Persistence Length of RNA†

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ABSTRACT: A set of 10 double-stranded RNAs ranging in length from 92 to 317 base pairs has been synthesized, and their rotational diffusion times were measured by transient electric birefringence. A hydrodynamic analysis of the birefringence decay data yields a helix persistence length of 720 ± 70 Å, based on a helix with an effective hydrodynamic diameter of 26 Å and a rise per base pair of 2.7 Å in the presence of Mg^{2+} ions. Thus, duplex RNA is somewhat stiffer than DNA, for which the persistence length is 450−500 Å. The measurements also suggest that (i) current hydrodynamic theory for the rotational decay times is applicable to RNA molecules that are longer than 100 base pairs and (ii) weak Mg^{2+}−RNA association decreases the rise per base pair slightly, by no more than 0.1 Å.

It is well-known that 2′-hydroxyl groups in RNA stabilize the double helix in the A form, which differs from the canonical B form helix in that the A form has a smaller rise per base pair and a substantial displacement of the base pairs from the helix axis (Saenger, 1984). It has been suggested that the A form helix is substantially stiffer than the B form. In particular, a detailed comparison of DNA and RNA mobilities in agarose gels found an RNA persistence length 1.5−1.9-fold longer than that for DNA (Edmonson & Gray, 1984). Furthermore, a sedimentation velocity study of rotavirus double-stranded RNAs yielded a persistence length that was approximately twice that of DNA (Kapahnke *et al.*, 1986). By contrast, electron microscope observations of duplex viral RNA revealed no major difference in the stiffness of RNA and DNA (Kleinschmidt *et al.*, 1964). Thus, whether the RNA helix is inherently stiffer than DNA remains an open question, one that is important to a broader understanding of the dynamics of helix structure. Knowledge of RNA stiffness is also needed as a basis for further hydrodynamic studies of bends and flexible joints in RNAs.

Two advances have made it possible to improve estimates of RNA stiffness. First, it is now possible to prepare, *in vitro*, large quantities of double-stranded RNAs of virtually any length. Methods have been described in which complementary transcripts made by phage polymerases are annealed to give duplex molecules (Tang & Draper, 1990; Gast & Hagerman, 1991), and this paper offers a further refinement of this approach. Second, there have been recent improvements in both the analysis and measurement of the rotational diffusion constants of linear and branched nucleic acids (Cooper & Hagerman, 1989; Gast & Hagerman, 1991; Amiri & Hagerman, 1994; Shen & Hagerman, 1994). In those studies, rotational decay times (inversely related to the diffusion constants) were obtained by measuring the rate of decay of the birefringence of a nucleic acid solution following the removal of a brief orienting pulse. This method, transient electric birefringence (TEB),[1] has also been used successfully to measure the persistence length of DNA (Elias & Eden, 1981; Hagerman, 1981; Lewis *et al.*, 1986) and to detect curvature in DNA (Hagerman, 1984; Levene *et al.*, 1986).

In a previous study of RNA using the TEB approach, the rotational decay times for three different double-stranded RNA molecules were used to obtain an initial estimate of 600 ± 100 Å for the RNA persistence length (Gast & Hagerman, 1991), a value that is 20−30% larger than the accepted value for DNA [for a review, see Hagerman (1988)]. In the current study, more detailed measurements have been performed on a set of 10 additional RNA lengths in an effort to further refine the persistence length for RNA. The central conclusion is that RNA is indeed stiffer than DNA, with a persistence length of ~720 ± 70 Å. It is also noted that the addition of Mg^{2+} ions results in a slight (~0.1 Å) reduction in the rise per base pair of the RNA helix.

MATERIALS AND METHODS

RNA Synthesis and Purification. Some of the RNAs for this study were prepared by a previously reported procedure (Tang & Draper, 1990). Briefly, a DNA restriction fragment is inserted into a plasmid cloning site between T7 and T3 RNA polymerase promoters oriented to transcribe complementary strands of the same sequence. Individual runoff transcripts are made with each polymerase, using a different restriction enzyme to linearize the DNA in each case. The transcripts are then purified from denaturing gels and annealed, and the single-strand tails are trimmed to the nearest G nucleotide with T1 RNase. The procedure leaves a five base overhang on each 3′-end of the resulting helix. To avoid the potential complication of single-stranded tails for hydrodynamic measurements, a polymerase chain reaction (PCR) method that yields blunt-ended duplexes was devised. The templates used for PCR were the same plasmids with both T7 and T3 promoters. Two pairs of DNA primers were used to amplify two DNA fragments, each starting with one of the promoters and ending at the desired 3′-nucleotide of

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[1] Abbreviations: TEB, transient electric birefringence; PCR, polymerase chain reaction.

