Sequence Dependence for the Energetics of Dangling Ends and Terminal Base Pairs in Ribonucleic Acid†

Naoki Sugimoto,‡ Ryszard Kierzek,§ and Douglas H. Turner*,‡

Department of Chemistry, University of Rochester, Rochester, New York 14627, and Institute of Bioorganic Chemistry, Polish Academy of Sciences, 60-704 Poznan, Noskowskiego 12/14, Poland

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ABSTRACT: Stability increments of terminal unpaired nucleotides (dangling ends) and terminal base pairs on the core helixes AUGCAU and UGCGCA are reported. Enthalpy, entropy, and free energy changes of helix formation were measured spectrophotometrically for 18 oligoribonucleotides containing the core sequences. The results indicate 3′ dangling purines add more stability than 3′ dangling pyrimidines. In most cases, the additional stability from a 3′ dangling end on an AU base pair is less than that on a GC base pair [Freier, S. M., Burger, B. J., Alkema, D., Neilson, T., & Turner, D. H. (1983) *Biochemistry 22*, 6198–6206]. The sequence dependence provides a test for the importance of dangling ends for various RNA interactions. Correlations are suggested with codon context effects and with the three-dimensional structure of yeast phenylalanine transfer RNA. In the latter case, all terminal unpaired nucleotides having stability increments more favorable than −1 kcal/mol are stacked on the adjacent base pair. All terminal unpaired nucleotides having stability increments less favorable than −0.3 kcal/mol are not stacked on the adjacent base pair. In several cases, this lack of stacking is associated with a turn in the sugar–phosphate backbone. This suggests stability increments measured on oligoribonucleotides may be useful for predicting tertiary structure in large RNA molecules. Comparison of the stability increments for terminal dangling ends and base pairs, and of terminal GC and AU base pairs, indicates the free energy increment associated with forming a hydrogen bond can be about −1 kcal/mol of hydrogen bond.

Unpaired terminal nucleotides (dangling ends) increase the stability of ribooligonucleotide double helixes (Martin et al., 1971; Romaniuk et al., 1978; Petersheim & Turner, 1983; Freier et al., 1985, 1986a). It has been suggested this effect is important in determining the stability of codon–anticodon associations (Grosjean et al., 1976; Yoon et al., 1976) and that it might be responsible for some codon context effects (Ayer & Yarus, 1986). While the extra stability conferred on terminal GC base pairs has been measured, little is known about the effect on terminal AU pairs. This paper reports thermodynamic parameters for dangling ends and terminal base pairs on the terminal AU pairs in the core sequences AUGCAU and UGCGCA. The results suggest sequence effects can be used to indicate the importance of dangling ends for various associations. Comparisons with the crystal structure of tRNA (Kim et al., 1974; Robertus et al., 1974) suggest the interactions measured with dangling ends may also be important for determining tertiary structure. In addition, the results support previous suggestions that both stacking and hydrogen bonds make significant contributions to the stability of base pairs (Freier et al., 1986a).

Materials and Methods

Oligonucleotide Synthesis. XAUGCAU (X = A, C, G, U) and UGCGCA were synthesized chemically on a solid support with phosphoramidite procedures (Kierzek et al., 1986) and purified by anion-exchange chromatography on diethylaminoethyl-Sephadex (A-25) with NaCl gradients in 7 M urea and 10 mM tris(hydroxymethyl)aminomethane pH 8.2. These oligomers do not have terminal phosphates.

AUGCAUZp, XAUGCAUZp, AUGCGCA, AUGCGCAUp, and UGCGCAZp (Z = A, C, G, U) were synthesized by successive additions of appropriate nucleoside 3′,5′-bisphosphates with T4 RNA ligase (Beckett & Uhlenbeck, 1984) to AUG (Sigma), XAUGCAU, or UGCGCA as described previously (Freier et al., 1986a). Except for AUGCGCA, these oligomers have a 3′ terminal phosphate.

