

Monovalent Cation Binding in the Minor Groove of DNA A-Tracts[†]

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ABSTRACT: The binding of five different monovalent cations to DNA oligomers containing A-tracts, runs of four or more contiguous adenine residues, has been assessed by capillary electrophoresis, using the Replacement Ion method. In this method, a nonbinding cation in the background electrolyte is gradually replaced by a binding cation, keeping the ionic strength of the solution constant. Monovalent cation binding reduces the effective charge of an A-tract-containing oligomer, decreasing its free solution mobility. The cations bind in the A-tract minor groove, because the binding site can be blocked by the minor groove binding drug netropsin. Li⁺, NH₄⁺, and Tris⁺ ions bind to A-tracts with similar affinities; the binding of Na⁺ ions is weaker, and K⁺ ion binding is highly variable. Each A-tract appears to bind one monovalent cation upon saturation of the binding site(s). For a given cation, the apparent dissociation constants depend on A-tract sequence and orientation, but not on the phasing of the A-tracts with respect to the helix repeat. Differences in the cooperativity of binding of the various cations to A-tracts with different sequences suggest that monovalent cation binding may be coupled with a conformational transition leading to the formation of the characteristic narrow minor groove A-tract structure.

X-ray diffraction and NMR studies, as well as molecular dynamics simulations, have shown that monovalent cations can bind in the minor groove of DNA A-tracts, runs of four or more contiguous adenine or thymine residues (reviews in refs 1–7). The grooves are only partially occupied by cations; the remainder of each groove is filled with water molecules (8–11). In the Dickerson–Drew dodecamer (12), which has an AATT sequence motif flanked by GC residues, monovalent cations are located at the ApT step in the minor groove (8–10, 13–16) and near the guanine residues in the major groove (3, 10, 17), two sites with relatively deep electronegative potentials (18). Since the bound cations can exchange readily with each other, with other cations in the bulk solution, and with water molecules in the grooves, the cations appear to remain hydrated in the bound state (5, 7, 19, 20).

Capillary electrophoresis (CE)¹ is another technique that can be used to assess monovalent cation binding to DNA A-tracts (21, 22). The free solution mobility of an analyte, μ , is determined by the ratio between its effective charge, Q , and its frictional coefficient, f , as shown in eq 1:

$$\mu = Q/f = QD_t/k_B T \quad (1)$$

where D_t is the translational diffusion coefficient of the analyte, k_B is Boltzmann's constant, and T is the absolute

temperature. Since the diffusion coefficients of small DNA oligomers containing the same number of base pairs are independent of sequence (23), differences in the free solution mobilities of different oligomers are a direct measure of cation binding.

In our first study (21), we showed that 20 bp oligomers containing phased A- or T-tracts migrated more slowly in Tris buffer solutions than a 20 bp oligomer without A-tracts, suggesting that the preferential binding of Tris⁺ ions decreased the effective net charge of the A-tract-containing oligomers. In a second study (22), we showed that the free solution mobility of a stably curved, 199 bp restriction fragment from the VP1 gene of simian virus 40 (SV40) decreased progressively with the increasing number of A-tracts in the “curvature module” in the center of the fragment, again indicating that A-tracts bind Tris⁺ ions. More recently (24), we have combined the CE measurements with estimates of the translational diffusion coefficients of the SV40 curvature module mutants to show that each A-tract in the curvature module is occupied ~30% of the time by Tris⁺ ions.

The previous studies were not designed to measure the affinity of cation binding to DNA A-tracts. Here we address this question using a variation of affinity capillary electrophoresis called the Replacement Ion (RI) method (25) to quantitate the binding of different cations to A-tracts with different sequences. In the RI method, a large, presumably nonbinding cation, such as the tetrapropylammonium (TPA⁺) ion, is gradually replaced by a binding cation, such as Li⁺, Na⁺, K⁺, NH₄⁺, or Tris⁺, keeping the total ionic strength of the solution constant. The constant ionic strength prevents the large variation in mobility with ionic strength (26, 27) from obscuring the small mobility differences due to cation binding. The RI method has previously been used to study

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¹ Abbreviations: BGE, background electrolyte; CE, capillary electrophoresis; DM, diethylmalonic acid; EOF, electroosmotic flow; m.u., mobility unit (1 m.u. = 1 × 10⁻⁴ cm²/Vs); ^{app}K_D, apparent dissociation constant; RI, replacement ion; TPA⁺, tetrapropylammonium ion.

Table 1: Sequences of Oligomers Containing 46% A+T, Descriptive Acronyms, and Li⁺ Ion Binding Constants

acronym	sequence	^{app} K _D (mM)	−Δμ _{max} (m.u.)
RA-46 (random)	CGCAGTGTCGACTAGACTACAGACG	nb ^a	—
A ₄ -46	CGCAAAAGCGTCGACACTAGTACTCG	51	0.0051
A ₅ -46	CGCAAAAGCGTCGCACTAGTACTCG	65	0.0054
A ₆ -46	CGCAAAAGCGTCGCTAGTACTCG	46	0.0082
A ₇ -46	CGCAAAAGCGTCGCTCGTACTCG	55	0.0090
A ₂ T ₂ -46	CGCAATTTCTATATGCTCCGCAGACCG	68	0.0060
A ₃ T ₃ -46	CGCAATTTCTATGCTCCGCAGACCG	78	0.0100
A ₄ T ₄ -46	CGCAAAATTTTCTGCTCCGCAGACCG	78	0.0130
A ₅ T ₅ -46	CGCAAAATTTTCTGCTCCGCAGACCG	74	0.0130
A ₃ in-46	CGCAAAAGCGTACGAAACTCTGTCTCG	100	0.0073
A ₄ in-46	CGCAAAAGCGTCGAAAACCTCTCTCG	41	0.0121
A ₅ in-46	CGCAAAAGCGTCGAAAACCTCTCTCG	71	0.0112
A ₅ out-46	CGCAAAAGCGTCGCTGAAAACCTCTCG	71	0.0109
T ₅ in-46	CGCTTTTTGCCGCTTTTTCAGCACG	100	0.0082
A ₂ T ₂ in-46	CGCAATTCGTCACAATTCTGCGACCG	128	0.0102
A ₃ T ₃ in-46	CGCAAAATTTTCGCCAAATTTTCGCCCG	97	0.0163
A ₅ =T ₅ in-46	CGCAAAAGCTCGTTTTTTCGCCACCG	67	0.0145
A ₅ =T ₅ out-46	CGCAAAAGCTCGCTTTTTTTCGCCCG	69	0.0106
A ₄ T ₁ in-46	CGCAAAATCGGTCAAAATCGGCTGCG	131	0.0084

