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Tertiary Structure in “Unfolded” RNA

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ABSTRACT: The nucleotide-by-nucleotide peak-fitting procedure developed in the previous year is now being applied to the structure of the “unfolded” RNA in the absence of divalent cations. Recent studies have shown that monovalent ions dramatically accelerate the rates at which the *Tetrahymena* ribozyme folds upon the addition of divalent cations. Monovalent ions act as counterions and are thought to stabilize RNA tertiary structure by neutralizing negatively charged backbone phosphates. Although even very high concentrations of monovalent ion are insufficient enough to stabilize the catalytically active conformation of the *Tetrahymena* group 1 intron ribozyme, monovalent ions do induce a collapse of the RNA. The effects of monovalent ions on ribozyme folding are complex; including the destabilization of kinetically trapped intermediate species. Although there are expectations of loosely formed structures at high monovalent ion concentrations, no structural information has yet been obtained regarding tertiary structure that may be present in the ensemble of ‘unfolded’ RNA molecules present in the absence of divalent cations. A single band analysis of footprinting data and whole data set analysis enable us to detect partial protection from hydroxyl radical cleavage. We are investigating the effect of monovalent ions on the structure of the P4-P6 domain of *Tetrahymena* ribozyme with high salt concentrations (up to 1M NaCl). There are partly protected sites within P4-P6 domain that indicate tertiary contacts within the domain that are consistent with those observed upon Mg^{2+} -dependent folding. The weakness of protection and distinct salt concentration dependency among sites may indicate the possibility of existence of multiple structural populations under these conditions.