Application of Improved Free-Energy Parameters of RNA Stability to Codon-Anticodon Interactions

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(Received June 5, 1989)

Synopsis. Free-energy parameters of the RNA duplex stability have been applied in order to investigate the complex between codon oligomers and anticodon loops in transfer RNA. The results suggested that the very large stability of the complex might be due to the stacking interactions of adjacent bases on the codon-anticodon helix and an additional hydrogen-bond formation of the core helix as well as a conformational change of the nucleic acid.

Even at present an interaction between a codon in the messenger RNA and an anticodon in a transfer RNA (tRNA), which is of central importance in the transfer of genetic information from DNA to proteins, still remains a mystery. It has often been found that the codon-anticodon complexes are more stable than found or predicted for two complementary trinucleotides.[1-3] The possible reason for the large stability of the complex was suggested to be the contribution of a base-pair formation between the 5′ adjacent codon base and the 3′ adjacent anticodon base on the helix.[4] However, this explanation can not be applied to complexes containing terminal mismatches or unpaired nucleotides (dangling ends).

Recently, improved thermodynamic parameters for RNA duplex stability[5-9] have been reported by using a nearest-neighbor model.[2] The improvements have permitted the parameters to provide breakthroughs in difficult structure predictions of tRNAs,[10] ribosomal RNAs,[10] a self-splicing intervening sequence,[11] and intermediate complexes of ribozymes.[12,13] In the present study we have applied the improved free-energy parameters of RNA stability to the interaction between codon oligomers and anticodon loops of tRNAs in order to determine the origin of the very large stability of the complexes.

Methods

The measured free-energy changes, ΔG_m, for the formation of codon-anticodon complexes were obtained from the observed association constants, K, for the binding of codon oligonucleotides to $tRNA^{Phe}$ [3,4,14] and $tRNA^{fMet}$ [15] at 273 K:

$$\Delta G_m = -RT\ln K, \quad (1)$$

where R is the gas constant and T is equal to 273 K. The values of ΔG_m for UUCA binding to the CmU-GmAAYA (m, 2′-*O*-methyl ribose; Y, a hypermodified base in $tRNA^{Phe}$) site of dodecamer[4] was also obtained by the same method.

The measured free-energy increments, $\Delta\Delta G_m$, for dangling ends of codon oligonucleotides were obtained by subtracting ΔG_m for codon trinucleotides from ΔG_m for codon tetranucleotides. For example, the value of $\Delta\Delta G_m$ for the AUGA codon oligomer binding to CmU-CAUAA (anticodon loop of $tRNA^{fMet}$) was calculated as being;

$$\Delta\Delta G_m = \Delta G_m(\mathrm{AUGA}) - \Delta G_m(\mathrm{AUG}) = (-21.7) - (-16.3) = -5.4\ \mathrm{kJ\ mol^{-1}},$$

where ΔG_m(AUGA) and ΔG_m(AUG) were the measured free-energy changes for the formation of codon-anticodon complexes of AUGA and AUG with CmUCAUAA, respectively.

The calculated free-energy changes, ΔG_c, for the formation of codon-anticodon complexes were obtained from nearest-neighbor thermodynamic parameters:[5-9]

$$\Delta G_c = \Delta H_c - T\,\Delta S_c, \quad (2)$$

where ΔH_c and ΔS_c are the enthalpy and entropy changes, respectively, and T is the temperature. For example, the value of ΔG_c for an AUG codon oligomer binding to CmUCAUAA (anticodon loop of $tRNA^{fMet}$) was calculated as follows: The calculated entropy change of a core helix formation for $\overrightarrow{\mathrm{CAU}}/\overleftarrow{\mathrm{GUA}}$ was

$$\Delta S_c(\mathrm{core}) = \Delta S_c(\mathrm{initi}) + \Delta S_c(\overrightarrow{\mathrm{CA}}/\overleftarrow{\mathrm{GU}}) + \Delta S_c(\overrightarrow{\mathrm{AU}}/\overleftarrow{\mathrm{UA}}) = (-45) + (-116) + (-65) = -226\ \mathrm{J\ K^{-1}\ mol^{-1}},$$