^a No binding.Table 2: Sequences of Oligomers Containing 62% A+T, Descriptive Acronyms, and Li⁺ Ion Binding Constants

acronym	sequence	^{app} K _D (mM)	Δμ _{max} (m.u.)
R-62 (random)	CGCTTACTAGATACTACTAGTACTAG	nb ^a	—
A ₄ T ₁ -62	CGCAAAATCTGATCATATGTACTTCG	50	0.0050
A ₄ T ₃ -62	CGCAAAATTTCTGATCATATGATCG	64	0.0050
A ₄ T ₄ -62	CGCAAAATTTTCTGATCATCAGATCG	68	0.0070
A ₄ T ₅ -62	CGCAAAATTTTTCGATCATCAGATCG	57	0.0075
A ₄ in-62	CGCAAAAGTGTCTAAAATCTGTTCTG	54	0.0096
A ₄ T ₁ in-62	CGTAAAATCTGTGAAAATCTGTCTCG	24	0.0133
A ₄ T ₂ in-62	CGCAAAATTCGTCAAAATTTCTGTCTG	73	0.0117
A ₄ T ₃ in-62	CGCAAAATTTGTCAAAATTTTCGTCG	58	0.0120
A ₄ T ₄ in-62	CGCAAAATTTTCGAAAATTTTCGCCG	64	0.0153
A ₄ T ₄ 'in-62	CGCCGCAAAATTTTCGAAAATTTTCG	58	0.0195
A ₄ T ₄ out-62	CGCAAAATTTTCGCGCAAAATTTTCG	54	0.0157
A ₄ T ₄ /T ₄ A ₄ in-62	CGCAAAATTTTCGTTTTAAACGCCG	100	0.0092
A ₃ T ₃ in-62	CGCAAAATTTTCGACAAATTTCTAGTCG	85	0.0085
A ₃ T ₃ out-62	CGCAAAATTTTCGAGTCAAAATTTCTACG	65	0.0090

^a No binding.

the nonspecific binding of monovalent cations to a random-sequence, 26 bp DNA oligomer (25). The random-sequence oligomer, and presumably all DNA oligomers, bind monovalent cations in a saturable manner, with apparent dissociation constants ranging from 71 mM for Tris⁺ to 173 mM for Na⁺ and K⁺. These bound cations appear to correspond to the strongly correlated, “tightly bound” ions recently postulated to be trapped on the DNA surface by the strong electrostatic field of the phosphate residues (28).

The sequence-specific binding of monovalent cations to DNA A-tracts would occur in addition to any nonspecific cation binding. Therefore, the difference in mobility between an A-tract-containing oligomer and a random-sequence oligomer containing the same number of base pairs can be attributed unambiguously to cation binding to the A-tract(s). Such mobility differences can be determined very accurately, because they are measured in the same solution at the same time under exactly the same ionic conditions. The mobility differences measured as a function of binding ion concentration are then used to calculate the apparent dissociation constants, as described previously (25).

The results indicate that Li⁺, NH₄⁺, and Tris⁺ ions bind to DNA A-tracts with similar affinities, Na⁺ ions bind more weakly, and the binding of K⁺ ions is variable. The binding affinities depend on A-tract sequence, sequence context, and

orientation but do not depend on the phasing of the A-tracts with respect to the helix repeat. Each A-tract appears to bind one monovalent cation upon saturation of the binding site(s). The sigmoidal binding curves observed in most of the mobility profiles suggest that monovalent cation binding in the A-tract minor groove may be coupled with a conformational transition leading to the characteristic narrow minor groove structure.

MATERIALS AND METHODS

DNA Samples. All DNA oligomers used in these studies contained 26 bp, with GC bp at the ends to minimize fraying. The oligomers contained one or two A-tracts of various lengths; the total A+T content was either 46 or 62%. The oligomers are identified in the following text by acronyms denoting the A-tract sequence and phasing (if more than one A-tract was present), followed by a hyphen and a number indicating the % A+T in the oligomer. The sequences of the oligomers and their acronyms are given in the first two columns of Tables 1 and 2. For clarity, the A-tracts in each oligomer are underlined.

All oligomers were synthesized by IDT (Integrated DNA Technologies, Coralville, IA) and purified by polyacrylamide gel electrophoresis. We prepared duplexes by mixing equi-

molar quantities of the complementary strands (1 $\mu\text{g}/\mu\text{L}$) in 10 mM Tris-chloride buffer (pH 8.0), heating them to 94 °C for 5 min, and slowly cooling them to room temperature. For sequences which had a significant tendency to form hairpins, it was necessary to extend the cooling period for several hours or overnight. All duplexes described here exhibited single bands in 9.3% T, 3% C polyacrylamide gels, prepared as described previously (29), and sharp, nearly Gaussian-shaped peaks in the CE electropherograms. Hence, no truncated failure sequences or unreacted single strands were present. Concentrated stock solutions of the duplexes in 10 mM Tris-HCl buffer were stored in a freezer at -20 °C; the samples were diluted with water to concentrations of 10–50 ng/ μL for the CE experiments. The mobilities were independent of concentration within this range.

Netropsin–DNA complexes were prepared by adding netropsin (4 μM) to samples containing a mixture of RA-46 and A₄T₁in-62 (2 μM each) and allowing the solutions to stand for 1 h at room temperature to reach equilibrium. The A-tract:netropsin molar ratio was 1:1, because oligomer A₄T₁in-62 contains two A-tracts.

Buffers. All buffers contained 200 mM diethylmalonic acid (DM) as the background electrolyte, titrated to pH 7.3, the pK_a of the second carboxyl group, with a concentrated solution of Tris base or the hydroxide of Li^+ , Na^+ , K^+ , NH_4^+ , or TPA^+ . Because the second carboxyl group in DM is half-ionized at pH 7.3, the total cation concentration in each solution was 300 mM; the ionic strength was 400 mM. Solutions with mixed cations were prepared by combining appropriate volumes of the individual buffer solutions. The actual cation concentration in each solution was calculated from the measured pH (Radiometer pHM82 Standard Meter) and the pK_a of DM, using the Henderson–Hasselbalch equation. Diethylmalonic acid [$(\text{CH}_3\text{CH}_2)_2\text{C}(\text{COOH})_2$] and the hydroxides of the various cations were purchased from Sigma-Aldrich (St. Louis, MO). Tris base was purchased from Research Products International Corp. (Mt. Prospect, IL).

Capillary Electrophoresis. Capillary zone electrophoresis measurements were carried out with a Beckman Coulter (Fullerton, CA) P/ACE System MDQ Capillary Electrophoresis System, run in the reverse polarity mode (anode on the detector side) with UV detection at 254 nm, as described previously (30). Migration times and peak profiles were analyzed using the supplied 32 Karat software. The capillaries were LPA (linear polyacrylamide) internally coated capillaries from Bio-Rad (Hercules, CA). Coated capillaries are used to minimize the electroosmotic flow (EOF) of the solvent; previous studies have shown that polyacrylamide coatings do not affect the observed mobilities (31). The capillaries were 40.0 cm in total length, with external diameters of 375 μm and internal diameters of 75 μm . The distance from the inlet to the detector, L_d , was 29.8 cm. The capillaries were mounted in a liquid-cooled cartridge thermostated at 20 ± 0.1 °C.

The capillary was conditioned at the beginning of each day by being rinsed with running buffer (called the background electrolyte, BGE) for 5 min at high pressure (25 psi, 0.17 Mpa). Between runs, the capillary was rinsed with the BGE at 25 psi for 2 min. The capillary was rinsed with deionized water at 25 psi for 5–10 min at the end of each day and filled with deionized water overnight. The DNA samples were hydrody-

namicly injected into the capillary by applying low pressure (0.5 psi, 0.0035 MPa) for 3 s. The injection volume was 22 nL, giving a sample plug 0.51 cm in length (1.7% of the total capillary length). Particular care was taken to equalize the liquid levels in the buffer reservoirs to prevent hydrodynamic flow between the two buffer chambers. The buffers in all vials were replaced after four to six runs. The electric field was typically 90–110 V/cm (applied voltage of 3.5–4.5 kV); the mobilities and mobility differences were independent of the applied field within this range. The current typically ranged between 40 and 70 μA for various experiments. Control experiments showed that heating within the capillary was negligible under these conditions.

