Origin of the intrinsic rigidity of DNA

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ABSTRACT

The intrinsic rigidities of DNA and RNA helices are generally thought to arise from some combination of vertical base-stacking interactions and intra-helix phosphate–phosphate charge repulsion; however, the relative contributions of these two types of interaction to helix rigidity have not been quantified. To address this issue, we have measured the rotational decay times of a ‘gapped-duplex’ DNA molecule possessing a central, single-stranded region, dT24, before and after addition of the free base, N⁶-methyladenine (m⁶A). Upon addition of m⁶A, the bases pair with the T residues, forming a continuous stack within the gap region. Formation of the gapped duplex is accompanied by a nearly 2-fold increase in decay time, values that are indistinguishable from the full duplex control for monovalent salt concentrations up to 90 mM. These results indicate that at least 90% of the rigidity of the dTₙ–dAₙ homopolymer derives from base pair stacking effects, with phosphate–phosphate interactions contributing relatively little to net helix rigidity at moderate salt concentrations.

INTRODUCTION

The intrinsic rigidity of the DNA double helix is usually modeled in terms of the resistance to distortion of vertical base stacking interactions (1–6). However, it has also been proposed that intra-helix phosphate interactions contribute substantially to helix rigidity, and that asymmetric neutralization of phosphate charges along one face of a helix, as might occur through DNA–protein interactions, can lead to substantial bending of the helix axis (7,8). Phosphate–phosphate interactions clearly do contribute to helix rigidity at low (i.e. millimolar) monovalent salt concentrations (9). Furthermore, Maher and coworkers have demonstrated that asymmetric phosphate charge neutralization can induce small bends (~5–10°) in the helix axis (9–12) for moderate monovalent and oligovalent salt conditions, and others have presented models for ion-coupled bending (13,14). However, the question remains as to whether partial charge neutralization per se is sufficient to produce the severe bends seen in many DNA–protein interactions.

Models for DNA bending through asymmetric charge neutralization basically involve a ‘release-from-constraint’ (fewer repulsive inter-phosphate interactions), which leads to the prediction that helix rigidity should be reduced regardless of whether neutralization is symmetric or asymmetric. Thus, the magnitude of the charge-neutralization effect can be gauged by measuring the accompanying reduction in persistence length for a partially charge-neutralized helix. We had reported previously that a DNA ‘meroduplex’, in which one strand of the helix was replaced by a continuous stack of non-covalently bound purines, possessed substantial helix rigidity, despite its having only one-half of the stoichiometric charge of its fully duplex counterpart (15). While those results argued against a dominant role of phosphate–phosphate interactions in conferring helix rigidity, it was not possible to quantify the relative rigidity of the meroduplex, due to the uncertain hydrodynamic properties of the meroduplex at its ends.

In the current study, we have overcome the inherent limitation of full-length meroduplex species by using gapped duplex helices (16) in which a central, single-stranded (dTₙ) region is converted to meroduplex with N⁶-methyladenine (m⁶A) (Figure 1). The methyl derivative of adenine is used to eliminate the possibility of triple helix formation via Hoogsteen interactions (15). Because the ends of both the gapped duplex and full duplex are identical in sequence and structure, the relative rigidity (compared to the full duplex) of the gapped duplex can be determined with much less uncertainty. Using transient electric birefringence to measure the rotational decay times of both meroduplex and full duplex DNA molecules, we find that the rigidity of the meroduplex within the gap region is essentially identical to the persistence length of an equivalent dAₙ–dTₙ segment.