Melting Curves. Extinction coefficients were calculated with the nearest-neighbor approximation (Richards, 1975). The calculated extinction coefficients ($\times 10^{-4}$ cm^{-1} M^{-1}) at 260 nm are for AUGCAUp, 5.09; for AUGCAUAp, 6.11; for AUGCAUCp, 5.49; for AUGCAUGp, 6.09; for AUGCAUUp, 5.82; for AAUGCAU, 5.76; for CAUGCAU, 5.63; for GAUGCAU, 6.06; for UAUGCAU, 5.83; for AAUGCAUUp, 7.20; for CAUGCAUGp, 6.86; for GAUGCAUCp, 6.85; for UAUGCAUAp, 6.72; for UGCGCA, 4.13; for UGCGCAAp, 5.18; for UGCGCACp, 4.78; for UGCGCAGp, 5.43; for UGCGCAUp, 4.91; for AUGCGCA, 5.34; and for AUGCGCAUp, 6.24. Total strand concentrations C_T were determined from the absorbance measured at 260 nm at 80 or 90 °C.

Absorbance vs. temperature melting curves were measured at 260 nm on a Gilford 250 spectrometer as described previously (Freier et al., 1983). The heating rate was 1 °C/min. The buffer was 1 M NaCl, 10 mM Na_2HPO_4, and 0.1 mM Na_2EDTA, pH 7. For each oligonucleotide, 12–15 absorbance vs. temperature profiles were measured over a 100-fold range in strand concentration.

Thermodynamic parameters for double-helix formation were obtained by two methods described previously (Petersheim & Turner, 1983; Freier et al., 1986a): (1) enthalpy and entropy changes derived from fitting individual melting curves to a two-state model were averaged and (2) reciprocal melting temperature T_M^{-1} was plotted vs. log C_T to give enthalpy and entropy changes.

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* Author to whom correspondence should be addressed.

‡ University of Rochester.

§ Polish Academy of Sciences.

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Table I: Thermodynamic Parameters of Helix Formation[a,b]

oligomer	log C_T parameters			temperature-independent parameters		
	$-\Delta H°$[c] (kcal/mol)	$-\Delta S°$[c] (eu)	T_M[c,d] (°C)	$-\Delta H°$[e] (kcal/mol)	$-\Delta S°$[e] (eu)	T_M[d,e] (°C)
core helix						
AUGCAU$_p$[f]	41.7	119.2	30.0	38.4	108.4	30.2
3′ dangling ends						
AUGCAUAp	53.2	152.0	39.1	51.2	145.7	39.2
AUGCAUCp	43.1	122.9	31.8	41.6	118.0	31.9
AUGCAUGp	53.2	152.0	39.4	51.3	145.9	39.6
AUGCAUUp	46.1	132.8	32.1	42.9	122.2	32.4
5′ dangling ends						
AAUGCAU	38.6	107.1	34.3	36.9	101.7	34.8
CAUGCAU	37.3	103.4	33.7	35.3	96.4	34.6
GAUGCAU	40.3	112.4	35.1	39.9	111.0	35.7
UAUGCAU	35.5	98.0	32.2	35.4	97.6	32.7
terminal base pairs						
AAUGCAUUp	59.8	169.7	45.0	57.7	163.0	45.2
CAUGCAUGp	73.7	206.3	54.8	71.5	199.6	54.9
GAUGCAUCp	72.6	201.9	57.2	75.3	209.8	57.1
UAUGCAUAp	67.7	195.0	44.5	64.6	184.9	44.6
core helix						
UGCGCA	50.8	137.6	52.7	51.9	141.1	52.7
3′ dangling ends						
UGCGCAAp	60.5	164.1	58.7	58.5	157.9	58.9
UGCGCACp	52.5	139.9	59.0	54.3	145.3	58.9
UGCGCAGp	61.8	167.7	59.2	59.3	159.9	59.4
UGCGCAUp	55.3	148.4	58.7	56.1	150.8	58.7
5′ dangling ends						
AUGCGCA	51.8	138.9	56.3	52.2	140.2	56.4
terminal base pairs						
AUGCGCAUp[f]	64.4	174.8	60.3	60.7	163.6	60.5

