Comparison of the Electrophoretic and Hydrodynamic Properties of DNA and RNA Oligonucleotide Duplexes

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ABSTRACT The electrophoretic behavior of defined DNA and RNA oligonucleotide duplexes from 10 to 20 bp in length has been investigated as a function of salt conditions, gel concentration, and temperature. The RNA oligomers migrated much more slowly than the DNA oligomers of the same sequence under all conditions. From sedimentation equilibrium and velocity measurements, the apparent partial specific volume in 0.1 M KCl, 20 mM NaP_i, pH 7, was determined as 0.56 ± 0.015 ml g^{-1} for DNA and 0.508 ml g^{-1} for RNA. The translational friction coefficients were determined and compared with the values calculated for cylinders. Taking into account the shape factors, the solution density, and partial specific volumes, the effective degree of hydration was estimated as 0.8–1 g g^{-1} DNA. There was no significant difference in the frictional coefficients of the DNA and RNA oligomers, indicating that the effective sizes of DNA and RNA are very similar in solution. The differential electrophoretic mobility of DNA and RNA must arise from the differences in interaction with counterions, which is probably a global property of the oligonucleotides.

INTRODUCTION

The conformations of nucleic acids, and DNA in particular, are strongly affected by hydration and ion binding. Thus, at the normal water activity in physiological salt solutions, DNA adopts the so-called B conformation, whereas under conditions of low water activity, such as in solution of alcohols or in fibers at <85% relative humidity, DNA adopts the more compact A conformation. Hydration is also an important factor in DNA-ligand interactions. When a ligand makes intimate specific contacts with the DNA, it is expected that water bound to the DNA will be displaced, with a concomitant increase in entropy. However, there are cases where water acts as a bridge between protein and DNA, such as in the trp repressor-operator complex (Otwinowski et al., 1988), the antennapedia homeodomain-DNA complex (Qian et al., 1993), and drug-DNA complexes (Brown et al., 1990; Lane et al., 1997). In such cases water is an integral part of the binding site.

There are various ways of measuring hydration, and each technique measures a different property. Thus, for example, x-ray crystallography reveals water molecules that are present at high occupancy, although there may be crystal packing influences on the locations of some water molecules. NMR can observe only a subset of water molecules, usually those that have a sufficiently long average residence time (Otting et al., 1991). Thermodynamic methods measure bulk water binding, although in some cases different qualities of bound water can be detected (Chalikian et al., 1994). Traditionally, hydrodynamic methods have been used to detect molecules of water that move with the solute, and there is an extensive literature on the hydration of proteins based on analytical centrifugation (cf. Cantor and Schimmel, 1980). In contrast, there is relatively little information of this kind on DNA and RNA. As far as nucleic acids are concerned, the hydrodynamic work has been carried out mainly on heterogeneous polynucleotides, although a combined study of electrophoresis and sedimentation velocity of restriction fragments has been reported (Kovacic and van Holde, 1977). Only a small amount of work based on rotational diffusion measurements by NMR has been reported (Eimer et al., 1990; Birchall and Lane, 1990; Gyi et al., 1996).

Another technique that depends on the size and shape of oligonucleotides is electrophoresis. This method has been used extensively to report on DNA bending in d(A)n tracts of DNA (Crothers, 1987). Recently it has been shown that DNA and RNA oligomers of essentially the same sequence have quite different electrophoretic mobilities (Ratmeyer et al., 1994; Gyi et al., 1996). In this experiment, the electrophoretic mobility depends on the net charge and the frictional coefficient. In addition, within the gel, there is presumably a sieving effect. The electrophoretic mobility of oligonucleotides is inversely proportional to the number of base pairs, and for sequences of the same number of base pairs the charge and size are nominally identical. However, RNA duplexes of the same sequence and length as DNA duplexes migrate much more slowly than would be expected from the difference in their effective Stokes radii (Ratmeyer et al., 1994; Lesnik and Freier, 1995; Gyi et al., 1996), suggesting that other factors come into play. Possibilities include the permanent neutralization of the negative phosphate charge by ion condensation and differential hydration. The degree of neutralization of the negative charge

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is proportional to the axial charge density, which should be higher for RNA (A-form) than DNA (B-form). Although this difference is small, an acrylamide gel can be expected to resolve molecules of the same size differing by only one unit of charge. Furthermore, RNA on average may be more hydrated than DNA by virtue of the C2′-hydroxyl in the minor groove of RNA that is not present in deoxyribose (Conte et al., 1996b; Egli et al., 1996; Wahl et al., 1996).

