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Contributions of Dangling End Stacking and Terminal Base-Pair Formation to the Stabilities of XGGCCp, XCCGGp, XGGCCYp, and XCCGGYp Helixes†

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ABSTRACT: The role of stacking in terminal base-pair formation was studied by comparison of the stability increments for dangling ends to those for fully formed base pairs. Thermodynamic parameters were measured spectrophotometrically for helix formation of the hexanucleotides AGGCCUp, UGGCCAp, CGGCCGp, GCCGGCp, and UCCGGAp and for the corresponding pentanucleotides containing a 5′-dangling end on the GGCCp or CCGGp core helix. In 1 M NaCl at 1×10^{-4} M strands, a 5′-dangling nucleotide in this series increases the duplex melting temperature (T_m) only 0–4 °C, about the same as adding a 5′-phosphate. In contrast, a 3′-dangling nucleotide increases the T_m at 1×10^{-4} M strands 7–23 °C, depending on the sequence [Freier, S. M., Burger, B. J., Alkema, D., Neilson, T., & Turner, D. H. (1983) *Biochemistry 22*, 6198–6206]. These results are consistent with stacking patterns observed in A-form RNA. The stability increments from terminal A·U, C·G, or U·A base pairs on GGCC or a terminal U·A pair on CCGG are nearly equal to the sums of the stability increments from the corresponding dangling ends. This suggests stacking plays a large role in nucleic acid stability. The stability increment from the terminal base pairs in GCCGGCp, however, is about 5 times the sum of the corresponding dangling ends, suggesting hydrogen bonding can also make important contributions.

Pairing of complementary bases and stacking of base pairs contribute to nucleic acid stability (Cantor & Schimmel, 1980; Bloomfield et al., 1974). Oligonucleotide helixes with terminal unpaired residues (dangling ends) provide useful model systems to study the role of stacking in nucleic acid stability (Martin et al., 1971; Romaniuk et al., 1978; Neilson et al., 1980; Alkema et al., 1981a,b; Petersheim & Turner, 1983; Freier et al., 1983a, 1984). "Pairing" effects can then be estimated by subtracting the free energy due to stacking from the total free energy of a base pair.

Previously we examined the sequence dependence of the stability increment provided by adding an unpaired nucleotide to the 3′ terminus of GGCC[1] and CCGG and found the melting temperature at 1×10^{-4} M strands increases 7–23 °C in 1 M NaCl (Freier et al., 1983a, 1984). We report below the effects of adding a dangling nucleotide to the 5′ end of GGCC or CCGG and of adding base pairs to both ends of either core. Comparison of these results provides an empirical measure of the contributions of stacking and pairing to the free energy of terminal base pairs.

Materials and Methods

Oligonucleotide Synthesis. AGGC, CGGC, UGG, GCCG, and UCCG were synthesized chemically by using phosphotriester procedures and were characterized by ^{1}H NMR (England & Neilson, 1976; Werstiuk & Neilson, 1976; Alkema et al., 1981a, 1982; Sinclair et al., 1984).

UGGCp was prepared by addition of pCp to UGG using T4 RNA ligase (Uhlenbeck & Cameron, 1977; England & Uhlenbeck, 1978). Following purification of the product, the 3′-phosphate was removed by incubation with calf alkaline phosphatase to yield UGGC. Conditions for the ligase reaction are given by Freier et al. (1983a); conditions for the phosphatase reaction are given below. The pentanucleotides XGGCCp and XCCGGp were synthesized from the respective tetranucleoside triphosphates by using the appropriate 5′–

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[1] For oligonucleotides, internal phosphates are not denoted; GGCC is GpGpCpC. If a molecule contains a terminal phosphate, it is explicitly indicated.

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3′-nucleoside bisphosphate and T4 RNA ligase.

After purification of the pentamers, the 3′-terminal phosphate was removed by incubation at 37 °C with 100 units/mL calf alkaline phosphatase in 0.05 M tris(hydroxymethyl)-aminomethane hydrochloride (Tris-HCl), pH 8.5. After the reaction was complete, boiling for 5 min removed all phosphatase activity. No magnesium was added to the phosphatase reaction because boiling in the presence of magnesium led to hydrolysis of the RNA oligomer.

The hexanucleotides AGGCCUp, CGGCCGp, UGGCCAp, GCCGGCp, and UCCGGAp were synthesized from the pentanucleoside tetraphosphates and 5′–3′-nucleoside bisphosphates by using T4 RNA ligase (Freier et al., 1983a). AGGCCU was obtained by reaction of AGGCCUp with calf alkaline phosphatase using conditions described above.

pGGCCU was synthesized from GGCCUp by using T4 polynucleotide kinase. The reaction contained 0.2 M Tris-HCl, pH 8.5, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 5 mM ATP, 0.5 mM pentamer, 50 μg/mL bovine serum albumin, and 10 units/mL T4 polynucleotide kinase. At this pH and enzyme concentration, wild-type polynucleotide kinase exhibits a 3′-phosphatase activity (Cameron & Uhlenbeck, 1977; Cameron et al., 1978) which led to removal of the 3′-phosphate of GGCCUp. This activity was confirmed by reaction of GGCCGp with polynucleotide kinase in the absence of ATP to yield GGCCG. In addition, by use of high-performance liquid chromatography (HPLC), pGGCCU synthesized from GGCCU with polynucleotide kinase coeluted with the product of the reaction described above.

