Effect of secondary structure of short double-stranded RNA on RNAi efficiency

Tatsuo Ohmichi, Hisae Karimata and Naoki Sugimoto

Abstract

The dangling nucleotides are very important for the RNA interference (RNAi). We investigate the effect of the dangling end on both the thermodynamic property of the RNA helix and efficiency of gene regulation in RNAi pathway. The result indicates that the four dangling adenines, which are more stable than two or three dangling residues, is not suitable for the RNAi efficiency.

Introduction

Recent studies of RNAi (RNA interference) have shown that long dangling ends are important for the RNAi functionality (Figure 1) (1). In the case of RNAi, short double-stranded RNAs (dsRNA) are formed with the 2-3 nt RNA dangling ends after cleavage with RNase III (2). Although the 2-3 nt RNA dangling end shows the RNAi efficiency, a short dsRNA without the dangling end displays no gene silencing activity (3). These observations clearly show that the dangling residues have the requisite energy contributions needed to stabilize the helix, thus resulting in the observed biological activities. However, the systematic energy contributions of long dangling ends have not been reported. Here, we report a quantitative thermodynamics in the stability of RNA-RNA duplexes containing long RNA dangling ends.

Materials and Methods

All melting curves were fitted with a curve fitting procedure to obtain three thermodynamic parameters, the enthalpy ($\Delta H^\circ$), entropy ($\Delta S^\circ$), and free energy changes ($\Delta G^\circ$) at 37 °C, for the formation of the nucleic acid duplex. We also evaluated them from plots of $T_m^{-1}$ vs. ln($C_t$), where $T_m$ is the melting temperature, and $C_t$ is the total strand concentration, respectively. Using the slope and vertical axis intercept, thermodynamic parameters were also analyzed according to the following equations;

$$T_m^{-1} = R \times \ln(C_t) / \Delta H^\circ + \Delta S^\circ / \Delta H^\circ$$

$$\Delta G^\circ = \Delta H^\circ - 310.15 \times \Delta S^\circ$$

where $R$ is the gas constant.

Results and Discussion

UV melting curves are used to measure the thermodynamic stability of the RNA-RNA helix with long dangling nucleotides. We measured $T_m$ as a function of duplex concentration and
calculated thermodynamic parameters by plotting $T_m^{-1}$ vs. $\log(C_t)$ (Figure 2) (4,5). The thermodynamic effects of one to four dangling adenines on r(AUGCAU) were measured. All RNA-RNA helixes with dangling nucleotides showed a two-state transition in 1 M NaCl. Differences of less than 10% were observed between parameters as determined by $T_m^{-1}$ vs. $\log(C_t)$ plots and curve fittings. Final thermodynamic parameters were determined from average values obtained with both methods.

The free-energy changes at 37°C on addition of the dangling ends was calculated (Table 1). The stabilization due to the dangling end was calculated using $\Delta G^*_{37} = \{\Delta G^*_{37} \text{(duplex with dangling ends)} - \Delta G^*_{37} \text{(core duplex)}\}/2$. The $\Delta G^*_{37}$ values for a single dangling adenine at the 3' terminus were -0.6 kcal mol$^{-1}$ (5'A/U3') per dangling end. These values are similar to those previously reported for 5'A/U3' (-0.7 kcal mol$^{-1}$) (6). This result confirms our choice of nucleic acid sequences as being suitable for the investigation of the effects of dangling length on duplex stability.

Interestingly, the results indicated that the RNA duplex was considerably stabilized by increasing the number of the dangling adenines. Although the calculated free energy change was the largest with the addition of the first dangling adenine, duplexes containing four adenines showed much higher stability than those containing a single adenine. The $\Delta G^*_{37}$ values for single, two, three and four dangling adenines at the 3' terminus of r(AUGCAU) were -0.6 (A1), -0.9 (A2), -1.2 (A3) and -1.1 kcal mol$^{-1}$ (A4), respectively. The results indicate that RNA stability can be achieved by increasing the length of the dangling end.

Finally, we investigated the effect of the number of the dangling adenine nucleotides on the efficiency of the RNAi. Our data indicate that the gene silencing efficiency of the dsRNA containing four dangling residues was lower than the corresponding dsRNA with two or three dangling residues. Thus, the results suggest that four dangling residues, which is more effective at stabilizing the helix than two or three dangling residues, is not suitable for the RNAi efficiency.

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**REFERENCES**