Sequence Specific Thermodynamic and Structural Properties for DNA·RNA Duplexes†

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ABSTRACT: DNA·RNA hybrid duplexes are found in many important biological processes and are involved in developing modes of disease treatment, such as antisense therapy, yet little is known about the sequence dependence of their structure and stability. The structure and thermodynamic stability of DNA·RNA hybrid model systems corresponding in composition and length and containing (1) all purine or all pyrimidine bases on each strand or (2) mixed purine and pyrimidine bases on each strand have been evaluated relative to pure RNA and DNA duplexes by thermal melting, CD, and electrophoresis analyses. The spread in free energies of denaturation of the homopurine·homopyrimidine systems covers over 14 kcal/mol of single strands, while the mixed sequence free energies vary by less than 4 kcal/mol. The RNA–homopurine·DNA–homopyrimidine hybrid resembles a corresponding pure RNA duplex in both structure and stability, whereas the DNA–homopurine·RNA–homopyrimidine hybrid resembles a corresponding pure DNA duplex. The mixed sequence hybrids show intermediate structure between the corresponding pure RNA and pure DNA duplexes and a stability closer to that of the pure DNA duplex. From these results and the evaluation of published hybrid data [Hall, K. B., & McLaughlin, L. W. (1991) *Biochemistry 30*, 10606–10613; Roberts, W. R., & Crothers, D. M. (1992) *Science 258*, 1463–1466], it can be predicted that a hybrid duplex containing more RNA purine bases will have a CD spectrum, and probably conformation, resembling that of A-form duplexes and will be more stable than a corresponding hybrid duplex with fewer RNA purine bases.

DNA·RNA hybrid duplexes normally occur in cells as a result of transcription or as Okazaki fragments in replication (Kornberg & Baker, 1992; Okazaki et al., 1973). Hybrid duplexes can also occur as a result of reverse transcription when cells are infected by retroviruses (Varmus, 1988). In addition, nonnatural cellular hybridization between a normal m-RNA and a complementary DNA oligomer is the key event in antisense therapy (Wickstrom, 1991). We are interested in the properties of DNA·RNA duplexes in order to enhance the stability of hybrids formed in antisense therapy and also to use the hybrids as receptors for drugs that could inhibit reverse transcription of retroviral RNA.

X-ray crystallographic investigations of chimeric RNA·DNA duplexes, with an RNA·DNA hybrid section and a pure DNA section, indicate that the global conformation of the molecules is the A-form (Wang et al., 1980; Egli et al., 1992). Although this agrees with observations that DNA can exist in both the A and B conformations while RNA strongly prefers the A-form, many pure DNA duplexes crystallize in an A conformation even without an RNA section (Saenger, 1984). NMR studies in solution (Haasnoot et al., 1983; Mellema et al., 1983; Chou et al., 1991; Jaishree et al., 1993; Hall, 1993; Lane et al., 1993) and X-ray fiber diffraction results (Arnott et al., 1986) have indicated more complex structures for mixed RNA/DNA systems, with most of the DNA sugars in a C2′-endo type conformation while the RNA sugars fall into the C3′-endo conformational family. A recent NMR study of a mixed sequence hybrid found the DNA sugars in an O4′-endo conformation, while the RNA sugars are in the normal C3′-endo conformation (Fedoroff et al., 1993).

Two detailed studies on the structure and thermodynamics of hybrid duplexes with one RNA and one DNA strand have yielded very interesting and somewhat different results. Hall and McLaughlin (1991) found that two heteroduplexes formed from complementary RNA and DNA pentamer strands (Table 1) were less stable than the DNA·DNA and RNA·RNA duplexes. CD and imino proton NMR spectra led to the conclusion that the conformations of both hybrid duplex structures were close to that of the pure RNA duplex. Roberts and Crothers (1992) studied hairpin duplexes in which the complementary stem strands of the hairpin were pure RNA, pure DNA, or hybrids and each strand sequence was all purine or all pyrimidine bases (Table 1). They found that the RR duplex was most stable, with the RD duplex next closest in stability. The DD duplex was less stable than the RR duplex by almost 10 kcal/mol, and the DR duplex was slightly less stable than DD. The total free energy difference between the RR and DR duplexes was over 11 kcal/mol and is considerably larger than the difference observed by Hall and McLaughlin (1991). Roberts and Crothers (1992) noted that their duplexes exhibited considerable differences in CD spectra and gel electrophoretic mobilities, but that the duplexes with similar stabilities, RR and RD or DD and DR, had more similar spectra and mobilities.