MATERIALS AND METHODS

Production of gapped duplex DNA molecules

Deoxyoligonucleotides were synthesized on a Biosearch Expedite 8909 automated synthesizer, and were cleaved and deprotected as described in (17). Crude oligonucleotides were resolved on denaturing polyacrylamide–urea gels (12 or 20% acrylamide; 29:1 w/w monomer:bis-acrylamide; 8 M urea; running buffer, 1× NNB: 133 mM Tris–HCl, pH 8.3; 44 mM boric acid, 2.5 mM Na-EDTA, 50°C), followed by purification on DE52 (Whatman) columns as described previously (15,17). The gapped duplex was formed by annealing two 38mers, 38L and 38R, to a 100mer, c(38L)-T₂₄-c(38R),

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Formation of meroduplex-gap molecules

Details of the formation of meroduplex-gap DNA molecules are essentially as described for the formation of meroduplex species, $[^{32}P]_{eh}d$T$_n$ (15), the principal difference being the use of a gapped-duplex substrate ($^{105}d$T$_{124}$) in the current work, in place of the fully single-stranded substrate, $d$T$_n$. Saturated solutions of $N^6$-methyladenine (Sigma) were mixed with small aliquots of $^{105}d$T$_{124}$ at room temperature (3- to 5-fold molar excess of meA over thymidylate monomer in the gap region). The mixtures were cooled to the measurement temperature (nominally 4°C) upon addition to the birefringence cell. As noted (15), meroduplex formation is complete within the time required for temperature equilibration of the sample before the start of the birefringence measurements.

Transient electric birefringence measurements

Procedures for performing and analyzing transient electric birefringence (TEB) experiments in this laboratory have been described (15,18–20). Detailed discussions of the sources of experimental error have been presented (15,19). All measurements were performed within a 2°C temperature range (3–5°C), and were corrected to 4.0°C. Temperature variation during an individual set of measurements was typically ±0.1°C, corresponding to a variation in the terminal decay time of 0.3%. TEB measurements typically utilized 4 μg DNA/35μl cell volume (~0.3 mM phosphate), and were performed in sodium phosphate buffer (NaPi) (pH 7.2; approximately 1.5 Na⁺ per phosphate), with added Na$_2$EDTA or MgCl$_2$ as specified. All TEB measurements used the following pulse configuration: pulselength, 1.0 μs; field strength, 10 kV/cm; pulse repetition frequency, 1 Hz. Decay curves represent averages of 128 to 512 individual decay transients, depending on signal strength. We have demonstrated previously that for either single-stranded or gapped-duplex DNA molecules, the birefringence decay times are strictly independent of field strength ($E \approx 10$ kV/cm) (15,16), and are independent of DNA concentration. Birefringence decay profiles were analyzed as described previously (18). Decay times and amplitudes were extracted from the biphasic decay profiles using the Levenberg–Marquardt method (21). The terminal (longer) decay times were used for the $\tau$ ratio analysis.

$\tau$ ratio analysis

A general analysis of the computational aspects of the $\tau$ ratio approach, including a description of the underlying hydrodynamic model, has been presented (15,19,20). A more specific analysis of the decay profiles of gap duplexes is given in (16). Reference parameters for the 38 bp elements flanking the single-stranded (gap) region were as follows: helix rise ($h$), $3.4$ Å/bp; persistence length ($P$), $450$ Å; hydrodynamic radius ($r_{hyd}$), $13$ Å. For the central gap region, $P$ is varied as indicated. The helix rise, $h$, within the gap was held fixed at $3.4$ Å/bp; this value has been established ($\approx 0.1$ Å/bp) for adenine stacks in free solution (16,22,23), meroduplex DNA (15), and single-stranded (stacked) poly(rA) and poly(dA) (16,24–26). For $P_{gap}/P_{helix}$ ratios greater than 0.9, reduction in the reference persistence length to 450 Å results in only a 0.5% variation in $\tau_{gap}/\tau_{helix}$ for a given $P_{gap}/P_{helix}$; thus, conclusions regarding the relationship between $\tau$ ratios and...
RESULTS

The meroduplex helix, $[\text{meA}]_n$–dT$_n$, possesses essentially the same intrinsic rigidity as the full duplex, dA$_n$–dT$_n$.

