CHEMISTRY LETTERS, pp. 345-348, 1991.

Stabilization Energy of the Catalytic Core of the Tetrahymena Ribozyme

Naoki SUGIMOTO* and Muneo SASAKI
Department of Chemistry, Faculty of Science, Konan University,
8-9-1 Okamoto, Higashinada-ku, Kobe 658

The stabilization energy of a catalytic core of the Tetrahymena ribozyme has been calculated according to a nearest-neighbor model for RNA duplex formation. The results suggested that the high activity of the mutant ribozyme lacking the P9 region was due to the formation of a stabilized secondary structure by the approach between the active and substrate-binding sites.

The Tetrahymena ribozyme catalyzes the making and breaking of covalent bonds not only in RNA but also in DNA.[1,2] For example, a 3'-terminal guanosine residue acts as a nucleophile to attack a phosphodiester bond in an oligopyrimidine as shown in Fig. 1. The mechanism of the ribozyme has been extensively studied.[3,4] Also, the detailed secondary structure required for the catalytic activity of the ribozyme is the subject of continued investigation.[5-8] Very recently, a model of a minimum secondary structure of the catalytic core of the ribozyme has been proposed.[9] Surprisingly, it has been also reported that the mutant ribozyme (ΔP9 mutant) lacking the P9 region but having the catalytic core is more reactive than the wild type.[9,10]

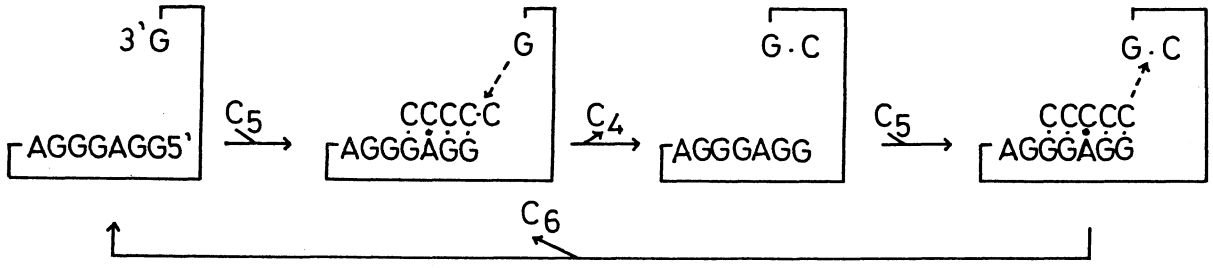

Fig. 1. The catalytic reaction scheme of the Tetrahymena ribozyme with an oligopyrimidine.

In this work, we have calculated the stabilization energy at 37°C of the catalytic core of the Tetrahymena ribozyme according to a nearest-neighbor model for RNA duplex and loop formations[11] in order to investigate the reason for the high reactivity of the ΔP9 mutant. We applied previously the nearest-neighbor model for the prediction of the stabilities of codon-anticodon complexes,[12] the Tetrahymena ribozyme-substrate complexes,[3,4] and its ribozyme structure,[7] suggesting that the model should be very useful for predicting the stabilities of any kind of RNAs.

The enthalpy change ΔH° and the entropy change ΔS° for the formation of the catalytic core of the ribozyme were obtained from nearest-neighbor thermodynamic parameters.[11] For example, the value of ΔS° for the formation of the P8 region in Fig. 2 was calculated as follows:

$$\Delta S^{\circ} = \Delta S^{\circ}(\text{initi}) + \Delta S^{\circ}(\text{GG/CU}) + \Delta S^{\circ}(\text{GG/UC}) + \Delta S^{\circ}(\text{GA/CU}) + \Delta S^{\circ}(\text{AA/UU}) + \Delta S^{\circ}(\text{AG/UC}) + \Delta S^{\circ}(\text{GA/CU})$$
$$= (-10.8) + (-35.5) + (-19.2) + (-35.5) + (-18.4) + (-19.2) + (-35.5)$$
$$= -174.1 \text{ cal } K^{-1} \text{ mol}^{-1},$$