The differences between the Hall and McLaughlin (1991) and Roberts and Crothers (1992) results are striking, but the systems used are quite different: pentamer strands for Hall and McLaughlin and hairpin 28-mers for Roberts and Crothers. As part of our efforts in the development of antisense

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Table 1: Oligomer Abbreviations and Sequences[a]

Abbreviation	Sequence	Abbreviation	Sequence
Group I: Homopurine.Homopyrimidine Sequences		**Roberts and Crothers (1992)**	
d(GA).d(CT)	5' $d(GAAG)_3$ 3' $d(CTTC)_3$	DD	5' GGAGAGGAGGGA$^{T}_{T}$ 3' CCTCTCCTCCCT$_{T}^{T}$
d(GA).r(CU)	5' $d(GAAG)_3$ 3' $r(CUUC)_3$	DR	5' GGAGAGGAGGGA$^{T}_{T}$ 3' CCUCUCCUCCCU$_{T}^{T}$
r(GA).r(CU)	5' $r(GAAG)_3$ 3' $r(CUUC)_3$	RR	5' GGAGAGGAGGGA$^{U}_{U}$ 3' CCUCUCCUCCCU$_{U}^{U}$
r(GA).d(CT)	5' $r(GAAG)_3$ 3' $d(CTTC)_3$	RD	5' GGAGAGGAGGGA$^{T}_{T}$ 3' CCTCTCCTCCCT$_{T}^{T}$
Group II: Mixed Sequences		**Hall and McLaughlin (1991)**	
d(GT).d(CA)	5' $d(GTTG)_3$ 3' $d(CAAC)_3$	D*D*	5' d(CTGTG) 3' d(GACAC)
d(GT).r(CA)	5' $d(GTTG)_3$ 3' $r(CAAC)_3$	D*R*	5' d(CTGTG) 3' r(GACAC)
r(GU).r(CA)	5' $r(GUUG)_3$ 3' $r(CAAC)_3$	R*R*	5' r(CUGUG) 3' r(GACAC)
r(GU).d(CA)	5' $r(GUUG)_3$ 3' $d(CAAC)_3$	R*D*	5' r(CUGUG) 3' d(GACAC)

[a] DD, DR, RR, and RD are from Roberts and Crothers (1992); RNA sequences are underlined. D*D*, D*R*, R*R*, and R*D* are from Hall and McLaughlin (1991).

therapy and in the use of hybrid duplexes as drug receptors in antiviral therapy, we have synthesized two groups of model dodecamer RNA and DNA strands (Table 1). Group I oligomers contain all purine or all pyrimidine bases on each strand, as in the hairpin duplexes studied by Roberts and Crothers (1992). Group II oligomers correspond to group I in composition and length, but the purine and pyrimidine bases are mixed on each strand, as in the studies of Hall and McLaughlin (1991). We find striking differences in thermodynamic stability for the two groups: the stabilities for group I duplexes resemble those for the hairpin duplexes of Roberts and Crothers (1992), while the stabilities for group II duplexes, when corrected for length differences, resemble those for the duplexes studied by Hall and McLaughlin (1991).

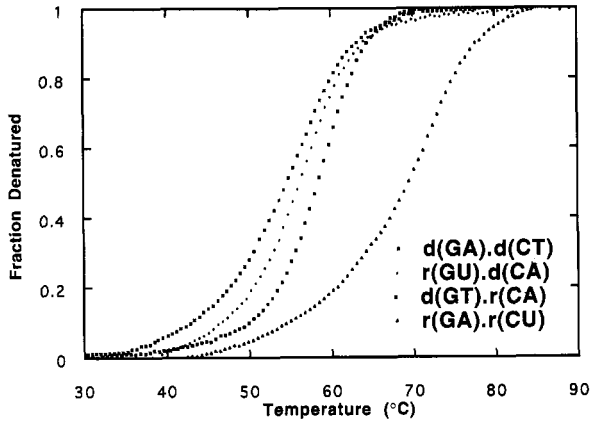

FIGURE 1: Typical melting curves for group I and group II duplexes. Measurements were taken in PIPES buffer, 1 M NaCl, and 8.3 × 10^{-6} M strand concentration. The curves were completely reversible, as observed by heating and cooling at a rate of 0.5 °C/min.

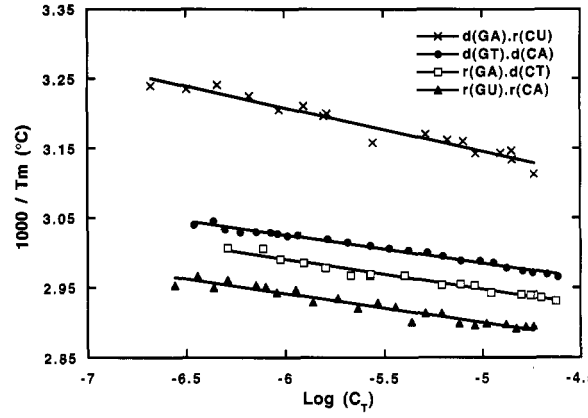

FIGURE 2: Examples of van't Hoff plots for group I and group II duplexes. Measurements were taken in PIPES buffer with 1 M NaCl at different oligomer strand concentrations. Thermodynamic parameters for these plots are in Table 2.

