Modeling the Electrophoresis of Short Duplex DNA: Counterions $K^+$ and Tris$^+$

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The electrophoretic mobility of short 18 and 20 bp duplex DNAs is modeled by an iterative boundary element procedure that numerically solves the coupled Poisson, low Reynolds Number Navier–Stokes, and ion transport equations. Both capped cylinder (CC) and “detailed” models derived from the secondary structure of the fragments are examined. Translation–rotation coupling is examined with regard to the transport of the detailed models, and it is concluded that this coupling has very little effect on either diffusion or electrophoresis. When the buffer consists primarily of KCl, the calculated mobility is about 4–6% larger than the experimental mobility for either the CC or “detailed” models, but when the buffer is Tris acetate, the discrepancy is significantly larger. This indicates that there is an association between Tris$^+$ and DNA beyond the classical electrostatic interactions accounted for in modeling. For 18 bp DNA in 0.04 M Tris acetate, a model in which the phosphate charges of DNA are reduced from $-1.0$ to $-0.45$ gives good agreement with experiment. Alternatively, a model in which 40% of the DNA phosphates are neutralized by Tris$^+$ cations specifically bound to the fragment also gives a mobility consistent with experiment.

Introduction

By the simple act of applying a voltage gradient across an aqueous mixture of biomolecules, different species in the mixture are subjected to different external forces due to the variation in charge the biomolecules, or polyions, carry. This, in turn, results in different species migrating at different rates and is the basis of all electrophoretic methods. Because of its simplicity and power, electrophoresis has become an invaluable separation technique in chemistry, biochemistry, and molecular biology. Although it is easy to understand and predict qualitatively the migration behavior of a particular polyion on the basis of its charge, it is much more difficult to predict quantitatively how fast that polyion will migrate under well-defined conditions as a result of the complex interplay of polyion, solvent, buffer and salt ions, and (if present) the gel support medium. As a consequence of this, electrophoresis has not been widely used to answer structural questions such as the size, conformation, and charge of a biomolecule and the complexes it forms with other species. Over the past few years, advances in both experiment and theory have revived interest in the potential use of electrophoresis as a structural probe. The introduction of free solution capillary electrophoresis\(^1\) and membrane-confined analytical electrophoresis\(^2\) have removed the complicating feature of the gel support medium, which has made interpretation more straightforward. It is also possible to carry these experiments out under conditions of low polyion concentration and low external field strength, which has simplified the problem further. In particular, a number of groups have examined the electrophoresis of short duplex DNAs of varying length under a variety of salt/buffer conditions.\(^3\)–\(^6\) There exists an extensive theory of electrophoresis summarized in a number of recent reviews.\(^7\)–\(^10\) In the present work, we shall be primarily interested in theory that is relevant to the following conditions: (a) dilute aqueous solutions of macromolecules of complex shape and charge distribution, (b) macromolecules that are not small compared to the thickness of the ion atmosphere that surrounds them, (c) low external field strengths, (d) the absence of a gel support medium, and (e) the macromolecules may be highly charged. Overbeek\(^11\) gave the general formulation of the coupled steady-state hydrodynamic and electrodynamic equations for the electrophoretic transport of a sphere with a weak, centrosymmetric charge distribution of uniform electrostatic surface potential, $\zeta$. His work showed that ion relaxation, the distortion of the ion atmosphere from its equilibrium distribution, starts with the nonlinear terms in the electrostatic potential ($\zeta^2$ and higher order terms). Thus, ion relaxation will be important if the polyion is highly charged, and the nonlinear Poisson–Boltzmann equation rather than the linearized version thereof is necessary to adequately describe the equilibrium electrostatic potential in the vicinity of the polyion. With the work of others,\(^12\)–\(^14\) the electrophoretic transport of spheres of uniform charge $\zeta$ is now well understood, and Stigter\(^15\),\(^16\) has carried out a similar analysis of long rods. In the past few years, the boundary element methodology has made it possible to model the electrophoretic transport of macromolecules of arbitrary shape and charge distribution.\(^17\) This methodology has been applied to a number of problems, including lysozyme,\(^18\) short DNA fragments,\(^19\) and RNAase.\(^20\) In a previous study of short DNA fragments, it was concluded that the electrophoretic mobility of a short DNA fragment was adequately explained by modeling if the salt solution was KCl, but not in Tris acetate.