where $\Delta S^{\circ}(\text{initi})$ was an entropy change for the initiation of making the first base pair in the region, and $\Delta S^{\circ}(\text{GG/CU})$, $\Delta S^{\circ}(\text{GG/UC})$, $\Delta S^{\circ}(\text{GA/CU})$, $\Delta S^{\circ}(\text{AA/UU})$, $\Delta S^{\circ}(\text{AG/UC})$, and $\Delta S^{\circ}(\text{GA/CU})$ were propagation entropy-changes for making each subsequent base pair. Similarly,

$$\Delta H^{\circ} = -61.7 \text{ kcal mol}^{-1}.$$

Therefore, the free-energy change at 37°C, ΔG°_{37}, was obtained as follows:

$$\Delta G^{\circ}_{37} = \Delta H^{\circ} - T\,\Delta S^{\circ}$$
$$= (-61.7) - (273+37) \times (-174.1 \times 10^{-3})$$
$$= -7.7 \text{ kcal mol}^{-1}.$$

In the calculation, since the stability of a G-U base-pair had been reported to be similar to that of an A-U base-pair,[13] the energy parameters for an A-U base-pair were used for a G-U base-pair. The estimated errors in the values of ΔG°_{37} were ±8%. The ΔG°_{37} values for the formations of the bulges, internal loops, hairpin loops, and terminal mismatches were also calculated on the basis of the nearest-neighbor thermodynamic parameters.[11] However, the stabilization energy for the formation of the base triplets was not able to be calculated, because the nearest-neighbor thermodynamic parameters for the triplets were not available so far. The obtained values of ΔG°_{37} were shown in Fig. 2 with the minimum secondary structure of the catalytic core of the Tetrahymena ribozyme proposed previously.[9] In Fig. 2, the 3'-terminal guanosine residue is an active site and the P1 region is a substrate-binding site.