The observed differences in stabilities are, thus, a consequence of the sequence differences between the groups.

MATERIALS AND METHODS

Materials. Oligomers were synthesized and purified as previously described (Ratmeyer et al., 1992).

T_m Studies. Cary 4 or 219 spectrophotometers interfaced to microcomputers were used to obtain the thermal denaturation data, as previously described (Li et al., 1991). The T_m data were fit with a nonlinear least-squares computer program that includes sloping base lines in the duplex and single-strand regions (Petersheim & Turner, 1983; Puglisi & Tinoco, 1989). Experiments were conducted in a buffer at pH 7.0 containing 10 mM PIPES, 1 mM EDTA, and NaCl concentrations up to 1.0 M. Oligomer concentrations were determined with extinction coefficients calculated from mononucleotide and dinucleotide data using the nearest-neighbor approximation (Fasman, 1975). Thermodynamic parameters were determined from van't Hoff plots as previously described (Li et al., 1991).

Circular Dichroism Spectra. CD spectra were obtained with a Jasco J-600 spectrophotometer interfaced to an IBM computer as previously described (Zuo et al., 1990). All CD experiments were performed at 15 °C in 1-cm path length cuvettes with a buffer adjusted to pH 7.0 containing 3.75 mM NaH_2PO_4, 1 mM EDTA, and 0.1 M NaCl. Phosphate buffer was substituted for the PIPES buffer used in the T_m experiments in order to measure the oligomer CD at lower wavelengths without interference from the buffer absorption.

Gel Electrophoresis. Electrophoresis experiments were conducted in gels containing 20% polyacrylamide (19.5% acrylamide/0.5% bisacrylamide) with a Bio-Rad miniProtean II gel apparatus, 7.3 × 10.2 cm glass slabs, and 0.75-mm spacers, as previously described (Kibler-Herzog et al., 1990). TBE buffer (0.09 M Tris, 0.09 M boric acid, and 0.2 mM EDTA, pH 8.3) was used in sample preparations and electrophoresis reservoirs.

RESULTS

Group I

T_m Comparisons and Effects of Salt Concentration. All group I duplexes (Table 1) give cooperative, reversible melting transitions (Figure 1) that allow the accurate determination of T_m values. Each duplex was observed by both CD and UV absorption methods to have a single melting transition with very similar curve shapes and T_m's. This result indicates two-state melting behavior for these duplexes. The order of stability for the duplexes under all conditions is r(G–A)·r(C–U) > r(G–A)·d(C–T) > d(G–A)·d(C–T) > d(G–A)·r(C–U) (Table 2). The stability of the RNA is significantly higher than that of the DNA, and the stability order is as observed by Roberts and Crothers (1992). The stability of the hybrid duplex with a purine RNA strand and a pyrimidine DNA strand, r(G–A)·d(C–T), is closer to that of the pure RNA duplex, while the stability of the hybrid duplex with a pyrimidine RNA strand and a purine DNA strand, d(G–A)·r(C–U), is closer to that of the pure DNA duplex. Linear plots of T_m for the duplexes vs log(Na^+ activity) have slopes between 10 and 13 (not shown), as expected for oligomers of this size (Olmsted et al., 1991).

Thermodynamic Parameters. T_m curves, such as those shown in Figure 1, were obtained at a range of oligomer strand concentrations and a 1:1 ratio of the two complementary strands to allow calculation of thermodynamic parameters for duplex formation. Parameters were determined by the van't Hoff method (Figure 2) and were calculated by the nearest-neighbor method for comparison (Table 2). Enthalpy and entropy values have wide variations among the sequences, but correlation of these values results in a smaller variation in the free energy values. The order of the free energy values, r(G–A)·r(C–U) < r(G–A)·d(C–T) < d(G–A)·d(C–T) < d(G–A)·r(C–U), is consistent with the stability conclusions drawn from the T_m data. r(G–A)·d(C–T) again resembles the pure RNA duplex and d(G–A)·r(C–U) the pure DNA duplex in free energy. This pattern of stability is also observed by Roberts and Crothers (1992). The spread in thermodynamic stabilities between r(G–A)·r(C–U) and d(G–A)·r(C–U) is over 14 kcal/mol of single strands, a remarkable difference for duplexes of such similar composition.