Kovacic and van Holde (1977) reported a hydrodynamic and electrophoretic analysis of restriction fragments ranging from 50 to 1700 bp, and showed that below ~200 bp, DNA behaves essentially as a rigid rod. However, the semiempirical equation used to fit the sedimentation data extrapolates to negative S values at small numbers of base pairs, and is therefore inadequate for oligonucleotides of less than ~20 base pairs. In this article we report an analysis of the hydrodynamic and electrophoretic behavior of a series of synthetic oligodeoxynucleotide duplexes from 10 to 20 base pairs in length that have been extensively characterized by other techniques. In addition, we have made a limited number of measurements on two RNA duplexes to provide a new comparison of hydration of RNA and DNA duplexes. The hydrodynamic data are used to interpret the different electrophoretic behaviors of RNA and DNA duplexes.

MATERIALS AND METHODS

The following oligonucleotides were purchased from Oswel (Southampton) and were purified by high-performance liquid chromatography as previously described (Brown and Brown, 1992; Gyi et al., 1996):

d(GAAGAGAAGC), d(CTTCTCTTCG) [dR.dY]
r(GAAGAGAAGC), r(CUUCUCUUCG) [rR.rY]
$d(CGCGAATTCGCG)_2$ [dA2T2]
$d(CGCAAATTTGCG)_2$ [dA3T3]
$d(CGCTTTAAAGCG)_2$ [dT3A3]
$r(CGCAAAUUUGCG)_2$ [rA3U3]
$d(CGCGTATATACGCG)_2$ [dTA3]
$d(CATGTGACGTCACATG)_2$ [ATF-2]
$d(CGTACTAGTTAACTAGTACG)_2$ [trp O].

These oligonucleotides have been examined in detail by CD, UV melting, and (except for d(TA3) and d(T3A3)) by NMR spectroscopy. All of the DNA oligomers adopt the B family of conformations under conditions very similar to those used in this study (Conte et al., 1996a; Lefèvre et al., 1987; Lane, 1991; Jenkins et al., 1993; Lane et al., 1997). The RNA oligomers adopt the A family of structures (Conte et al., 1996b; Gyi et al., 1996). Both the DNA and RNA oligomers show small but significant deviations from the canonical B and A form structures.

The oligonucleotides were dissolved in 0.1 M KCl, 0.02 M Na-phosphate, pH 7; filtered through 0.2-μm membranes; and annealed slowly from 85°C.

Ultracentrifugation was carried out on a Beckman XLA instrument at 20°C. The density of the buffer was calculated to be 1.0077 g ml^{-1}. All experiments were carried out at three different concentrations, and three different rotor speeds. Under the conditions of the experiments, all oligonucleotides were completely in the duplex forms. For equilibrium runs, the absorbance at 260 nm was analyzed as a function of distance, using the Origen software according to the ideal equation:

$$A(r) = A(r_0)\exp[M(1 - v\rho)\omega^2 r^2/2RT] \quad (1)$$

The apparent mass of a solute under equilibrium conditions is given by

$$M_{app} = M(1 - v\rho) \quad (2)$$

where M is the true molecular mass, v is the partial specific volume, ρ is the solution density, ω is angular speed, and r is the distance from the axis of rotation. It was found that the ideal noninteracting model was adequate to describe all of the data. If M is known independently, then v can be calculated from Eq. 2. The partial specific volume was also determined by measuring the apparent mass in both H_2O and D_2O. For nucleic acids, exchange of H with D leads to an increase in mass of 1%; hence, from Eq. 1, the ratio of the apparent masses, r, is given by

$$r = M(H)/M(D) = M(1 - v\rho_H)/1.01M(1 - v\rho_D) \quad (3)$$

where ρ_H and ρ_D are the density of the protiated and deuterated solvents, respectively. v is then obtained as

$$v = (1 - 1.01r)/(\rho_H - 1.01r\rho_D) \quad (4)$$

For sedimentation velocity measurements, experiments were performed at different concentrations and rotor speeds, and the results were analyzed using the Svedberg program (Philo, 1994). This program also supplies estimates of the translational diffusion coefficient.

