Bulge Loops Used To Measure the Helical Twist of RNA in Solution†

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ABSTRACT: Bulge loops are commonly found in helical segments of cellular RNAs. When incorporated into long double-stranded RNAs, they may introduce points of flexibility or permanent bend that can be detected by the altered electrophoretic gel mobility of the RNA. We find that a single A_n or U_n bulge loop near the middle of a long RNA helix significantly retards the RNA during polyacrylamide gel electrophoresis if $n \geq 2$. The mobility of an RNA containing two A_2 bulges varies periodically with the number of base pairs between the bulges. We interpret this to mean that A_2 bulges form torsionally stiff bends in the helix; the gel mobility reaches a minimum when the total helical twist between the bulges rotates the arms of the molecule into a cis conformation. The gel mobilities are proportional to the predicted end-to-end distance of the RNA if the average RNA helical repeat is 11.8 ± 0.2 bp/turn and there is no helical twist (3 ± 9°) associated with the bulge (data obtained in 0.15 M Na^+). Other sizes and sequences of bulges have very different effects on RNA helix conformation and flexibility. U_2 bulges bend the helix to a much smaller degree than A_2 bulges, while longer A or U bulge sequences probably allow bends of 90° or more; all of these may be fairly flexible joints. This simple gel method for measuring the helical repeat of an RNA sequence and determining the helix conformation near a bulge loop will provide useful information for modeling the folding of complex RNA structures containing these motifs.

The helical twist of a duplex nucleic acid is one of several fundamental parameters needed to describe the helix structure. It is now generally appreciated that the precise repeat of 10.0 base pairs per turn found in B-form DNA fibers (Arnott, 1970) is not an invariant feature of duplex DNA: several solution methods have shown that the helical repeat of B-form DNA is an average of 10.4 bp/turn and varies with sequence (Wang, 1979; Rhodes & Klug, 1981; Shore & Baldwin, 1983), while single-crystal X-ray diffraction studies have revealed large, sequence-dependent variations in the helical twist (Drew et al., 1988). Only X-ray methods have been brought to bear on the helical structure of RNA, in studies of either fibers (Arnott et al., 1968) or a small number of single crystals (Holbrook et al., 1978; Ladner et al., 1975; Westhof et al., 1985; Dock-Bregeon et al., 1988), and these studies suggest that RNA helices can adopt a range of conformations. RNAs of biological interest incorporate frequent mismatches, non-canonical pairings, bulges, and loops, which may add to the variability of RNA helical structure. Structural details of these features are of increasing interest as attempts are made to model the folding of large RNAs (Kim & Cech, 1987; Brimacombe, 1988; Stern et al., 1989).

In this paper we describe a method for determining the helical repeat of an RNA sequence in solution. The experiments have been inspired by the observation that bent DNA molecules electrophoreses more slowly in polyacrylamide gels than predicted on the basis of their contour length (Diekmann & Wang, 1985). If two bends are introduced into a DNA helix, the gel mobility varies periodically as the phasing between the bends takes the molecule from a cis conformation (slower mobility) to trans (faster mobility) (Zinkel & Crothers, 1987; Salvo & Grindley, 1987). We thought it possible that a bulge loop in an RNA helix might bend the helix sufficiently to alter the RNA gel mobility and enable us to measure the helical repeat of specific sequences inserted between two bulges. This turns out to be correct; in addition, the experiments give us some information on the conformation and flexibility of helices containing different-size and -sequence bulges.

Experimental Procedures

RNAs were prepared by transcribing derivatives of pT7/T3-18 (BRL), a plasmid containing both T7 and T3 RNA polymerase promoters separated by a cloning polylinker. Synthetic DNAs were prepared on automated synthesizers by either *O*-methyl or cyanoethyl phosphoramidite chemistry and cloned into pT7/T3-18 by standard methods. The length of A-tract inserts was confirmed by dideoxy sequencing of the plasmid DNAs. Purified plasmids were cut with either *Pvu*II for transcription with T3 RNA polymerase (BRL) or *Ssp*I for transcription with T7 RNA polymerase (purified in this laboratory). Cut DNAs were transcribed, and the RNA was purified by phenol extraction and ethanol precipitation. For most experiments the full-length RNA transcript was first cut from a denaturing gel as previously described (Draper et al., 1988).

Duplex RNA was formed by mixing equimolar amounts of two transcripts in 0.2 M NaCl, 10 mM Tris, pH 7.6, and 1 mM Na_2EDTA, incubating for 15 min at 65 °C and then incubating an additional 15 min at 37 °C; 20 units of T_1 RNase (Boehringer Mannheim) was added, and incubation was continued for 75 min at 37 °C. When RNAs purified only by reverse-phase columns are hybridized in this way, gels always show a faint band that runs near the "bulgeless" RNAs we have prepared (see Figure 3). When the RNA transcripts are purified from denaturing gels, this band does not appear. The mobility of this band is independent of the length of the cloned DNA or the way transcripts from different plasmids are mixed and thus serves as a useful internal control.