Circular Dichroism Spectra. CD spectra (Figure 3A) of d(G–A)·d(C–T) and r(G–A)·r(C–U) have characteristic B-form and A-form shapes (Cantor & Schimmel, 1980), respectively. r(G–A)·d(C–T) has a CD spectrum that resembles that of the pure RNA duplex, while d(G–A)·r(C–U) has a CD spectrum supporting its similarity to the pure DNA duplex. The negative CD band at 210 nm in the pure RNA spectrum provides a useful basis for comparison. r(G–A)·d(C–U) has a very similar band, but it is much reduced in the d(G–A)·r(C–U) spectrum. The pure DNA has only a small dip in its CD spectrum at the same wavelength. Note that neither hybrid duplex falls into a pure B-form or pure A-form CD pattern. The CD spectra of the hairpin duplexes

Table 2: Thermodynamic Parameters for Duplex Formation by Group I and Group II Oligomers[a]

		van't Hoff method				nearest-neighbor calculation			
abbreviation	T_m[b] (°C)	$-\Delta G°_{37}$ (kcal/mol)	$-\Delta G°_{25}$ (kcal/mol)	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (kcal/mol·deg)	$-\Delta G°_{37}$ (kcal/mol)	$-\Delta G°_{25}$ (kcal/mol)	$-\Delta H°$ (kcal/mol)	$-\Delta S°$ (kcal/mol·deg)
d(G–A)·d(C–T)	55.6	13.2	16.2	89.5	0.246	13.5	16.5	89.5	0.245
d(G–A)·r(C–U)	43.3	9.91	12.4	73.8	0.206				
r(G–A)·r(C–U)	72.2	22.2	26.8	141	0.383	17.1	20.5	107	0.290
r(G–A)·d(C–T)	65.4	17.4	20.9	108	0.292				
d(G–T)·d(C–A)	61.5	16.3	20.1	114	0.315	13.9	16.7	86.2	0.233
d(G–T)·r(C–A)	60.0	15.9	19.8	117	0.326				
r(G–U)·r(C–A)	72.1	19.3	22.9	112	0.299	17.0	20.4	106	0.287
r(G–U)·d(C–A)	57.4	15.5	19.3	112	0.311				

[a] Thermodynamic parameters were determined in PIPES buffer with 1 M NaCl. ΔG was calculated at 25 and 37 °C to allow direct comparison with values calculated by the nearest-neighbor method, where tables are at 37 °C (Turner et al., 1988) for RNA and 25 °C (Breslauer et al., 1986) for DNA. [b] T_m values were taken at 8.3×10^{-6} M strand concentrations and 1 M NaCl.