In the present work, we would like to examine more closely the predicted behavior of DNA in KCl and Tris acetate to better understand why there are differences in the electrophoretic mobility under different salt/buffer conditions. More realistic models of the DNA fragments based on the secondary structure will be examined in an attempt to identify specific structural models that explain the observed mobilities and also to identify those structural features that influence mobility. The work of Stellwagen and co-workers is of particular relevance to the present work since it explores the nature of the complexes DNA...
forms with buffer ions that are invariably present in any electrophoresis experiment.\textsuperscript{3,21} They conclude that complex formation occurs not only between DNA and buffer, but between DNA and neutral salts such as histidine as well. The nature of the poorly understood interaction between DNA and buffer is of great practical importance since the buffer composition can greatly influence band distortion and splitting in electrophoresis.\textsuperscript{4}

The outline of this paper is as follows. In the next section, a brief general review of the boundary element methodology is provided and the coupling of the various transport equations described. We then describe the detailed DNA models and how they are constructed. The Results section is subdivided into two parts. The first subsection discusses the electrophoresis of short DNA in KCl and the second the more complex problem of DNA in Tris acetate. In the latter subsection, some detailed models are considered which contain Tris\textsuperscript{+} cations specifically “bound” to DNA in our attempt to reproduce the experimental mobility. Finally, we summarize the main conclusions of the present study.

Modeling

A. Continuum Transport Model. In the boundary element (BE) modeling procedure, the polyion surface is represented as a structure consisting of \(N\) interconnected plates, and the assumption is made that physical quantities such as electrostatic potential and hydrodynamic stress force are uniform over individual plates. Systematic error arising from discretizing the surface into a finite number of plates can be accounted for by carrying out a series of calculations in which \(N\) is varied and extrapolating to the limit \(1/N \to 0.\textsuperscript{19,22}\) The resulting transport properties obtained in this way are called the “extrapolated shell” values. In past studies of the electrophoretic mobility of short DNA fragments, the fragments were modeled as capped cylinders designed to reproduce experimental translational diffusion constants. The upper object in Figure 1 consists of 96 plates designed to reproduce the experimental mobility. For clay particles and also certain micelles, the shear surface is located at \(1 \pm 1\) \(\text{Å}\) from the particle/water interface.\textsuperscript{23} In the fluid domain exterior to \(S_h\), the fluid is assumed to obey the low Reynolds Number Navier–Stokes and solvent incompressibility equations

\[
\eta \nabla^2 \mathbf{v}(\mathbf{x}) - \nabla p(\mathbf{x}) = -s(\mathbf{x}) \quad (1a)
\]

\[
\nabla \cdot \mathbf{v}(\mathbf{x}) = 0 \quad (1b)
\]

where \(\mathbf{v}(\mathbf{x})\) and \(p(\mathbf{x})\) are the local fluid velocity and pressure at \(\mathbf{x}\) and \(s(\mathbf{x})\) is the force per unit volume on the fluid at \(\mathbf{x}\) due to external forces. It shall be assumed further that on \(S_h\), the fluid obeys “stick” hydrodynamic boundary conditions, or in other words, the fluid and particle velocities match on \(S_h\).

To generate \(S_h\), we start with an all-atom representation of the polyion. How this structure is generated is discussed in the next section. For simplicity, a uniform hydrodynamic radius, \(\sigma_h\), is assigned to all atoms of the polyion. Then a probe sphere of the same radius is “rolled” over the polyion\textsuperscript{24} to produce a candidate for \(S_h\); call it \(S^\ast\). A particular choice of \(\sigma_h\) uniquely defines \(S^\ast\), and once \(S^\ast\) is defined, the translational diffusion constant, \(D_h\), for that structure can be estimated as discussed later. That choice of \(\sigma_h\) which yields the experimental \(D_h\) can be deduced by iteration, and this also serves to define \(S_h\).