All the gel mobility data presented here were taken from RNAs electrophoresed in 12% acrylamide gels containing 75 mM Na_2HPO_4 and 5 mM Na_2EDTA and adjusted to pH 7.0. Gels 20 cm long by 0.8 mm thick were run at 150 V for 18 h, stained with ethidium bromide, and photographed over a UV light box.

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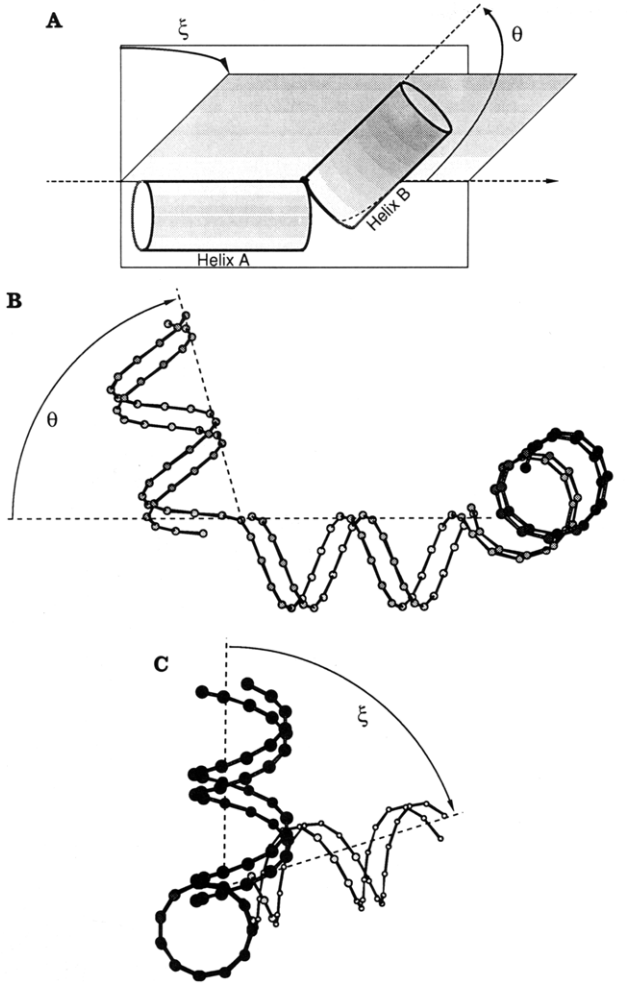

FIGURE 1: RNA helix bend conformations. (A) Angles used to describe a helix bend. Helical segment A is held in the plane of the page, while segment B is bent at the connecting joint by an angle θ and the plane containing B is rotated by ξ away from the plane containing A. (B and C) Diagrams of a helix with bends at two positions. Balls represent backbone phosphates at approximately the positions of an A-form helix. Each of the three helical segments contains 22 bp with a helical repeat of 11 bp/turn. One of the strands has been broken in two places, and the same bending operation has been performed at each location. The two views of the molecule illustrate the two angles θ and ξ.

THEORY

Figure 1A defines the parameters we use to describe bends in an RNA helix. We consider two cylindrical segments connected by a joint at their ends. Two motions orient segment B with respect to A. First, there can be a hinge-like bend, θ, which maintains the cylinder axes and the joint in the same plane. Second, the plane containing the axis of B and the joint may be rotated by ξ about a line parallel to the axis of cylinder A and containing the joint; this is a helical twist associated with the joint itself.[1] If an RNA helix contains two identical bends, then a three-dimensional configuration of the three helical segments such as the one shown in Figure 1B,C is obtained. The molecule illustrated contains two bulges in the same strand, with an integral number of helical repeats in the middle helical segment. The "joint" between the segments is considered to be at the phosphate opposite the bulged bases (though θ and ξ are not equivalent to motions of the phosphate bonds). It is important to note that the rotation ξ, when made with the same handedness at each of the two bulges, produces a torsion angle between the end helical segments of just ξ. The overall torsion angle between these segments is $\phi = 2\pi b/R + \xi$ radians, where b is the number of base pairs in the middle segment and R is the helical repeat in units of base pairs per turn.

