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Free Energy Contributions of G·U and Other Terminal Mismatches to Helix Stability†

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ABSTRACT: Thermodynamic parameters of helix formation were measured spectroscopically for seven hexaribonucleotides containing a GC tetramer core and G·U or other terminal mismatches. The free energies of helix formation are compared with those for the tetramer core alone and with those for the hexamer with six Watson–Crick base pairs. In 1 M NaCl, at 37 °C, the free energy of a terminal G·U mismatch is about equal to that of the corresponding A·U pair. Although other terminal mismatches studied add between −1.0 and −1.6 kcal/mol to ΔG°_{37} for helix formation, all are less stable than the corresponding Watson–Crick pairs. Comparisons of the stability increments for terminal G·U mismatches and G·C pairs suggest when stacking is weak the additional hydrogen bond in the G·C pair adds roughly −1 kcal/mol to the favorable free energy of duplex formation.

The wobble hypothesis includes a special stability of terminal G·U mismatches among the eight possible non-Watson–Crick mismatches of the four standard ribonucleotides (Crick, 1966). G·U mismatches are observed in the stems of tRNA cloverleaf structures (Sussman & Kim, 1976; Johnston & Redfield, 1981; Sprinzl et al., 1985) as well as in proposed double-helical regions in 5S rRNA (Fox & Woese, 1975; Kime & Moore, 1983; Noller, 1984; Erdmann et al., 1985), 16S RNA (Woese et al., 1983), viroids (Steger et al., 1984), and the excised intervening sequence from the rRNA of *Tetrahymena thermophila* (Cech et al., 1983). Frequently, these G·U mismatches are at the ends of proposed helical regions (Ninio, 1973; Clark, 1978; Mizuno & Sundaralingam, 1978). Al-

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Table I: Thermodynamic Parameters of Helix Formation[a]

oligomer	log C_t parameters			"temperature-independent" parameters		
	$-\Delta H°$ (kcal/mol)[b,c]	$-\Delta S°$ (eu)[b,c]	T_M (°C)[c,d]	$-\Delta H°$ (kcal/mol)[b,c]	$-\Delta S°$ (eu)[b,c]	T_M (°C)[d,e]
UGGCCGp	53.0	143.3	54.7	49.8	133.7	54.9
UCCGGGp	47.7	129.8	48.5	47.4	129.0	48.6
GCCGGUp	58.2	158.1	57.0	59.3	161.2	57.0
GGCGCU	56.4	154.7	52.9	55.9	153.0	53.0
GCCGGAp	45.3	123.4	46.7	47.2	129.0	47.2
GCCGGGp	46.5	125.8	49.2	48.3	131.5	49.6
UGCGCU	47.7	131.2	46.3	46.2	126.4	46.4
GCCGGUAp	73.4	200.0	63.5	70.3	190.5	63.6

[a] Measurements were in 1 M NaCl, 0.005 M Na_2HPO_4, and 0.5 mM Na_2EDTA, pH 7. [b] Although estimated errors in $\Delta H°$ and $\Delta S°$ are ±5%, additional significant figures are given to allow accurate calculation of T_M. Due to correction of $\Delta H°$ and $\Delta S°$, errors in $\Delta G°$ at the T_M are ±2% (Freier et al., 1985). [c] From plots of reciprocal melting temperature vs. log C_t. [d] Calculated for 10^{-4} M oligomer concentration. [e] Temperature-independent thermodynamic parameters. These are the average of those from plots of T_M^{-1} vs. log C_t and those from fits of individual melting curves to a two-state model with sloping base lines.

though there are limited studies on the effect of internal G·U mismatches on duplex stability (Uhlenbeck et al., 1971; Lomant & Fresco, 1975; Romaniuk et al., 1979a,b; Alkema et al., 1982), no experiments have been reported on the effect of terminal G·U mismatches on oligonucleotide stability. We report below thermodynamic parameters of helix formation for four oligoribonucleotides containing terminal G·U mismatches. The free energies of stabilization of these terminal G·U mismatches are compared to those for A·U and G·C pairs and to other terminal mismatches.