To calculate an electrophoretic mobility from a modeling study, it is necessary to know the hydrodynamic and electrical forces acting on the polyion, which, in turn, requires knowledge of the fluid velocity field around the polyion and the hydrodynamic stress forces on \(S_h\). From eq 1a, however, it is seen that this also requires knowledge of \(s(\mathbf{x})\). In the present case, we anticipate that these forces arise primarily from the interactions of the charge contained in the fluid with the local electric field. Under these conditions, \(s(\mathbf{x}) = -\rho(\mathbf{x})\nabla \Lambda(\mathbf{x})\), where \(\rho(\mathbf{x})\) is the charge density and \(\Lambda(\mathbf{x})\) is the electrodynamic potential at position \(\mathbf{x}\) in the fluid domain.

To determine \(\Lambda(\mathbf{x})\), we divide space into a region of low dielectric constant, \(\epsilon_i\), characteristic the polyion interior, and a high dielectric region, \(\epsilon_o\), characteristic of the bulk fluid. Let the surface \(S_d\) denote the boundary separating the low and high dielectric regions. Let \(\rho(\mathbf{x})\) denote the fixed charge density of the polyion interior at position \(\mathbf{x}\). In the present work, this charge distribution is discrete and can be represented as a sum of delta functions. In general, the local charge density, \(\rho(\mathbf{x})\), is related to the electrodynamic potential, \(\Lambda(\mathbf{x})\), by Poisson’s equation\textsuperscript{25}

\[
\nabla(\epsilon(\mathbf{x})\nabla \Lambda(\mathbf{x})) = -4\pi \rho(\mathbf{x}) \quad (2)
\]

both inside and outside of \(S_d\). On \(S_d\), the normal derivative of \(\Lambda\) is discontinuous but satisfies the boundary condition

\[
\epsilon_i (\nabla \Lambda(\mathbf{x}_i) \cdot \mathbf{n})_{\text{interior}} = \epsilon_o (\nabla \Lambda(\mathbf{x}_e) \cdot \mathbf{n})_{\text{exterior}} \quad (3)
\]

where \(\mathbf{x}_i\) is a point on \(S_d\), \(\mathbf{n}\) is a local outward unit normal to \(S_d\) into the domain characterized by dielectric constant \(\epsilon_o\) at \(\mathbf{x}_e\), and the “interior” (“exterior”) subscripts indicate that the derivative is evaluated interior (exterior) to \(S_d\).
Past studies of spherical polyions\textsuperscript{13,14} as well as detailed model studies of lysozyme\textsuperscript{18} have shown that the electrophoretic mobility is insensitive to the choice of the interior dielectric constant, $\varepsilon_i$. Because of this, we can expect the electrophoretic mobility to depend only weakly on the choice of $\varepsilon_i$ or the precise definition of $S_d$. In the present work, we set $\varepsilon_i = 4.26.27$ and the exterior dielectric constant, $\varepsilon_o$, to the value appropriate for water (80.36 and 78.3 at 20 °C and 25 °C, respectively).\textsuperscript{28} To generate the surface $S_d$, we follow a procedure similar to that discussed above for $S_b$. $S_d$ is equated to a solvent-accessible surface that is generated by “rolling” a probe sphere of radius $\sigma_o$ over the poliony surface.\textsuperscript{28} A radius of 1.4 Å is chosen, which corresponds to the distance of closest approach of the oxygen atoms in water.\textsuperscript{29} For the poliony atoms, a wide range of choices are available such as the Pauling van der Waals radii\textsuperscript{30} or the PARSE radii.\textsuperscript{31} In the present work, we adopt a uniform radius of $\sigma_o = 1.4$ Å since this approach is simple, physically reasonable, and undoubtedly adequate for the problem at hand.

