/cosolute molecules are released in the docking reaction, which is consistent with the surface of the RNA having a higher affinity for water than for PEG. Similar results were observed for dextran 12 000 (Supporting Information). Sucrose and glycerol each showed little effect on GAAA tetraloop−receptor docking (Figure 1d and Supporting Information), suggesting that these polar cosolutes with vicinal hydroxyl groups have affinities similar to water for the RNA surface. Glycerol was previously observed to have a smaller effect than PEG on the stability of a DNA G-quartet.[19] In contrast with our results, sucrose and glycerol usually stabilize protein folds and protein complexes.[17,20] Thus, this RNA differs from most proteins in its interactions with these cosolutes.

Previous studies have shown that proteins can be differentially affected by the same cosolute; for example, urea is preferentially bound relative to water to β-lactoglobulin, whereas myoglobin is preferentially hydrated.[17] Since proteins vary dramatically in their hydrophobic/hydrophilic character, distribution of charges, and distribution of hydrogen bonding groups, different protein surfaces can have very different affinities for a particular cosolute. By contrast, there is much less variation in these chemical properties between RNA surfaces. Thus, on the basis of the GAAA tetraloop−receptor data, PEG 400 and dextran 12 000 are predicted to stabilize most other RNA−RNA interactions.

In summary, the GAAA tetraloop−receptor was used as a model system to explore how both *P* and various cosolutes affect RNA−RNA interactions. FRET measurements of tetraloop−receptor docking showed that *P* up to 2.5 kbar slightly destabilizes docking ($\Delta\Delta G^{\circ}_{dock} < 0.6$ kcal/mol), yielding a ΔV of 5−9 mL/mol for the docking reaction. PEG and dextran cosolutes increased docking, suggesting a significant release of water/cosolute molecules is associated with docking. In contrast to many protein systems, this RNA−RNA interaction was not significantly stabilized by sucrose or glycerol. This study provides valuable insight into the role of hydration in GAAA tetraloop−receptor docking and demonstrates the potential for probing thermodynamics of RNA−RNA interactions with *P* and nondenaturing cosolutes.

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Supporting Information Available: Materials and methods, glycerol and sucrose data, and control experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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