Materials and Methods

Oligonucleotide Synthesis. GCCGGp, UCCGGp, and UGGCCp were synthesized previously (Freier et al., 1985). Terminal phosphates were removed from the pentanucleotides with calf alkaline phosphatase. Hexanucleotides were synthesized from the appropriate pentanucleoside tetraphosphate by addition of a 3′–5′ nucleoside bisphosphate with T4 RNA ligase. Details of the enzymatic synthesis and product purification are given elsewhere (Freier et al., 1983, 1985; Beckett & Uhlenbeck, 1984). UGCGCU and GGCGCU were synthesized chemically on solid support by phosphoramidite procedures and purified by ion exchange chromatography on DEAE-Sephadex with a NaCl gradient in 7 M urea/10 mM tris(hydroxymethyl)aminomethane (Tris), pH 8.2.

Oligonucleotide Solutions. Thermodynamic parameters were measured in 1 M NaCl, 0.005 M Na_2HPO_4, and 0.5 mM disodium ethylenediaminetetraacetate (Na_2EDTA), pH 7. Oligonucleotide concentrations (C_t) are strand concentrations and were calculated from the high-temperature absorbance at 280 nm. Single-strand extinction coefficients were calculated from extinction coefficients for dinucleoside monophosphates and nucleotides as described previously (Freier et al., 1983). In units of 10^4 M^{-1} cm^{-1}, the calculated extinction coefficients are as follows: UGCGCU, 2.48; GGCGCU, 3.03; GCCGGUp, 3.16; UGGCCGp, 3.09; UCCGGGp, 3.36; GCCGGAp, 3.12; GCCGGGp, 3.64; GCCGGUAp, 3.35.

Melting Curves and Thermodynamic Parameters. All melting curves were measured at 280 nm as described previously (Freier et al., 1983).

Thermodynamic parameters of helix formation were obtained from absorbance vs. temperature profiles by two methods: (1) enthalpies and entropies from fits of individual melting curves to a two-state model with sloping base lines were averaged, and (2) linear plots of reciprocal melting temperature (T_M^{-1} vs. log C_t) yielded enthalpies and entropies (Borer et al., 1974):

$$T_M^{-1} = (2.3R/\Delta H°) \log C_t + \Delta S°/\Delta H° \quad (1)$$

We report thermodynamic parameters from plots of T_M^{-1} vs. log C_t and temperature-independent thermodynamic parameters, which are the average of methods 1 and 2. [See Petersheim and Turner (1983) and Freier et al. (1985) for details.] Estimated errors are ±5% in the temperature-independent entropies and enthalpies and ±2% in $\Delta G°$ at the T_M (Freier et al., 1985). Errors in $\Delta G°$ at other temperatures are propagated from the errors in $\Delta H°$ and $\Delta G°_{T_M}$, assuming no correlation in these parameters.

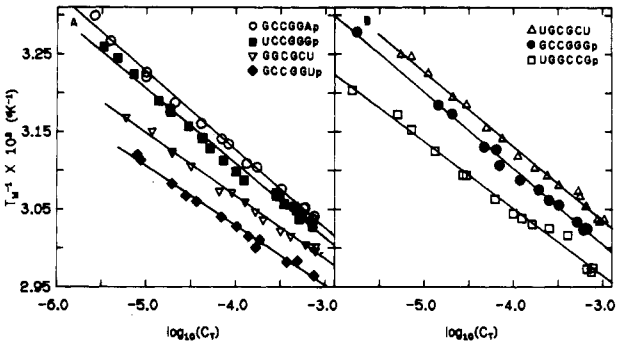

FIGURE 1: Reciprocal melting temperature vs. log concentration for (A) GCCGGAp (○), UCCGGGp (■), GGCGCU (▽), and GCCGGUp (♦) and for (B) UGCGCU (△), GCCGGGp (●), and UGGCCGp (□) in 1 M NaCl, 0.005 M Na_2HPO_4, and 0.5 mM Na_2EDTA, pH 7.