The sedimentation constant, S, is given by

$$S = M(1 - v\rho)/N_A \cdot f \quad (5)$$

where N_A is Avogadro's number, and f is the frictional coefficient. f can be obtained if both S and M_{app} are measured.

For a particle of hydrated radius a, the frictional coefficient is given by

$$f = 6\pi\eta aF \quad (6)$$

where F is the asymmetry factor for a nonspherical object. There are analytical expressions for F for ellipsoids of revolution, and semiempirical expressions for cylinders based on hydrodynamic theory of interacting beads (Tirado and Garcia de la Torre, 1979). The hydrated radius is then taken to be that of a sphere of mass m, partial molal volume v, and hydration h, as

$$a = (3M(v + h)/4\pi N_A)^{1/3} \quad (7)$$

Hence, if F can be calculated, then an estimate of h can be obtained. A more detailed analysis of the frictional behavior was made using the bead-modeling program Hydro (Garcia de la Torre et al., 1993). Atomic coordinates were taken from NMR-determined structures, and bead radii corresponding to the van der Waals radius of the appropriate atom types were used. The limitations of the bead models have been discussed in detail, but different macroscopic models give good agreement with the experimental data (Eimer et al., 1990; Garcia de la Torre et al., 1994).

Additional estimates of the translational diffusion coefficients were obtained for dA2T2 and trp O by pulsed-field gradient NMR spectroscopy (Stilbs, 1987).

Electrophoresis experiments were carried out using 10–20% acrylamide minigels (Atto Corp) in Tris-borate EDTA, Tris-borate-Mg (pH 8), or Tris-phosphate (pH 7.5) at either room temperature (23°C) or 5°C. Gels were preelectrophoresed for 20 min before loading approx. Duplex (30 pmol) was dissolved in the run buffer supplemented with 25% glycerol. Tracking dye (bromophenol blue) was not mixed with the samples, but was run separately in the outermost lanes. Gels were run at 120–200 V until the dye approached the end of the gel. Samples were stained with ethidium bromide and were visualized by fluorescence of the intercalated dye.

RESULTS

Electrophoretic mobility

The electrophoretic mobility was measured under a variety of conditions to determine the relative influence of the sieving effect and ion concentration. Gels were prepared at three concentrations (10%, 15%, and 20%) in both Tris-

borate-EDTA (TBE) and Tris-borate-magnesium, and run at both room temperature and at 5°C. The results are summarized in Table 1.

As expected, the mobility of the DNA oligonucleotides decreases with increasing length, as demonstrated in Fig. 1. For a series of oligomers whose charge and linear dimensions increase with increasing number of base pairs, the dominant resistance should arise from the sieving effect. This was confirmed by noting that the absolute mobility increased markedly with decreasing gel concentration (not shown). For example, between 15% and 20% acrylamide, the mobility decreased between 50% and 70%. The relative mobility was essentially unaffected by temperature or by the presence of magnesium (Table 1). However, the absolute mobility decreased by 30–40% in the presence of 1 mM Mg^{2+} in a 20% gel at 5°C, with all other conditions of gel concentration, buffer components, and temperature kept constant. Thus the mobility depends on the ionic composition of the mobile phase.