where ΔS_c(initi) was the entropy change for a helix initiation associated with the formation of the first base pair in the duplex,[6] $\Delta S_c(\overrightarrow{\mathrm{CA}}/\overleftarrow{\mathrm{GU}})$ and $\Delta S_c(\overrightarrow{\mathrm{AU}}/\overleftarrow{\mathrm{UA}})$ were the propagation entropy changes during the formation of each subsequent base pair;[5] the arrows point from the 5′ to the 3′ direction. Similarly, ΔH_c(core)=−67.8 kJ mol^{-1}. Therefore, according to Eq. 2, ΔG_c(core) at 273 K was −6.1 kJ mol^{-1} for the core helix formation. Since the core helix had two dangling bases of 5′U and 3′A in the anticodon loop, $\Delta G_c(\overrightarrow{\mathrm{UC}}/\overleftarrow{\mathrm{G}})$ and $\Delta G_c(\overrightarrow{\mathrm{A}}/\overleftarrow{\mathrm{AU}})$ (−0.4 and −2.9 kJ mol^{-1},[7] respectively) had to be added to ΔG_c(core) in order to obtain the total free-energy change, ΔG_c. Consequently, ΔG_c at 273 K was −9.4 kJ mol^{-1} for AUG binding to the anticodon loop of $tRNA^{fMet}$.

Note that in the ΔG_c calculation for a codon binding to the anticodon of $tRNA^{Phe}$ or the CmUGmAAYA site of the dodecamer, two assumptions were used: One was that the stabilization energy of 2′-*O*-methyl ribose was equal to that of the unmodified ribose, and the other was that a dangling Y base which was the hypermodified base in $tRNA^{Phe}$ had the same stabilization energy as the dangling purine base, since the nearest-neighbor energy parameters for the dangling Y

base were not available. These assumptions were reasonable, since they had been applied to the prediction of secondary structures of tRNAs, and the most probable structure, determined by the calculation, was almost consistent with that from phylogenetic data.[10,11]

The calculated free-energy increments, $\Delta\Delta G_c$, by stacking the dangling bases of the codon oligonucleotides were obtained from the literature.[6-9] The values of $\Delta\Delta G_c$ by the fourth base-pair formation between the dangling bases of the codon oligomer and the anticodon loop (shown in parentheses in Table 1) were calculated by the same method for ΔG_c described above. For example, the complex between AUGA and CmUCAUAA had the possibility of an additional A–U base-pair formation on the G–C base pair. The free-energy increment, $\Delta\Delta G_c$, for this fourth base pair was

$$\begin{aligned}\Delta\Delta G_c &= \Delta H(\overrightarrow{GA}/\underleftarrow{CU}) - T\,\Delta S(\overrightarrow{GA}/\underleftarrow{CU}) \\ &= (-55.7) - (273) \times (-148 \times 10^{-3}) \\ &= -15.3\ \text{kJ mol}^{-1},\end{aligned}$$

where $\Delta H(\overrightarrow{GA}/\underleftarrow{CU})$ and $\Delta S(\overrightarrow{GA}/\underleftarrow{CU})$ were the enthalpy- and entropy-changes for forming the A–U base pair on the G–C base pair.

Results and Discussion

The measured free-energy changes, ΔG_m, and the calculated free-energy changes, ΔG_c, for the formation of codon–anticodon complexes are listed in Table 1. The values of $\Delta\Delta G_c$ (the differences between the calculated free-energy changes of helix formation of the tetranucleotide with anticodon and the calculated free-energy changes of helix formation of the trinucleotide with anticodon) are also listed in Table 1 along with the values of $\Delta\Delta G_m$.

In Table 1, the values of $\Delta\Delta G_c$ in parentheses are quite different from those of $\Delta\Delta G_m$, not only for UUCA binding to $tRNA^{Phe}$ but also for AUGA, UAUG, and GUGA binding to $tRNA^{fMet}$ which can form the fourth base pair. This result suggests that there is either the very weak or no hydrogen-bond formation. It has been shown that hydrogen bonding and stacking compete in a base pair.[16] Stacking interactions are more favorable when hydrogen-bonding interactions are less favorable. Therefore, stacking of dangling nucleotides might contribute to the large stability of the codon–anticodon complex.