For highly charged polyions such as DNA fragments, the distortion of the ion atmosphere from its equilibrium value in response to a perturbing electric and/or flow field must be accounted for in the calculation of electrostatic mobilities.\textsuperscript{11} In addition to the Navier–Stokes (eq 1) and Poisson (eq 2) equations, it is necessary to solve a steady-state ion transport equation for each ion present as well

\[
\nabla \cdot \mathbf{j}_a = 0 \quad (4a)
\]

\[
\mathbf{j}_a = n_a \mathbf{v} - D_a \nabla n_a + \frac{n_a D_a \mathbf{f}_a}{k_B T} \quad (4b)
\]

where $\mathbf{j}_a$ is the local current density of species $\alpha$, $n_a$ the local concentration of that species, $\mathbf{v}$ the fluid velocity, $D_a$ the diffusion constant of an $\alpha$ ion, $\mathbf{f}_a$ the local external force on an $\alpha$ ion, $k_B$ Boltzmann’s constant, and $T$ the absolute temperature. It should be emphasized that eqs 1, 2, and 4 are coupled and must be solved simultaneously. This is done by an iterative boundary element procedure described in detail elsewhere.\textsuperscript{17}

B. Generation of Atomic Structures of Duplex DNA Fragments. The atomic structures of the duplex DNA fragments (both 18-mer and 20-mer) are generated using the BIOPOLYMERIC/BUILD module of the SYBYL commercial molecular mechanics package.\textsuperscript{32} Hydrogens are added, and charges are assigned using the Gasteiger-Huckel method.\textsuperscript{33} The resulting structure is refined using MAXIMIN2, SYBYL’s energy minimizer interfaced with the standard TRIPOS force field.\textsuperscript{34} Energy minimization is carried out following the Powell method,\textsuperscript{35} which belongs to the conjugate gradient family of descent methods. During the minimization procedure, the total energy occasionally increases from one step to the next, but the conformational energy, in general, converges after approximately 2000 iterations. The coordinates are translated and rotated so that the poliony center of mass is located at the origin of a molecule-fixed reference frame and the helix axis is oriented along the $x$ axis of this reference frame.

In some of the modeling of the 18 bp DNA fragment, Tris\textsuperscript{+} cations are explicitly included in the structure to mimic specific binding of counterions (Tris\textsuperscript{+} = C(CH\textsubscript{3}OH)\textsubscript{3}NH\textsubscript{+}). The cation is generated and minimized using SYBYL as described above for the DNA fragments. Then a Tris\textsuperscript{+} is manually docked near the DNA with the amino nitrogen of Tris\textsuperscript{+} to the oxygens of a particular phosphate group on the DNA. Optimization of the resulting structure is then carried out using the ANNEAL function of SYBYL and convergence of the resulting structure is achieved after approximately 10 000 iterations. This procedure is then repeated for additional Tris\textsuperscript{+}. Between 14 and 22 Tris\textsuperscript{+} were docked to the 18bp DNA in this manner.

For BE modeling, a simplified charge distribution is used for the poliony interior. At the positions of the O1P and O2P atoms of DNA, charges of $-0.5$ (in protonic units) are placed, and in models containing Tris\textsuperscript{+} explicitly, and $+1.0$ charges are placed at the positions of the amino nitrogen. It is occasionally necessary to displace these charges in order to ensure they lie inside the poliony surface, $S_d$. How this surface is defined is described in the following subsection. To determine if some position vector, $\mathbf{s}$, lies inside or outside $S_d$, we apply Gauss’s law and numerically evaluate a surface integral. Let $\mathbf{x}$ be some point on $S_d$, and define $y = x - s$ and $\gamma = |y|$. From the property $\mathbf{\nabla} \cdot (1/4\pi \gamma) = -\delta(x - s)$, where $\delta$ is the delta function, integrating this over the interior domain of the poliony, $V_m$, and making use of Gauss’s law to convert a volume integral to a surface integral,\textsuperscript{25} one obtains

\[
\Phi(S_d, s) = \oint_{S_d} \frac{\mathbf{\nabla} \cdot \mathbf{n}(x)}{4\pi \gamma} \, dS_x
\]

where $\mathbf{n}(x)$ is the outward unit normal to $S_d$ (pointing out of $V_m$ and into the fluid domain), and $\Phi(S_d, s)$ equals 1 if $s$ lies within $V_m$ and it equals 0 otherwise. If a charge lies outside $S_d$, it is retracted (moved inward toward the helix axis of the fragment) until $\Phi(S_d, s)$ nearly equals one.