The residual EOF in the capillary was measured every day by a fast pressure-assisted method (32), using 40 mM acrylamide as the analyte. The EOF of a new capillary was typically $\sim 5 \times 10^{-6}$ cm^2/Vs . When the EOF increased to $\sim 1 \times 10^{-5}$ cm^2/Vs , indicating deterioration of the capillary coating, the capillary was replaced. Since the EOF was negligibly small, DNA electrophoretic mobilities, μ , were calculated from eq 2:

$$\mu = L_d/Et \quad (2)$$

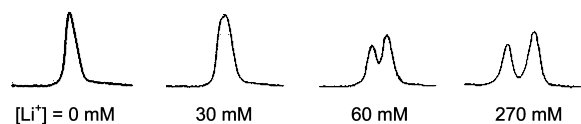
where L_d is the distance to the detector (in centimeters), E is the electric field strength (in volts per centimeter), and t is the time required for the sample to migrate from the inlet to the detector (in seconds). The average standard deviation of the mobilities measured for a given DNA sample on the same or different days was typically $\pm 0.2\%$.

In this case, the quantity of greatest interest was not the apparent absolute mobility, μ , but the difference in mobility between an A-tract-containing oligomer and a random-sequence oligomer of the same size, as shown by eq 3:

$$\Delta\mu = \mu(\text{A-tract oligomer}) - \mu(\text{random oligomer}) \quad (3)$$

Defined in this manner, the $\Delta\mu$ values are negative when cations are bound because the mobility of the A-tract-containing oligomer is smaller than the mobility of the random-sequence control. For the sake of brevity, all mobilities and mobility differences are expressed in mobility units, m.u. (1 m.u. = 1×10^{-4} cm^2/Vs).

Measurement of Cation Binding Using the Replacement Ion (RI) Method. In the Replacement Ion (RI) method (25), the ionic strength of the solution is kept constant by gradually replacing a nonbinding ion in the BGE with a binding ion and comparing the mobility of binding and nonbinding oligomers as a function of binding ion concentration. In this work, TPA^+ was used as the nonbinding ion, Li^+ , Na^+ , K^+ , NH_4^+ , and Tris^+ were used as the binding ions, and oligomer RA-46 was used as the nonbinding oligomer. If cation binding does not occur, no mobility differences are observed between the test oligomer and oligomer RA-46. When binding occurs, the mobility differences gradually increase (in absolute value) with an increase in cation concentration; the mobility differences observed as a function of binding ion concentration are called mobility profiles, for brevity. The dissociation constants (K_D) were determined either from the midpoints of the transitions in the mobility profiles or by nonlinear curve fitting, using SigmaPlot. A three-parameter Hill equation, shown in eq 4, was usually used to fit the data.

A. RA-46 and A₄T₄'in-62

B. RA-46 and R-62

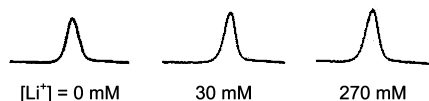


FIGURE 1: Electropherograms observed for mixtures of RA-46 with (A) A₄T₄'in-62 and (B) R-62. The leftmost electropherogram in each row corresponds to the mobility pattern observed when TPA⁺ was the only cation in the BGE; the electropherograms to the right in each row were obtained with the indicated Li⁺ concentration in the BGE. The ordinate corresponds to optical density in arbitrary units; the abscissa corresponds to the time required for the peak(s) to migrate to the detector. The scales are the same for all electropherograms.

$$\Delta\mu = \frac{\Delta\mu_{\max} [M^+]^n}{K_D^n + [M]^n} \quad (4)$$

where $\Delta\mu$ is the mobility difference observed at a ligand concentration of $[M^+]$, $\Delta\mu_{\max}$ is the mobility difference that would be observed upon saturation of the binding site(s), K_D is the dissociation constant, and n is the cooperativity parameter (33). Since the dissociation constants are population averages that could reflect the binding of cations to different positions within an A-tract or to different A-tracts in the same oligomer, the results are described as apparent dissociation constants, ${}^{\text{app}}K_D$, in the following text. The ${}^{\text{app}}K_D$ values calculated from the mobility profiles or the transition midpoints agreed with each other and could usually be reproduced within $\pm 10\%$ in replicate experiments; the mobility differences observed upon saturation of the binding site(s), $\Delta\mu_{\max}$, could also usually be reproduced within $\pm 10\%$.

RESULTS AND DISCUSSION

Characterization of Oligomers by Capillary Electrophoresis. Samples containing the random-sequence oligomer RA-46 and an A-tract-containing oligomer were electrophoresed in BGEs containing TPA⁺ as the nonbinding ion, a cation such as Li⁺ as the binding ion, or mixtures of the two ions. The mobility profiles were usually measured from pure TPA⁺ to pure Li⁺; identical results were obtained if the titration was carried out in the opposite direction. Typical electropherograms observed for the titration of oligomer A₄T₄'in-62 with Li⁺ are illustrated in Figure 1A. Oligomers RA-46 and A₄T₄'in-62 migrated as a single peak in pure TPA⁺ (leftmost panel). The peak gradually resolved into two peaks with an increase in Li⁺ concentration, as shown in the panels to the right. For the sake of clarity, the Li⁺ concentration is indicated beneath each panel. Separate control experiments, carried out either by "spiking" (increasing the concentration of one of the oligomers and repeating the measurement) or by measuring the oligomers separately in solutions containing only Li⁺ ions, showed that the more slowly migrating peak observed at high Li⁺ concentrations was A₄T₄'in-62. Hence, Li⁺ ions bind preferentially to A₄T₄'in-62, reducing its effective charge and thereby reducing its mobility. Similar

