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Effects of Terminal Mismatches on RNA Stability: Thermodynamics of Duplex Formation for ACCGGGp, ACCGGAp, and ACCGGCp†

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ABSTRACT: The thermodynamics of ACCGGCp, ACCGGAp, and ACCGGGp helix formation have been measured in 1 M NaCl. The terminal mispairs stabilize the CCGG core duplex at 10^{-4} M in the order AC ≈ AA < AG ≈ AU. The data reported provide thermodynamic parameters for RNA structure prediction and suggest useful approximations for including other mismatches in current algorithms.

The importance of base mismatches in RNA interactions has become increasingly evident since the G·U wobble pair was first proposed (Crick, 1966). Mismatches are present in essentially all known and predicted RNA structures (Rich & Rajbhanday, 1976; de Brujin & Klug, 1983; Furdon et al., 1983; Noller, 1984; Dams et al., 1983; Traub & Sussman, 1982; Fox & Woese, 1975; Studnicka et al., 1981; Salser, 1977; Cech et al., 1983; Auron et al., 1982). Despite this prevalence, contributions of mismatches have been largely ignored in predictions of RNA stability due to lack of measured parameters (Tinoco et al., 1971; Borer et al., 1974; Salser, 1977; Zuker & Stiegler, 1981). Nevertheless, there is evidence mismatches add significant stability to double helices (Alkema et al., 1981). Previous attempts to incorporate such effects have been based on indirect estimates of the thermodynamic parameters for mismatches (Papanicolaou et al., 1984). This paper reports thermodynamic parameters for duplex formation by ACCGGGp, ACCGGAp, and ACCGGCp. Comparison with previous results for CCGG (Petersheim & Turner, 1983) provides parameters for AG, AA, and AC terminal mismatches.

Materials and Methods

Oligonucleotide Synthesis. ACCGGXp's (X = A, C, or G) were synthesized by successive addition of nucleoside

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3′,5′-bisphosphates (pXp) to ACC (Sigma). Each monomer was joined to the acceptor oligonucleotide by repeated application of the following sequence: removal of the 3′ acceptor phosphate with calf alkaline phosphatase (CAP) (Fernley, 1971), inactivation of CAP by immersion in boiling H_2O for 6 min, addition of pXp to acceptor by adding T4 RNA ligase reaction components directly to the boiled phosphatase solution, and purification of products. Phosphatase reaction conditions were 40 units/mL CAP (Boehringer-Mannheim, molecular biology grade), 100 μg/mL bovine serum albumin (BSA),[1] 300 mM Tris-HCl, pH 8.6, and 4 mM oligomer. Final reaction conditions in the ligase step (Uhlenbeck & Cameron, 1977; England & Uhlenbeck, 1978) were 150 mM Tris-HCl, pH 8.6, 20 mM $MgCl_2$, 10 mM DTT, 50 μg/mL BSA, 5–10 mM ATP, 8 mM pXp, 2 mM acceptor, and 30–60 units/mL T4 RNA ligase (P-L Biochemicals). Synthesis of ACCGp did not include the phosphatase reaction. All reactions were incubated at 37 °C. Phosphatase reactions were complete within 2 h, while ligase reactions required 4–19 h for 100% yield.

Purification of ACCGGp and ACCGGCp was by anion-exchange chromatography with NaCl/7 M urea and TEAB gradients, respectively (Petersheim & Turner, 1983; Petersheim, 1982; Freier et al., 1983b; Hickey & Turner, 1985). ACCGp, ACCGGAp, and ACCGGGp were isolated with Waters Associates Sep-Paks. Sep-Paks were first washed and equilibrated with 10 mL each of 100% CH_3CN, 1% (v/v) CH_3CN with 10 mM ammonium acetate, and 100% H_2O with 10 mM ammonium acetate. Reaction mixtures were loaded and then washed with aqueous 10 mM ammonium acetate until the net eluant absorbance was less than 0.05/cm. Oligonucleotides were eluted from the Sep-Pak with 20% (v/v) CH_3CN and 10 mM ammonium acetate and then lyophilized to dryness.