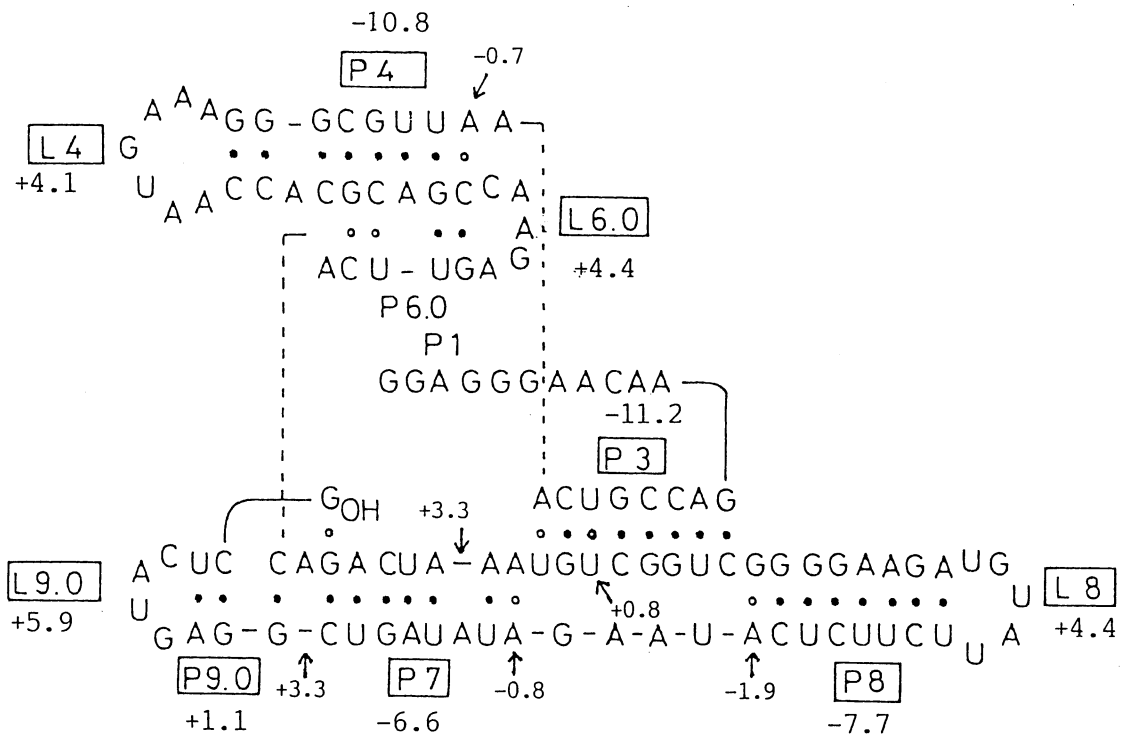

Fig. 2. Secondary Structure of the catalytic core of the Tetrahymena ribozyme and its stabilization energy.

The values of ΔG°_{37} for the stem-regions of P3, P4, P7, and P8 are much negative. The result shows that these stem-regions are relatively stable, even considering the unstabilities of the loop-region adjacent to each stem and the bulges in each stem. The formation of the stems mainly contribute to the stability of the catalytic core of the ribozyme.

However, the value of ΔG°_{37} for the stem of P9.0 is +1.1 kcal mol^{-1}. In addition, the ΔG°_{37} value for L9.0 region is +5.9 kcal mol^{-1}. Therefore, the secondary structure of the region of P9.0 and L9.0 is very unstable. The wild type of the ribozyme has the long stem regions of P9, P9.1, P9.2, and P9.2a.[7] On the other hand, the ΔP9 deletion mutant of the Tetrahymena ribozyme having the P9.0 and L9.0 regions is shorter than the wild type by 35 nucleotides and is more reactive than the wild type.[9,10] The unstable secondary structure of P9.0 and L9.0 in Fig. 2 can be changed to the stable structure in

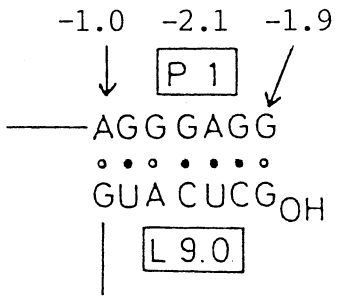

Fig. 3. The stable structure of the P9.0 and L9.0 regions of the catalytic core of the Tetrahymena ribozyme.

Fig. 3. The ΔG°_{37} value in Fig. 3 is much negative and the secondary structure is more promising. Furthermore, this secondary structure can explain the high reactivity of the mutant: that is, the structure leads the 3'-terminal G_{OH} to close proximity of the substrate-binding site of the P1 region and then the target phosphodiester bond of the substrate.

This work was partly supported by the Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture, Japan.

References

1) A. J. Zaug and T. R. Cech, Science, **229**, 1060 (1985).
2) D. Herschlag and T. R. Cech, Nature, **344**, 405 (1990).
3) N. Sugimoto, R. Kierzek, and D.H. Turner, Biochemistry, **27**, 6384 (1988).
4) N. Sugimoto, M. Tomka, R. Kierzek, P. C. Bevilacqua, and D. H. Turner, Nucleic Acids Res., **17**, 355 (1989).
5) T. R. Cech, Gene, **73**, 259 (1988).
6) J. M. Burke, Gene, **73**, 273 (1988).
7) N. Sugimoto, A. Tanaka, and M. Sasaki, Chem. Express, **4**, 613 (1989).
8) N. Sugimoto, N. Monden, and M. Sasaki, Chem. Express, **5**, 585 (1990).
9) A. A. Beaudry and G. F. Joyce, Biochemistry, **29**, 6534 (1990).
10) D. L. Robertson and G. F. Joyce, Nature, **344**, 467 (1990).
11) D. H. Turner, N. Sugimoto, and S. H. Freier, Ann. Rev. Biophys. Biophys. Chem., **17**, 167 (1988).
12) N. Sugimoto and M. Sasaki, Bull. Chem. Soc. Jpn., **62**, 3745 (1989).
13) N. Sugimoto, R. Kierzek, S. M. Freier, and D. H. Turner, Biochemistry, **25**, 5755 (1986).

(Received October 24, 1990)