The RNA oligomers had a much smaller mobility than that of the analogous DNA sequence (Fig. 1), despite possessing the same formal negative charge. The lower mobility observed for RNA is in agreement with other work for duplexes ranging from 12 to 16 bp (Ratmeyer et al., 1994; Lesnik and Freier, 1995). The mobility of the RNA oligomers relative to that of the corresponding DNA oligomers was the same in Tris-phosphate-EDTA buffer and TBE buffer, indicating that there is no specific effect of the buffer anion on relative mobility. RNA differs chemically from DNA in two ways. First the substitution of U for T and the resultant replacement of a methyl group with a hydrogen atom makes the major groove less hydrophobic. Second, the substitution of D-ribose for 2′-deoxy-D-ribose introduces a hydroxyl group in place of a hydrogen atom, making the minor groove of RNA much more hydrophilic. These chemical changes are responsible for large differences in structure. The effect of the methyl groups on electrophoretic mobility has been tested in both DNA and RNA, and has been shown to decrease the mobility slightly compared with a hydrogen atom at the C5 position of U (Gyi et al., unpublished data). Thus the presence of the methyl groups in DNA actually decreases the difference in mobility. The lower mobility of RNA compared with DNA must therefore be a consequence of the presence of the C2′-OH group and/or its influence on conformation. As Fig. 1 shows, the RNA decamer has a mobility similar to that of the DNA 16-mer. However, because RNA is expected to be shorter than a DNA duplex of the same number of base pairs, and therefore to generate less friction, the observed difference in mobility cannot be due to the difference in linear dimensions. The difference in mobility between RNA and DNA must therefore be a function of the hydrated shape, size, and effective charge. To determine the hydrodynamic contribution to the frictional properties of the oligonucleotides, we have used analytical ultracentrifugation.

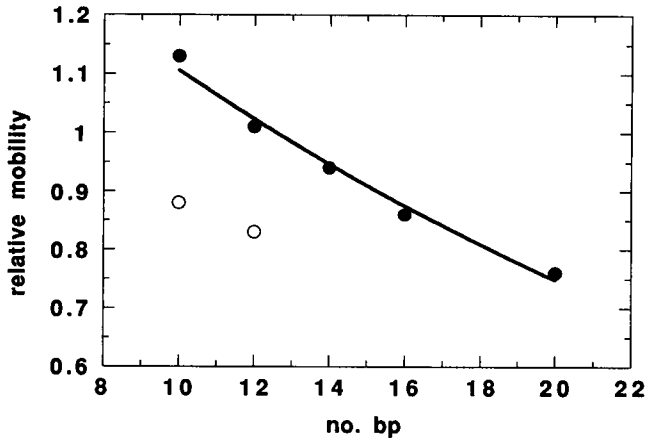

FIGURE 1 Electrophoretic mobility versus number of base pairs. Electrophoretic mobilities were measured in 20% acrylamide/TBE at 5°C as described in the Methods and normalized to the mobility of A3T3. The line is an exponential fit of mobility versus the number of base pairs for the DNA oligomers. ●, DNA; ○, RNA

TABLE 1 Relative electrophoretic mobilities of oligonucleotides

	Relative mobility		
Molecule	TBM (*RT*)	TBM (5°C)	TBE (5°C)
dR.dY	ND	1.13	1.13
dT3A3	1.03	ND	1.02
dA3T3	1.0	1.0	1.0
dAT3	0.90	ND	0.94
ATF-2	0.86	ND	0.86
trp O	0.77	ND	0.76
rR.rY	ND	0.92	0.88
rA3U3	ND	0.81	0.83

Mobilities were determined in 20% polyacrylamide gels as described in the Methods, and have been normalized to the mobility of dA3T3.

Hydrodynamics

Fig. 2 shows a typical absorbance versus radial distance profile from an equilibrium run of the trp O oligonucleotide, and the best fit to Eq. 1. The residual deviations demonstrate that the oligonucleotide behaves essentially as an ideal solute. Analogous results were obtained with the other oligonucleotides. This is because the concentration of oligonucleotides is low, and at relatively high ionic strengths the electrostatic interactions between oligonucleotide duplexes are minimal. Kovacic and van Holde (1977) also reported a negligible concentration dependence of the sedimentation constant for larger oligonucleotides under similar conditions.

The chemical and apparent molecular masses of the different oligonucleotides obtained from equilibrium centrifugation are given in Table 2. The effective partial specific volume was determined from the ratio of the two masses, according to Eq. 2. For the DNA samples, the average value of v is 0.56 ± 0.015 g ml^{-1}. We have also determined the apparent partial molal volume for the trp O sequence by equilibrium centrifugation in D_2O and H_2O as described in