Extinction Coefficients. Extinction coefficients at 280 nm were calculated from published dimer and monomer data by using the nearest-neighbor approximation (Richards, 1975). Extinction coefficients (in units of 10^{-4} M^{-1} cm^{-1}) are the following: ACCGGCp, 2.95; ACCGGGp, 3.34; ACCGGAp, 2.82. ACCGGXp strand concentrations were calculated at 80° (X = C) and 90° (X = A, G) from absorbance vs. temperature data.

Melting Curves. Melting curves were obtained as described in Freier et al. (1983b) except that a minimum of 170 absorbance vs. temperature data points per curve were taken at a heating rate of 1 °C/min. The buffer was 1 M NaCl, 1 mM EDTA, and 10 mM sodium cacodylate, pH 7. No corrections were made for thermal expansion (Albergo & Turner, 1981; Petersheim & Turner, 1983).

Analysis of Melting Curves. Absorbance vs. temperature data were analyzed as previously described (Petersheim & Turner, 1983; Freier et al., 1983a,b). Fits of melting curves to a two-state model with linear upper and lower base lines provided temperature-dependent thermodynamic parameters. From these fitted parameters, a melting temperature, T_m, was determined for each strand concentration, c_T. Plots of T_m^{-1} vs. log c_T then provided another measure of $\Delta H°$ and $\Delta S°$ (Borer et al., 1974):

$$\frac{1}{T_m} = \frac{2.303R}{\Delta H°} \log c_T + \frac{\Delta S°}{\Delta H°} \quad (1)$$

[1] Abbreviations: ATP, adenosine 5′-triphosphate; BSA, bovine serum albumin; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; TEAB, triethylammonium bicarbonate; Tris, tris(hydroxymethyl)-aminomethane.

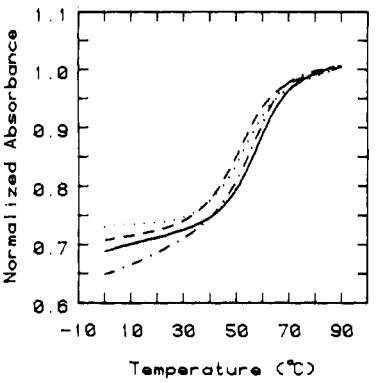

FIGURE 1: Normalized absorbance at 90° vs. temperature for 358 μM ACCGGUp (—), 335 μM ACCGGGp (···), 425 μM ACCGGAp (···), and 352 μM ACCGGCp (---). Buffer is 1 M NaCl, 10 mM sodium cacodylate, and 1 mM EDTA, pH 7.

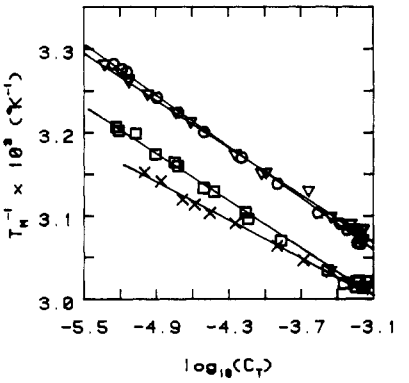

FIGURE 2: T_m^{-1} vs. log c_T for ACCGGUp (Petersheim & Turner, 1983) (×), ACCGGGp (□), ACCGGAp (○), and ACCGGCp (▽). Buffer is 1 M NaCl, 10 mM sodium cacodylate, and 1 mM EDTA, pH 7.

A third measure of $\Delta H°$ and $\Delta S°$ was obtained by averaging the results of the curve fitting for each oligomer. These averages differed by less than 5% from values obtained from eq 1. They have therefore been averaged with the log c_T values to provide "temperature-independent" thermodynamics.