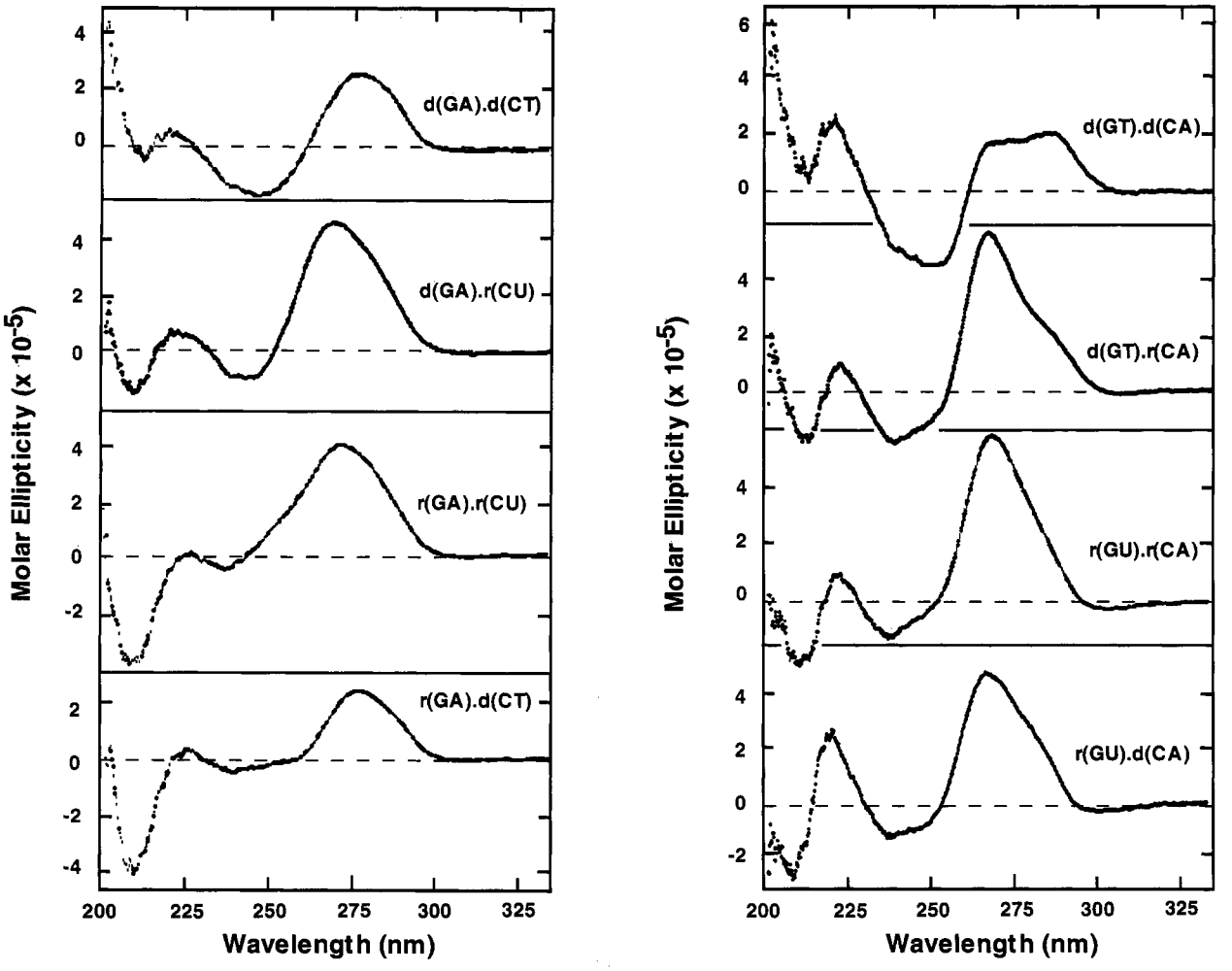

FIGURE 3: CD spectra of group I (A, left) and group II (B, right). Measurements were taken at 15 °C in NaH_2PO_4 buffer, 0.1 M NaCl, and 2.1×10^{-6} M strand concentration.

of Roberts and Crothers (1992) show similar correlations.

Gel Electrophoresis. Conformational similarities of the duplexes can also be monitored by polyacrylamide gel electrophoresis. Quite large variations in mobilities are observed, with the mobilities of the duplexes as follows: d(G–A)·d(C–T) > d(G–A)·r(C–U) > r(G–A)·d(C–T) > r(G–A)·r(C–U) (Figure 4). The mobilities follow a pattern similar to that observed in the T_m and CD studies, where d(G–A)·r(C–U) and r(G–A)·d(C–T) have mobilities that resemble those for the pure DNA and RNA duplexes, respectively. The mobility pattern of DNA > hybrids > RNA has also been observed by Bhattacharyya et al. (1990) and by Roberts and Crothers (1992).

Group II

T_m Comparisons and Effects of Salt Concentration. As with group I duplexes, group II (Table 1) duplexes have cooperative, reversible melting transitions (Figure 1) that allow the accurate determination of T_m values. All group II duplexes gave single UV and CD melting transitions, which indicate two-state melting behavior as with group I duplexes. The order of stability for the duplexes is r(G–U)·r(C–A) > d(G–T)·d(C–A) > d(G–T)·r(C–A) > r(G–U)·d(C–A). The stability of the RNA duplex is greater than that of the DNA duplex, as observed by Hall and McLaughlin (1991). The orders of stability for the two heteroduplexes from group II