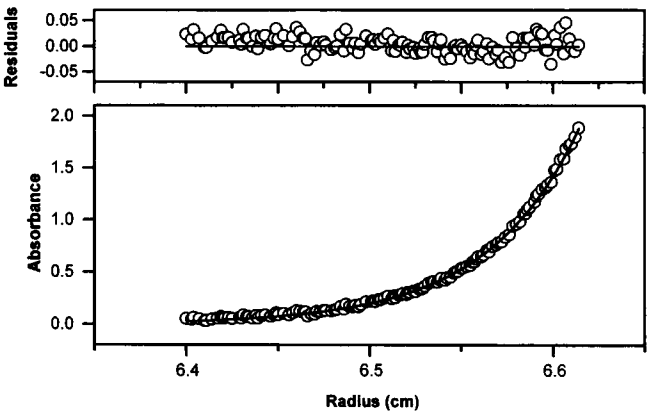

FIGURE 2 Equilibrium centrifugation. Absorbance at 260 nm versus radial distance for Trp O. The loading absorbance was 1.2 A_{260} units. The rotor was run at 37,000 rpm and 20°C. The line is the best fit of the experimental points to Eq. 1, assuming a single species, ideal solution. The top portion of the plot shows the residuals.

the Methods. From Eq. 4 we obtained a value of 0.593 ± 0.005 g ml^{-1} by this method, which is 2.6% higher than the value determined by using the known molecular weight, but is within the margin of experimental error. Thus the partial specific volumes are well determined. The mean value of v for the two RNA molecules was 0.508 g/ml, which is significantly smaller than the value for DNA. These values are comparable to those reported for polynucleotides (Ralston, 1993).

A typical sedimentation profile is shown in Fig. 3. The sedimentation constants showed no significant effect on concentration over a range of approximately fourfold, again because under these conditions there are no significant interparticle effects. Control experiments showed that indistinguishable values were obtained at 0.5 M KCl. The sedimentation coefficients are given in Table 2. According to Kovacic and van Holde (1977), the sedimentation coefficient for short oligonucleotides should obey a logarithmic dependence on the number of base pairs, as shown in Fig. 3 *B* and using the data in Table 2, and their data for 50–200 bp. The slope and intercept are significantly different from the values given by Kovacic and van Holde (1977), whose equation predicts much smaller sedimentation coefficients than observed for the range of 10–20 bp.

The translational friction coefficients were calculated using the apparent (buoyant) masses and the sedimentation and diffusion coefficients, as shown in Fig. 4. The frictional coefficients derived from the diffusion constants measured by dynamic light scattering for 8-, 12-, and 20-bp fragments (Eimer and Pecora, 1991) are plotted for comparison. There is very good agreement between the two methods, and the data are well represented by the linear equation

$$f = 1.75 + 0.104\, nbp \qquad (8)$$

where *nbp* is the number of base pairs.

We have calculated the expected translational friction coefficient for the DNA, using both the Perrin equations and the formulae for a circular cylinder (Tirado and Garcia de la Torre, 1979). For the cylindrical model, the length was calculated as 3.4 times the number of base pairs, and the diameter was varied from 15 Å to 26 Å to obtain the best agreement with all of the DNA data. The dotted lines in Fig. 4 show f calculated for three diameters, 17, 21, and 25 Å. The data are best represented with a diameter of 21–23 Å (best fit for the DNA data = 22.6 ± 1.4 Å), which is slightly larger than the value of 20.5 ± 1 Å estimated from light scattering data (Eimer et al., 1990; Eimer and Pecora, 1991), but smaller than the 27 Å estimated from sedimentation of longer oligonucleotides (Kovacic and van Holde, 1977). Calculations based on ellipsoids of the same volume and maximum length gave equal agreement with the data, for a diameter of the short axis of 22 Å. Garcia de la Torre et al. (1994) noted that a double helix with a diameter of 20 Å made up of beads, each representing one nucleotide, also gave good agreement with hydrodynamic data. This indicates that although the translational frictional coefficients are sensitive to the linear dimensions of biaxial objects, they are not strongly dependent on the details of the model.

Fig. 4 also shows that the frictional coefficients of the two RNA duplexes are the same, within experimental error, as the corresponding DNA duplexes. This is in contrast with the electrophoretic mobility (Fig. 1). Therefore the electrophoretic mobility difference cannot be due to differences in hydrodynamic friction.