To obtain the helical repeat, we compare our gel mobility data to the end-to-end length h of the bulge-containing RNAs. Addition of vectors corresponding to the three helical segments in Figure 1 gives

$$h^2 = (2a^2 \sin^2 \theta)(1 - \cos \phi) + (b + 2a \cos \theta)^2 \quad (1)$$

where a is the number of base pairs in the two end segments and h is in units of base pairs (the diameter of the helix has been ignored). The length of a control molecule with the same sequence except for the bulges is $2a + b$. If D_{bulge} and D_{cntrl} are the distances of gel migration for the bulge-containing and control RNAs, respectively, then plotting

$$\frac{D_{bulge}}{D_{cntrl}} = \frac{h}{2a + b} + Y \quad (2)$$

as a function of b gives a very good fit to the experimental data. In principle, only ϕ (a function of R and ξ) and the offset parameter Y are variables to be adjusted. Since θ has not been experimentally measured and there is no theory that precisely predicts the gel mobility of bent nucleic acid helices, we find it best to allow a and θ to be adjustable parameters along with R, ξ, and Y. The least-squares best fit of all five of these parameters was found by multiple regression (Bevington, 1969). We find that other equations containing $1 - \cos \phi$ can be fit to the data, as long as adjustable parameters are included that allow for the attenuation of the curve amplitude as b increases. For all the equations tried, the least-squares best fit values of R and ξ are very similar (less than $\pm$ 0.1 bp for R and $\pm 3°$ for ξ).

RESULTS

Preparation of Duplex RNA. The transcription method we use to prepare double-stranded RNAs is shown in Figure 2. By cloning a DNA sequence in between oppositely oriented T7 and T3 promoters in a plasmid, we can prepare RNA transcripts of either DNA strand. To make RNA helices containing bulges, we prepare two plasmids, one containing extra base pairs at the bulge position. The four possible transcripts from the two plasmids can be combined to make four different helix sequences: two helices with perfectly complementary strands and two bulge-containing versions of the same helices. Thus from one pair of plasmids we obtain helices with either A or U bulges and the appropriate "bulgeless" controls. By making long transcripts and then trimming the ends with T_1 RNase, we avoid problems that might arise from the tendency of phage polymerases to randomly add nucleotides at the ends of transcripts (Milligan et al., 1987; Draper et al., 1988).

Bulges Retard Duplex RNA Electrophoresis in Gels. We first examined the effect of a single bulge on duplex RNA gel mobility. (The bulge is located between base pairs 35 and 36 in a 92-bp RNA and is in the same sequence context as the left-hand bulge in Figure 2.) A gel photograph is shown in

[1] This is not a complete discussion of bulge conformations, since we have ignored the possibility of rotating helix B about a line parallel to the helix axis and extending through the joint. In calculating the end-to-end distance of the bent helix, we disregard the finite diameter of the helix, and with it this additional motion. Since the relationship of gel mobility to helix dimension and configuration is not precisely understood, it is pointless to include a more detailed description of the bulge in our calculations. Our approximations do not have any effect on the measurement of R and ξ, the parameters that determine the dihedral angle between two bulges in a molecule.