Purification of Oligonucleotides. For several of the syntheses described above, yields were 100%. In those cases, products were separated from excess mononucleotide reactants by using a C-18 Sep-Pak cartridge (Waters). The reaction mixture was applied to the cartridge, and monomers were eluted with 0.01 M triethylammonium acetate, pH 4.8. Product oligomers eluted with 20% acetonitrile. Lyophilization removed the solvent and any residual triethylammonium acetate.

When product yields were less than 100%, products were purified on DEAE-Sephadex as described previously (Freier et al., 1983a). Product peaks from the Sephadex column were acidified with acetic acid to pH 4.8 and desalted by use of a Sep-Pak cartridge as described above. Purities of all oligomers were confirmed by HPLC.

Oligonucleotide Solutions. Concentrations (C_T) are strand concentrations and were determined as described previously (Freier et al., 1983a). In units of 10^4 M^{-1} cm^{-1}, calculated extinction coefficients at 280 nm, 90 °C, are as follows: AGGCCp, 2.61; CGGCCp, 2.78; UGGCCp, 2.54; GCCGGp, 2.91; UCCGGp, 2.63; AGGCCUp, 2.90; CGGCCGp, 3.34; UGGCCAp, 2.70; GCCGGCp, 3.25; UCCGGAp, 2.84. It was assumed neither salt concentration, pH, nor terminal phosphates affected the extinction coefficient at 90 °C. In 1 M NaCl, at 90 °C and strand concentrations greater than 1×10^{-4} M, CGGCCGp and GCCGGCp are not totally single stranded. For those oligonucleotides in 1 M NaCl, the calculated extinction coefficient was applied at 98 °C.

Most melting curves were measured in 0.01 M sodium cacodylate and 0.001 M ethylenediaminetetraacetic acid (EDTA), pH 7, either with or without 1 M NaCl. Melting curves for the CCGG family were measured in 0.01 M sodium phosphate, 0.5 mM Na_2EDTA, and 1 M NaCl, pH 7. The pH 8.2 melting curves were measured in 0.01 M sodium pyrophosphate, 0.001 M EDTA, and 1 M NaCl, pH 8.2. Pyrophosphate was chosen because of its small temperature dependence of pK_a (Good & Izawa, 1972).

Thermodynamic Parameters. Absorbance vs. temperature profiles (melting curves) were obtained as described previously (Freier et al., 1983a). Details of the thermodynamic analysis are given elsewhere (Petersheim & Turner, 1983; Freier et al., 1983a,b) so only a brief description is given here. For each oligomer, at least 12 melting curves ranging 100-fold in concentration were measured. Each melting curve was fit to a two-state transition model with linear sloping base lines. "Temperature-independent" parameters are the average of two methods: (1) the enthalpies and entropies obtained from the fits were averaged, and (2) reciprocal melting temperature was plotted vs. log C_T (Borer et al., 1974).

Temperature-dependent thermodynamic parameters were obtained from plots of $\Delta H°$ vs. T_m and $\Delta S°$ vs. ln T_m where $\Delta H°$, $\Delta S°$, and T_m are the values obtained from the fit of each curve to a two-state transition with linear sloping base lines. Heat capacity changes were calculated from slopes of $\Delta H°$ vs. T_m and $\Delta S°$ vs. ln T_m as described elsewhere (Freier et al., 1983a).

The thermodynamic parameters most precisely determined are the T_m's of the individual melting curves. Consequently, free energies near these T_m's can be determined with great precision. Plots of $1/T_m$ vs. log C_T typically cover a 100-fold concentration range so the slopes and intercepts of these plots can also be measured reproducibly. The error estimates on $\Delta H°$ and $\Delta S°$ obtained from plots of $1/T_m$ vs. log C_T are, therefore, ±5%; error estimates on $\Delta G°$ near the T_m are less than ±2%.

Although enthalpies and entropies obtained from fits of duplicate curves can vary as much as 10%, parameters averaged over several fits are reproducible, so error estimates for fitted parameters are also ±5% on $\Delta H°$ or $\Delta S°$ and ±2% on $\Delta G°$ near the T_m. Temperature-independent parameters are the average of those obtained from plots of $1/T_m$ vs. log C_T and those from fits, so the precision in these values is also about ±5% for $\Delta H°$ or $\Delta S°$ and ±2% for $\Delta G°$ near the T_m. It should be pointed out that in some cases parameters from plots of $1/T_m$ vs. log C_T differ from fitted parameters (as much as 35% in the worst case), so although temperature-independent thermodynamic parameters can be precisely measured, these two-state parameters may not accurately describe the transition.