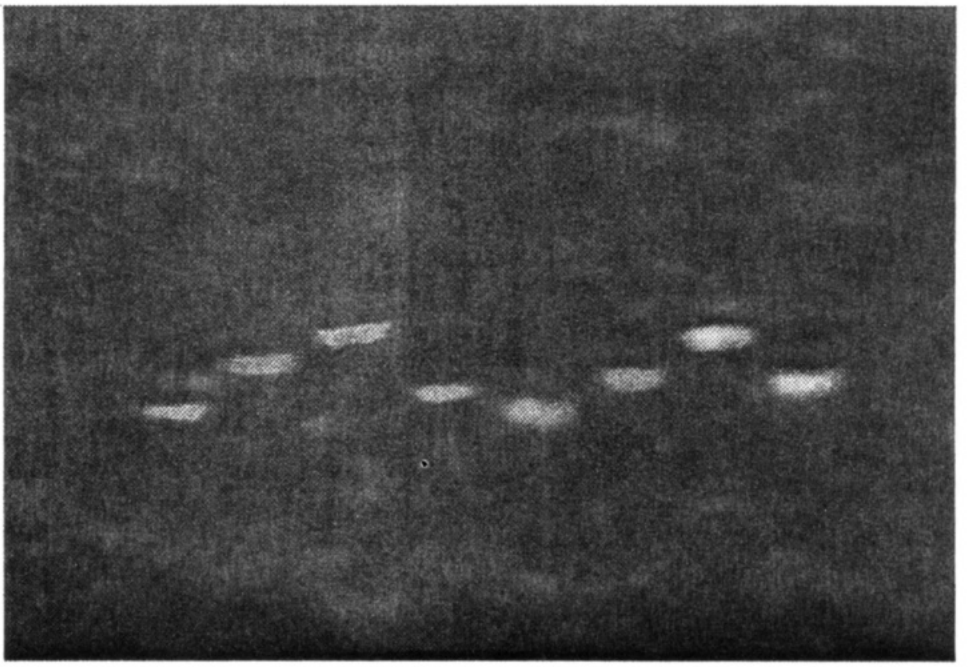

FIGURE 4: Photograph of polyacrylamide gel for group I and group II oligomers. From left to right, the duplexes and relative electrophoretic mobilities are as follows: (1) d(G–A)·d(C–T), 1.0; (2) r(G–A)·d(C–T), 0.87; (3) r(G–A)·r(C–U), 0.80; (4) d(G–A)·r(C–U), 0.95; (5) d(G–T)·d(C–A), 1.0; (6) r(G–U)·d(C–A), 0.90; (7) r(G–U)·r(C–A), 0.80; (8) d(G–T)·r(C–A), 0.91. Gels were stained with ethidium bromide.

and those from Hall and McLaughlin are not the same. The stabilities of these hybrid duplexes are similar, however, and the differences in T_m values are close to experimental error when considering the relatively broad T_m curves and low T_m values of pentamers in the comparisons. In both group II and the Hall and McLaughlin (1991) duplexes (Table 1), the pure RNA duplexes are considerably more stable than all of the DNA-containing sequences (Table 2). Linear plots of T_m vs log(Na^+ activity) have slopes between 10 and 12 (not shown), as expected for oligomers of this size and as observed for group I duplexes.

Thermodynamic Parameters. Thermodynamic parameters were determined for group II duplexes in the same manner as described for group I duplexes (Table 2 and Figure 2). The order of the free energy values, r(G–U)·r(C–A) < d(G–T)·d(C–A) < d(G–T)·r(C–A) < r(G–U)·d(C–A), is consistent with the stability conclusions drawn from the T_m data. The pure RNA has the lowest melting free energy, while the DNA and hybrid duplexes have similar, higher free energies, as also observed by Hall and McLaughlin (1991).

Circular Dichroism Spectra. CD spectra (Figure 3B) of d(G–T)·d(C–A) and r(G–U)·r(C–A) show similarities to the characteristic B-form and A-form shapes of the pure DNA and RNA duplexes of group I, but the group II sequences have fine structure not seen with the group I set. The CD spectra of the group I hybrid duplexes resemble spectra for either the pure RNA or the pure DNA duplexes, as shown above, while the spectra of the group II hybrid duplexes do not fall neatly into a pattern of similarity. For example, the negative CD band at 210 nm of both hybrid duplexes seems to resemble that of the pure RNA. On the other hand, the CD peak at 220 nm for r(G–U)·d(C–A) resembles that of the pure DNA duplex, while the peak for d(G–T)·r(C–A) resembles that of the pure RNA duplex. Moreover, at 265 nm the CD peaks for the hybrids seem to be mixtures of those found for the pure DNA and RNA duplexes. The conclusion from the CD data is that the spectra of the group II hybrid duplexes are mixtures of the pure DNA and RNA spectra, with a greater similarity to the pure RNA spectra. Hall and McLaughlin (1991) also concluded that the CD spectra from both of their hybrid duplexes resembled that of the pure RNA duplex.

Gel Electrophoresis. The mobilities of the group II duplexes on a polyacrylamide gel also give an indication of duplex conformation, as discussed for group I. The mobility pattern is as follows: d(G–T)·d(C–A) > d(G–T)·r(C–A) > r(G–U)·d(C–A) > r(G–U)·r(C–A) (Figure 4). As with group I, the mobility pattern of DNA > hybrid > RNA is observed. These results suggest that the global conformations of the hybrid duplexes are similar and are between those of the pure DNA and RNA duplexes.

DISCUSSION

DNA·RNA hybrid duplexes are found in many important biological processes (Kornberg & Baker, 1992; Okazaki et al., 1973; Varmus, 1988) and are involved in developing modes of disease treatment, such as antisense therapy (Wickstrom, 1991). At present, little is known about the sequence dependence of the structure and stability of such hybrids. Two studies on the structure and stability of hybrid duplexes have yielded very interesting and somewhat different results. The systems used in the two studies, however, were quite different: a homopurine·homopyrimidine hairpin 28-mer (Roberts & Crothers, 1992) and a mixed purine·pyrimidine pentamer (Hall & McLaughlin, 1991). The goal of our efforts was to investigate a homopurine·homopyrimidine sequence and a mixed sequence in hybrid systems corresponding in composition and length. Stability and thermodynamic parameters were determined by T_m experiments, while CD and gel electrophoresis experiments were used to investigate structure. Hybrid duplexes were investigated with pure DNA and pure RNA duplexes for comparison.