[a] Measurements were in 1 M NaCl, 10 mM Na_2HPO_4, and 0.1 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. [c] From plots of reciprocal melting temperature vs. log C_T. [d] Calculated for 10^{-4} M strand concentration. [e] Temperature-independent thermodynamic parameters are the average of those from plots of T_M^{-1} vs. log C_T and those from averaging fits of individual melting curves to a two-state model with sloping base lines. [f] From Sugimoto et al. (1986).

Results

Temperature-Independent Thermodynamic Parameters. Plots of reciprocal melting temperature vs. log C_T are shown in Figure 1 and in the supplementary material (see paragraph at end of paper regarding supplementary material). Enthalpy and entropy changes derived from these plots are in Table I. These parameters were averaged with those derived from fits of individual melting curves to obtain "temperature-independent" parameters which are also listed in Table I. The agreement between values derived from log C_T plots and from fitting melting curves is a measure of the two-state character of the helix–coil transitions (Petersheim & Turner, 1983; Albergo et al., 1981; Hickey & Turner, 1985). All oligomers listed in Table I melt in an essentially two-state manner, except for AUGCAUp and AUGCAUUp. For these oligomers, the two analysis methods give $\Delta H°$'s that differ by 16% and 14%, respectively. Thus comparisons with the $\Delta H°$'s measured for these oligomers should be treated with caution. For both oligomers, however, the difference in the $\Delta G°_{37}$ derived from the two analysis methods is less than 2%. Thus comparisons of free energy changes can be made with confidence.

Temperature-Dependent Thermodynamic Parameters. The enthalpy and entropy changes derived by fitting individual melting curves are not constant but rather change consistently as the melting temperature increases. Thus the fitted $\Delta H°$ and $\Delta S°$ values were plotted vs. T_M and ln T_M, respectively, to provide heat capacity changes $\Delta C_p°$ between helix and coil. These are listed in Table II, along with enthalpy and entropy changes for helix formation extrapolated to 37 °C.

Inspection of Table II indicates $\Delta C_p°$ is especially large for the oligomers with 5′ dangling ends. This suggests something special about these transitions. One possible origin for this extra heat capacity is a temperature-dependent aggregation. These helixes also do not have terminal phosphates to inhibit aggregation. The results raise the possibility that oligomers with 5′ dangling ends may be particularly prone to aggregation.

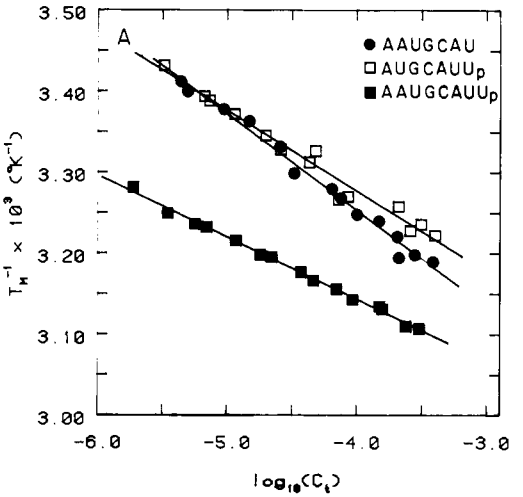

FIGURE 1: Reciprocal melting temperature vs. log (concentration) for AAUGCAU (●), AUGCAUUp (□), and AAUGCAUUp (■) in 1 M NaCl, 10 mM Na_2HPO_4, and 0.1 mM Na_2EDTA, pH 7.