Temperature-dependent thermodynamic parameters address the possible non-two-state character of the coil to helix transition. These parameters, however, are obtained from plots of $\Delta H°$ vs. T_m and $\Delta S°$ vs. ln T_m, and these plots have significant scatter [see Petersheim & Turner (1983) for representative plots]. Heat capacity changes obtained from such plots are probably reliable only within ±50%. Temperature-dependent enthalpies, entropies, and free energies are also less reproducible than the temperature-independent parameters, especially at temperatures different from the measured T_m's. We estimate the errors in temperature-dependent values near the T_m to be ±10% for $\Delta H°$ and $\Delta S°$ and ±5% for $\Delta G°$. Errors can be even larger, however, away from the T_m of the oligonucleotide.

Results

Temperature-Independent Thermodynamics. Plots of T_m^{-1} vs. log C_T are reported in Figures 1 and 2. Thermodynamic parameters derived from these plots are listed in Table I. They were averaged with those obtained from fits to yield the temperature-independent enthalpies and entropies in Table II. Thermodynamic parameters derived from fits are listed in the supplementary material (see paragraph at end of paper re-

Table I: Thermodynamic Parameters of Helix Formation Obtained from Plots of Reciprocal Melting Temperature vs. log Concentration

		1 M NaCl			0.01 M Na^+		
oligonucleotide	pH	$-\Delta H°$ (kcal/mol)[a]	$-\Delta S°$ (eu)[a]	T_m (°C)[b]	$-\Delta H°$ (kcal/mol)[a]	$-\Delta S°$ (eu)[a]	T_m (°C)[b]
GGCC[c]	7	35.8	98	34.4	32.7	92	24.2
GGCCp[c]	7	41.3	116	33.3	30.4	86	19.6
pGGCC[c]	7	39.2	107	38.6	29.0	81	17.4
AGGCCp	7	39.0	107	38.1	21.9	56	19.3
pGGCCU	7	46.0	122	55.4	40.0	112	33.8
AGGCCUp	7	52.0	140	55.7	40.2	112	34.6
AGGCCU	7	48.2	128	55.3			
CGGCCp	7	30.6	80	38.0	32.2	91	20.4
CGGCCGp	7	54.1	143	63.3	40.9	110	44.7
UGGCCp	7	36.5	101	34.1	24.6	68	10.5
UGGCCAp	7	59.9	164	55.2			
pGGCC	8.2	37.8	103	38.0			
GGCCp	8.2	35.2	97	33.4			
CCGG[d]	7	34.2	96	27.1			
pCCGG[c]	7	35.3	97	32.6			
GCCGGp	7	32.5	89	30.6			
GCCGGCp	7	62.7	166	67.2			
UCCGGp	7	37.1	104	29.5			
UCCGGAp	7	51.9	142	50.1			

[a] Although the estimated errors in $\Delta H°$ and $\Delta S°$ are ±5%, additional significant figures are given to allow accurate calculation of T_m. [b] Calculated for 1×10^{-4} M oligomer concentration. [c] From Freier et al. (1983a). [d] From Petersheim & Turner (1983).

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

		1 M NaCl			0.01 M Na^+		
oligonucleotide	pH	$-\Delta H°$ (kcal/mol)[b]	$-\Delta S°$ (eu)[b]	T_m (°C)[c]	$-\Delta H°$ (kcal/mol)[b]	$-\Delta S°$ (eu)[b]	T_m (°C)[c]
GGCC[d]	7	35.9	98	35.0	33.1	93	24.5
GGCCp[d]	7	39.0	109	33.5	31.6	89	20.1
pGGCC[d]	7	39.4	108	38.8	29.7	84	17.8
AGGCCp	7	37.9	103	38.3	28.2	78	21.0
pGGCCU	7	45.9	121	55.6	41.1	115	33.9
AGGCCUp	7	51.2	137	55.8	43.4	123	34.7
AGGCCU	7	48.1	128	55.4			
CGGCCp	7	35.1	94	39.3	32.0	91	20.8
CGGCCGp	7	55.4	146	63.3	47.3	130	44.7
UGGCCp	7	35.6	97	34.5	27.4	78	11.6
UGGCCAp	7	54.8	148	55.9			
pGGCC	8.2	39.2	108	38.3			
GGCCp	8.2	36.3	100	33.7			
CCGG[e]	7	34.5	96	27.4			
pCCGG[d]	7	36.4	101	32.9			
GCCGGp	7	34.0	93	31.0			
GCCGGCp	7	64.2	170	67.2			
UCCGGp	7	37.0	104	29.7			
UCCGGAp	7	51.5	141	50.3			

[a] Obtained from absorbance vs. temperature profiles as described under Materials and Methods. [b] Although the estimated errors in $\Delta H°$ and $\Delta S°$ are ±5%, additional significant figures are given to allow accurate calculation of T_m. [c] Calculated for 1×10^{-4} M oligomer concentration. [d] From Freier et al. (1983a). [e] From Petersheim & Turner (1983).

garding supplementary material). For some oligonucleotides, especially in 0.01 M Na^+, enthalpies and entropies from plots of T_m^{-1} vs. log C_T are up to 35% less negative than those obtained from fits. This was observed in low salt for several pentanucleotides in the GGCCYp series and may indicate the transition is not two state (Freier et al., 1983a). Fortunately, $\Delta G°$ near 37 °C and the T_m's at 1×10^{-4} M strand are less sensitive than $\Delta H°$ and $\Delta S°$ to analytical methods, so oligomer stabilities can be compared with confidence.