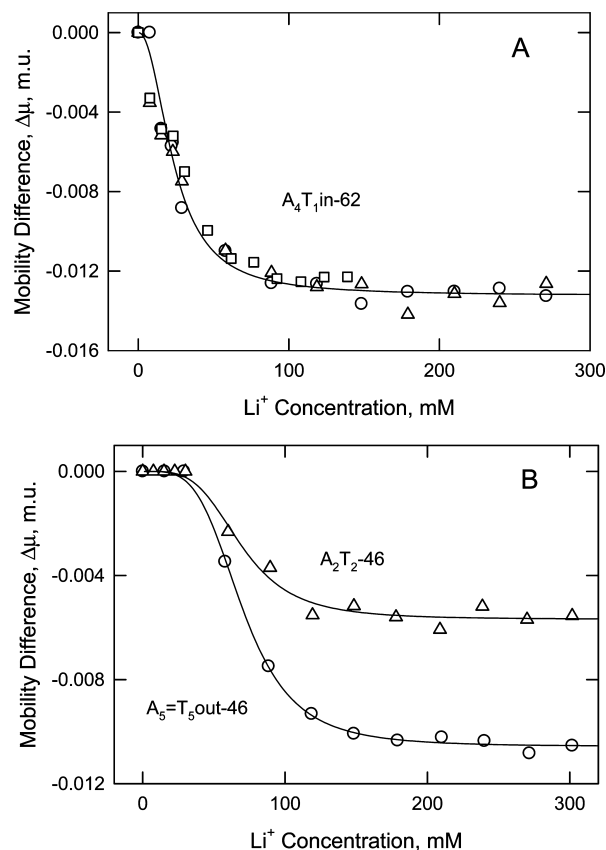


FIGURE 2: Typical mobility profiles observed for A-tract-containing oligomers, using Li⁺ as the binding ion. Mobility profiles are defined as the mobility difference ($\Delta\mu$) between an A-tract-containing oligomer and RA-46, plotted as a function of binding ion concentration. (A) Oligomer A₄T₄'in-62: (○ and △) two independent titrations in BGEs containing 300 mM cation and (□) a BGE containing 150 mM cation. (B) Mobility profiles observed for oligomers A₂T₂-46 (△) and A₅=T₅out-46 (○). The BGE contained 300 mM cation. The curved lines were drawn with eq 4; the corresponding ${}^{\text{app}}K_D$ and $\Delta\mu_{\max}$ values are given in Tables 1 and 2. All mobility differences are given in mobility units.

mobility patterns, differing only in quantitative detail, were observed for most of the other A-tract-containing oligomers.

Completely different results were observed when the sample contained two random-sequence oligomers, as shown in Figure 1B. Oligomers RA-46 and R-62 comigrated in pure TPA⁺, in BGEs containing various Li⁺:TPA⁺ ratios, and in pure Li⁺, indicating that neither oligomer preferentially binds monovalent cations. It is possible that both oligomers bind Li⁺ ions in a non-sequence-specific manner, as observed previously (25). However, any non-sequence-specific binding must be the same for both oligomers, because they comigrate at all Li⁺ concentrations. Hence, the mobility differences observed between the random-sequence control, RA-46, and an A-tract-containing oligomer can be attributed unambiguously to preferential cation binding by the A-tract(s) in the test oligomer.

Mobility Profiles. Migration times corresponding to the maxima observed in the electropherograms of RA-46 and the A-tract-containing oligomers were converted to mobilities using eq 2 and the mobility differences, $\Delta\mu$, calculated from eq 3. The mobility differences were plotted as a function of the concentration of the binding cation, giving mobility profiles such as those illustrated in Figure 2. The mobility profiles were very reproducible, as shown for Li⁺ ions

binding to oligomer A₄T₁in-62 in Figure 2A. Figure 2A also shows that the mobility profiles measured in BGEs containing two different cation concentrations (150 and 300 mM) were superimposable over the common range of Li⁺ concentrations. Hence, the ^{app}K_D values measured here are independent of the particular buffer concentration chosen for the study.

Although the individual mobility profiles were very reproducible, the shapes of the mobility profiles depended on A-tract sequence. For some oligomers, such as A₄T₁in-62 (Figure 2A), the mobility differences increased monotonically (in absolute value) with an increase in Li⁺ ion concentration, leveling off at a constant plateau value at high Li⁺ concentrations. This plateau value is defined as $\Delta\mu_{\max}$. The titration curves observed for most of the other oligomers, such as A₂T₂-46 and A₅=T₅out-46 (Figure 2B), were qualitatively different. The mobility differences were zero until a threshold concentration of Li⁺ was reached, after which the mobility differences increased (in absolute value) until a constant plateau value was reached at high Li⁺ concentrations. Figure 2B also shows that larger values of $\Delta\mu_{\max}$ were observed for oligomers containing two A-tracts rather than one, indicating that greater numbers of Li⁺ ions bind to oligomers with two A-tracts.

Identification of the Cation Binding Site. To identify the location of the monovalent cation binding site in the A-tract-containing oligomers, netropsin was added to a solution containing RA-46 and A₄T₁in-62 and titrations were carried out using Tris⁺ as the binding ion. Netropsin binds to DNA in the A-tract minor groove with an affinity in the low nanomolar range, depending somewhat on A-tract sequence (34–39). Surface plasmon resonance measurements (40) have shown that the on rate for netropsin binding to an AATT site is $3.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$; the off rate is $7.5 \times 10^{-2} \text{ s}^{-1}$. Hence, a DNA–netropsin complex is expected to be stable for the duration of a CE measurement. However, because netropsin contains two positively charged residues, netropsin binding decreases the net charge of an A-tract-containing oligomer. Hence, a mixture of RA-46 and the A₄T₁in-62–netropsin complex exhibits two peaks in TPA⁺, a rapidly migrating peak due to RA-46 and a slower peak corresponding to the A₄T₁in-62–netropsin complex (not shown). Within experimental error, the widths of the peaks (full width at half-height) were independent of the TPA⁺:Tris⁺ ratio in the BGE.

The mobility profile observed for oligomer A₄T₁in-62 (without netropsin) is illustrated in the top curve in Figure 3. The mobility difference increased gradually (in absolute value) with an increase in Tris⁺ concentration before leveling off at a plateau value of $-0.012 \pm 0.001 \text{ m.u.}$ at high Tris⁺ concentrations. By contrast, the mobility profile observed for the A₄T₁in-62–netropsin complex was nearly independent of Tris⁺ concentration, as shown by the lower mobility profile in Figure 3. The average value of $\Delta\mu$ observed for the A₄T₁in-62–netropsin complex at Tris⁺ concentrations greater than 15 mM was $-0.028 \pm 0.001 \text{ m.u.}$ The constancy of this value is strong evidence for the integrity of the A₄T₁in-62–netropsin complex at high Tris⁺ concentrations. The conclusion must be drawn that Tris⁺ ions do not bind preferentially to the A₄T₁in-62–netropsin complex, most likely because the minor groove binding site is blocked by netropsin. Similar results are observed with Li⁺ as the binding ion (not shown).

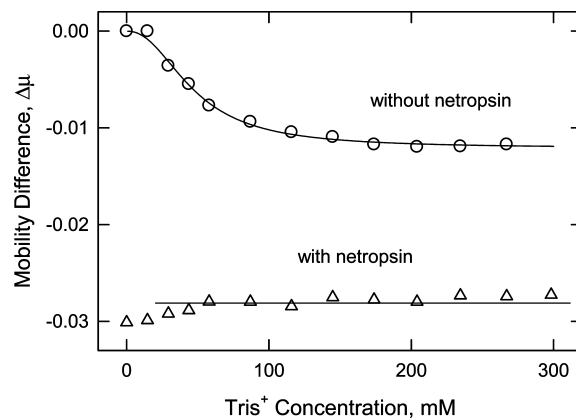


FIGURE 3: Effect of netropsin on the mobility profiles observed for oligomer A₄T₁in-62, using Tris⁺ as the binding ion: (○) without netropsin and (△) with netropsin added to the DNA. The curved line describing the $\Delta\mu$ values of oligomer A₄T₁in-62 (top profile) was calculated from eq 4; the horizontal line (bottom profile) corresponds to the average $\Delta\mu$ observed for the A₄T₁in-62–netropsin complex at $>15 \text{ mM}$ Tris⁺.