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Table 1: Rotational Relaxation Times of Double-Stranded RNAs[a]

length (bp)	ends[b]	fast $\tau_{20,w}$ (μs)	slow $\tau_{20,w}$ (μs)	amplitude of slow fraction
317	3′ overhangs	0.805	7.31	0.650
288	3′ overhangs	0.673 ± 0.128	6.63 ± 0.24	0.747
243	3′ overhangs	0.44 ± 0.11	4.03 ± 0.05	0.895
203	3′ overhangs	0.45 ± 0.08	2.48 ± 0.06	0.973
186	3′ overhangs	0.22 ± 0.01	2.02 ± 0.04	0.979
132	3′ overhangs		1.00 ± 0.01	1
119	blunt		0.746 ± 0.003	1
116	blunt		0.686 ± 0.002	1
109	blunt		0.620 ± 0.003	1
92	blunt		0.455 ± 0.001	1
92	3′ overhangs		0.433 ± 0.002	1
136[c]	blunt		1.08 ± 0.05	
180[c]	blunt		1.90 ± 0.06	
226[c]	blunt		3.46 ± 0.08	

[a]Measurements were made in buffer containing 7.5 mM NaP_i, 0.125 mM NaEDTA, and 1 mM $MgCl_2$. Errors reported are the averages of three sets of measurements, except for the 317 bp molecule, for which only one set was made. [b]The overhanging end sequences are $^{5'GGGAGA}_{GAUUUCCCUCU}$ and $^{UCUCCCUAUAG}_{AGAGGG5'}$, and the blunt ends terminate with $^{5'GGGAGA}_{CCCUCU}$ and $^{UCUCCC}_{AGAGGG5'}$. [c]Data taken from Gast and Hagerman, (1991); measurements were made under the same conditions.

the transcript. The PCR-generated DNAs were individually transcribed approximately as described previously (Gluick & Draper, 1994), and the products were purified from denaturing gels in a Bio-Rad Model 422 electroelution cell and annealed. The final product was purified from a denaturing gel, electroeluted from the gel, precipitated with ethanol, and spin-dialyzed against the buffer needed for measurements using Centricon filters with a 10 000 molecular weight cutoff. In all of the preparative procedures, standard precautions were taken to prevent nuclease or metal ion contamination (i.e., sterile solutions, baked glassware, and water purified through a Barnstead Nanopure unit).

Transient Electric Birefringence Measurements. Rotational decay times for the RNAs from 132 to 317 base pairs in length were measured in a previously described electric birefringence instrument (Hagerman, 1985). Approximately 15 μg of RNA in a volume of 170 μL was used. Measurements on the shorter RNAs were made in a new birefringence instrument with an 80 μL cell and faster response time (~20 ns), which will be described elsewhere. Smaller RNA samples (7−12 μg) were used for these experiments. In all cases, a 2 kV pulse (1−2 μs) was applied across a 2 mm electrode gap, and data from 64 pulses were averaged for each measurement. The temperature was held constant within 0.2 °C (2.2−3.5 °C), with the reported decay times being corrected for both viscosity and temperature to 20 °C, using the correction $\tau_{20,w} = (\eta_{20,w}/\eta_T)(T/293)\tau_{obs}$, where T is the temperature of measurement in kelvin and η is the viscosity of water at the subscript temperature. The buffer used was 7.5 mM NaP_i, 0.125 mM Na_2EDTA, and various concentrations of $MgCl_2$.

Rotational relaxation times were determined by fitting decay curves to the sum of two exponential decays; for all but the two longest molecules used in this study, the longer decay component contributed >90% of the signal amplitude. Fitting was carried out using Kaleidagraph 3.0.4 (Abelbeck Software).