Several trends are apparent. In 1 M NaCl, addition of two 5′ ends stabilizes the core helix by only 0–4 °C. The effect is similar to that of 5′-phosphates alone. This result contrasts with that for 3′-dangling ends, which increases the T_m 7–23 °C depending on the sequence (Freier et al., 1983a). Terminal base pairs increase T_m even more, from 20 °C for a U·A pair on GGCC to 40 °C for a G·C pair on CCGG.

Temperature-Dependent Thermodynamics. Temperature dependent thermodynamic properties are listed in Table III. These parameters are most reliable near the T_m of the oligomer studied and are listed for 37 °C, roughly an average T_m for the oligomers studied as well as a physiologically relevant temperature. The data in Table III support the same trends observed in Tables I and II.

As described above, heat capacity changes upon helix formation were calculated from the temperature dependence of $\Delta H°$ and $\Delta S°$, and are listed in Table IV. For oligomers with a melting temperature below 50 °C, the average $\Delta C_p°$ divided by chain length is −80 cal K^{-1} (2 mol of nucleotides)$^{-1}$. This is consistent with the $\Delta C_p°$'s of −40 to −100 cal K^{-1} (2 mol of nucleotides)$^{-1}$ determined spectroscopically for other oligomers (Petersheim & Turner, 1983; Freier et al., 1983a; Hickey & Turner, 1985) and calorimetrically for homopolynucleotides and DNA (Ross & Scruggs, 1965; Neumann & Ackerman, 1967; Krakauer & Sturtevant, 1968; Rawitscher et al., 1968; Hinz et al., 1970; Shiao & Sturtevant, 1973; Suurkuusk et al., 1977; Filimonov & Privalov, 1978). For oligomers that melt above 50 °C, $\Delta C_p°$ averages only −40 cal K^{-1} (2 mol of nucleotides)$^{-1}$. The most likely explanation of this heat capacity increase upon helix denaturation is the excess heat capacity due to single-strand unstacking. This excess

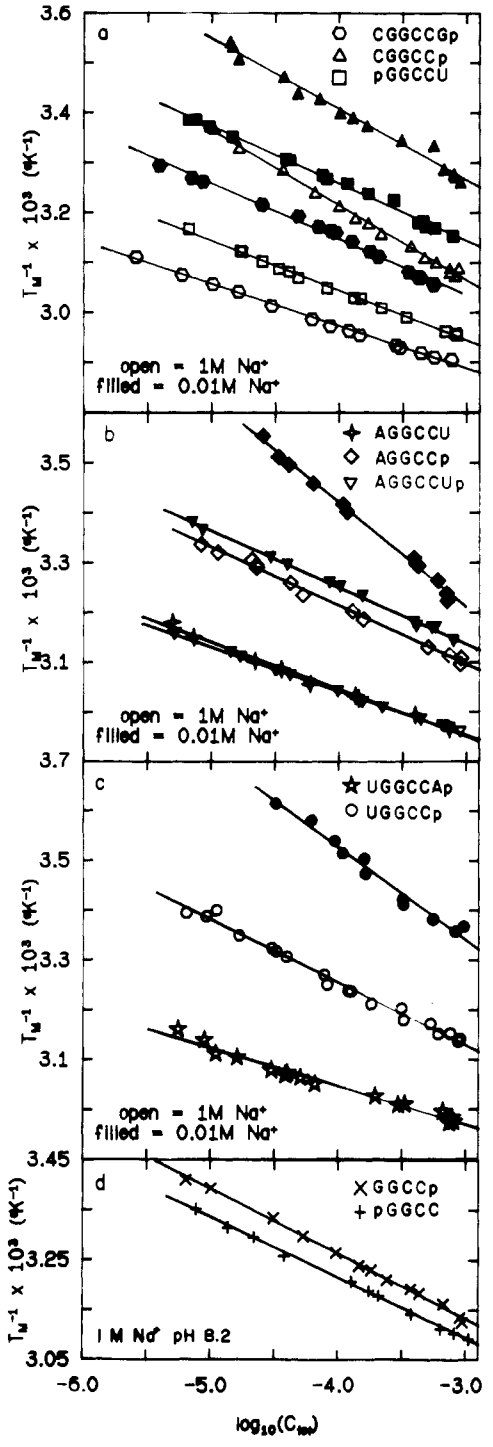

FIGURE 1: Reciprocal melting temperature vs. log C_T for several oligoribonucleotides with a GGCC core. The solid symbols are low salt (0.01 M sodium cacodylate and 0.001 M EDTA, pH 7); the open symbols are high salt (1 M NaCl, 0.01 M sodium cacodylate, and 0.001 M EDTA, pH 7): (hexagons) CGGCCGp; (△) CGGCCp; (□) pGGCCU; (clovers) AGGCCU; (◇) AGGCCp; (▽) AGGCCUp; (stars) UGGCCAp; (○) UGGCCp; (×) GGCCp; (+) pGGCC in 1 M NaCl, 0.01 M sodium pyrophosphate, and 0.001 M EDTA, pH 8.2.