Binding of Li⁺ Ions to A-Tracts with Different Sequences. Li⁺ ions were used to investigate monovalent cation binding to A-tracts with different sequences. Thirteen of the tested oligomers failed to bind monovalent cations; the electropherograms observed for these samples exhibited a single peak at all Li⁺ concentrations. The acronyms of these oligomers, which denote their A-tract sequences, are A₃-46, A₃-62, A₄-62, A₅-62, A₄T₂-62, A₃in-62, A₄in-62, A₂T₂in-62, A₁T₄in-62, A₂T₄in-62, A₃T₄in-62, T₂A₂in-62, and T₄A₄in-62. Several conclusions may be drawn from these results. First, A₃-tracts are too short to bind monovalent cations, even when two A₃-tracts occur in phase with the helix repeat. Other studies have also found that an A₃-tract is too short to exhibit the characteristic A-tract structure (16, 41, 42). Second, single A₄- and A₅-tracts do not bind monovalent cations when embedded in an AT-rich oligomer. Third, an A_nT_m sequence motif in a relatively short A-tract must have $n \geq m$, to bind monovalent cations. The combined results suggest that monovalent cation binding is a sensitive reporter of whether a given A/T motif has the potential to form the narrow minor groove characteristic of the A-tract structure.

The ^{app}K_D values determined for the binding of Li⁺ ions to the other tested A-tract-containing oligomers are given in the third columns of Tables 1 and 2. Several trends can be noted within the data. First, the ^{app}K_D values observed for the various oligomers are independent of A-tract phasing, as shown for oligomer pairs A₅in-46 and A₅out-46 (both ^{app}K_D = 71 mM), A₅=T₅in-46 and A₅=T₅out-46 (average ^{app}K_D = $68 \pm 1 \text{ mM}$), A₃T₃in-62 and A₃T₃out-62 (average ^{app}K_D = $75 \pm 10 \text{ mM}$), and A₄T₄in-62 and A₄T₄out-62 (average ^{app}K_D = $59 \pm 5 \text{ mM}$). Second, the ^{app}K_D values are also independent of whether the oligomer contains one or two A-tracts. The ^{app}K_D value observed for oligomer A₅-46 (65 mM) is essentially equal to the ^{app}K_D values observed for oligomers A₅in-46, A₅out-46, A₅=T₅in-46, and T₅=A₅in-46 (average value, $70 \pm 2 \text{ mM}$). In addition, the ^{app}K_D value observed for oligomer A₄T₄-62 (68 mM) is close to the average value observed for oligomers A₄T₄in-62, A₄T₄'in-62, and A₄T₄out-62 (average ^{app}K_D = $59 \pm 4 \text{ mM}$). Third, the ^{app}K_D values depend on A-tract orientation. The ^{app}K_D values observed for oligomers A₅in-46 and A₄T₄in-62 ($67 \pm 3 \text{ mM}$) are smaller than the ^{app}K_D values observed for

oligomers T₅in-46 and A₄T₄/T₄A₄in-62 (both 100 mM). In addition, oligomers A₄T₁in-62 and A₄T₄in-62 bind monovalent cations, while the corresponding oligomers, A₁T₄in-62 and T₄A₄in-62, do not.

Finally, oligomers containing A_n-tracts (e.g., A₄-46, A₆-46, and A₄in-46) have smaller ^{app}K_D values (46 ± 4 mM) than oligomers containing A_nT_n-tracts of equal length (A₂T₂-46, A₃T₃-46, and A₂T₂in-46; average ^{app}K_D = 91 ± 26 mM). This result could be taken to indicate that the ApT step interferes with cation binding. However, oligomer A₄T₁in-62 has the lowest ^{app}K_D of all tested oligomers (24 mM), suggesting that a thymine residue following an A-tract actually facilitates cation binding. It is known that the A-tract minor groove progressively narrows in the 5' → 3' direction, reaching a minimum at 4–5 bp (43–47). Since monovalent cation binding appears to occur preferentially at the narrowest end of the minor groove (46, 47), the relatively deep electrostatic potential at an ApT step (18) may increase the affinity of monovalent cations for binding to an A₄T₁-tract. Oligomers with longer T-tracts might have larger apparent dissociation constants because a T_n-tract following an ApT step widens the minor groove somewhat (46). In agreement with this hypothesis, the average ^{app}K_D observed for oligomers A₄T₂in-62, A₄T₃in-62, A₄T₄in-62, and A₄T₄'in-62 was 63 ± 6 mM, much larger than the ^{app}K_D of 24 mM observed for oligomer A₄T₁in-62.

The plateau value of the mobility differences observed at high Li⁺ concentrations, Δμ_{max}, is a measure of the relative concentration of Li⁺ ions bound to the A-tract minor groove upon saturation of the binding site(s). The Δμ_{max} values are given in the last columns of Tables 1 and 2. Significantly, the Δμ_{max} values observed for oligomers A₄in-46, A₅in-46, A₂T₂in-46, A₃T₃in-46, A₄T₁in-62, A₄T₃in-62, and A₄T₄in-62, each of which contains two A-tracts, were 2.0 ± 0.4 times greater than the Δμ_{max} values observed for the corresponding oligomers with one A-tract: A₄-46, A₅-46, A₂T₂-46, A₃T₃-46, A₄T₁-62, A₄T₄-62, and A₄T₄'-62. Hence, each A-tract binds the same number of monovalent cations upon saturation of the binding site(s). In addition, the ratio of the Δμ_{max} values observed for oligomer pairs A₅in-46 and A₅out-46, A₅=T₅in-46 and A₅=T₅out-46, A₃T₃in-62 and A₃T₃out-62, and A₄T₄in-62 and A₄T₄out-62 was 1.0 ± 0.1. Hence, the extent of monovalent cation binding in the A-tract minor groove at saturation is independent of the phasing of the A-tracts with respect to the helix repeat.

Comparison of the Binding of Different Cations to DNA A-Tracts. To analyze the dependence of cation binding on the type of monovalent cation, mobility profiles were measured for two representative oligomers, A₄T₁in-62 and A₄T₄'in-62, using Li⁺, Tris⁺, NH₄⁺, Na⁺, and K⁺ as the binding ions. The mobility profiles observed for oligomer A₄T₁in-62 are illustrated in panels A and B of Figure 4. The mobility profiles observed in solutions containing Li⁺ or NH₄⁺ ions were nearly hyperbolic in shape, while the mobility profiles observed for the other three cations were clearly sigmoidal. In addition, the Δμ_{max} value observed with NH₄⁺ as the binding ion was ~30% greater than that observed for the other cations. For oligomer A₄T₄'in-62, shown in Figure 5, the mobility profile observed in solutions containing NH₄⁺ as the binding ion was nearly hyperbolic in shape; the mobility profiles observed with Li⁺, Tris⁺, and