Discussion

The data in Table I can be used to derive free energy increments for dangling ends and terminal base pairs next to AU base pairs (Freier et al., 1985, 1986a). These are listed in Table III along with increments on GC pairs determined previously (Freier et al., 1985, 1986a). One trend is common to both cases: 3′ dangling purines add more stability than 3′

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

oligomer	$-\Delta H°_{37}$[c] (kcal/mol)	$-\Delta S°_{37}$[d] (eu)	$-\Delta C_p°$ (cal mol^{-1} K^{-1})
core helix			
AUGCAUp[e]	37.8	107.1	379
3′ dangling ends			
AUGCAUAp	49.3	137.8	60
AUGCAUCp	40.7	114.9	67
AUGCAUGp	49.5	140.5	186
AUGCAUUp	41.6	117.5	234
5′ dangling ends			
AAUGCAU	38.6	107.4	572
CAUGCAU	39.2	109.4	717
GAUGCAU	46.2	131.7	1076
UAUGCAU	42.0	119.0	667
terminal base pairs			
AAUGCAUUp	54.8	152.3	195
CAUGCAUGp	68.2	194.9	80
GAUGCAUCp	76.3	214.2	88
UAUGCAUAp	61.2	172.4	67
core helix			
UGCGCA	52.4	143.4	48
3′ dangling ends			
UGCGCAAp	54.6	145.4	115
UGCGCACp	52.8	140.0	181
UGCGCAGp	54.8	143.0	108
UGCGCAUp	53.2	140.6	214
5′ dangling ends			
AUGCGCA	47.0	124.2	348
terminal base pairs			
AUGCGCAUp[e]	53.5	140.5	178

[a] Measurements were in 1 M NaCl, 10 mM Na_2HPO_4, and 0.1 mM Na_2EDTA, pH 7. [b] Although estimated errors in $\Delta H°$ and $\Delta S°$ at the T_M are ±10%, additional significant figures are given to allow accurate calculation of T_M. [c] From plots of $\Delta H°$ vs. T_M. [d] From plots of $\Delta S°$ vs. ln T_M. [e] From Sugimoto et al. (1986).

Table III: Excess Stabilization (in Kilocalories per Mole) by Dangling Ends and Terminal Base Pairs in 1 M NaCl

	$-\Delta\Delta G°_{37}$ for core helix			
added terminus	AUGCAUp[a]	UGCGCA[a]	GCGC or GGCC[b]	CCGG[b]
5′Ap	0.3	0.3	0.2	0.5
5′Cp	0.3		0.3	
5′Gp	0.3		0.0	0.2
5′Up	0.2		0.1	0.1
3′Ap	0.6	0.7	1.8	1.1
3′Cp	0.1	0.5	0.8	0.4
3′Gp	0.6	0.7	1.7	1.3
3′Up	0.1	0.6	1.2	0.6
5′Ap + 3′Up (pair)	1.2 (0.9)	0.9 (0.9)	1.6	1.9
5′Cp + 3′Gp (pair)	2.4 (1.8)		2.3	
5′Gp + 3′Cp (pair)	2.7 (2.3)		3.3	3.4
5′Up + 3′Ap (pair)	1.2 (1.1)		1.6	1.6

[a] $\Delta\Delta G°_{37}$ 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 core. Temperature-independent thermodynamic parameters were used to calculated $\Delta\Delta G°_{37}$. Estimated errors in $\Delta\Delta G°_{37}$ are about 0.1 kcal/mol. The values in parentheses were based on the nearest-neighbor parameters of Freier et al. (1986c). [b] From Freier et al. (1986a). For cases where measurements were made on both GCGC and GGCC cores, the average is reported.

dangling pyrimidines. This may be the reason the base 3′ to the anticodon in tRNA is always a purine (Grosjean et al., 1986; Sprinzl & Gauss, 1984). In order to provide the stability of 5 base pairs for the original codon–anticodon association, it has been suggested the initial genetic code had the form RRY RRY RRY (Crick et al., 1976) or RNY RNY RNY (Eigen & Schuster, 1978; Shepard, 1981), where R is a purine, Y is a pyrimidine, and N is any nucleotide. In both schemes, the complex between messenger RNA and the tRNA carrying the polypeptide chain is further stabilized by a 3′ dangling purine from the messenger strand. This may have been selected because it provides the most stable codon–anticodon complex in the absence of other factors. As other factors became available for stabilizing the complex, dangling end effects may have facilitated the conversion to a purer 3-base code. In this respect, it is interesting that A is never found in the 5′-position of the anticodon. For each 3′ dangling end X, the omitted sequence $5'^{A}_{UX}3'$ provides the smallest free energy increment from the dangling end.