In the absence of added meA, the gapped duplex, 100dT$_{24}$, is substantially more flexible than its full duplex (100 bp) counterpart, due to the dT$_{24}$ segment at the center of the molecule. As is evident from Figure 2, the terminal decay times for the gapped species are nearly a factor of two smaller than the decay times for the duplex control molecule (16), both in the absence and presence of divalent cations. However, the addition of supersaturating solutions of the free base, meA, converts the gapped duplex to a meroduplex form with terminal birefringence decay times that are identical to those of the full duplex control molecule. The standard deviation ($\sigma$) for all $\tau$ ratios in Table 1 is 1.5%, which is equivalent to an uncertainty of 8% in the persistence length of the meroduplex segment (Figure 3). It should be noted that the current approach does not provide a precise measure of the absolute persistence length; rather, it demonstrates the absence of any significant difference between the gapped duplex (+ adenine) and the full duplex.

Despite the quantitative agreement between the meroduplex and the duplex decay times, the terminal phase amplitudes ($\alpha$) for the meroduplex species are slightly smaller than the amplitudes for the duplex, particularly for the lowest Na$^+$ concentration and in the presence of Mg$^{2+}$. This observation may be indicative of a slight increase in flexibility for the meroduplex, or some other structural perturbation, but in view of the identical decay times, such effects must be relatively minor. We have also noticed (data not shown) that in the presence of Mg$^{2+}$ ions, reduction in Na$^+$ concentration to 8 mM is not

Figure 2. Birefringence decay profiles for the gapped duplex, 100dT$_{24}$, in the presence (filled circle) or absence (square) of N$^6$-methyladenine. Decay profiles for full-duplex molecules are also displayed (circle). Solid lines represent two-exponential fits to the data for the gapped duplex (see Materials and Methods); dashed lines represent fits to the data for the full duplex species. (A) Na$^+$ of 40 mM, no magnesium; (B) Na$^+$ of 60 mM, 2 mM Mg$^{2+}$.

Table 1. Terminal decay times for the gapped duplex and full duplex control molecules in the presence of N$^6$-methyladenine

<table>
<thead>
<tr>
<th>[Na$^+$] (mM)</th>
<th>[Mg$^{2+}$] (mM)</th>
<th>$\tau_{gp}$ + $\alpha$ $^a$ (\mus)</th>
<th>$\sigma_{gp}$ + $\lambda$ $^a$</th>
<th>$\tau_{helix}$ (\mus)</th>
<th>$\sigma_{helix}$</th>
<th>$\tau$ ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>—</td>
<td>1.19 $^a$</td>
<td>0.85</td>
<td>1.18</td>
<td>0.95</td>
<td>1.01</td>
</tr>
<tr>
<td>43</td>
<td>—</td>
<td>1.11</td>
<td>0.82</td>
<td>1.12 $^a$</td>
<td>—</td>
<td>0.99</td>
</tr>
<tr>
<td>60</td>
<td>—</td>
<td>1.10</td>
<td>0.73</td>
<td>1.07</td>
<td>0.77</td>
<td>1.03 ± 0.012</td>
</tr>
<tr>
<td>92</td>
<td>—</td>
<td>1.06 ± 0.015</td>
<td>0.66 ± 0.012</td>
<td>1.07 ± 0.022</td>
<td>0.59</td>
<td>1.00 ± 0.027</td>
</tr>
<tr>
<td>60</td>
<td>2</td>
<td>1.09</td>
<td>0.76</td>
<td>1.09</td>
<td>0.84</td>
<td>1.00</td>
</tr>
</tbody>
</table>

$^a$All values for $\tau$ are corrected to 4.0°C; measurements were performed between ~3 and 5°C.

$^b$gp + $\lambda$ refers to the gapped duplex, 100dT$_{24}$, in the presence of N$^6$-methyladenine (meA). helix refers to the full duplex control DNA with a central dT$_{24}$–dA$_{24}$ block, also in the presence of meA, the latter to control for changes in solution viscosity.

$^c$Fractional amplitude of the slow phase in the birefringence decay curve.

$^d$Standard errors of the mean values are not reported if less than 0.01. All reported values represent averages of at least five sets of measurements.