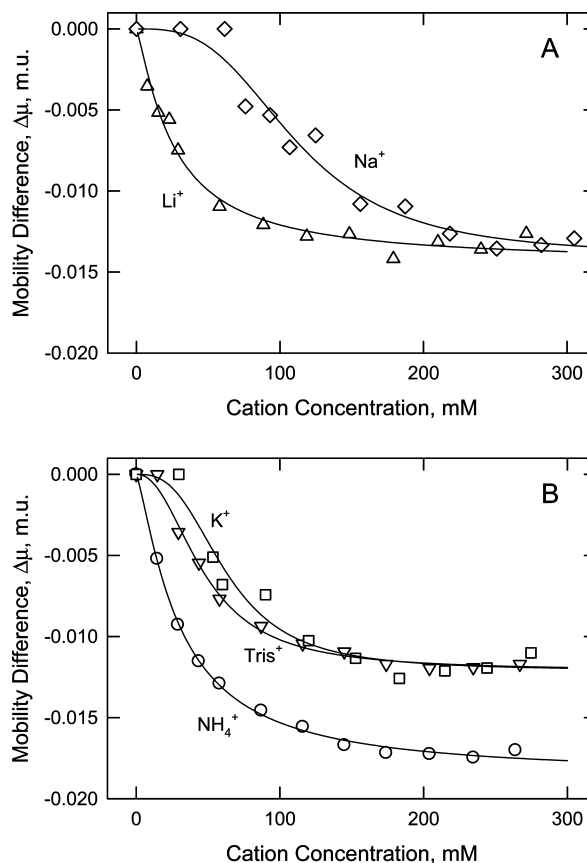


FIGURE 4: Mobility profiles observed for the binding of different cations to oligomer A₄T₁in-62: (A) (Δ) Li⁺ and (◇) Na⁺ and (B) (○) NH₄⁺, (▽) Tris⁺, and (□) K⁺. The curved lines were calculated from eq 4; the fitting parameters are given in Table 3.

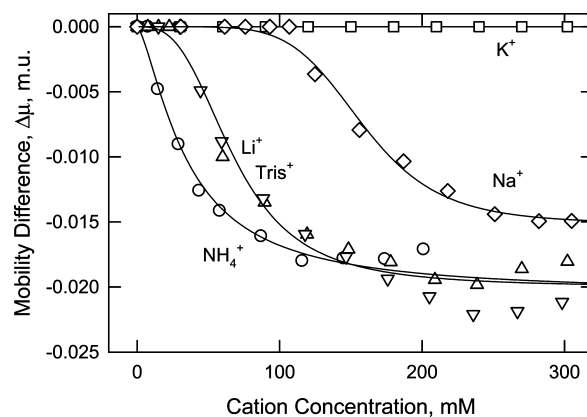


FIGURE 5: Mobility profiles observed for the binding of various cations to oligomer A₄T₄'in-62: (○) NH₄⁺, (Δ) Li⁺, (▽) Tris⁺, (◇) Na⁺, and (□) K⁺. The curved lines were calculated from eq 4; the fitting parameters are given in Table 3.

Na⁺ as the binding ions were sigmoidal. K⁺ ions did not bind to oligomer A₄T₄'in-62 in solutions containing 300 mM cation.

The mobility profiles in Figures 4 and 5 were analyzed using eq 4. The curved lines in Figures 4 and 5 were drawn with these fitting parameters; the ^{app}K_D and Δμ_{max} values and the cooperativity parameters, *n*, are given in Table 3. NH₄⁺, Li⁺, and Tris⁺ ions have similar affinities for binding to oligomer A₄T₁in-62, with an average ^{app}K_D of 34 ± 9 mM; the binding of K⁺ ions is somewhat weaker (^{app}K_D = 63 mM), and the binding of Na⁺ ions is weaker still (^{app}K_D = 112 mM). For oligomer A₄T₄in-62, NH₄⁺ ions have the

Table 3: Comparison of Binding Constants Observed for Different Cations

cation	A ₄ T ₁ in-62			A ₄ T ₄ 'in-62		
	^{app} K _D (mM)	−Δμ _{max} (m.u.)	N	^{app} K _D (mM)	−Δμ _{max} (m.u.)	N
NH ₄ ⁺	30	0.0186	1.3	35	0.0205	1.4
Li ⁺	26	0.0145	1.2	68	0.0200	3.0
Tris ⁺	47	0.0121	2.2	68	0.0200	3.0
Na ⁺	112	0.0140	3.1	159	0.0151	5.9
K ⁺	63	0.0121	2.8	>300	—	—

strongest binding affinity (^{app}K_D = 35 mM), Li⁺ and Tris⁺ ions bind more weakly (^{app}K_D = 68 mM), Na⁺ ions binds still more weakly (^{app}K_D = 159 mM), and K⁺ ions do not bind in solutions containing 300 mM cation. The results are in reasonable agreement with those of Denisov and Halle (48), who found that the level of monovalent cation binding to an A₂T₂-tract decreased in the following order: NH₄⁺ ≫ K⁺ ∼ Rb⁺ ∼ Cs⁺ > Na⁺. However, the results also indicate that monovalent cation binding to DNA A-tracts depends on A-tract sequence as well as the identity of the cation.

The average value of Δμ_{max} observed for the binding of Li⁺, Tris⁺, Na⁺, and K⁺ ions to oligomer A₄T₁in-62 was −0.013 ± 0.001 m.u. The level of binding of NH₄⁺ ions was ∼30% higher, suggesting that this cation may have additional binding sites on this oligomer. For oligomer A₄T₄'in-62, the average value of Δμ_{max} was −0.019 ± 0.002 m.u. (when binding was observed). The combined results indicate that the extent of the mobility decrease observed upon saturation of the binding site(s) varies modestly with A-tract sequence and cation identity.

By contrast, the cooperativity of binding depends significantly on A-tract sequence and cation identity, as shown in columns 4 and 7 of Table 3. The binding of NH₄⁺ ions to both oligomers, and Li⁺ ions to oligomer A₄T₁in-62, was essentially noncooperative, since the cooperativity parameter *n* was close to 1. However, higher *n* values were observed for the other cations, suggesting that cation binding may be coupled with another effect, such as a conformational transition. It is possible that, with TPA⁺ as the only cation in the BGE, many A-tract-containing DNA oligomers exist in an equilibrium between the normal B-form conformation and a conformation with the narrow minor groove characteristic of A-tract-containing oligomers (12, 49, 50); this conformation will be called the B' conformation, for the sake of brevity. If the B' conformation is the only one capable of binding monovalent cations, cation binding would pull the equilibrium toward this conformation. Molecular dynamics (MD) simulations (51–53) have shown that the narrow minor groove characteristic of DNA A-tracts cannot exist for any significant length of time without cations to neutralize part of the negative charge on the phosphate residues; cation binding in the minor groove draws the phosphate residues closer together and stabilizes the B' conformation. The results in Table 3 indicate that some cations, especially NH₄⁺ and Li⁺, are more effective than others in pulling the equilibrium toward (and/or stabilizing) the B' conformation. In addition, some A-tract sequences, such as A₄T₁, bind cations in the minor groove with a higher affinity than other A-tract sequences, suggesting that the equilibrium between the B and B' conformations and/or the effect of monovalent cations on the B ↔ B' conformational equilibrium is sequence-specific.

