Nucleic Acids Chemistry beyond the Watson-Crick Double Helix (80) : Validation of the nearest-neighbor model for Watson-Crick RNA duplexes under molecular crowding condition

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Secondary structures of RNA were shown to affect the kinetics and yield of the transcription and translation reactions significantly depending on their stabilities. Therefore, the stability prediction of RNA secondary structures in intracellular condition is beneficial to understand biological reactions involved in gene expression. Majority of the RNA secondary structural motifs are double helices formed by the Watson-Crick base pairing and loop structures. It was established that stability of a nucleic acid duplex depends on the adjacent base pairs and the total duplex stability can be estimated by a sequence dependent manner, known as nearest-neighbor (NN) model.[1] Hence, NN parameters for RNA duplex under cell-mimicking molecular environment is valuable to predict stability and secondary structures of RNAs in cell.

In this work, we systematically analyzed the thermodynamic parameters ($\Delta H°$, $\Delta S°$ and $\Delta G°_{37}$) forming RNA oligomeric duplexes of varying length and base composition in a crowding condition of 40 wt% polyethylene glycol 200 (PEG 200) with ionic concentration similar to that found in cells. We found that sequences having identical NN pairs showed similar stabilities and thermodynamic parameters (Figure 1), which indicates the validity of the NN model even in the crowding condition. From thermodynamic data of the studied sequences, we determined NN parameters for the RNA duplex stability following linear least square analysis.[2] Our results suggested that compared to NN parameters of DNA duplexes, RNA NN parameters were less destabilized by the crowding condition. The determined NN parameters for RNA duplexes could be helpful for predicting RNA structures *in vivo* and also for designing RNA-based therapeutics.

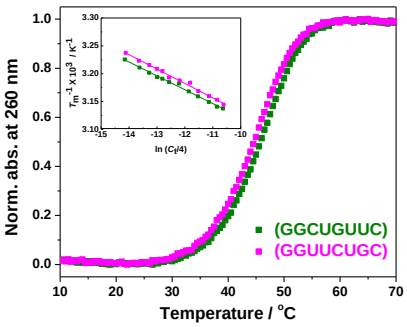

Figure 1. Normalized UV melting curves of pairs of 100 μM RNA duplex having the same NN frequencies in 0.1 M NaCl, 10 mM Na_2HPO_4 (pH 7.0), and 1 mM Na_2EDTA in the presence of 40 wt% PEG 200. Inset shows T_m^{-1} vs. ln (C_t/4) plots for the sequences.

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