Other trends in Table III depend on whether the dangling end is on a GC or AU base pair. For GC pairs, a 5′ dangling end always provides less stability than the corresponding 3′ dangling end. This is not true for dangling ends on AU pairs. In particular, the smallest stability increment of −0.1 kcal/mol is associated with adding a 3′ dangling pyrimidine next to a U to give a $5'^{A}_{UY}3'$ sequence. When 3′ dangling ends, X, are added next to GC pairs, the stability increment is always larger for the 5′CX3′ than the 5′GX3′ sequence. For AU pairs, the increments for 5′UR3′ and 5′AR3′ sequences are essentially the same within experimental error. These changes in trends reflect to some extent another trend: For most sequences, a 3′ dangling end on a GC pair enhances stability more than the same 3′ dangling end on an AU pair. The only exceptions are 5′RY3′ sequences where the stability increments are the same within experimental error for R = A or G. The larger stability increments on GC pairs presumably are due to more favorable electronic interactions. This may be due to the larger dipole moments for guanine and cytosine relative to adenine and uracil (Devoe & Tinoco, 1962; Pullman & Pullman, 1968). The stability increment for a 3′ dangling A on a GU pair is intermediate between a 3′ A on a GC and an AU pair (Freier et al., 1986b), consistent with the suggested rationale.

It has been suggested that codon context effects for suppression of amber (UAG) mutations may be due to dangling end effects (Ayer & Yarus, 1986). In particular, greater suppression is observed for a UAGR sequence than for UAGY, even when the tRNA is unable to form an RU base pair with the 3′ dangling R. If dangling end effects are responsible, then the results in Table III suggest context effects on suppression of ochre (UAA) and opal (UGA) mutations should be smaller than observed for amber mutations. Although comparable data for ochre and opal mutations are not available, suppression of opal mutations in one study was weaker than for amber mutations (Miller & Albertini, 1983). This is the trend predicted from the results in Table III. In general, the results in Table III suggest dangling end effects should be quite sequence dependent. This sequence dependence can thus provide one experimental test for the importance of dangling end effects for various biological processes.

Sequence dependence in the three-dimensional structure of tRNA suggests the interactions measured by dangling end effects may also be important in determining RNA tertiary structure. In the cloverleaf structure of tRNA, there are 12 nucleotides immediately adjacent to base-paired regions (Holley et al., 1965). These include nucleotides in hairpin and multibranched loops. In the crystal structure of yeast phenylalanine tRNA, the bases of seven of these nucleotides are stacked on the adjacent base pair in the secondary structure and five are not (Kim et al., 1974; Robertus et al., 1974; Quigley & Rich, 1976; Cantor & Schimmel, 1980). From the results in Table III, all five unstacked nucleotides (U8, A9, A21, C48, and C60) are expected to have $\Delta G°$'s less favorable than −0.3 kcal/mol as dangling ends. Four out of