RESULTS AND DISCUSSION

RNAs Used in the Current Study. Ten different double-stranded RNA lengths were prepared for this study, as described in Materials and Methods and listed in Table 1. Some of the RNAs were made by a previously described procedure, in which long RNA transcripts were annealed and the ends were trimmed with T1 RNase. This procedure left a five base single-stranded overhang at each 3′-end. Other RNAs were made by a newer method, in which DNA templates of precisely the length needed for transcription were prepared by PCR; this avoided the use of nucleases and yielded blunt ends. A nondenaturing gel of the RNAs used is shown in Figure 1. None of the duplex RNAs behaved anomalously in polyacrylamide gel electrophoresis, as plots of the logarithm of RNA length as a function of gel mobility were linear (not shown). No consistent difference between the mobilities of RNAs with and without the 3′ single-stranded tails could be detected.

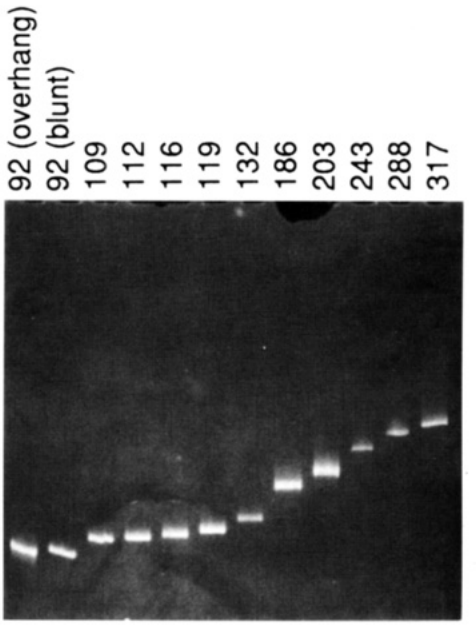

FIGURE 1: Nondenaturing gel electrophoresis of the duplex RNAs used in this study. The gel was 8% polyacrylamide made in 0.1 M Tris−borate buffer with 1 mM Na_2EDTA; staining was with ethidium bromide. Duplex RNA lengths in base pairs are indicated above the lanes.

Determination of the Persistence Length of RNA from the Birefringence Decay Times of the Set of RNA Molecules. Plots of the normalized birefringence decay curves are displayed in Figure 2 for three different RNA molecules. Most of the molecules demonstrate biphasic decay curves;

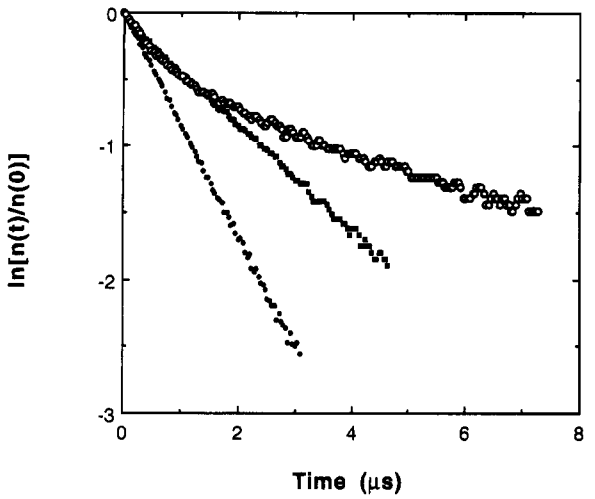

FIGURE 2: Field-free decay of birefringence for duplex RNA. Lengths are (●), 119, (■), 186, and (○) 317 base pairs. The buffer contains 1 mM $MgCl_2$, as in the legend to Table 1.

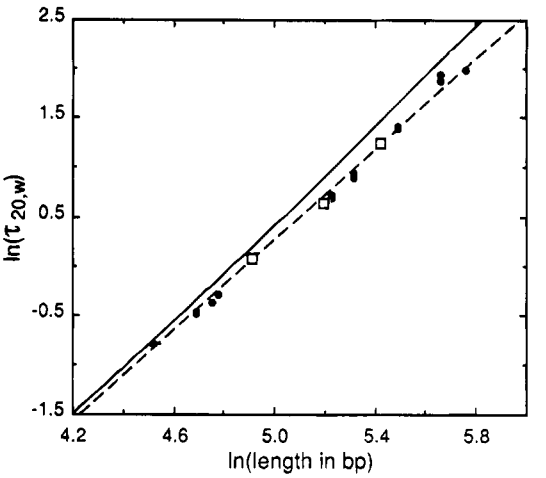

FIGURE 3: $\tau_{20,w}$ as a function of RNA helix length: (●) RNA molecules prepared for this study; (□), data from Gast and Hagerman (1991). The solid line was calculated from the Broersma equation with a hydrodynamic diameter of 26 Å and a rise per base pair of 2.7 Å; it presumes an infinitely stiff helix. The dashed line is a best fit of the Hagerman and Zimm (1981) equations to the data, using the same values for the helix diameter and rise per base pair; the fitted persistence length is 720 Å. See text for discussion.