C. Plate Structures for Detailed Models of Duplex DNA Fragments. The surface generation procedure is begun by first constructing a “protocylinder”, and an example is shown in Figure 2. It consists of $n_b$ “barrels”, and each barrel contains $n_c$ triangular plates. In addition, the ends of the protocylinder are capped by $n_c$ triangular plates so that the total number of plates comprising the structure is

\[
N = n_b n_c + 2n_c
\]

For the example shown in Figure 1, $n_b = 20$, $n_c = 16$, and $N = 352$. In the present work, we choose to set $n_b$ equal to the number of base pairs in the DNA fragment being modeled and also to set the barrel length to a uniform spacing of $h = 3.4$ Å. Origin vectors, $\mathbf{o}_j$ ($j = 1$ to $n_b-1$) are defined and placed at the edges of each barrel except for the edges formed by the endcaps. If the axis of the protocylinder lies along the $x$ axis and the protocylinder is centered at the origin, then the $y$ and $z$ components of $\mathbf{o}_j$ equal zero, and $\mathbf{o}_j = h(j - n_b/2)$. Three position vectors define the vertices of each triangular plate in the structure. Let $\mathbf{y}_{mk}$ denote the $m$-th ($m = 1-3$) position vector associated with plate $k$ ($k = 1$ to $N$), and to each $\mathbf{y}_{mk}$ is assigned an origin vector. All $\mathbf{y}_{mk}$’s associated with plates comprising

Figure 2. Protocylinder. This cylinder model is used to derive a SAS surface. Points on the surface of the cylinder are retracted until overlap with any atomic coordinate occurs.
the endcaps are assigned the nearest of origins 1 or $n_b - 1$, and the origins assigned to all other plate vectors satisfy the condition \((\mathbf{ o})_k = (y_{mk})_o\). Also, let \(p_{mk}\) denote the unit vector extending from the origin assigned to the \(m\)-th position vector of plate \(k\) to \(y_{mk}\).

To define some polyion surface, \(S\), we begin by choosing some point along

\[ r_{mk}(\lambda) = \mathbf{ o}_j + \lambda p_{mk} \]  

(7)

where \(\mathbf{ o}_j\) is the origin vector assigned to \(y_{mk}\) (or \(p_{mk}\)) and \(\lambda\) is a scaling factor to be determined. Let \(\sigma_i\) be some exclusion radius of the \(i\)-th atom in the all-atom representation of the polyion and \(\sigma_{\text{probe}}\) some “probe” (solvent or ion) radius. In the generation of \(S\), for example, \(\sigma_i = \sigma_{\text{probe}} = 1.4\) Å. Starting with a fairly large value of \(\lambda\) (20 Å in the case of DNA), we scan all atoms to see if \((r_1 - r_{mk}(\lambda))^2 > (\sigma_i + \sigma_{\text{probe}})^2\). If this condition is satisfied, then \(\lambda\) is reduced, and the procedure continued until a \(\lambda\) is found where “overlap” first occurs. This defines a particular position on the polyion surface and is done to an accuracy of about 0.1 Å. The above procedure is repeated for all possible plate vectors in the structure. Physically, one can view the procedure as placing the polyion in a large protocylinder and then “deflating” the protocylinder (along the \(p_{mk}\) vectors) until the deflated surface conforms closely to the actual polyion surface. The lower object in Figure 1 was generated in this manner for 20 bp DNA.

D. Transport Properties of Detailed Model Structures.

The detailed DNA models considered in the present work are complex in shape, and care must be exercised in determining their transport properties. Given the propeller-like quality of the detailed DNA models (lower object in Figure 1), it can be expected that translation parallel to the helix axis, for example, induces a rotational motion of the model structure. This “translation—rotation coupling” and the consequences it has on resistance and mobility of uncharged model structures has been discussed in detail by Happel and Brenner\(^{36}\) and others.\(^{37-38}\) A brief but lucid description of the problem is given by Kim and Karilla.\(^{39}\) Consider first a “case 1” transport problem in which the overall motion of the model can be decomposed into a uniform rotation with angular velocity \(\omega\) about some body fixed origin, \(\mathbf{ o}\), and a uniform translation (of point \(\mathbf{ o}\) fixed within the particle) with velocity \(\mathbf{ u}_o\). It is assumed the fluid is at rest except for the perturbation produced by the motion of the particle itself and its associated ion atmosphere. The total force, \(\mathbf{ z}^{(1)}\), and total torque, \(\mathbf{ t}_o^{(1)}\), exerted by the particle on the surrounding fluid are related to \(\mathbf{ u}_o\) and \(\mathbf{ o}\) by\(^{37}\)

\[ \mathbf{ z}^{(1)} = \mathbf{ z}_r \mathbf{ u}_o + \mathbf{ z}_o^{T} \cdot \mathbf{ o} \]  