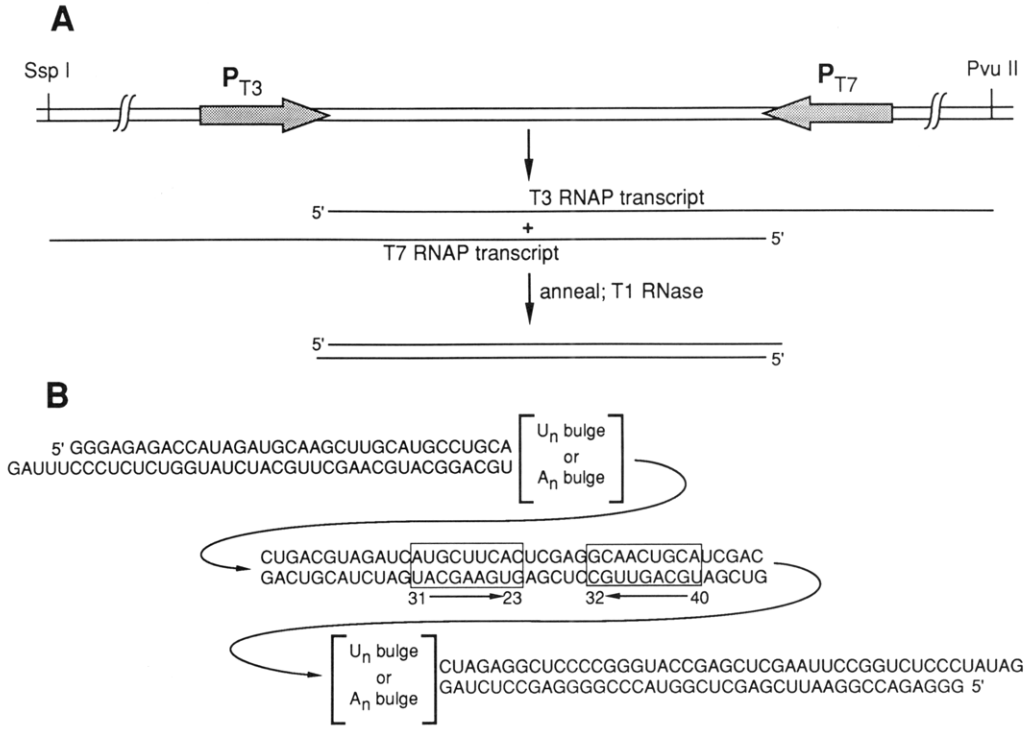

FIGURE 2: (A) Preparation of double-stranded RNAs by transcription from plasmids containing phage polymerase promoters. See Results for a description. (B) RNA duplex sequence used in this study. Shown is the RNA containing two bulges and the longest insert tested (40 bp). U_n bulges are inserted into the upper strand and A_n bulges into the lower strand. Boxes indicate sequences that are successively deleted to give smaller numbers of base pairs between the two bulges.

Table I: Electrophoretic Mobilities of Bulge- and Nick-Containing RNAs[a]

bulge size	relative mobility	
	A	U
1	0.98	1.00
2	0.90	0.96
3	0.81	0.84
5	0.81	0.84
nick	0.97	

[a] Electrophoresis was in 12% acrylamide and sodium phosphate buffer, as described under Experimental Procedures. The row marked nick is the mobility of an A_2 bulge containing RNA which has been digested with T_2 RNase, relative to that of a control (bulgeless) RNA similarly digested with T_2 RNase.

Figure 3, and mobilities relative to those of bulgeless controls are listed in Table I. A single-base U bulge does not affect the RNA electrophoretic mobility, while a single-base A bulge reduces the mobility by a very small amount. Two- and three-base A or U bulges retard the helix electrophoresis by increasing amounts, but five-base bulges have virtually the same effect as three-base bulges. Increasing bulge size might increase both the average bend angle and the flexibility of the RNA at the bulge site. Both effects are expected to slow gel migration, and the experiment of Figure 3 alone cannot distinguish contributions of bend and flexibility to the gel mobility.

As a control we prepared an RNA with a nick on one strand (by digesting an A_2 bulge with T_2 RNase) and found that its mobility (relative to a bulgeless helix digested with T_2 RNase) is different from that of any of the bulge-containing RNAs (Table I). This shows that the T_1 RNase treatment does not nick near the bulge site.

Gel Mobility of Duplex RNA Containing Two Large Bulges Depends Strongly on the Number of Bases between the Bulges. We next prepared a series of RNAs containing increasing numbers of base pairs between two bulges. We chose a larger

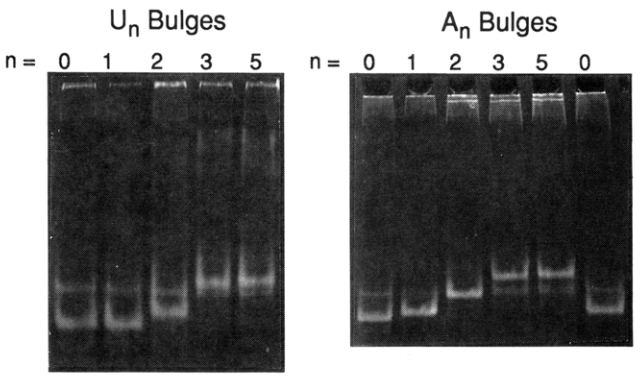

FIGURE 3: Gel electrophoresis of RNAs containing one bulge loop. The 92-bp RNAs described under Results were electrophoresed in 12% polyacrylamide gels and sodium phosphate buffer (see Experimental Procedures). The RNAs were either perfect duplexes (n = 0) or contained a single A_n or U_n bulge of increasing length, as indicated.

number of bases for the bulge, to give the maximum possible variation in gel mobility. Inadvertently, the series was prepared with one U_3 and one U_5 bulge, though this should not affect the outcome of the experiment. We expected to see a periodic variation in gel mobility with increasing length of the intervening helix, and this is in fact observed (Figure 4). Although the gel mobility minima occur roughly at the expected multiples of 11–12 base pairs, they are very broad, and it is impossible to estimate the helical repeat with any accuracy; curves predicted for helical repeats ranging from 11.4 to 13.8 bp/turn with bulge-associated twists of −60° to 100°, respectively, fit the data equally well. We presume that the gel mobility of these RNAs is dominated by an extreme bend angle and/or flexibility at the bulge positions, which reduce the phasing information available from the experiment. This point is considered further under Discussion.