Temperature-dependent thermodynamic parameters are also reported. They were obtained from plots of $\Delta H°$ vs. T_M and $\Delta S°$ vs. ln T_M, where $\Delta H°$ and $\Delta S°$ are the parameters obtained from the fit of each curve to a two-state model with linear sloping base lines. Heat-capacity changes are obtained from the slopes of these plots (Petersheim & Turner, 1983; Freier et al., 1983). Estimated errors in the temperature-dependent free energies, enthalpies, and entropies are ±5, ±10, and ±10%, respectively, at the T_M; errors in $\Delta C_P°$ are ±50% (Freier et al., 1985). Errors in $\Delta H°$ and $\Delta S°$ at other temperatures are propagated from the error in $\Delta C_P°$ and the errors at the T_M.

Results

Temperature-Independent Thermodynamic Parameters. Plots of reciprocal melting temperature (T_M^{-1}) vs. log C_t are shown in Figure 1. Enthalpy and entropy changes obtained from these plots are in Table I. These parameters were also averaged with those derived from fits of individual melting curves to obtain the temperature-independent parameters in Table I.

Temperature-Dependent Thermodynamic Parameters. Fitted enthalpy and entropy changes are slightly dependent on T_M, so thermodynamic parameters should be extrapolated to a common temperature before comparison. Enthalpy and

Table II: Temperature-Dependent Thermodynamic Parameters of Helix Formation[a]

oligomer	$-\Delta H^\circ_{37}$ (kcal/mol)[b,d]	$-\Delta S^\circ_{37}$ (eu)[c,d]	$-\Delta C^\circ_P$ (cal mol^{-1} K^{-1})
UGGCCGp	42.8	110.4	250
UCCGGGp	45.6	123.6	139
GCCGGUp	55.7	149.7	232
GGCGCU	48.4	129.0	421
GCCGGAp	42.9	116.2	716
GCCGGGp	42.8	113.6	585
UGCGCU	43.0	115.8	175
GCCGGUAp	60.3	159.5	282

[a] Measurements were in 1 M NaCl, 0.005 M Na_2HPO_4, and 0.5 mM Na_2EDTA, pH 7. [b] From plots of ΔH° vs. T_M. [c] From plots of ΔS° vs. ln T_M. [d] Although estimated errors in ΔH° and ΔS° at the T_M are ±10%, additional significant figures are given to allow accurate calculation of T_M. Estimated errors in ΔG° at the T_M are ±5% (Freier et al., 1985).

Table III: Excess Stabilization (in kcal/mol) by Terminal Base Mismatches in 1 M NaCl

added terminus	$-\Delta\Delta G^\circ_{37}$[a] for core helix GGCC	CCGG	GCGC
G·U mismatches			
5'Up + 3'pGp	1.5	1.4	
5'Gp + 3'pUp		2.3	
5'Gp + 3'pU			1.9
other mismatches			
5'Ap + 3'pAp		1.1[b]	
5'Ap + 3'pCp		1.1[b]	
5'Ap + 3'pGp		1.6[b]	
5'Gp + 3'pAp		1.3	
5'Gp + 3'pGp		1.5	
5'Up + 3'pU			1.2
Watson–Crick pairs[c]			
5'Ap + 3'pUp	1.6	1.9	
5'Ap + 3'pU	1.5		1.7
5'Cp + 3'pGp	2.3		2.2
5'Gp + 3'pCp		3.4	3.3
5'Up + 3'pAp	1.6	1.6	

[a] $\Delta\Delta G^\circ_{37}$ is half the difference between the free energy of helix formation for the molecule containing the core helix plus the added termini and the free energy of helix formation for the tetramer core. Temperature-independent thermodynamic parameters were used to calculate $\Delta\Delta G^\circ_{37}$. Propagation of the errors in ΔH° and $\Delta G^\circ_{T_m}$ lead to errors in $\Delta\Delta G^\circ_{37}$ of about 0.1 kcal/mol. [b] From Hickey and Turner (1985b). [c] From Petersheim and Turner (1983) and Freier et al. (1985, 1986).

entropy changes at 37 °C obtained from plots of ΔH° vs. T_M or ΔS° vs. ln T_M are reported in Table II. The slope of either plot is the heat capacity change upon helix formation and is listed in Table II. The magnitude of these heat capacity changes is similar to that observed previously and is probably due to single-strand stacking (Freier et al., 1985; Hickey & Turner, 1985a).