Results

Temperature-Independent Thermodynamic Parameters. Figure 1 shows representative normalized absorbance vs. temperature curves for ACCGGUp (Petersheim & Turner, 1983), ACCGGCp, ACCGGAp, and ACCGGGp in 1 M NaCl. Figure 2 shows plots of T_m^{-1} vs. log c_T for these hexamers. Thermodynamic parameters derived from these plots and from fits of the melting curves are listed in Table I.

Temperature-Dependent Thermodynamic Parameters. The heat capacity difference between products and reactants, $\Delta C_p°$, can be obtained by plotting the fitted parameters, $\Delta H°$ and $\Delta S°$, according to

$$\Delta H°(T_m) = \Delta H°(T_0) + \Delta C_p°(T_m - T_0) \quad (2)$$

$$\Delta S°(T_m) = \Delta S°(T_0') + \Delta C_p° \ln (T_m/T_0') \quad (3)$$

Here T_0 and T_0' are arbitrary reference temperatures. Plots of $\Delta H°$ and $\Delta S°$ vs. T_m and ln T_m, respectively, are linear within experimental error [see supplementary material (see paragraph at end of paper regarding supplementary material)]. The $\Delta C_p°$'s calculated with eq 2 and 3 differ by less than 2% for each oligomer, and the averages are listed in Table I. The values per mole stack are similar to those for oligomers with 5′- and 3′-dangling ends (Petersheim & Turner, 1983; Freier et al., 1983b), poly(A)·poly(U) (Rawitscher et al., 1965; Ross

Table I: Thermodynamic Parameters for Helix Formation of CCGG Core Oligomers in 1 M NaCl, 0.001 M EDTA, and 0.01 M Sodium Cacodylate, pH 7

	log c_T parameters		temperature-independent parameters		
oligonucleotide	$-\Delta H°$ (kcal/mol of duplex)	$-\Delta S°$ [cal/(K·mol of duplex)]	$-\Delta H°$ (kcal/mol of duplex)	$-\Delta S°$ [cal/(K·mol of duplex)]	T_m at 1×10^{-4} M (°C)
CCGG[a]	34.2	95.6	34.5	96.4	27.4
ACCGGp[a]	39.0	107.7	39.4	108.9	36.7
ACCGGCp	48.5	134.7	47.8	132.4	44.2
ACCGGAp	44.6	122.1	43.8	119.8	44.4
ACCGGGp	48.4	131.2	49.0	132.8	50.8
ACCGGUp[a]	59.8	165.3	56.2	154.2	52.6

	temperature-dependent thermodynamic parameters						
oligonucleotide	$-\Delta H°$ at 37 °C (kcal/mol of duplex)	$-\Delta S°$ at 37 °C [cal/(K·mol of duplex)]	$-\Delta G°$ at 37 °C (kcal/mol of duplex)	$-\Delta H°$ at 50 °C (kcal/mol of duplex)	$-\Delta S°$ at 50 °C [cal/(K·mol of duplex)]	$-\Delta G°$ at 50 °C (kcal/mol of duplex)	$-\Delta C_p°$ [cal/(K·mol of duplex)]
CCGG[a]	38.3	108.7	4.6	43.4	124.5	3.1	385
ACCGGp[a]	40.3	111.6	5.7	45.2	126.9	4.2	375
ACCGGCp	45.0	122.9	6.9	49.1	135.7	5.2	310
ACCGGAp	40.5	108.8	6.7	45.6	125.4	5.1	399
ACCGGGp	44.5	118.5	7.7	49.4	134.1	6.1	381
ACCGGUp[a]	47.1	126.3	8.0	52.6	144.3	6.0	430

[a] Petersheim & Turner (1983).