We also made and tested duplex RNAs that are the Watson–Crick complements of the RNAs used in Figure 4,

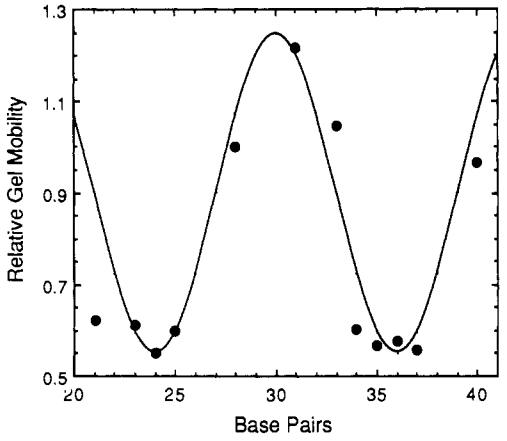

FIGURE 4: Relative gel mobility of RNAs containing two bulges (U_3 and U_5). The ratio of the distances of bulge-containing and bulgeless RNA migration, D_{bulge}/D_{cntrl} (relative gel mobility), is plotted as a function of the number of base pairs between the two bulges b (base pairs). The curve drawn is the function $1 - \cos(2\pi b/R)$ scaled to approximately fit the data. R, the helical repeat, has been set equal to 12. The curve is not a least-squares fit and is meant only to show that the periodicity of the data is approximately that expected for an RNA helix.

i.e., RNAs with A_3 and A_5 bulge sequences. The relative gel mobilities showed a slightly smaller range of values, 0.73–1.15, but the curve was qualitatively similar in that no sharp peak in the mobility was found as the middle helix was lengthened (data not shown).

Behavior of Duplex RNA Containing a Pair of Two-Base Bulges Depends Strongly on Bulge Sequence. Reasoning that smaller bulge sizes would have a more defined conformation and so improve resolution of the helix repeat, we prepared a series of RNAs with two U_2 or A_2 bulges. The gel mobilities of these molecules are shown in Figure 5. All RNAs with U_2 bulges run more slowly than the controls, but with virtually no periodic dependence on the length of the middle helix. The RNAs containing two A_2 bulges behave much differently. The gel mobility data can be fit to eq 2 by use of a helical repeat of $R = 11.8 \pm 0.2$ bp/turn and a helical twist associated with the bulge of $\xi = 3 \pm 9°$. The data seem to show some reproducible irregularities, which may be a consequence of sequence-dependent variations in the helical repeat.

These measurements have been made with Na^+ as the sole cation. The same set of RNAs electrophoresed in buffers containing Tris or multivalent cations can show substantial negative twist associated with the bulge. A significant negative twist associated with single-base bulges in DNA has been measured (Rice & Crothers, 1989). Cation effects on the bulge conformation and helix repeat will be presented in detail elsewhere.

The best fits to the data are obtained by use of a value of a, the number of base pairs in the arms, that is too small by a factor of 3. Since we do not have a theoretically derived relation between RNA configuration and gel mobility, we do not find it surprising that the curve is not fit with the correct value of a. For the same reason, we do not consider the fit of $\theta \approx 48°$ to be a reliable estimate of the bending associated with the bulge.

Discussion

Flexing and Bending of an RNA Helix at a Bulge Defect. The gel mobility data provide some insight into the behavior of bulge loops in RNA helices. NMR studies of single-base bulges in DNA have shown that purines tend to stack in between the adjacent base pairs, bending the helix, while pyrimidines are frequently excluded from the helix and exposed to solvent [see, for example, Kalnik et al. (1989a,b)]. This fits our observation that an A bulge affects RNA gel mobility slightly, while the U bulge does not. However, we cannot rule out the possibility that the U bulge also bends the helix significantly. "A tracts" in DNA are estimated to bend the helix by 22° but only retard the DNA gel mobility when several phased tracts are present (Koo & Crothers, 1988). A single-base G bulge bends a DNA helix by ≈20° (Woodson & Crothers, 1988); it is therefore possible that a single-base U bulge in RNA bends the helix without affecting the gel mobility significantly.