Stability Increments for G·U and Other Terminal Mismatches. The free energy increment for adding a G·U or other terminal mismatch to a tetramer core can be calculated from the free energy of helix formation for a hexamer containing the terminal mismatch and the free energy of helix formation for the tetramer core. For example, $\Delta\Delta G^\circ$ for a terminal G·U on GCGC is $(1/2)[\Delta G^\circ(\text{GGCGCU}) - \Delta G^\circ(\text{GCGC})]$. Free energies of helix formation for the tetramer cores GGCC, CCGG, and GCGC have been previously reported (Petersheim & Turner, 1983; Freier et al., 1983, 1986) and were used with the data in Table I to calculate stability increments for terminal mismatches. These are listed in Table III. Increments for terminal Watson–Crick pairs are also listed.

Terminal G·U mismatches stabilize the helix by 1.4–2.3 kcal/mol. In all four cases studied, the free energy contribution of a terminal G·U mismatch is approximately equal to the free energy increment for the corresponding terminal A·U pair and is about 1 kcal/mol less favorable than that of the corresponding G·C pair. This observation contrasts with that for other mismatches. In the case of terminal A·X, G·X, and U·U mismatches, the free energy of the terminal mismatch is always less than that of the corresponding Watson–Crick pairs.

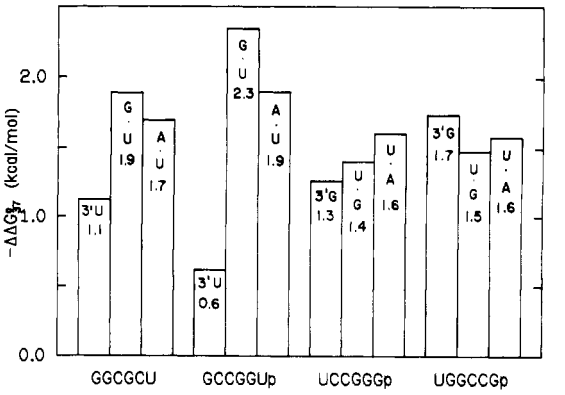

FIGURE 2: Free energy increments at 37 °C for adding 3′ dangling ends or terminal G·U or A·U pairs to a GCGC, GGCC, or CCGG tetramer core. The left-hand column represents the free energy increments for the dangling ends; the center column represents the free energy increment for the G·U pair; the right-hand column is the free energy increment for the terminal A·U pair. The data are from Table III, Petersheim and Turner (1983), and Freier et al. (1983, 1985, 1986).

Discussion

Nearest-Neighbor Free Energy Parameters for G·U and Other Terminal Mismatches. One goal of thermodynamic studies of oligonucleotides is to develop a set of parameters that will predict the stabilities of RNA secondary structures. Most frequently, a nearest-neighbor model is used, and recent experiments indicate it is adequate to predict duplex stability (Freier et al., 1986; Turner et al., 1986). Current parameters (Tinoco et al., 1973; Salser, 1977), however, assign no favorable free energy to terminal G·U base pairs or other terminal mismatches. It is clear from Table III that terminal mismatches contribute significantly to duplex stability, so inclusion of them will improve structure predictions. In the nearest-neighbor approximation, 48 free energy parameters are necessary for terminal mismatches. Single measurements of 10 of these parameters are reported in Table III.

The results in Table III suggest rules to predict the other nearest-neighbor free energy parameters for terminal mismatches until measurements of all 48 are made. For terminal G·U mismatches, the free energy increment is essentially identical with that of the corresponding A·U pair in the same sequence (see Figure 2). For other mismatches, one approximation is to sum the effects of the two dangling ends (Hickey & Turner, 1985b). As shown in Figure 3, this is reasonable for a py·py and a pu·py mismatch but not for the A·A mismatch in ACCGGAp. The limited data in Figure 3 suggest pu·pu mismatches are better approximated by the free energy of the 3′ end alone made more favorable by 0.2 kcal/mol for the effect of the 5′-phosphate (Freier et al., 1983).