for these curves, the longer decay component is used to extract information pertaining to persistence length (Hagerman & Zimm, 1981; Hagerman, 1981). The analysis of the terminal decay component is based on the "equilibrium-ensemble" approach (Hagerman & Zimm, 1981), which has received substantial support from both theoretical studies (Roitman & Zimm, 1981a,b; Roitman, 1984; Allison & Nambi, 1992) and independent, non-hydrodynamic determinations of the persistence length of DNA by ligase-catalyzed cyclization (Shore *et al.*, 1981; Taylor & Hagerman, 1990; Levene & Crothers, 1986).

An important control experiment for the birefringence studies is the demonstration that the decay times are independent of the magnitude of the orienting electric field. For four field strengths, from 3.25 to 9.25 kV/cm, used to orient the 186 bp RNA molecule, there was no detectable influence of the field on the decay time. It should also be noted that no field strength effects were observed in the birefringence decay of DNA molecules longer than any of the RNA molecules used in the current study (Hagerman, 1981). A second control determined the effect of single-stranded tails on the decay times. The 92 bp RNA was employed for this study, since the influence of the tails would be largest for this short molecule. A comparison of the decay times for the 92 bp species, with or without single-stranded tails, is presented in Table 1. The tails decrease the birefringence decay times by about 5%. The effect is also seen in the absence of $MgCl_2$ (not shown); therefore, the dependence does not arise from tight binding of ions to the single strands. The effect of tails on any of the longer RNAs listed in Table 1 will be smaller and within the experimental error of the measurements. Thus, no further corrections have been made for end effects.

The birefringence decay times ($\tau_{20,w}$) and the corresponding terminal amplitude are collected in Table 1 for each of the 10 double-stranded RNAs, and a plot of $\tau_{20,w}$ (slow) as a function of RNA length is shown in Figure 3. The lower line in the graph is a least-squares fit of the data (excluding the 92 bp molecule; see the following) to eqs 20−22 of Hagerman and Zimm (1981). The fitted value of the persistence length is 720 ± 70 Å and is essentially unchanged by the inclusion of the data of Gast and Hagerman (1991). The fitting was performed using values of 26 Å for the RNA hydrodynamic diameter (Record *et al.*, 1975; Hagerman, 1981) and 2.7 Å for the rise per base pair, as found by fiber diffraction of reovirus RNA (Saenger, 1984; Arnott *et al.*, 1967). The upper line in Figure 3 is the predicted dependence of the relaxation time for an infinitely stiff helix with these values of the diameter and rise per base pair (Broersma, 1960).

The persistence length of DNA has been variously estimated as ~500 Å by the same rotational diffusion measurements (Elias & Eden, 1981; Hagerman, 1981), 470 Å by flow dichroism (Rizzo & Schellman, 1981), and ~475 Å by cyclization (Shore *et al.*, 1981), as reviewed by Hagerman (1988). Thus, the conclusion from our measurements is that the duplex RNA persistence length is about 50% larger than that of DNA.

Applicability of Hydrodynamic Theory to Short Helices. An important consideration in the current analysis is the range of helix lengths over which the hydrodynamic theory used to calculate the persistence length is applicable. The relations between birefringence decay times and length, derived from the Monte Carlo simulations of Hagerman and Zimm (1981) and expressed as functions of persistence length (P), axial rise, and effective hydrodynamic diameter, are valid for $L/P < 5$, which is clearly satisfied for all the molecules in the current study.