Three- to five-base bulges of either A or U behave similarly and, when two bulges are present, induce a wide range of gel mobilities that depend on the phasing between the bulges. In building models we find that a three-base bulge can allow large bends of up to ~90°. It is difficult to predict what effect two large bends may have on the gel mobility of a duplex RNA, since it may adopt "U"- or (for $\theta > 90°$) "N"-shaped conformations in which the end-to-end distance is irrelevant to the gel mobility. For instance, if three equal-size helix segments are joined by 90° bends, then for ϕ between +60° and −60° the largest dimension of the molecule is a constant. This is a potential explanation for the "flattening" of the curves in Figure 4 when the intervening helix is close to an integral number of turns. Torsional flexibility at the bulge might also be expected to produce a flattened curve by generating a broad distribution of torsion angles that is only weakly dependent on the length of the middle helix, though this would also reduce

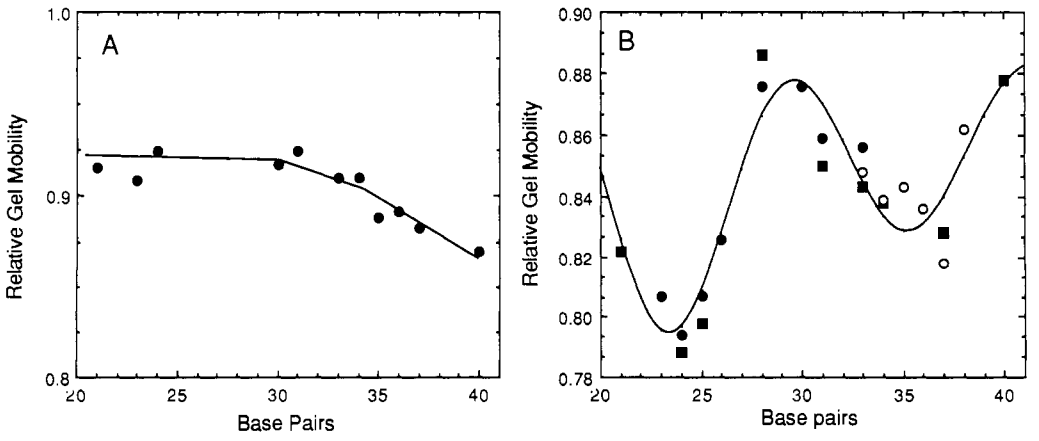

FIGURE 5: Relative gel mobility of RNAs containing two U_2 or A_2 bulges. (A) RNAs containing two U_2 bulges. (B) RNAs containing two A_2 bulges. Data have been taken from three different gels, as indicated by the different symbols. The curve has been fit to eq 2, with $R = 11.8$, $\xi = 2.9°$, $\theta = 47.8°$, $a = 12.1$ bp, and $Y = -0.0345$.

the gel mobility difference between bulges in and out of phase. Both extreme bend angle and torsional flexibility may contribute to the behavior seen.

U_2 and A_2 bulges must have very different effects on helix conformation, which may stem from the fact that adenine bases stack together much more strongly than do uracil bases. If U_2 bulge bases do not stack with the adjacent base pairs, they can be exposed to solvent with only a slight bending of the helix axis; they might also allow some torsional flexibility. These effects can account for the lack of gel mobility dependence on bulge phasing. Since A_2 bulges, in contrast, give small but periodic variations in the gel mobility, they must significantly bend the helix in a way that is at least torsionally rigid. In building models of A_2 bulges, we find it difficult to maintain all the bases along the bulge-containing strand in a fully stacked conformation, but several configurations in which the bulged bases are partially stacked with each other and with the adjacent base pairs can be built. It may be that one of these configurations is sufficiently preferred to give the bulge a well-defined structure in solution. Our preliminary observation that ξ depends on the counterions present implies that the preferred structure depends strongly on the way backbone phosphates are neutralized in the vicinity of the bulge.

The results with A_2 bulges strictly argue only that the bulge has little torsional flexibility; hinge-like flexibility (i.e., a range of θ values) does not alter the dihedral angle present in the bent RNA and is therefore not detectable in these experiments. Most backbone motions at a bulge will change both bend and torsion angles; thus, one might expect torsional stiffness to imply that there is a unique bend angle as well. The question of hinge-like motions can be resolved by transient electric birefringence experiments, which measure θ directly (Hagerman, 1984).

While two-base bulges are not common in RNA structures, there are some that are conserved near critical regions of the large-subunit ribosomal RNAs (Noller, 1984; Gutell & Fox, 1989). For instance, a bulge in the "GTPase center" of the ribosome is highly conserved as either AA or GU (positions 1089–1090 in the *Escherichia coli* 23S rRNA sequence), and there is an AC bulge sequence conserved near the peptidyl transferase center (nucleotides 2439–2440). How the bend and twist introduced by a two-base bulge vary with the bulge sequence and solution conditions are thus potentially relevant to the biological function of ribosomal RNAs.

Comparison with Other Studies. Two recent studies have found that bulges of increasing length retard electrophoresis of DNA fragments by progressively greater factors; bulges up to seven nucleotides have been examined (Hsieh & Griffith, 1989; Bhattacharyya & Lilley, 1989). This is in contrast to our observation that three- and five-base bulges decrease RNA gel mobility by nearly the same amount. Whether the slower gel mobilities are due to increasing bend angle, increasing helix flexibility, or both is still an open question; therefore, we do not know to what aspect of DNA and RNA structure we should attribute these differences in gel behavior.