five of these nucleotides occur at turns in the sugar–phosphate backbone. In contrast, four of the seven stacked nucleotides (A14, m_2^2G26, A44, and A73) would have $\Delta G°$'s more favorable than −1.0 kcal/mol as dangling ends. This assumes the two methyl groups on m_2^2G make the free energy of stacking more favorable, as expected (D'Andrea et al., 1983; Olsthoorn et al., 1980; T'so, 1974; Pörschke & Eggers, 1972). Another stacked nucleotide, T54, is also likely to have a $\Delta G°$ for stacking of about −1 kcal/mol due to methylation. None of these nucleotides occur at turns in the sugar–phosphate backbone. The last two stacked nucleotides, C_m32 and A38, would have $\Delta G°$'s of −0.5 and −0.3 kcal/mol, respectively, as dangling ends. Although these $\Delta G°$'s are small, both bases are found stacked at the end of the anticodon stem. This stacking may be enhanced because both bases are hydrogen bonded through water molecules to a Mg^{2+} ion located inside the anticodon loop (Teeter et al., 1980). Thus, a rule consistent with available data is that a base is likely to be stacked on the base pair adjacent in the secondary structure if the $\Delta G°$ for the equivalent dangling end is more favorable than −1.0 kcal/mol. It is not likely to be stacked on the adjacent base pair if the $\Delta G°$ for the equivalent dangling end is less favorable than −0.3 kcal/mol. For intermediate $\Delta G°$'s, stacking will likely be determined by other factors. In addition, turns in the sugar–phosphate backbone are favored by weak stacking interactions.

An interesting test of the above rule is provided by U8 in tRNA. In the secondary structure of yeast phenylalanine tRNA, U8 is a 3′ dangling end in a $5'\overset{A}{UU}3'$ sequence. In the crystal structure, U8 is unstacked. From Table III, the $\Delta\Delta G°$'s associated with a 3′ dangling U in the sequences 5′UU3′, 5′AU3′, 5′GU3′, and 5′CU3′ are −0.1, −0.6, −0.6, and −1.2 kcal/mol, respectively. Thus the sequence CU is least favorable for the unstacked conformation observed in the crystal structure. In the 281 known tRNA sequences (Sprinzl & Gauss, 1984), CU is found in this position only 8 times. Thus the suggested rule is also consistent with phylogenetic data for tRNA. This suggests the parameters reported in Table III may be useful for predicting RNA tertiary structure. Conformation of this requires determination of more three-dimensional structures for RNAs.

Predictions of RNA stability and secondary structure are most often based on a nearest-neighbor model (Tinoco et al., 1971). This model treats terminal and internal base pairs the same. It has been suggested, however, that terminal pairs may be less stable than internal pairs because the hydrogen bonds are exposed to water (Levitt, 1972). Conversely, Papanicolaou et al. (1984) found it necessary to increase the stability of terminal GC pairs by 0.8 kcal/mol in order to improve predictions of RNA secondary structure. On the basis of the nearest-neighbor model, the results in Table III provide single direct measurements for the free energy of propagation for five terminal base pairs. These free energy changes can be compared with those derived for the corresponding sequences from a nearest-neighbor analysis of 45 oligoribonucleotides (Freier, 1986c). The nearest-neighbor values are listed in parentheses in Table III. In all cases, the terminal base pairs are slightly more stable than expected from the nearest-neighbor analysis. The difference, however, is within experimental error, except for CAUGCAUGp. The terminal GC pairs for CAUGCAUGp are 0.6 kcal/mol more stable than expected from the nearest-neighbor analysis of Freier et al. (1986c). The terminal GC pairs of GAUGCAUCp (see Table III) and other oligomers (Freier et al., 1986a) do not have enhanced stability, however. Thus extra stability is not a

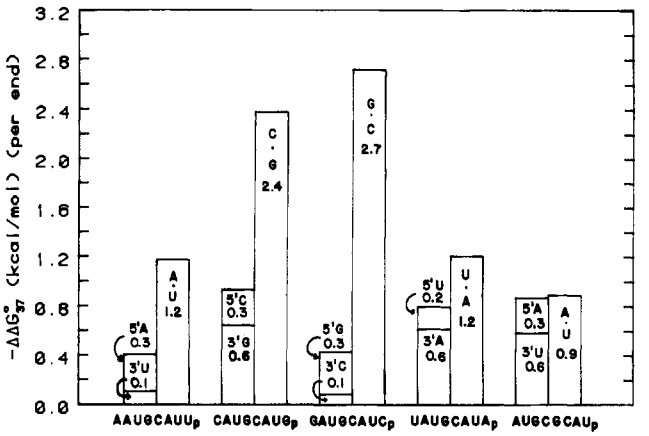

FIGURE 2: Free energy increments at 37 °C for adding a terminal base pair or dangling end to an AUGCAU or UGCGCA core. The left-hand column represents free energy increments for 5′ and 3′ dangling ends; the right is the free energy of base pair formation. The free energy increments are from Table III.