$^e$Not separately determined; the value was obtained by interpolation using $\tau_{helix}$ values for 32 mM and 60 mM Na$^+$. 

relative gap rigidity within this region are insensitive to the choice of $P_{helix}$ [see (19) for further discussion of this point].
accompanied by an increase in decay time for the meroduplex, in contrast to a slight (<9%) increase for the duplex. This last observation may indicate that, at low monovalent salt concentrations, there is a slight added effect on rigidity, or perhaps some other conformational effect, due to the second phosphodiester backbone in the duplex; again, this effect is small.

**DISCUSSION**

In a previous work (15), we demonstrated that the addition of free purine base (m^A) to single polydeoxypyrimidine strands (dT_n; n = 40, 60, 81, 110 nt) leads to the formation of meroduplex complexes, [m^A]-dT_n, in which the purines form stacked base pairs with the thymine bases. These meroduplexes were shown to possess a persistence length that was within ~20% of the persistence length for the full duplex (dA_n-dT_n) controls. However, due to the uncertain hydrodynamic character of the ends of the meroduplex, which lack the second phosphodiester backbone, it was not possible to further refine the estimate for its intrinsic rigidity. More recently, we have developed a gapped-duplex system for measuring the intrinsic (relative) rigidities of single-stranded DNA and RNA molecules (16). This latter method enables one to measure the physical properties of a localized non-helix segment of DNA (or RNA) in the context of flanking duplex regions. Thus, the hydrodynamic properties of the ends of the molecule are identical to those of the full duplex controls.

In the current instance, we have applied the gapped DNA methodology to refine the estimate for the relative rigidity of the meroduplex, finding it to be essentially as rigid (and linear) as its duplex counterpart, despite an effective 50% charge neutralization of the central gapped region. Since the effective persistence length of the central, poly dT_24 strand (P_gap) is only ~20–30 Å (16), the increase in the rigidity of the gap region upon addition of m^A represents more than a 10-fold increase in P (more than a 12-fold increase, for P_helix = 450 Å; given an uncertainty of 8% in P/P_helix).

These observations argue against any model in which DNA rigidity is substantially determined by inter-phosphate repulsive interactions at moderate monovalent or divalent salt concentrations. In particular, ‘release-from-constraint’ models, in which the helix contour is substantially bent by asymmetric charge neutralization (8), necessarily predict significant reductions in persistence length for either symmetric or asymmetric neutralization, contrary to current observations. We would argue that other effects, such as asymmetric solvation along the protein–DNA interface, or bends involving specific ion-coordination events (13,14) must play an important role in protein-induced bending of DNA. Although ion pair formation and counterion release provide additional energy of stabilization, the latter effects appear to contribute slightly <10% to overall helix rigidity.

Our results are not inconsistent with the observations of Maher and co-workers (10–12), and of Okonogi et al. (27), who demonstrated that asymmetrically placed blocks of (neutral) methylphosphonates led to small, but detectable, DNA curvature or reduced (apparent) persistence length. Although a significant portion of the observed curvature probably derives from asymmetric solvation of the alkyl groups, charge neutralization undoubtedly plays some role. Indeed, because DNA possesses significant intrinsic flexibility, either asymmetric charge neutralization or solvation (or a combination thereof) may give rise to a modest local curvature. The issue of solvation effects is underscored by the reductions in the midpoint of thermal melting profiles (T_m) observed for the neutral phosphonate species [e.g. (27)]. In particular, any simple release from ion–ion repulsive constraints should render the partially neutralized helix more stable; thus, the reduced T_m’s are likely to be due to altered solvation of the alkyl groups.

Finally, it should be emphasized that the rigidity of the gap-meroduplex is not a consequence of the reduced effective rise for the dT_n element [from ~5 Å/nt to 3.4 Å/nt (16)] upon addition of m^A. We have reported (16) that single-stranded dA_n, with the same vertical base–base separation (~3.2 Å/nt), possesses a persistence length of only ~80 Å, more than 5-fold lower than the persistence length of the gap-meroduplex species.

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