Concentration of Bound Cations at Saturation. The average mobility difference observed at high cation concen-

trations, Δμ_{max}, can be used to estimate the number of cations bound to the A-tract(s) upon saturation of the binding site(s). The oligomers studied here contained 50 charged phosphate residues, since the 5'-ends were not phosphorylated. The electrophoretic mobility observed for the random-sequence oligomer RA-46 in the Tris-DM buffers used here was 1.570 m.u. or, equivalently, 0.0314 m.u. per charged residue. However, counterion condensation theory (54) suggests that the effective charge of DNA is reduced to 24% of the formal charge in solutions containing monovalent cations. Therefore, the mobility observed for oligomer RA-46 corresponds to 0.0075 m.u. per charged residue (0.0314 m.u. × 0.24). The average mobility difference observed for the A₄T₁in-62–netropsin complex at high Tris⁺ concentrations was −0.028 ± 0.001 m.u. (Figure 3), or −0.014 m.u. per A-tract. The observed mobility reduction is therefore equivalent to a decrease of two charges per A-tract. Since each netropsin contains two positive charges, one netropsin is bound to each A-tract. A similar 1:1 binding stoichiometry has also been observed by others (36–39, 55).

The average value of Δμ_{max} observed for the binding of Li⁺, Tris⁺, NH₄⁺, Na⁺, and K⁺ ions to oligomers A₄T₁in-62 and A₄T₄'in-62 (Table 3) was −0.016 ± 0.003 m.u. (when binding was observed) or, equivalently, −0.008 m.u. per A-tract. From the above discussion, this mobility difference corresponds to the binding of 1.1 ± 0.2 cations to each A-tract upon saturation of the binding site(s).

CONCLUDING REMARKS

The results described here have shown that monovalent cations bind to the A-tract minor groove, because the binding site can be blocked by netropsin, a minor groove binding drug. Not all A-tracts bind monovalent cations: A₃-tracts are too short, and isolated A₄- and A₅-tracts, especially when embedded in AT-rich oligomers, often do not bind cations. The ^{app}K_D values observed for oligomers that do bind cations in the minor groove indicate that monovalent cation binding is sequence-specific. The ^{app}K_D values are independent of the number of A-tracts in the oligomer and the phasing of the A-tracts but vary with A-tract orientation and the presence or absence of an ApT step in the A-tract. In addition, oligomers containing A_{2n}-tracts exhibit a higher affinity for monovalent cations than oligomers containing A_nT_n-tracts. The Δμ_{max} values, which indicate the extent of cation binding upon saturation of the binding site(s), are 2-fold larger when an oligomer contains two A-tracts rather than one; therefore, each A-tract binds the same number of cations at saturation. The affinity of monovalent cation binding depends both on A-tract sequence and on cation identity. When binding is observed, the affinity decreases in the following order: NH₄⁺ ∼ Li⁺ ∼ Tris⁺ > K⁺ > Na⁺. Finally, differences in the cooperativity of binding to various A-tracts suggest that

monovalent cations can shift the equilibrium between the normal B conformation and the B' conformation toward the latter, leading to the narrow minor groove structure characteristic of DNA A-tracts.