For shorter molecules, those relations were regarded as increasingly uncertain for axial ratios less than 20, corresponding to an RNA length of ~185 bp. Five of the RNA molecules listed in Table 1 are shorter than this value; however, it is apparent that the same curve describes both the longer and shorter lengths to within the precision of the measurements. Exclusion of these shorter molecules from the analysis still yields optimal fits in the 700−800 Å range, but with larger uncertainty. (Since the 92 bp RNA decay time does not come as close to the fitted line as the points for the next four longer RNAs, it was omitted from the persistence length estimation.) These observations suggest that the original Hagerman−Zimm model, using a rigid-

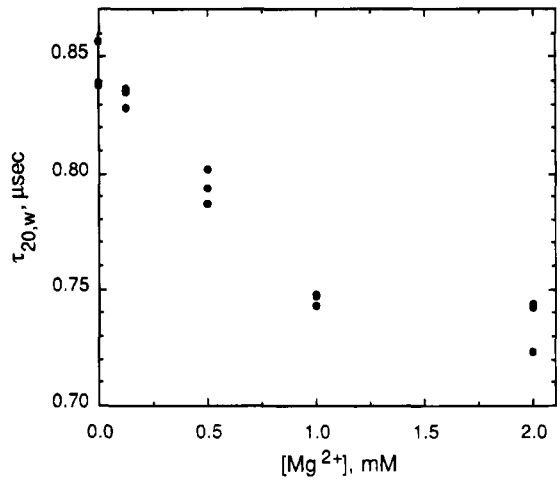

FIGURE 4: Dependence of $\tau_{20,w}$ on Mg^{2+} concentration for the 119 base pair RNA.

cylinder limit developed by Broersma (1960), may provide an adequate representation for the shorter RNA molecules. However, such agreement should not be taken as evidence for the validity of the cylinder model of Broersma, since the fit in the 100−320 bp range is relatively insensitive to the choice of rigid-cylinder models [a comparison of models is given by Tirado *et al.* (1984)]. In fact, replacement of the Broersma cylinder model (Broersma, 1960) with the more recent model of Tirado and Garcia de la Torre (1980) over that range yields a slightly (~9%) lower value for P; this difference is within the uncertainty of the methods. A careful examination of this issue must await a much more complete data set in the 50−100 bp range.

Dependence of τ on Mg^{2+} Concentration. In order to determine whether Mg^{2+} has any special effect on duplex RNA structure, birefringence decay times were measured as a function of Mg^{2+} concentration. Results for the 119 base pair RNA are shown in Figure 4. There is about a 15% drop in $\tau_{20,w}$ with increasing Mg^{2+} concentrations; the effect saturates at about 1 mM. The same decrease was seen for all of the lengths examined (92−119 base pairs) and did not depend on the presence or absence of the 3′ single-stranded tail (not shown). Detailed measurements of Mg^{2+} association with poly(A)−poly(U) have been made by Krakauer (1971, 1974). At the low Na^+ concentration in use in our experiments, the RNA should be half-saturated with Mg^{2+} (one Mg^{2+} bound for every four phosphates) at only ~20 μM Mg^{2+}. Since the rotational relaxation times are affected only at an order of magnitude higher concentration, a second, weaker mode of Mg^{2+} association with RNA is suggested.

Mg^{2+} could affect the rotational decay times of RNA by changing the dimensions of the helix (for instance, coordination to phosphates could alter the rise per base pair) or by affecting the persistence length. The data cannot be accommodated by increasing P from 720 Å to much higher values; either the helix radius or the rise per base pair must be altered. Since τ scales as approximately L^3 at these shorter lengths, only a 0.1 Å increase in h is needed to account for the entire increase in decay time upon the removal of Mg^{2+}. This should be considered an upper limit to any length difference caused by Mg^{2+}, since it is difficult to sort out the exact contributions of the three variables to these small changes.

CONCLUSIONS

On the basis of an analysis of the birefringence decay times of 9 double-stranded RNAs ranging from 109 to 317 bp in length, it appears that the persistence length of RNA in the presence of Mg^{2+} (700−750 Å) exceeds that of DNA by approximately 50%. Moreover, Mg^{2+} ions appear to lead to a slight but detectable decrease in the rise per base pair of RNA. The structural basis for the greater stiffness of RNA compared to DNA can only be speculated on at present; the larger charge density of RNA, the tendency for interstrand base stacking in RNA vs intrastrand stacking in B form helices, and hydrogen bonding of the 2′-OH are all factors that potentially contribute (Saenger, 1984).

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