Table II: Excess Thermodynamic Parameters due to Terminal Base Oppositions on a CCGG Core in 1 M NaCl[a]

	temperature-dependent parameters at 37 °C			
3′-pXp	$-\Delta\Delta H°$(3′-pXp)[b] (kcal/mol)	$-\Delta\Delta H°$(ACCGGXp) (kcal/mol)	$-\Delta\Delta S°$(3′-pXp)[b] [cal/(K·mol)]	$-\Delta\Delta S°$(ACCGGXp) [cal/(K·mol)]
pCp	1.6	3.4	4.0	7.1
pAp	3.5	1.1	7.8	0.1
pGp	4.6	3.1	10.9	4.9
pUp	2.4	4.4[d]	5.5	8.8[d]

	temperature-independent parameters at 37 °C		
3′-pXp	$-\Delta\Delta G°$(3′-pXp)[b] (kcal/mol)	$-[\Delta\Delta G°$(5′-pA) + $\Delta\Delta G°$(3′-pXp)][c] (kcal/mol)	$-\Delta\Delta G°$(ACCGGXp) (kcal/mol)
pCp	0.4	0.9	1.1
pAp	1.1	1.7	1.1
pGp	1.3	1.8	1.6
pUp	0.6	1.2	1.9[d]

[a] Buffer also includes 10 mM sodium cacodylate and 1 mM EDTA at pH 7. $\Delta\Delta H°$(3′-pXp) = $^1/_2[\Delta H°$(CCGGXp) − $\Delta H°$(CCGG)] and similarly for $\Delta\Delta G°$ and $\Delta\Delta S°$ values. [b] Petersheim & Turner (1983) Freier et al. (1983). [c] $\Delta\Delta G°$(5′-pA) = 0.53 kcal/mol of strand (Petersheim & Turner, 1983). [d] Petersheim & Turner (1983).

& Scruggs, 1965; Krakauer & Sturtevant, 1968; Neumann & Ackermann, 1969; Suurkuusk et al., 1977; Filimonov & Privalov, 1978), and A_7U_7p (Hickey & Turner, 1985). Temperature-dependent values of $\Delta H°$ and $\Delta S°$ were determined from plots according to eq 2 and 3 and are listed in Table I for 37 and 50 °C.

Discussion

The purpose of this work is to determine the duplex stabilization due to terminal mismatches in the series ACCGGXp. The results provide insight into incorporation of such mismatches in prediction of RNA structure and stability.

The relative effect of various terminal pairings is illustrated by the increment provided to the T_m of CCGG at 10^{-4} M (see Table I): AU (25.3 °C) > AG (23.7 °C) > AA (17.3 °C) ≈ AC (17.1 °C). This order is similar to the stabilities observed for internal pairings in DNA oligonucleotides: AT > AA ≈ AC (Tibanyenda, 1984) and GA > AC (Patel et al., 1984a,b). The stability conferred by an AG mismatch is almost the same as for an AU pair, particularly at high temperatures (see Figure 1 and Table I). Thus, terminal mismatches are significant contributors to RNA stability. The ordering of stabilities correlates well with the natural occurrence of the mismatches. AG mismatches are the only ones to occur with more than statistical frequency at helix ends in rRNA (Traub & Sussman, 1982; Noller, 1984). AG also occurs at the helix–loop junction of almost all tRNA D loops that have only three base pairs in the helix (Lomant & Fresco, 1975) and is the most prevalent pairing other than m_2^2GA between positions 26 and 44 in tRNAs with short extra arms (Mikelsaar, 1981).

The free energy increment due to a single terminal mismatch, $\Delta\Delta G°$(AX), can be calculated for any temperature from the results in Table I by using $\Delta\Delta G°$(AX) = $^1/_2$-[$\Delta G°$(ACCGGXp) − $\Delta G°$(CCGG)]. Here the assumption is made that a 3′-terminal phosphate has little effect on the thermodynamics, as previously shown for other oligomers (Petersheim & Turner, 1983; Freier et al., 1983b; Hickey & Turner, 1985). The increments in $\Delta G°$ (in kcal/mol) at 37 °C are AG (−1.6), AA (−1.1), and AC (−1.1). Evidently, 0.5 kcal/mol is enough to provide an evolutionary advantage for AG. The $\Delta\Delta G°$'s for base pairs at 37 °C range from −0.9 to −3.5 kcal/mol (Freier et al., 1984; Borer et al., 1974). Thus, terminal mismatches contribute as much to stability as some base pairs. However, the range in $\Delta\Delta G°$'s for terminal mismatches is probably less than for base pairs. The 3′-dangling Up in CCGGUp adds −0.4 kcal/mol to duplex stability at 37 °C (Freier et al., 1983b), and an additional 5′-pyrimidine is