(8a)

\[ \mathbf{ t}_o^{(1)} = \mathbf{ t}_o^{T} \mathbf{ u}_o + \mathbf{ t}_o^{T} \cdot \mathbf{ o} \]  

(8b)

where \(\mathbf{ z}_r\), \(\mathbf{ z}_o\), and \(\mathbf{ t}_o\) are the translation, rotation, and translation—rotation coupling resistance tensors, the “\(r\)” subscript on certain vector and tensor quantities denotes that they are origin dependent, and the “\(T\)” superscript denotes transposition. The total forces and torques can be determined by the BE procedure.\(^{35}\) By translating (but not rotating) along three orthogonal directions and then rotating (but not translating) about three orthogonal directions, it is straightforward to compute the three tensors \(\mathbf{ z}_r\), \(\mathbf{ z}_o\), and \(\mathbf{ t}_o\). The connections between these resistance tensors and the corresponding mobility or diffusion tensors have been established for uncharged model structures.\(^{36-39}\)

We can anticipate that these resistance—mobility relationships will also be valid for charged biomolecules even though charge effects will alter the \(\mathbf{ z}_r\), \(\mathbf{ z}_o\), and \(\mathbf{ t}_o\) tensors.

To adequately treat steady-state electrophoresis, we also need to consider a “case 2” transport problem, in which our model is held stationary in a fluid at rest but placed in a constant external electric field, \(\mathbf{ e}\).\(^{41}\) The corresponding force, \(\mathbf{ z}^{(2)}\), and torque, \(\mathbf{ t}_o^{(2)}\), exerted by the particle on the fluid can be written

\[ \mathbf{ z}^{(2)} = -\mathbf{ Q} \cdot \mathbf{ e} \]  

(9a)

\[ \mathbf{ t}_o^{(2)} = -\mathbf{ P}_o \cdot \mathbf{ e} \]  

(9b)

where \(\mathbf{ Q}\) and \(\mathbf{ P}_o\) can be thought of as the “effective charge” and “effective electric dipole” tensors, respectively. If, for example, we had a weakly charged polyion of net charge \(q\) and permanent electric dipole vector \(\mathbf{ p}_o\) (relative to origin \(\mathbf{ o}\)) in the limit of zero salt, then \(\mathbf{ Q} = q \mathbf{ I}\) (\(I\) is the 3 × 3 identity tensor), and \(\mathbf{ P}_o = \mathbf{ p}_o \cdot \mathbf{ e}\) (where \(\mathbf{ e}\) is the Levi—Civita triaxic tensor\(^{41}\)). In steady-state electrophoresis, the net force exerted by the particle on the fluid is zero. At sufficiently low external fields, it is valid to retain only those terms linear in \(\mathbf{ u}_o\) and \(\mathbf{ e}\). Adding eqs 8a and 9a and setting them to zero gives

\[ \mathbf{ Q} \cdot \mathbf{ e} = \mathbf{ z}_r \cdot \mathbf{ u}_o + \mathbf{ z}_o^{T} \cdot \mathbf{ o} \]  

(10)

The second term involving \(\omega\) is new\(^{40}\) and is included here because of the possible presence of translation—rotation coupling. As discussed elsewhere,\(^{36}\) there exists a unique point in any rigid body, the “center of reaction”, where \(\mathbf{ z}_o\) is symmetric. For certain bodies such as ellipsoids and cylinders, \(\mathbf{ z}_o\) vanishes at the center of reaction, which means that the translational and rotational motions are uncoupled. For the “detailed” models considered here, we do not expect \(\mathbf{ z}_o\) to vanish. Happel and Brenner\(^{36}\) and also Kim and Karilla\(^{40}\) describe straightforward procedures for determining the center of reaction. Relative to this origin, we can anticipate that the motion of the particle is “steady” to a good approximation (see pages 197-205 of ref 36) and that the total torque can be set to zero, which gives

\[ \mathbf{ P}_o \cdot \mathbf{ e} = \mathbf{ z}_r \cdot \mathbf{ u}_o + \mathbf{ z}_o^{T} \cdot \mathbf{ o} \]  