heat capacity can be estimated from thermodynamic parameters for single-strand unstacking. Using $\Delta H°$, $\Delta S°$, and σ for unstacking of poly(C) and poly(A) (Freier et al., 1981; Turner et al., 1981), we calculate ϕC_p to be between −60 and −100 cal K^{-1} (mol of stack)$^{-1}$ at 37 °C. Above 50 °C, ϕC_p will be lower due to decreased stacking in the single strands. The qualitative agreement between these estimates of ϕC_p and the observed $\Delta C_p°$ for oligonucleotides suggests that temperature-dependent stacking of single strands is the primary cause of the observed $\Delta C_p°$.

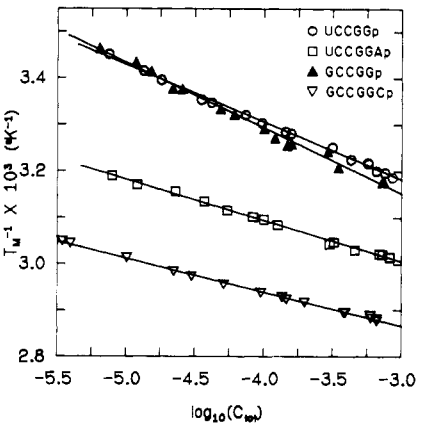

FIGURE 2: Reciprocal melting temperature vs. log C_T for oligoribonucleotides with a CCGG core in 1 M NaCl, 0.01 M sodium phosphate, and 0.5 mM EDTA, pH 7. (○) UCCGGp; (□) UCCGGAp; (▲) GCCGGp; (▽) GCCGGCp.

Salt Effects on Helix Stability. All the oligonucleotides studied are less stable in 0.01 M Na^+ than in 1 M Na^+; $\Delta T_m/\Delta \log [Na^+]$ ranges from 6 to 11 °C. The dependence of the free energy of helix formation on salt concentration can be used to estimate Δi, the number of ions released upon helix denaturation (Record et al., 1981; Freier et al., 1983a, 1984). For the oligomers in Table III, Δi is 0.06–0.11 ion per phosphate.

Effects of pH on Helix Stability. Little is known about the ionization constants of terminal phosphates on oligonucleotides. They are expected to be similar to nucleotide monomers, except the negative charges of the phosphodiester linkages may increase pK_a's slightly. The pK_a's for 2′- and 3′-mononucleotides range between 5.9 and 6.2; the corresponding 5′-nucleotides have pK_a's about 0.3 unit higher (Jencks & Regenstein, 1968). At pH 7, therefore, it is possible the terminal phosphates on pGGCC and GGCCp are not equally charged. To see if this was responsible for the higher T_m observed for pGGCC, thermodynamic parameters for pGGCC and GGCCp were measured in 1 M NaCl at pH 8.2 where both terminal phosphates should be fully ionized. Thermodynamic parameters measured at pH 8.2 are identical with those at pH 7. Thus, the increased stability of pGGCC over GGCCp in 1 M NaCl is not a charge effect.

Discussion

To gain insight into the relative importance of stacking and pairing for helix stability, the formation of the pentamer or hexamer double helix from single strands can be divided into two processes (see Figure 3). The first is formation of the core helix of four bases, leaving the terminal bases unstacked ($\Delta G°_3$ or $\Delta G°_7$ in Figure 3). The second is stacking of the terminal bases onto the core helix, $\Delta G°_2$ or $\Delta G°_6$. $\Delta G°_2$ and $\Delta G°_6$ are, respectively, twice the free energy of stacking a base on or twice the free energy of adding a terminal base pair to the end of an RNA helix. The thermodynamic cycles in Figure 3 can be used to obtain $\Delta G°_2$ and $\Delta G°_6$:

$$\Delta G°_2 = \Delta G°_1 - \Delta G°_3 \qquad \Delta G°_6 = \Delta G°_5 - \Delta G°_7 \qquad (1)$$

$\Delta G°_1$ and $\Delta G°_5$ are the free energies of helix formation for the pentamer and hexamer, respectively, and can be measured directly. $\Delta G°_3$ and $\Delta G°_7$ cannot be measured directly. We approximate them with $\Delta G°_4$, the free energy for helix formation of the tetramer core.