Within a few kilojouls per mole (that is, within estimated errors) these $\Delta\Delta G_m$ are equal to the $\Delta\Delta G_c$ calculated by the assumption that the adjacent bases on a codon-anticodon core helix do not make a base pair but, rather, are stacking. The good correspondence between $\Delta\Delta G_c$ and $\Delta\Delta G_m$ is also found in the cases of the other 3′ and 5′ end bases of codon oligomers which can not form the fourth Watson–Crick base-pairs with the anticodon loops. These results show that an adjacent base on a codon-anticodon core helix stabilizes the helix by the stacking interaction, though this stabilization is not sufficiently large to provide a good explanation for the very large stability of the codon-anticodon complex. Therefore, the larger stability of ΔG_m than ΔG_c might come from an unusual base-pair formation of the codon–anticodon core helix.

Because the free-energy change for a codon–anticodon helix initiation is positive, the large stability of ΔG_m

Table 1. The Measured and Calculated Free-Energy Changes of the Complex Formation between Codon Oligomers and Anticodon Loops in the tRNA in Solution

Anticodon loop	Codon oligonucleotide	ΔG_m[a]	ΔG_c[b]	$\Delta\Delta G_m$	$\Delta\Delta G_c$[c]
		kJ mol^{-1}	kJ mol^{-1}	kJ mol^{-1}	kJ mol^{-1}
CmUGmAAYA	UUC	−17.2	−12.6		
($tRNA^{Phe}$)	UUCA	−26.8		−9.6	7.1
					(−13.0)
CmUGmAAYA	UUU	−13.4	−7.1		
($tRNA^{Phe}$)	UUUU	−13.0		0.4	−0.4
	UUUU	−13.0		0.4	−0.8
CmUCAUAA	AUG	−16.3	−9.4		
($tRNA^{fMet}$)	AUGA	−21.7		−5.4	4.4
					(−15.3)
	AUGU	−16.3		0	−2.3
	AUGC	−15.5		0.8	−1.5
	UAUG	−15.9		0.4	−0.6
					(−8.6)
CmUCAUAA	GUG	−16.3	−9.4		
($tRNA^{fMet}$)	GUGA	−20.9		−4.5	−4.4
					(−15.3)
CmUGmAAYA	UUCA	−25.5	−25.9		
(Dodecamer)					

a) From the data of the observed binding constants in Refs. 3, 4, 14, and 15. Estimated errors in ΔG_m are within ± 10%. b) Estimated errors in ΔG_c are within ± 5%. c) The values in parentheses were calculated according to the assumption that the dangling bases of the codon oligomer and the anticodon loop were able to make the fourth base-pair.

for helix formation is mainly due to the formation of Watson-Crick base pairs and a G-U base pair. However, as shown in Table 1, the measured stability of ΔG_m is significantly larger than the calculated stability of ΔG_c, which was obtained by the free-energy value of not only the base pairs, but also the stacking interaction of the adjacent bases in a codon oligomer. The differences in the stability between ΔG_m and ΔG_c are -4.6, -6.3, -6.9, and -6.9 kJ mol^{-1} for UUC and UUU binding to $tRNA^{Phe}$ and for AUG and GUG binding to $tRNA^{fMet}$, respectively. The value for each binding suggests a possible explanation for the large stability of the complex: An additional hydrogen bond contributes to the stability of a codon-anticodon helix, since it is known that the addition of a hydrogen bond for terminal and interior base pairs in RNA oligomers changes the free energy for duplex formation by -4 to -7 kJ mol^{-1} [16] and, especially, the value of -7 kJ mol^{-1} is consistent with the free energy increment recently predicted for a hydrogen bond formation between base pairs in the absence of competing stacking interactions.[17] Furthermore, the stability of ΔG_m for UUCA binding to dodecamer is well predicted by the calculated free-energy change, ΔG_c, which does not contain the stability by an additional hydrogen-bond formation. Therefore, the results and the fact that the dodecamer can not form a loop suggest that the additional hydrogen bond on the codon-anticodon helix might come from the other base in an anticodon loop of tRNA as well as a conformational change of the nucleic acid during the formation of the complex.

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