In X-ray diffraction studies of RNA fibers, two conformations have been detected: an 11-fold helix formed at low salt (A form) and a 12-fold helix at higher salt (A′ form) [Arnott et al., 1968; reviewed by Arnott (1970)]. Single-crystal studies of tRNAs show that there is considerable variation in helical twist between base pairs, predicting helical repeats from about 9 to 14 bp/turn (Holbrook et al., 1978; Westhof et al., 1985; Westhof & Sundaralingham, 1986). Our measurement of 11.8 bp/turn for a random RNA sequence therefore seems quite reasonable. The interesting questions to pursue are how the helical twist might vary with sequence and with the noncanonical pairings frequently found in RNAs.

After our work was completed, a paper appeared that attempted to measure the helical repeat between two A_5 bulges in a 60-bp RNA (Bhattacharyya et al., 1990). The data presented by these workers are very similar to our data with larger bulges, in that the plot of gel mobility is flattened between 10 and 13 base pairs; their estimate of RNA helical repeat is therefore subject to the same degree of uncertainty as for the data we show in Figure 4 (i.e., more than ± 1 bp/turn). In addition, Bhattacharyya et al. (1990) estimate the helical repeat from the position of one maximum in the gel mobility. At least two maxima or minima in the gel mobility are needed to separate the contributions of helical repeat and bulge-associated twist. Since these workers ignore the possibility of bulge-associated twist, the value they calculate for the helical repeat may be substantially in error.

References

Arnott, S. (1970) *Prog. Biophys. Mol. Biol. 21*, 267–319.

Arnott, S., Fuller, W., Hodgson, A., & Prutton, I. (1968) *Nature 220*, 561–564.

Bevington, P. R. (1969) *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill, New York.

Bhattacharyya, A., & Lilley, D. M. (1989) *Nucleic Acids Res. 17*, 6821–6840.

Bhattacharyya, A., Murchie, A. I. H., & Lilley, D. M. (1990) *Nature 343*, 484–487.

Brimacombe, R. (1988) *Biochemistry 27*, 4207–4214.

Diekmann, S., & Wang, J. C. (1985) *J. Mol. Biol. 186*, 1–11.

Dock-Bregeon, A. C., Chevrier, B., Pdjarny, A. D., Moras, D., deBear, J. S., Gough, G. R., Gilham, P. T., & Johnson, J. E. (1988) *Nature 335*, 375–378.

Draper, D. E., White, S. A., & Kean, J. M. (1988) *Methods Enzymol. 164*, 221–237.

Drew, H. R., McCall, M. J., & Calladine, C. R. (1988) *Annu. Rev. Cell Biol. 4*, 1–20.

Gutell, R. R., & Fox, G. E. (1989) *Nucleic Acids Res. 16*, r175–r269.

Hagerman, P. J. (1984) *Proc. Natl. Acad. Sci. U.S.A. 81*, 4632–4636.

Holbrook, S. R., Sussman, J. L., Warrant, R. W., & Kim, S.-H. (1978) *J. Mol. Biol. 123*, 631–660.

Hsieh, C.-H., & Griffith, J. D. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 4833–4837.

Kalnik, M. W., Norman, D. G., Swann, P. F., & Patel, D. J. (1989a) *J. Biol. Chem. 264*, 3702–2712.

Kalnik, M. W., Norman, D. G., Zagorski, M. G., Swann, P. F., & Patel, D. J. (1989b) *Biochemistry 28*, 294–303.

Kim, S.-H., & Cech, T. R. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 8788–8792.

Koo, H.-S., & Crothers, D. M. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1763–1767.

Ladner, J. E., Jack, A., Robertus, J. D., Brown, R. S., Rhodes, D., Clark, B. F. C., & Klug, A. (1975) *Proc. Natl. Acad. Sci. U.S.A. 72*, 4414–4418.

Milligan, J. F., Groebe, D. R., Witherell, G. W., & Uhlenbeck, O. C. (1987) *Nucleic Acids Res. 15*, 8783–8798.

Noller, H. F. (1984) *Annu. Rev. Biochem. 53*, 119–162.

Rhodes, D., & Klug, A. (1981) *Nature 292*, 378–382.

Rice, J. A., & Crothers, D. M. (1989) *Biochemistry 28*, 4512–4516.

Salvo, J. J., & Grindley, N. D. F. (1987) *Nucleic Acids Res. 15*, 9771–9797.

Shore, D., & Baldwin, R. (1983) *J. Mol. Biol. 170*, 983–1007.