Group I Stability. The key observations from the group I duplexes are as follows: (1) the hybrid duplex with a pyrimidine DNA strand is of lower stability than the pure RNA duplex; and (2) the hybrid duplex with a purine DNA strand is lower in stability than both the RNA and DNA

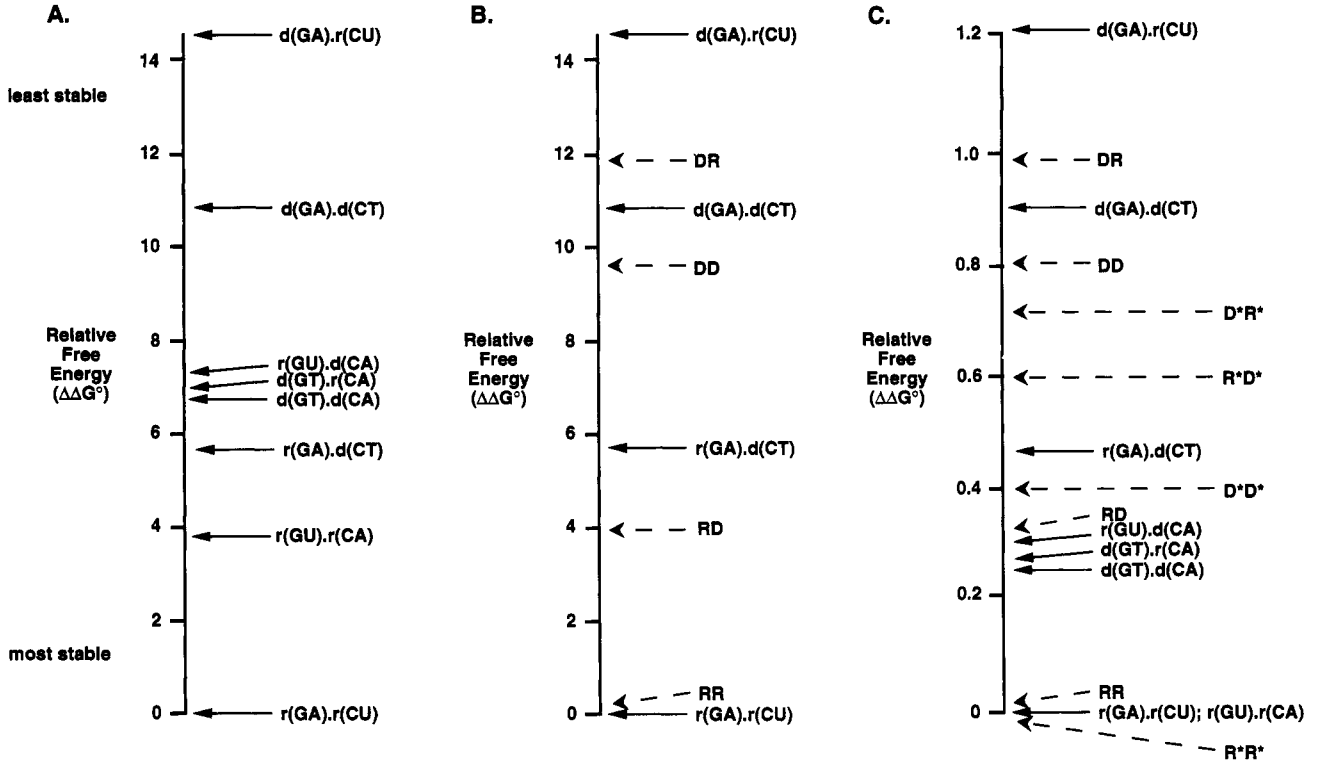

FIGURE 5: Relative free energies ($\Delta\Delta G°_{25}$) in kilocalories/mol of single strands for duplex formation relative to the most stable duplex: (A) values for group I and group II duplexes; (B) values for group I duplexes as well as those from Roberts and Crothers (1992) (dashed arrows); (C) values for group I and group II duplexes as well as those from Roberts and Crothers (1992) (short dashed arrows) and Hall and McLaughlin (1991) (long dashed arrows). Since the duplexes from these different studies vary in length, the results are presented on a per base pair basis. All sets are referenced relative to the RNA duplex that is the most stable in each set.

duplexes. Relative free energy values [free energies of the duplexes minus the free energy of the most stable duplex (Figure 5A)] of group I and those from Roberts and Crothers (1992) (Figure 5B) have striking similarity.