Free Energy Increments for 3′ Dangling Ends Next to G·U Mismatches. Free energy increments for dangling ends next to G·C pairs have been reported (Freier et al., 1986). What contribution will be made by a dangling end next to a G·U mismatch? In GCCGGUAp, each 3′ dangling A contributes about −1.0 kcal/mol to duplex stability at 37 °C (see Table I). The predicted increments are −1.7 and −0.6 kcal/mol,

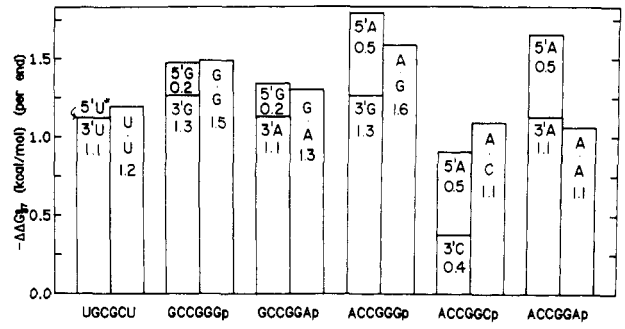

FIGURE 3: Free energy increments at 37 °C for adding dangling ends or terminal G·X, A·X, or U·U mismatches to a GCGC or CCGG tetramer core. The left-hand column represents the free energy increments for the dangling ends; the right-hand column is the free energy increment for the terminal mismatch. The dangling end data are from Petersheim and Turner (1983) and Freier et al. (1983, 1985, 1986). Data for terminal mismatches are from Hickey and Turner (1985b) and Table III. (*) This increment for a 5′-U was measured on GGCC. The lengths of the bars are exact whereas the values inside the bars are rounded to two places.

respectively, for a 3′ dangling A on a G·C or A·U pair (Freier et al., 1986; N. Sugimoto and D. H. Turner, unpublished results). These reults suggest helix stabilization by 3′ dangling ends next to G·U mismatches will be similar to that for 3′ ends next to Watson–Crick pairs. In a fragment from 16S rRNA, the terminal G_{597}·U_{606} mismatch is followed by a 3′ dangling A_{607}, which is sensitive to double-helix-specific nuclease (Kean & Draper, 1985). This suggests similar stacking effects are present in large RNA molecules.

Comparison to Previous Estimates of Terminal Mismatch Stability. Free energies of terminal pairs and mismatches have been derived by Papanicolaou et al. (1984) by fitting predictions of tRNA and 5S rRNA secondary structures to consensus structures. Their free energy changes for terminal G·U mismatches are less favorable by an average of 0.6 kcal/mol at 25 °C compared to the measured values [calculated as for Table III from data in Table I, Petersheim & Turner (1983), Freier et al. (1983, 1986), and Hickey & Turner (1985b)]. Other mismatches differ in a random fashion with an average magnitude of the difference of 0.4 kcal/mol.

Contributions of Stacking and Hydrogen Bonding to Stability of Terminal G·U Mismatches and G·C Pairs. Comparison of the free energy of a terminal mismatch or pair to the stability increment for the corresponding 3′ dangling end can be used to estimate the relative importance of base stacking and pairing to helix stability (Petersheim & Turner, 1983; Freier et al., 1985, 1986). Such comparisons are illustrated in Figure 2. For UCCGGGp and UGGCCGp, stacking of the 3′ dangling G provides essentially the same stability as the terminal G·U mismatch. When stacking is weak, however, as in GCCGGUp, hydrogen bonding appears to be important. A similar result is observed for Watson–Crick pairs (Freier et al., 1985, 1986).

Since G·U mismatches and G·C pairs have two and three hydrogen bonds, respectively, the difference in free energy increments for terminal G·U mismatches and G·C pairs provides an estimate for the free energy of a hydrogen bond. For GCCGGUp, UGGCCGp, and GGCGCU, this difference is −1.1, −0.8, and −1.4 kcal/mol, respectively, corresponding to the conversion from a G·U to a G·C pair. This analysis assumes the contributions of stacking and other factors are identical in both sequences and may be most appropriate for GCCGGUp and GCCGGCp where stacking is weak. It also neglects the fact that different kinds of hydrogen bonds are present in G·U mismatches and G·C pairs. The value of about −1 kcal/mol of hydrogen bond, however, agrees with that observed for hydrogen bonds in other terminal pairs (Freier et al., 1986).