expected to provide little additional stability (Freier et al., 1985). Thus, the range in $\Delta\Delta G°$'s for terminal mismatches is probably about 1 kcal/mol. Since a few kilocalories per mole often distinguish competing structures in large RNA molecules (Auron et al., 1982; Cech et al., 1983), inclusion of mismatches is likely to improve predictions of structures.

If a nearest-neighbor model (Tinoco et al., 1971) is valid, then inclusion of mismatches in structure prediction algorithms requires determination of 48 parameters. It is unlikely all these parameters will be measured until a breakthrough is achieved in RNA synthesis. However, current results suggest some approximations that may be useful in the interim. One approximation is that a mismatch adds the same stability increment regardless of the adjacent base pair. Consistent with this assumption is the observation that, at 10 mM strand concentration, two AA mismatches add 15 °C to the melting temperature of AGCU (Alkema et al., 1981) and are predicted to add 13 °C to the melting temperature of CCGG at 10 mM. A second potentially useful assumption is that a mismatch can be approximated by the sum of the parameters for the corresponding 5′- and 3′-dangling ends. A substantial number of these parameters have been measured (Petersheim & Turner, 1983; Freier et al., 1983b; S. M. Freier, D. Alkema, A. Sinclair, T. Neilson, and D. H. Turner, unpublished results). From steric considerations, this approximation seems intuitively reasonable for pyrimidine–pyrimidine and pyrimidine–purine oppositions, but poor for purine–purine oppositions. Table II contains data for initial testing of this assumption. For AC, $\Delta\Delta G°$(5′-A) + $\Delta\Delta G°$(3′-C) = −0.9 kcal/mol compared to the experimental $\Delta\Delta G°$ of −1.1 kcal/mol. This is reasonable agreement, considering experimental errors. For AA and AG mismatches, the predicted $\Delta\Delta G°$'s are too negative by 0.6 and 0.2 kcal/mol, respectively, relative to the measured values (see Table II). This is the direction expected since it is unlikely both 5′- and 3′-purines can occupy their most favorable stacking positions simultaneously. In fact, as shown in Table II, AA and AG mismatches add only 0 and 0.3 kcal/mol, respectively, more stability than the corresponding 3′-dangling ends. Thus, purine–purine mismatches may be better approximated by the appropriate 3′-dangling end than by a sum of 5′- and 3′-dangling ends. While more data are clearly required, it is likely the approximations suggested above will be an improvement over the current practice of assigning no free energy increment to terminal mismatches.

The enthalpy and entropy changes associated with AX pairs are listed in Table II, along with those previously measured for corresponding 3′-dangling X's (Petersheim & Turner, 1983; Freier et al., 1983b). Comparisons of these parameters are consistent with steric hindrance affecting stacking for purine–purine but not purine–pyrimidine oppositions. In particular, for AU and AC pairs, $\Delta\Delta H°$ and $\Delta\Delta S°$ are both more negative than for the corresponding 3′-dangling end. This is expected if the 5′-A does not interfere with stacking of the 3′-Xp. For AA and AG mismatches, however, $\Delta\Delta H°$ and $\Delta\Delta S°$ are more positive than the corresponding 3′-dangling end. This is consistent with addition of the 5′-A disrupting the most favorable stacking of the 3′-Xp.

The thermodynamic parameters in Tables I and II can be compared with those derived from fitting predictions of tRNA and 5S RNA secondary structures to the known structures (Papanicolaou et al., 1984). Extrapolation to terminal mismatches on GC pairs using Table I of Papanicolaou et al. gives $\Delta G°$'s (presumably at 25 °C) of −1.1 kcal/mol for AG and AA and −1.5 kcal/mol for AC. The measured values in the ACCGGXp series are −1.9, −1.2, and −1.3 kcal/mol, respectively. This is surprisingly good agreement considering the disparity of methods.