REFERENCES

- Halle, B., and Denisov, V. P. (1998) Water and monovalent ions in the minor groove of B-DNA oligonucleotides as seen by NMR. *Biopolymers* 48, 210–233.
- McFail-Isom, L., Sines, C. C., and Williams, L. D. (1999) DNA structure: Cations in charge? *Curr. Opin. Struct. Biol.* 9, 298–304.
- McConnell, K. J., and Beveridge, D. L. (2000) DNA structure: What's in charge? *J. Mol. Biol.* 304, 803–820.
- Hud, N. V., and Plavec, J. (2003) A unified model for the origin of DNA sequence-directed curvature. *Biopolymers* 69, 144–159.
- Marincola, F. C., Casu, M., Saba, G., and Lai, A. (2001) ^{23}Na MNR relaxation studies of the Na-DNA/drug interaction. *ChemPhysChem* 2, 569–575.
- Egli, M. (2002) DNA-cation interactions: Quo vadis? *Chem. Biol.* 9, 277–286.
- Subriana, J. A., and Soler-López, M. (2003) Cations as hydrogen bond donors: A view of electrostatic interactions in DNA. *Annu. Rev. Biophys. Biomol. Struct.* 32, 27–45.
- Tereshko, V., Minasov, G., and Egli, M. (1999) A 'hydrat-ion' spine in a B-DNA minor groove. *J. Am. Chem. Soc.* 121, 3590–3595.
- Woods, K. K., McFail-Isom, L., Sines, C. C., Howerton, S. B., Stephens, R. K., and Williams, L. D. (2000) Monovalent cations sequester within the A-tract minor groove of [d(CGCGAATTCGCG)]₂. *J. Am. Chem. Soc.* 122, 1546–1547.
- Howerton, S. B., Sines, C. C., Vanderveer, D., and Williams, L. D. (2001) Locating monovalent cations in the grooves of B-DNA. *Biochemistry* 40, 10023–10031.
- Marincola, F. C., Denisov, V. P., and Halle, B. (2004) Competitive Na^+ and Rb^+ binding in the minor groove of DNA. *J. Am. Chem. Soc.* 126, 6739–6750.
- Dickerson, R. E., and Drew, H. R. (1981) Structure of a B-DNA dodecamer. II. Influence of base sequence on helix structure. *J. Mol. Biol.* 149, 761–786.
- Shui, X., McFail-Isom, L., Hu, G. G., and Williams, L. D. (1998) The B-DNA dodecamer at high resolution reveals a spine of water on sodium. *Biochemistry* 37, 8341–8355.
- Shui, X., Sines, C. C., McFail-Isom, L., VanDerveer, D., and Williams, L. D. (1998) Structure of the potassium form of CGCGAATTCGCG: DNA deformation by electrostatic collapse around inorganic cations. *Biochemistry* 37, 16877–16887.
- Young, M. A., Jayaram, B., and Beveridge, D. L. (1997) Intrusion of counterions into the spine of hydration in the minor groove of B-DNA: Fractional occupancy of electronegative pockets. *J. Am. Chem. Soc.* 119, 59–69.
- Snoussi, K., and Leroy, J.-L. (2002) Alteration of A·T base-pair opening kinetics by the ammonium cation in DNA A-tracts. *Biochemistry* 41, 12467–12474.
- Ponomarev, S. Y., Thayer, K. M., and Beveridge, D. L. (2004) Ion motions in molecular dynamics simulations on DNA. *Proc. Natl. Acad. Sci. U.S.A.* 101, 14771–14775.
- Lavery, R., and Pullman, B. (1985) The dependence of the surface electrostatic potential of B-DNA on environmental factors. *J. Biomol. Struct. Dyn.* 2, 1021–1032.
- Das, R., Mills, T. T., Kwok, L. W., Maskel, G. S., Millett, I. S., Doniach, S., Finkelstein, K. D., Herschlag, D., and Pollack, L. (2003) Counterion distribution around DNA probed by solution X-ray scattering. *Phys. Rev. Lett.* 90, 188103.
- Várnai, P., and Zakrzewska, K. (2004) DNA and its counterions: A molecular dynamics study. *Nucleic Acids Res.* 32, 4269–4280.
- Stellwagen, N. C., Magnúsdóttir, S., Gelfi, C., and Righetti, P. G. (2001) Preferential counterion binding to A-tract DNA oligomers. *J. Mol. Biol.* 305, 1025–1033.
- Stellwagen, E., Lu, Y. J., and Stellwagen, N. C. (2005) Curved DNA molecules migrate anomalously slowly in free solution. *Nucleic Acids Res.* 33, 4425–4432.
- Stellwagen, N. C., Magnúsdóttir, S., Gelfi, C., and Righetti, P. G. (2001) Measuring the translational diffusion coefficients of small DNA molecules by capillary electrophoresis. *Biopolymers* 58, 390–397.
- Lu, Y. J., and Stellwagen, N. C. (2008) Monovalent cation binding by curved DNA molecules containing variable numbers of A-tracts. *Biophys. J.* 94, 1719–1725.
- Stellwagen, E., Dong, Q., and Stellwagen, N. C. (2007) Quantitative analysis of monovalent counterion binding to random-sequence, double-stranded DNA using the Replacement Ion method. *Biochemistry* 46, 2050–2058.
- Stellwagen, E., and Stellwagen, N. C. (2002) The free solution mobility of DNA in Tris-acetate-EDTA buffers of different concentrations, with and without added NaCl. *Electrophoresis* 23, 1935–1941.
- Stellwagen, E., and Stellwagen, N. C. (2003) Probing the electrostatic shielding of DNA with capillary electrophoresis. *Biophys. J.* 84, 1855–1866.
- Tan, Z.-J., and Chen, S. J. (2006) Ion-mediated nucleic acid helix-helix interactions. *Biophys. J.* 91, 518–536.
- Holmes, D. L., and Stellwagen, N. C. (1991) Estimation of polyacrylamide gel pore size from Ferguson plots of normal and anomalously migrating DNA fragments I. Gels containing 3% N,N'-methylenebisacrylamide. *Electrophoresis* 12, 253–263.
- Dong, Q., Stellwagen, E., Dagle, J. M., and Stellwagen, N. C. (2003) Free solution mobility of small single-stranded oligonucleotides with variable charge densities. *Electrophoresis* 24, 3323–3329.
- Stellwagen, N. C., Gelfi, C., and Righetti, P. G. (1997) The free solution mobility of DNA. *Biopolymers* 42, 687–703.
- Williams, B. A., and Vigh, G. (1996) Fast, accurate mobility determination method for capillary electrophoresis. *Anal. Chem.* 68, 1174–1180.
- Wyman, J., and Gill, S. J. (1990) *Binding and Linkage*, University Science Books, Mill Valley, CA.
- Marky, L. A., and Breslauer, K. J. (1987) Origins of netropsin binding affinity and specificity: Correlations of thermodynamic and structural data. *Proc. Natl. Acad. Sci. U.S.A.* 84, 4359–4363.
- Ward, B., Rehfuess, R., Goodisman, J., and Dabrowiak, J. C. (1988) Determination of netropsin-DNA binding constants from footprinting data. *Biochemistry* 27, 1198–1205.
- Rentzperis, D., and Marky, L. A. (1995) Interaction of minor groove ligands to an AAATT/AATTT site: Correlation of thermodynamic characterization and solution structure. *Biochemistry* 34, 2937–2945.
- Lah, J., and Vesnaver, G. (2004) Energetic diversity of DNA minor-groove recognition by small molecules displayed through some model ligand-DNA systems. *J. Mol. Biol.* 342, 73–80.
- Freyer, M. W., Buscaglia, R., Chasman, D., Hyslop, S., Wilson, W. D., Chaires, J. B., and Lewis, E. A. (2007) Binding of netropsin to several DNA constructs: Evidence for at least two different 1:1 complexes formed from an -AATT-containing ds-DNA construct and a single minor groove binding ligand. *Biophys. Chem.* 126, 186–196.
- Degtyareva, N. N., Fresia, M. J., and Petty, J. T. (2007) DNA conformational effects on the interaction of netropsin with A-tract sequences. *Biochemistry* 46, 15136–15143.
- Nguyen, B., Tanious, F. A., and Wilson, W. D. (2007) Biosensor-surface plasmon resonance: Quantitative analysis of small molecule-nucleic acid interactions. *Methods* 42, 150–161.
- Diekmann, S. (1986) Sequence specificity of curved DNA. *FEBS* 3244, 53–56.
- Koo, H.-S., Wu, H.-M., and Crothers, D. M. (1986) DNA bending at adenine·thymine tracts. *Nature* 329, 501–506.
- Katahira, M., Sugeta, H., Kyogoku, Y., Fujii, S., Fujisawa, R., and Tomita, K. (1988) One- and two-dimensional NMR studies of DNA containing the oligo(dA)oligo(dT) tract. *Nucleic Acids Res.* 16, 8619–8632.
- Katahira, M., Sugeta, H., and Kyogoku, Y. (1990) A new model for the bending of DNAs containing oligo(dA) tracts based on NMR observations. *Nucleic Acids Res.* 18, 613–618.
- Chuprina, V. P., Lipanov, A. A., Fedoroff, O. Y., Kim, S.-G., Kintanar, A., and Reid, B. R. (1991) Sequence effects on local DNA topology. *Proc. Natl. Acad. Sci. U.S.A.* 88, 9087–9091.
- Steffl, R., Wu, H., Ravindranathan, S., Sklenář, V., and Feigon, J. (2004) DNA A-tract bending in three dimensions: Solving the dA4T4 vs. dT4A4 conundrum. *Proc. Natl. Acad. Sci. U.S.A.* 101, 1177–1182.
- Hud, N. V., Sklenář, V., and Feigon, J. (1999) Localization of ammonium ions in the minor groove of DNA duplexes in solution and the origin of DNA A-tract bending. *J. Mol. Biol.* 286, 651–660.

48. Denisov, V. P., and Halle, B. (2000) Sequence-specific binding of counterions to B-DNA. *Proc. Natl. Acad. Sci. U.S.A.* 97, 629–633.
49. Nelson, H. C. M., Finch, J. T., Luisi, B. F., and Klug, A. (1987) The structure of an oligo(dA) • oligo(dT) tract and its biological implications. *Nature* 330, 221–226.
50. DiGabriele, A. D., and Steitz, T. A. (1993) A DNA dodecamer containing an adenine tract crystallizes in a unique lattice and exhibits a new bend. *J. Mol. Biol.* 231, 1024–1039.
51. Feig, M., and Pettitt, B. M. (1999) Sodium and chlorine ions as part of the DNA solvation shell. *Biophys. J.* 77, 1769–1781.
52. Hamelberg, D., McFail-Isom, L., Williams, L. D., and Wilson, W. D. (2000) Flexible structure of DNA: Ion dependence of minor-groove structure and dynamics. *J. Am. Chem. Soc.* 122, 10513–10520.
53. Hamelberg, D., Williams, L. D., and Wilson, W. D. (2001) Influence of the dynamic positions of cations on the structure of the DNA minor groove: Sequence-dependent effects. *J. Am. Chem. Soc.* 123, 7745–7755.
54. Manning, G. S. (1978) The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Q. Rev. Biophys.* 11, 179–246.
55. Goodsell, D. S., Kopka, M. L., and Dickerson, R. D. (1995) Refinement of netropsin bound to DNA: Bias and feedback in electron density map interpretation. *Biochemistry* 34, 4983–4993.

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