Stabilities of G·U and Other Terminal Mismatches in Natural RNAs. The data in Table III can be compared to predictions regarding the sequence dependence of terminal G·U mismatch stability. Examination of stacking geometries around the G_4·U_{67} base pair in the amino acid acceptor stem of yeast $tRNA^{Phe}$ and the distribution of G·U pairs at the ends of helical stems in the cloverleaf structure of tRNA led Mizuno and Sundaralingum (1978) to propose a terminal G·U pair will be more stable if it contains a 5′-G and a 3′-U than if it is a 5′-U and a 3′-G. In addition, they suggested the stabilization would be greatest if the 5′-G is next to a pyrimidine. The data in Table III confirm these predictions. First, terminal G·U pairs in $\overset{GG\rightarrow}{UC\leftarrow}$ and $\overset{GC\rightarrow}{UG\leftarrow}$ sequences are more stable than in $\overset{UG\rightarrow}{GC\leftarrow}$ and $\overset{UC\rightarrow}{GG\leftarrow}$, respectively. Second, the terminal pair in GCCGGUp is more stable than the terminal pair in GGCGCU. The small stability increment observed for 5′ dangling G stacking (Freier et al., 1985, 1986), however, suggests these differences may not be due to favorable stacking interactions of the 5′-G.

Terminal G·U pairs occur in the association of codons and wobble codons to tRNAs. For initiator tRNAs, the wobble codon containing a terminal G·U mismatch binds as tightly as the codon with a terminal A·U pair (Uhlenbeck et al., 1970; Freier & Tinoco, 1975). For three elongation tRNAs, the terminal G·U pair of the wobble codon–tRNA complex is less stable than the corresponding G·C pair (Eisinger et al., 1970; Schimmel et al., 1972; Uhlenbeck, 1972). These results agree with predictions of Table III.

Terminal mismatches also affect predicted stabilities of complexes between messenger and ribosomal RNAs. For example, terminal mismatches are predicted to add −2.1 kcal/mol to the free energy of the proposed pairing between the ribosome binding site of the replicase gene in R17 and the 3′ terminus of *Escherichia coli* 16S rRNA (Shine & Dalgarno, 1974). This represents 30% of the calculated base pairing free energy and corresponds to a 30-fold increase in the association constant at 37 °C.

Inclusion of a favorable free energy for terminal G·U pairs will affect free energy changes calculated for RNA secondary structure. Not only will the G·U pair at the end of a helical stem make a favorable contribution to the stability of the stem, but removal of two residues from the adjacent loop will often reduce the unfavorable loop free energy. For example, the calculated ΔG°_{37} of helix formation for alternate helix IV between residues 688–690 and 699–701 of *E. coli* 16S rRNA (Kean & Draper, 1985) is −0.2 kcal/mol if only two G·C pairs are allowed in the stem. If a G·U pair is also included in the helix, the free energy of helix formation is −2.5 kcal/mol. This 40-fold increase in the predicted helix association constant clearly influences structure predictions.

On the basis of observed mismatch frequency in native RNAs, it has been proposed A·G mismatches are especially stable (Woese et al., 1983), particularly if they contain a 5′-A and a 3′-G at the end of a helix (Traub & Sussman, 1982). The terminal A·G mismatch in ACCGGGp is more stable than other mismatches (see Figure 3 and Table III), but the difference is within experimental error for the G·G mismatch in GCCGGGp.

The observation of G·U pairs at the ends of proposed helical regions in natural RNAs and the observed contribution of G·U and other terminal mismatches to helix stability in oligo-

nucleotides demonstrate the importance of these non-Watson–Crick structures. Presumably, predictions of RNA secondary structure will improve if free energies of terminal mismatches are explicitly included in the calculations.

Registry No. UGGCCGp, 101696-79-1; UCCGGGp, 101696-80-4; GCCGGUp, 101696-81-5; GGCGCUp, 101696-82-6; GCCGGAp, 101696-83-7; GCCGGGp, 101696-84-8; UGCGCU, 101696-85-9; GCCGGUAp, 101696-86-0; G, 73-40-5; U, 66-22-8.

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