Table IV: Empirical Estimates of $\Delta\Delta G°_{37}$ (in Kilocalories per Mole) for Hydrogen Bonds in Terminal Base Pairs

oligomer	$-\Delta\Delta G°_{37}$ for empirical pairing[a]	$-\Delta\Delta G°_{37}$ per hydrogen bond: from corrected empirical pairing[b]	$-\Delta\Delta G°_{37}$ per hydrogen bond: from (GC − AU)[c]
AAUGCAUUp	0.8	1.4	
CAUGCAUGp	1.5	1.1	1.2
GAUGCAUCp	2.3	1.4	1.5
UAUGCAUAp	0.4	1.2	
AUGCGCAUp	0.1	1.0	(1.4)[d]

[a] Empirical pairing is defined as $\Delta\Delta G°_{emp}$(pairing) = $\Delta\Delta G°_{emp}$(bp) − $\Delta\Delta G°_{emp}$(3′ dangle) − $\Delta\Delta G°_{emp}$(5′ dangle). [b] The stabilization per H bond was calculated with the assumption that the configurational $\Delta\Delta G°_{37}$ for fixing the 5′-nucleotide in a base pair is 1.9 kcal/mol. For a base pair with n H bonds, the stabilization per H bond is defined as $\Delta\Delta G°$(H bond) = $(1/n)[\Delta\Delta G°_{emp}$(pairing) − 1.9]. [c] These values are calculated by subtracting the free energy increment for a terminal AU pair from the free energy increment for the corresponding terminal GC pair. [d] $\Delta\Delta G°_{37}$ of GUGCGCAC was predicted by using nearest-neighbor parameters of Freier et al. (1986c).

property of most terminal GC pairs. The melting temperature of CAUG at 9.2×10^{-3} M (Romaniuk et al., 1978) is 9 °C higher than predicted by the nearest-neighbor parameters of Freier et al. (1986c), whereas the melting temperature of UCAUGA is 9 °C lower than predicted (Kierzek et al., 1986). The results suggest the sequences CAUG or AUG may have unusual context effects not included in the nearest-neighbor model. For most cases, however, terminal base pairs on short helixes seem thermodynamically the same as internal pairs.

As shown in Figure 2, the free energy increments for three out of five of the terminal base pairs reported here are considerably larger than the sums of the free energy increments for their corresponding dangling ends. This suggests pairing effects, most likely hydrogen bonding, make a contribution to terminal base pair stability. Stability increments for terminal base pairs and dangling ends on GC pairs have been used previously to derive estimates for the free energy increment associated with a hydrogen bond (Freier et al., 1986a). Two measures were used. In the first, the increments for terminal base pairs were reduced by the increments for the corresponding dangling ends to give a value for "empirical pairing". This value was corrected for configurational effects to give the $\Delta G°$ due to hydrogen bonds. In the second, the difference between free energy increments for terminal GC

and AU pairs was taken as a measure of the $\Delta G°$ contributed by a hydrogen bond. The latter procedure presumes similar stacking contributions for GC and AU pairs. The results in Table III suggest that terminal A and G dangling ends or U and C dangling ends make similar contributions to the stability of a helix. Thus the results in Table I were analyzed with both methods suggested by Freier et al. (1986a) to provide additional estimates for the $\Delta G°$ contributed by a hydrogen bond. The results are listed in Table IV. Both methods suggest a hydrogen bond contributes roughly −1 kcal/mol to the stability of a base pair, in agreement with the conclusions of Freier et al. (1986a).

Supplementary Material Available

Five figures showing reciprocal melting temperature vs. log C_T (5 pages). Ordering information is given on any current masthead page.

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