A simplification resulting from use of eq 1 and the above approximation is that $\Delta G°_2$ and $\Delta G°_6$ thus calculated do not

Table III: Temperature-Dependent Thermodynamic Properties of Helix Formation

		1 M NaCl			0.01 M Na^+		
oligonucleotide	pH	$-\Delta H°$(37 °C) (kcal/mol)[a,c]	$-\Delta S°$(37 °C) (eu)[b,c]	T_m (°C)[d]	$-\Delta H°$(37 °C) (kcal/mol)[a,c]	$-\Delta S°$(37 °C) (eu)[b,c]	T_m (°C)[d]
GGCC[e]	7	38.6	106	36.7	37.1	106	24.2
GGCCp[e]	7	37.7	105	33.9	43.3	127	21.3
pGGCC[e]	7	38.9	106	40.6	36.1	105	17.9
AGGCCp	7	36.6	99	38.3	40.6	117	19.8
pGGCCU	7	40.2	103	55.4	42.7	120	35.8
AGGCCUp	7	45.6	119	57.3	46.7	134	34.1
AGGCCU	7	45.3	120	53.3			
CGGCCp	7	36.3	98	40.4	37.4	109	20.0
CGGCCGp	7	53.8	142	63.0	52.2	146	46.8
UGGCCp	7	35.7	98	34.8	43.8	131	12.1
UGGCCAp	7	44.7	114	56.8			
pGGCC	8.2	39.8	110	35.9			
GGCCp	8.2	37.8	105	32.7			
CCGG[f]	7	38.3	109	28.0			
pCCGG[e]	7	38.8	109	31.2			
GCCGGp	7	37.6	105	31.5			
GCCGGCp	7	61.5	162	68.2			
UCCGGp	7	38.5	108	30.4			
UCCGGAp	7	44.8	119	52.2			

[a] Obtained from plots of $\Delta H°$ vs. T_m as described under Materials and Methods. [b] Obtained from plots of $\Delta S°$ vs. ln T_m as described under Materials and Methods. [c] Although the estimated errors in $\Delta H°$ and $\Delta S°$ are ±10%, additional significant figures are given to allow accurate calculation of T_m. [d] Calculated for 1×10^{-4} M oligomer concentration. [e] From Freier et al. (1983a). [f] From Petersheim & Turner (1983).

Table IV: Changes in Heat Capacity[a] upon Helix Formation

		$-\Delta C_p°$	
oligonucleotide	pH	1 M NaCl	0.01 M Na^+
GGCC[b]	7	705	327
GGCCp[b]	7	402	552
pGGCC[b]	7	261	331
AGGCCp	7	187	435
pGGCCU	7	307	164
AGGCCUp	7	253	60
AGGCCU	7	173	
CGGCCp	7	672	385
CGGCCGp	7	111	183
UGGCCp	7	431	629
UGGCCAp	7	302	
pGGCC	8.2	251	
GGCCp	8.2	274	
CCGG[c]	7	385	
pCCGG	7	399	
GCCGGp	7	327	
GCCGGCp	7	138	
UCCGGCCp	7	253	
UCCGGA	7	439	

[a] From plots of $\Delta H°$ vs. T_m and $\Delta S°$ ln T_m as described under Materials and Methods. Error estimates on $\Delta C_p°$ are ±50%. Heat capacity is in units of calories per mole per degree kelvin. [b] From Freier et al. (1983a). [c] From Petersheim & Turner (1983).

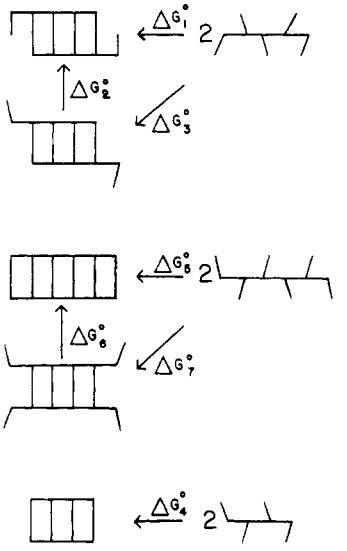

FIGURE 3: Thermodynamic cycles for calculating stabilization free energies of dangling ends and terminal base pairs.

include contributions from residual stacking in the single strands. Thus, only interstrand interactions contribute to calculated free-energy increments for addition of a base stack or a base pair to an RNA double helix.

The data in Table II and the process described above were used to calculate free energies for adding a 5′- or 3′-dangling nucleotide or a terminal base pair to the GGCC or CCGG core helixes. These numbers are $0.5\Delta G°_2$ or $0.5\Delta G°_6$ defined above and are listed in Table V.

A striking feature from Table V is that, in general, free-energy increments are smaller for 5′- than for 3′-dangling ends. This difference has been observed previously in T_m measurements of short oligonucleotides (Romaniuk et al., 1978; Neilson et al., 1980; Alkema et al., 1981a,b; Turner et al., 1981; Petersheim & Turner, 1983). Moreover, the results in Table III suggest the favorable $\Delta G°$ due to a 3′-dangling end on GGCC or CCGG is associated with a favorable $\Delta H°$ and unfavorable $\Delta S°$, whereas it appears the opposite holds for 5′-dangling ends. A possible explanation is suggested by the geometry of A-form RNA. Figure 4 compares the stacking geometry of a 5′-dangling uridine in UGGCC to that of a 3′-dangling adenosine in GGCCA. The 5′-U does not overlap the G·C pair below it. In contrast, the 3′-A has significant overlap with the 5′-G on the opposite strand. Thus, if the dangling ends roughly continue RNA geometry, strong interaction with the opposite strand is expected for a 3′-A, but not for a 5′-U. This is consistent with the measured increments in thermodynamic parameters. Stacking is expected to provide a favorable $\Delta G°$ due to a favorable $\Delta H°$, reflecting increased bonding due to electronic interactions (Freier et al., 1981).