As shown by the data in Table 2, the hydrodynamic radius of the equivalent ellipsoid or cylinder is much larger than the anhydrous radius. This is partly a consequence of molecular asymmetry, and partly due to the shell of hydration that cosediments with the solute particles. A DNA duplex of 6–8 bp is essentially isotropic, so that the deviation of the frictional coefficient from that of an anhydrous sphere can be attributed to additional material that cosediments with the oligonucleotide (i.e., water and counterions). The value of f/f_0 for 6–8 bp is 1.42 (cf. Eqs. 6 and 7). The difference in diameter between the anhydrous sphere and the hydrodynamic sphere is 7.4 Å, which is equivalent to an effective hydration of ~0.8–1 g/g DNA, i.e., ~25–35 water molecules per base pair. Other estimates of hydration have given ~40 water molecules per base pair in the first shell (Saenger, 1984). This hydration will contain a contribution from the preferential hydration as a function of ionic strength. However, under these conditions, this effect should be small (Record et al., 1978).

The two RNA oligomers have also been examined by CD and NMR spectroscopy (Conn, 1996; Conte et al., 1996b; Gyi et al., 1996), and they are both within the A family of conformations. An A-form RNA duplex should be 20–25% shorter and have a diameter 10–15% larger than a B-form DNA duplex with the same number of base pairs. However, as the length affects the frictional coefficient more than the diameter, the cylinder model predicts that the friction coefficient should be smaller for the RNA duplex than for the analogous DNA duplex. Comparison of cylinder models of DNA and RNA using an axial rise of 2.8 Å for the RNA,

TABLE 2 Hydrodynamic properties

Molecule	*nbp*	*M* (Da)	M_{app}	*v* (ml/g)	$S_{20,w}$ (S)	10^6 $D_{20,w}$ (cm^2 s^{-1})	*RT S/D*	10^8 f_t (g/s)	a_0 (Å)	a_h
dR.dY	10	6033	2540	0.575	1.50	1.40	2611	2.86	11.1	15.2
rR.rY	10	6283	3075	0.507	1.81	1.37	3218	2.89	10.8	15.3
dT3A3	12	7264	3080	0.572	1.72	ND	ND	2.97	11.8	15.8
dA3T3	12	7264	3256	0.548	1.65	1.22	3295	3.29	11.7	17.5
dA2T2	12	7266	3279	0.545	1.77	1.45	2974	2.94	11.6	15.6
rA3U3	12	7564	3680	0.510	2.03	1.30	3747	3.06	11.5	16.5
dAT3	14	8496	3911	0.538	1.85	1.17	3748	3.49	12.1	18.8
ATF-2	16	9697	4200	0.563	2.1	1.11	4608	3.49	13.0	18.5
trp O	20	12186	5096	0.578	2.2	1.1*	4872	3.85	14.1	20.4

M is the chemical molecular mass; M_{app} is the buoyant mass from equilibrium centrifugation; *v* is the partial specific volume; *S* and *D* are the sedimentation and diffusion coefficients, respectively; *RT S/D* is the buoyant mass calculated from the ratio of *S* and *D*; f_t is the translational friction coefficient; a_0 is the radius of the anhydrous sphere; a_h is the radius calculated from *f*. Estimated error on M_{app} = 2%; error on *v* ≈ 3% and 5% on $S_{20,w}$.
*Diffusion coefficient from NMR.

and bead model calculations using the program Hydro (Garcia de la Torre, 1993), based on the atomic coordinates, indicate that the translational friction coefficients for the RNA and DNA should be similar (within 10%). This is in agreement with the observed values (Table 1). However, to make the frictional coefficient of the RNA oligomers equal to those of their DNA analogs (cf. Fig. 4), the effective diameter of the equivalent cylinder for RNA must be increased to 25–26 Å, and would be consistent with a somewhat higher degree of hydration than for the DNA. The rotational correlation times of the two RNA species determined by NMR are, within experimental error, the same as the correlation times for the DNA analogs when measured by proton cross-relaxation. When ^{31}P NMR relaxation rate constants are used, the RNA duplexes have slightly smaller rotational correlation times than the DNA analogs (Gyi et al., 1996; Conte et al., unpublished data). This is in closer agreement with the prediction of the rigid cylinder model. Unfortunately, the influence of internal motions on NMR-derived correlation times makes a detailed analysis of the hydrodynamic behavior of DNA and RNA duplexes more difficult (Eimer et al., 1990; Birchall and Lane, 1990; Gar-