The effects of the replacement of a *DNA* purine strand with an *RNA* purine strand are observed in the relative stabilities of d(G–A)·r(C–U) to r(G–A)·r(C–U) and of d(G–A)·d(C–T) to r(G–A)·d(C–T). Such changes stabilize the hybrid duplexes by 1.2–0.4 kcal/mol of base pairs. Comparison of the relative stabilities of r(G–A)·d(C–T) to r(G–A)·r(C–U) and d(G–A)·d(C–T) to d(G–A)·r(C–U) isolates the effects of replacing a *DNA* pyrimidine strand with an *RNA* pyrimidine strand. The RNA pyrimidine strand replacement is stabilizing by 0.5 kcal/mol of base pairs for the formation of the RNA duplex and destabilizing by 0.3 kcal/mol of base pairs for the formation of the hybrid duplex. The results of Roberts and Crothers (1992) are quite similar (Figure 5B). Overall, an RNA purine strand is stabilizing to a duplex, while a pyrimidine RNA strand can be stabilizing when paired with another RNA strand or destabilizing when paired with a DNA strand.

Group II Stability. The key observation is that the stabilities of the hybrid group II duplexes resemble the stability of the pure DNA but are less than that of the pure RNA duplex (Figure 5). Hall and McLaughlin (1991) also found the RNA duplex to be the most stable and the three DNA-containing duplexes to be similar; however, they found the hybrid duplex D*R* to be less stable than R*D* (Figure 5C). When sequence differences and experimental variations (considering that pentamers have broad melting curves and low T_m's) are taken into account, the patterns of results for the two sets of heterosequence oligomers are quite similar. A primary source for the differences in order of stability could also be the sequence differences between the group II and the Hall and McLaughlin (1991) duplexes. The ratio of pyrimidine/purine RNA bases is 1 in d(G–T)·r(C–A) and 1.5 in D*R*; e.g. although the Hall and McLaughlin (1991) duplexes are of mixed sequence, they are actually between groups I and II in composition.

As observed for the stability of the group I duplexes, the more pyrimidine RNA bases that are present, the more destabilized a heteroduplex becomes. On the basis of this result, D*R* should be less stable than D*D*, as observed by Hall and McLaughlin (1991). In the same manner, R*D*, which has a 0.7 ratio of pyrimidine/purine RNA bases, should be and is more stable than D*R* due to its lower RNA pyrimidine content. On the basis of this reasoning, R*D* should be more stable than D*D*; however, R*D* is observed by Hall and McLaughlin (1991) to be less stable than D*D*. The restricted ranges of T_m and free energy values for the heterosequence duplexes can account for these differences in order.

Comparison of Groups I and II. The two RNA duplexes are the most stable for both groups, with the group I RNA being slightly more stable than the group II RNA (Figure 5A). It is destabilizing to replace the mixed sequence RNA with either one strand or two strands of DNA. It is similarly destabilizing to replace the group I RNA with a DNA pyrimidine strand. However, it is even more destabilizing to replace the group I RNA with a purine DNA strand or with two DNA strands—the replacement of only the purine strand being the more deleterious. A hybrid duplex of a mixed purine and pyrimidine sequence, which has a higher probability in biological systems, will be more stable when it contains a greater amount of RNA purine bases (Figure 5A).

Structure. Although CD spectra and gel electrophoresis mobilities do not give detailed structural information, they can provide information about the global conformations of

the hybrid duplexes, especially on a comparative basis. Both group I and group II duplexes were shown to have structures intermediate between the RNA A-form and DNA B-form structures by CD and gel electrophoresis experiments. To some degree, the group I duplexes are more similar to either the A-form or B-form, depending on whether they have an RNA or a DNA purine strand, respectively. A prediction from these results is that a hybrid duplex containing more purine RNA segments will have a conformation with more A-form similarities than B-form similarities and will also be more stable.

The two pure RNA duplexes from groups I and II have similar T_m and ΔG values for the melting transition (Table 2). The spread of results in group I is, however, much larger than that in group II. The T_m of the group I d(G–A)·r(C–U) hybrid in Table 2, for example, is almost 17 °C less than that for the group II d(G–T)·r(C–A) hybrid, and the free energy for duplex formation is over 7 kcal/mol of single strands more positive. Thus, it is clear that there are dramatic differences among the stabilities of RNA, DNA, and hybrid duplexes that are magnified in some sequences. We are investigating additional hybrid duplexes, as well as pursuing theoretical approaches to explain the observed energetic differences.

Finally, it is worth considering these results from the standpoint of antisense interactions, which require formation of DNA·RNA hybrid duplexes (Wickstrom, 1991). The two hybrids in group I differ enormously in stability, and this result suggests that RNA sequences with long pyrimidine repeats should be avoided as antisense targets whenever possible. On the other hand, long RNA purine repeats should be good targets for DNA antisense oligomers from the duplex stability standpoint. Hybrids with mixed purine–pyrimidine sequences are more similar in stability and have stabilities between those of the two hybrids in group I. Sequence variations in the RNA target in this case have a much smaller effect on duplex stability.

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