Although 3′-dangling ends stabilize RNA duplexes more than 5′-dangling ends, the opposite has been reported for DNA. Mellema et al. (1984) report dTCG forms a stable miniduplex, whereas dCGT does not. Similarly, 5′-dangling thymidines on $d(CG)_3$ or $d(GC)_3$ increase stability more than 3′-dangling thymidines (M. Senior, R. Jones, and K. Breslauer, unpublished results). Thus, trends observed for RNA oligonucleotides cannot be extrapolated to DNA helixes, presum-

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

added terminus	$-\Delta\Delta G°_{37}$[a,b] for the core helix	
	GGCC	CCGG
5′-phosphate	0.2	0.3
3′-phosphate	−0.1	
5′-Ap + 3′-p	0.2	0.5
5′-Cp + 3′-p	0.2	
5′-Gp + 3′-p		0.2
5′-Up + 3′-p	0.0	0.1
3′-pAp	1.8	1.1
3′-pCp	0.8	0.4
3′-pGp	1.7	1.3
3′-pUp	1.2	0.6
5′-p + 3′-pU	1.4	
5′-Ap + 3′-pUp (pair)	1.6 (1.7)	1.9 (2.2)
5′-Ap + 3′-pU (pair)	1.5 (1.7)	
5′-Cp + 3′-pGp (pair)	2.3 (2.3)	
5′-Gp + 3′-pCp (pair)		3.4 (3.3)
5′-Up + 3′-pAp (pair)	1.6 (2.1)	1.6 (2.2)

[a] $\Delta\Delta G°_{37}$ is half the difference between the $\Delta G°_{37}$ of helix formation for the molecule containing the core helix plus the added termini and the $\Delta G°_{37}$ of helix formation for the tetramer core. Temperature-independent thermodynamic parameters from Table II were used to calculate $\Delta\Delta G°_{37}$. [b] The numbers in parentheses are helix propagation parameters determined by fitting experimental free energies of helix formation for RNA oligomers to the nearest-neighbor model (S. M. Freier, A. Sinclair, T. Neilson, and D. H. Turner, unpublished experiments).

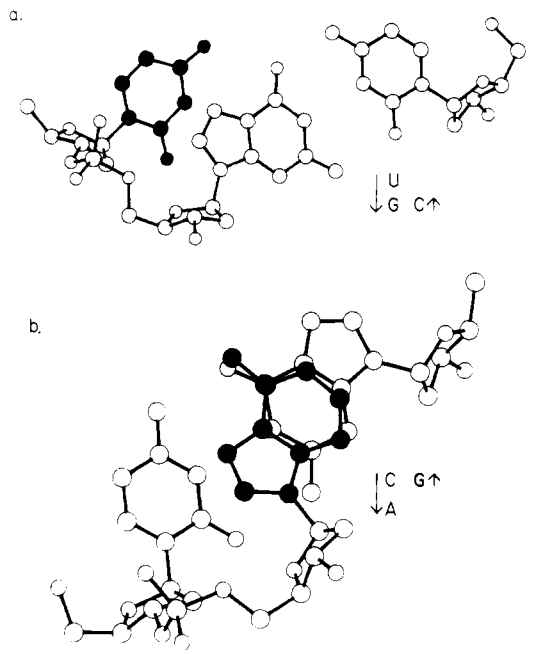

FIGURE 4: Stacking geometry of (a) a 5′-dangling uridine on a G·C base pair and (b) a 3′-dangling adenosine following a C·G base pair. The view is along the helix axis. The atoms of the dangling base are filled in and are above the plane of the base pair. Coordinates are for A-form DNA (S. Arnott, unpublished results).

ably because the geometries are different.

A surprising result from Table V is 5′-terminal phosphates stabilize GGCC, CCGG, and GGCCU duplexes by 0.2–0.3 kcal/mol at 37 °C. pGGCC has the same stability at pH 8.2 and 7, indicating it is not a charge effect. The effect may be related to the observation that 5′-nucleotides have less configurational freedom than 3′-nucleotides or nucleosides (Sundaralingam, 1973, 1975). Possibly the 5′-phosphate forces the sugar into a conformation favorable for base pairing. Alternatively, there may be some weak direct interaction between the 5′-phosphate and the opposite strand.