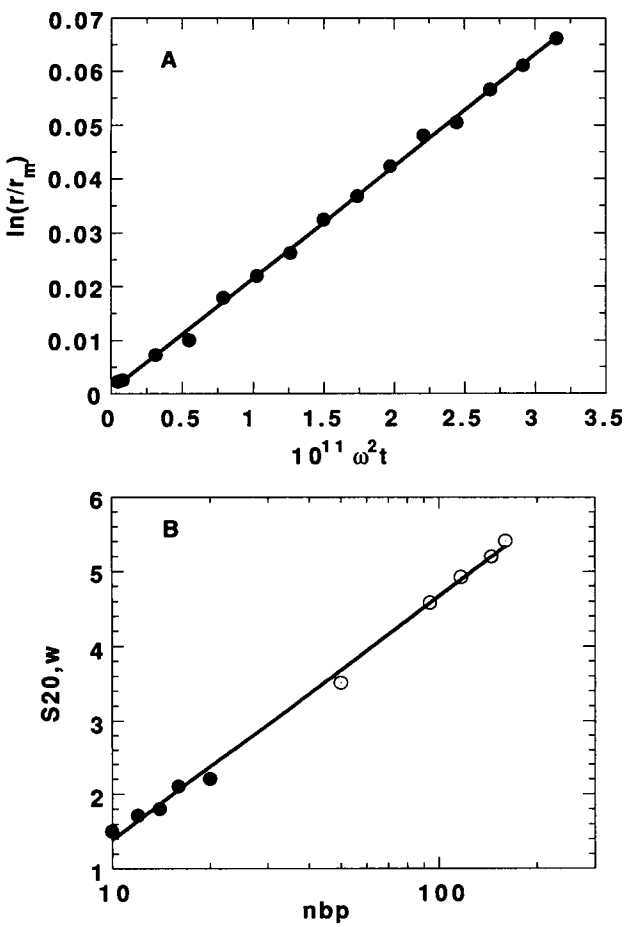

FIGURE 3 Sedimentation velocity. A solution of A3U3 at 20°C at a loading absorbance of 1.2 A_{260} units is shown. The rotor speed was 60,000 rpm. (*A*) ln(*r*/*rm*) versus $\omega^2 t$. The line is the linear regression fit to the experimental points using the Origen program, with s = 2.08 S, and a correlation coefficient of 0.9992. (*B*) $S_{20,w}$ versus number of base pairs of DNA. ●, This study; ○, from Kovacic and van Holde (1977). The line is the linear regression fit: $S = -1.906 + 3.283 \log(nbp)$ with R = 0.9981.

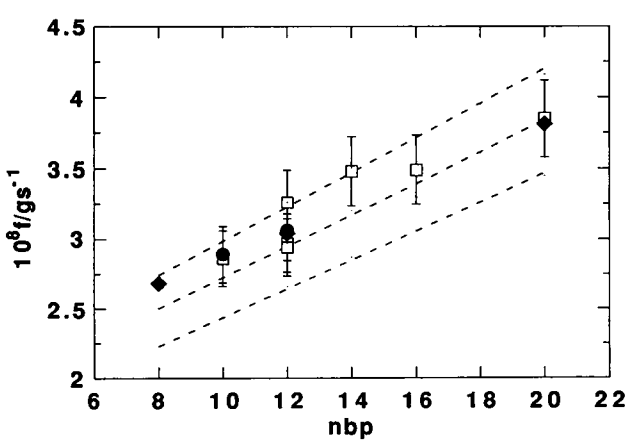

FIGURE 4 Dependence of the translational friction coefficient on the number of base pairs. The frictional coefficients were determined as described in the text. □, DNA; ●, RNA; ♦, DNA oligomers determined by dynamic light scattering from Eimer and Pecora (1991). The three lines were calculated for cylinders of length 3.4*n* and diameters of 17, 21, and 25 Å (*bottom to top*).

cia de la Torre et al., 1994). Nevertheless, the two independent methods show that the hydrodynamic properties of the DNA and RNA analogs are very similar. Therefore the lower electrophoretic mobility of RNA cannot be attributed to a substantially larger frictional coefficient than the DNA analog.