Stern, S., Powers, T., Changchien, L.-M., & Noller, H. F. (1989) *Science 244*, 783–790.
Wang, J. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 200–203.
Westhof, E., & Sundaralingham, M. (1986) *Biochemistry 25*, 4868–4878.
Westhof, E., Dumas, P., & Moras, D. (1985) *J. Mol. Biol. 184*, 119–145.
Woodson, S. A., & Crothers, D. M. (1988) *Biochemistry 27*, 3130–3141.
Zinkel, S. S., & Crothers, D. M. (1987) *Nature 328*, 178–181.

Articles

Thermodynamics of Double- and Triple-Helical Aggregates Formed by Self-Complementary Oligoribonucleotides of the Type rA_xU_y†

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ABSTRACT: The thermal denaturation of a series of oligoribonucleotides of the form rA_xU_y (x = 5 or 7 and y = 3–11) has been characterized by means of IR spectroscopy, UV spectroscopy, and DSC. IR spectra proved the occurrence of double- and triple-helical regions at various contents of uracil residues in the nucleotide. From DSC measurements transition enthalpies, entropies, and free enthalpies were derived. The effect of fraying in terminal base pairs of symmetrical nucleotides ($x = y$) was quantified. Thermodynamic excess parameters due to dangling ends (5′A and 3′U), terminal AU base pairs, and UAU base triplets were obtained by comparing DSC results from different nucleotides. Empirical values for contributions of base stacking and pairing to the stability of terminal AU base pairs have been estimated: for nucleotides under study with a high degree of fraying at the ends of the helix the major stabilization effect comes from base stacking. The size of the cooperative unit λ in most nucleotides under study is larger than 1; i.e., in these cases intermolecular cooperation takes place. Through deconvolution of DSC data maximum populations of intermediate states $F_{I,max}$ were obtained. On the basis of these results all nucleotides under study were proved to melt in multistate manner. $F_{I,max}$ increases with the number of base pairs, decreases through dangling ends, and shows approximately constant values for triple-helical aggregates of the series rA_5U_y as well as rA_7U_y.

The knowledge of thermodynamic parameters for RNA[1] secondary structure stability is an essential base for better understanding of the function of different RNA structures like tRNA, mRNA, or rRNA. Typically, these structures contain short helical segments which are interrupted by internal loops, mismatches, or bulge nucleotides and terminated by hairpins or dangling ends (Cantor & Schimmel, 1980; Crawford & Stauffer, 1980; Gutell et al., 1985). The contribution of these various structural features to the stability of RNA has been subject to intensive research since the beginning of the seventies (Martin et al., 1971; Gralla & Crothers, 1973; Uhlenbeck et al., 1973; Tinoco et al., 1973; Borer et al., 1973, 1974; Breslauer et al., 1975). By means of improved synthetic techniques more interesting sequences could be realized and became the subject of different studies in the last few years (Petersheim & Turner, 1983; Freier et al., 1983, 1985, 1986a,b; Hickey & Turner, 1985; Sugimoto et al., 1987a,b; Turner et al., 1987; Groebe & Uhlenbeck, 1988, 1989; Longfellow et al., 1990). As a main result of these studies it is generally accepted that for a wide range of various sequences the prediction of RNA stabilities has become possible.

For most oligoribonucleotides the excess stabilization of double helices by dangling ends is well-known as an effect due to enthalpic contributions (Petersheim & Turner, 1983; Freier et al., 1983; Sugimoto et al., 1987a). Favorable entropic contributions have only been obtained for nucleotides in which 5′ dangling ends are added next to GC pairs (Freier et al., 1985, 1986a). However, self-complementary oligoribonucleotides of the general type rA_xU_y (Martin et al., 1971) seem to be an exception: the reason for stabilization of a symmetrical core helix by dangling ends appears to be an unusual entropic effect even in cases of a 3′ dangling U base due to these results.

Data which lead to this surprising conclusion have been determined by analysis of UV melting profiles and therefore depend on the assumption of a two-state process governing the helix to coil transition of the oligoribonucleotide. However, oligoribonucleotides with a high content of AU base pairs actually do not melt in a two-state manner (Breslauer et al., 1975). Therefore, a more careful analysis of thermal denaturation processes of oligoribonucleotides like rA_xU_y needs model-independent data which can be obtained from calorimetric measurements (Breslauer, 1986; Marky & Breslauer, 1987; Sturtevant, 1987; Ackermann, 1989).

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[1] Abbreviations: UV, ultraviolet; DSC, differential scanning calorimetry; FTIR, fourier transform infrared spectroscopy; IR, infrared; A, adenine; U, uracil; RNA, ribonucleic acid; tRNA, transfer ribonucleic acid; mRNA, messenger ribonucleic acid; rRNA, ribosomal ribonucleic acid; bp, base pair(s).

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