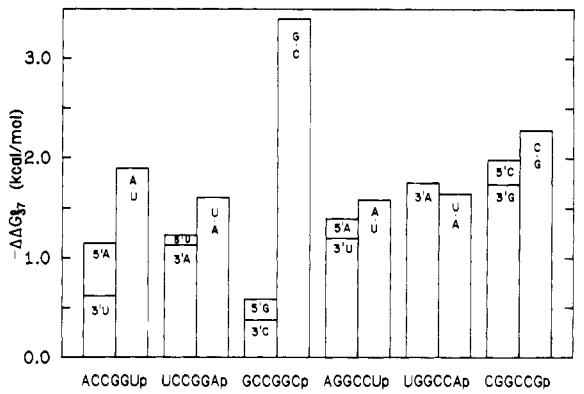

FIGURE 5: Free-energy increments at 37 °C for adding a terminal base pair or dangling end to a GGCC or CCGG tetramer core. The left-hand column represents the stacking free energy; the right is the free energy of base-pair formation. The stacking contributions were estimated by using the data in Table II. For example, the stabilization due to stacking of a terminal A·U base pair in AGGCCUp is $\Delta\Delta G°$(5′-A) + $\Delta\Delta G°$(3′-U) = $^1/_2[\Delta G°$(AGGCCp) + $\Delta G°$-(GGCCUp) − $2\Delta G°$(GGCC)].

The stability increments from adding a 5′-Ap or -Cp to GGCCp are essentially the same as for adding a 5′-phosphate to GGCC. Moreover, the measured stabilities of AGGCCU and pGGCCU are identical. This is consistent with the observation by Alkema et al. (1981b) that the T_m's of UGCA and GCA are identical at 8 mM. Apparently, the 5′-nucleoside of a terminal dangling end or base pair makes little or no contribution to stability when it is adjacent to a pG. This is not always the case when the 5′-nucleoside is adjacent to a pC. A 5′-Ap on CCGGp adds significantly greater stability than a 5′-phosphate on CCGG. For ACCGGUp and GCCGGCp, respectively, the sum of free-energy increments for a 5′-phosphate and a 3′-dangling end account for only roughly 60% or 20% of the free energy of a terminal base pair.

The data for base pairs in Table V provide single direct measurements of the free energy of helix propagation for six sequences. These parameters can be compared with those determined by fitting experimental free energies of helix formation for RNA oligomers to the nearest-neighbor model (Freier et al., 1984; Borer et al., 1974), as listed in parentheses in Table V. The data in Table V are small differences of large numbers and have associated with them an error of about 0.2 kcal/mol. Error estimates on the nearest-neighbor parameters are also about 0.2 kcal/mol. Considering these uncertainties, there is reasonable agreement between the single determinations and the nearest-neighbor parameters. Terminal base pairs do not seem significantly different from average base pairs.

Figure 5 demonstrates how the values in Table V can be used to provide an empirical measure of the contribution of stacking to the stabilities of terminal base pairs. This is an empirical estimate because it is not known if the geometries of the dangling ends are the same as the base pairs. For the terminal base pairs in AGGCCUp, UGGCCAp, and CGGCCGp, respectively, 90%, 110%, and 90% of the free energy is attributable to stacking; for ACCGGUp, UCCGGAp, and GCCGGCp, respectively, stacking accounts for 60%, 80%, and 20% of terminal base-pair free energy. These comparisons underestimate the pure bonding contribution of stacking because the stacking free energy includes the unfavorable entropy change associated with restriction of backbone bonds (Longuet-Higgins & Zimm, 1960; Dewey & Turner, 1979; Freier et al., 1981). Evidently, in most cases, pairing contributes little to the stability of terminal base pairs.

However, when stacking is particularly weak as in GCCGGCp, pairing makes an important contribution to stability. This may indicate that strong stacking interactions favor geometries that are not optimal for hydrogen bonding. This type of competition has been suggested by energy minimization studies of DNA (Levitt, 1978), and the predicted distortions of helix geometries have been detected experimentally (Hogan et al., 1978; Dickerson, 1983).

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Supplementary Material Available

Table of thermodynamic parameters of helix formation for the GGCC and CCGG families of oligomers. Parameters are average values from fits of melting curves to a two-state model with sloping base lines (1 page). Ordering information is given on any current masthead page.

Registry No. AGGCCp, 97073-26-2; pGGCCU, 97073-27-3; AGGCCUp, 97073-28-4; AGGCCU, 97073-29-5; CGGCCp, 97073-30-8; CGGCCGp, 97073-31-9; UGGCCp, 97073-32-0; UGGCCAp, 97102-10-8; pGGCC, 87640-15-1; GGCCp, 87640-16-2; GCCGGp, 97073-33-1; GCCGGCp, 97073-34-2; UCCGGp, 97073-35-3; UCCGGAp, 97073-36-4; pCCGG, 76873-96-6; Ap, 84-21-9; Cp, 84-52-6; Gp, 117-68-0; Up, 84-53-7; pAp, 1053-73-2; pCp, 2922-94-3; pGp, 3237-37-4; pUp, 2922-95-4; cytosine, 71-30-7; guanine, 73-40-5; adenine, 73-24-5; uracil, 66-22-8.

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