The results presented above demonstrate that terminal mismatches are significant contributors to RNA stability and suggest reasonable approximations for including mismatches in algorithms for RNA structure prediction. Further refinement of parameters for mismatches will require synthesis of a large number of appropriate oligonucleotides.

Supplementary Material Available

Two figures each for ACCGGCp, ACCGGAp, and ACCGGGp showing $\Delta H°$ vs. T_m and $\Delta S°$ vs. ln (T_m) (6 pages). Ordering information is given on any current masthead page.

Registry No. ACCGGCp, 96413-46-6; ACCGGAp, 96413-47-7; ACCGGGp, 96413-48-8.

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Poly(dA)·Poly(dT) Exists in an Unusual Conformation under Physiological Conditions: Propidium Binding to Poly(dA)·Poly(dT) and Poly[d(A-T)]·Poly[d(A-T)]†

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ABSTRACT: The binding of propidium to poly(dA)·poly(dT) [poly(dA·dT)] and to poly[d(A-T)]·poly[d(A-T)] [poly[d(A-T)$_2$]] has been compared under a variety of solution conditions by viscometric titrations, binding studies, and kinetic experiments. The binding of propidium to poly[d(A-T)$_2$] is quite similar to its binding to calf thymus deoxyribonucleic acid (DNA). The interaction with poly(dA·dT), however, is quite unusual. The viscosity of a poly(dA·dT) solution first decreases and then increases in a titration with propidium at 18 °C. The viscosity of poly[d(A-T)$_2$] shows no decrease in a similar titration. Scatchard plots for the interaction of propidium with poly(dA·dT) show the classical upward curvature for positive cooperativity. The curvature decreases as the temperature is increased in binding experiments. A van't Hoff plot of the observed binding constants yields an apparent positive enthalpy of approximately +6 kcal/mol for the propidium–poly(dA·dT) interaction. Propidium binding to poly[d(A-T)$_2$] shows no evidence for positive cooperativity, and the enthalpy change for the reaction is approximately −9 kcal/mol. Both the magnitude of the dissociation constants and the effects of ionic strength are quite similar for the dissociation of propidium from poly(dA·dT) and from poly[d(A-T)$_2$], suggesting that the intercalated states are similar for the two complexes. The observed association reactions, under pseudo-first-order conditions, are quite different. Plots of the observed pseudo-first-order association rate constant vs. polymer concentration have much larger slopes for propidium binding to poly[d(A-T)$_2$] than to poly(dA·dT). These results are interpreted in terms of a fairly standard B conformation for poly[d(A-T)$_2$] which can bind propidium in a manner similar to DNA. Poly(dA·dT), however, must have some unusual structural features under normal conditions but can be converted to an intercalated B conformation with the unusual binding results described above. The polymer conformational transition is characterized by a large positive enthalpy and entropy values which result in a relatively small free-energy change for the transition.

The success of the Watson & Crick (1953) B-form model for deoxyribonucleic acid (DNA)[1] conformation in predicting biological and chemical properties of DNA has led to an ongoing analysis of DNA structure and possible structural changes. Early studies produced the related A, C, and D variations of the double helix (Arnott et al., 1982). More recently, the dramatically different left-handed Z-form helix (Wang et al., 1980) with its potential importance in gene expression (Nordheim et al., 1981) has attracted attention. Dickerson and co-workers (Dickerson et al., 1982), on the other hand, have shown that even random-sequence DNA can have structural variations which could serve as recognition signals in the biological functions of the nucleic acid.

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[1] Abbreviations: CD, circular dichroism; DNA, deoxyribonucleic acid; NMR, nuclear magnetic resonance; PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); poly(dA·dT), poly(dA)·poly(dT); poly[d(A-T)$_2$], poly[d(A-T)]·poly[d(A-T)]; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; 2D, two dimensional; NOE, nuclear Overhauser effect.

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