DISCUSSION

The apparent partial specific volumes of the oligonucleotides estimated by centrifugation are within the range previously reported for polynucleotides (Ralston, 1993). The value obtained for the RNA nucleotides is significantly smaller than for the DNA, which is due to the higher mass per nucleotide, and may also reflect the different interaction with water. The apparent hydration is on the order of 0.8–1 g/g nucleotide for the DNA, which is somewhat higher than is typically observed by this method for proteins. This is to be expected, as double-stranded nucleic acids have a larger surface-to-volume ratio than globular proteins, and are decorated with hydrophilic sites. It is also in agreement with other methods of assessing hydration (Saenger, 1984), and indicates that a significant shell of water adds to the hydrodynamic drag of the particles.

The translational frictional coefficients for the DNA series vary as expected for the number of base pairs, indicating that the size is the dominant factor in determining the drag. However, for longer fragments of DNA, it is probable that the shape would affect the relative frictional drag to a greater extent. As the frictional coefficient varies approximately as the cube root of the number of base pairs, this effect varies only 26% between 10 and 20 bp.

The frictional coefficients (and rotational correlation times) of the RNA molecules are only slightly different from those of the analogous DNA oligonucleotides, as expected from the calculations based on the known shapes, although the effective diameter of the RNA duplexes may require a slightly greater degree of hydration. This is compatible with the more hydrophilic minor groove of RNA compared with DNA (Egli et al., 1996; Wahl et al., 1996). This indicates that the observed substantial differences in electrophoretic mobility cannot be ascribed to the differences in shape. Indeed, as the hydrodynamic frictional coefficient includes the effects of hydration, it is likely that the difference in electrophoretic behavior between RNA and DNA can be ascribed largely to differential ion condensation or ionic atmospheres. That electrostatics are very important in nucleic acids is evident from the observation that the thermodynamic stabilities of oligonucleotides in general, and the present ones in particular (Conn, 1996), are strongly dependent on the ionic strength. Furthermore, the electrophoretic mobility was substantially reduced in the presence of magnesium cations, which have a much greater influence on thermodynamic properties than monovalent salts.

The A conformation adopted by RNA and the B conformation adopted by DNA in solution have slightly different axial charge densities. Therefore the number of charges neutralized by condensed Na^+ or K^+ also differ for RNA and DNA. According to Manning (1978), the fraction of the negative charge on the phosphates that is neutralized by counterions depends only on the axial charge density, which is greater for A-form RNA than for B-form DNA. This implies that the RNA has a lower net charge than DNA for the same number of base pairs. However, Manning's theory is strictly applicable to polymers. For the oligonucleotides studied here, the molecular shapes are more nearly isotropic. In the limit of a spherical molecule (which is approached for the 10-bp duplexes), the fraction of the neutralized charge should be zero. Furthermore, calculations of the electrostatic field around a short DNA duplex in either the A or B form (Mills et al., 1992) showed that although the number of counterions in a shell immediately surrounding the duplex was greater for the A form, the number of counterions in a slightly wider shell was the same for both.

During electrophoresis, the more mobile counter and coions will tend to redistribute around the slower-moving macromolecule. In particular, the shell of counterions closest to the solute will tend to move in the opposite direction, and therefore slow down the net migration of the macromolecule. This will have two effects: one electrostatic, the other due to the hydration that is associated with the counterions (Overbeek and Wiersema, 1967). It is probable that differences in electrophoretic mobility of short oligonucleotides of the A and B families can be ascribed to differential ion atmospheres, which depend on the global conformation and fixed charge distribution of the duplex.

DNA.RNA hybrid molecules display an electrophoretic mobility intermediate between DNA and RNA duplexes of the same sequence (Ratmeyer et al., 1994; Lesnik and Freier, 1995; Gyi et al., 1996). The present work indicates that this difference is not due to differences in hydrodynamic friction coefficient. However, the actual conformations of the DNA, RNA, and DNA.RNA hybrid duplexes are significantly different (Gyi et al., 1996), particularly in the axial rise, *Dz* (Gyi et al., unpublished calculations). This will result in different axial charge densities and presumably, therefore, electrodynamic properties of the duplexes.

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