(11)

Solving eq 11 for \(\omega\) and using this result in eq 10, we obtain

\[ \mathbf{ u}_o = \mathbf{ M}_r \cdot \mathbf{ e} \]  

(12)

where \(\mathbf{ M}_r\) is the electrophoretic mobility tensor

\[ \mathbf{ M}_o = \mathbf{ D}_{o,3} \cdot (\mathbf{ Q} - \mathbf{ z}_o^{T} \cdot \mathbf{ z}_o^{T} \cdot \mathbf{ p}_o) / k_B T \]  

(13)

the “−1” superscript indicates matrix inversion, and

\[ \mathbf{ D}_{o,3} = k_B T / 3 \text{Tr}(\mathbf{ z}_r^{-1}) \]  

(15a)

\[ D_i^c = \frac{1}{3} \text{Tr}(\mathbf{ D}_{o,3}) \]  

(15b)

is the origin-dependent translational diffusion tensor referred to origin \(o\). \(\mathbf{ D}_{o,1}\) has been discussed in detail previously (for arbitrary choice of origin) by Garcia de la Torre and coworkers.\(^{37,38}\) Within a numerical constant, \(\mathbf{ D}_{o,3}\) also equals the mobility tensor, \(a\), of Kim and Karilla.\(^{39}\) The second term on the right-hand side of eq 14 represents the contribution of translation-rotation coupling. It will prove convenient to also define the following scalar quantities

\[ D_i^{\text{mac}} = \frac{k_B T}{3} \text{Tr}(\mathbf{ z}_r^{-1}) \]  

(15a)

\[ D_i^c = \frac{1}{3} \text{Tr}(\mathbf{ D}_{o,3}) \]  

(15b)
where “Tr” denotes summation over the diagonal terms of the tensor. As discussed by García de la Torre and co-workers,\textsuperscript{37,38} the translational diffusion tensor is only meaningful when referred to a specific point, the center of diffusion, \( \mathbf{d} \). They go on and give specific equations for determining \( \mathbf{d} \) as well as the translational diffusion tensor in a reference frame with \( \mathbf{d} \) chosen as the origin, \( \mathbf{D}_d \). In general, the center of diffusion will be different from the center of reaction discussed previously.\textsuperscript{37−39}

The translational diffusion constant, \( D_t \), measured in an experiment such as light scattering\textsuperscript{42} is

\[
D_t = \frac{1}{3} \text{Tr}(\mathbf{D}_d) \tag{16}
\]

For the models considered in this work, the starting origin is chosen to correspond to the center of mass of the fragment and the helix axis is oriented along the \( x \) axis of a molecule-fixed reference frame. The center of reaction, \( \mathbf{o} \), is then determined following the procedure of Happel and Brenner\textsuperscript{16} or Kim and Karilla.\textsuperscript{39} From an analysis of a 20 bp DNA detailed model discussed below, \( D^m_c \), \( D^t_c \), and \( D_t \) equal each other to within 0.1%. A careful choice of the origin (placing it near the actual center of diffusion) accounts for \( D^o_c \approx D_t \). The fact that \( D^m_c \approx D^t_c \) demonstrates that translation−rotation coupling is negligible for the detailed DNA models considered in this work despite their propeller-like nature.

Finally, DNA does not have a significant permanent dipole moment.\textsuperscript{43,44} It does have a induced dipole moment,\textsuperscript{43,44} but that gives rise to a torque on the polyion that varies as the square of the external field \( E \). Since we are restricting ourselves to terms linear in the electric and/or flow fields, we can neglect the contribution of the electric dipole, the \( \mathbf{P} \) term, in eq 13. When translation−rotation coupling is negligible, eq 13 simplifies to

\[
\mathbf{M} = \mathbf{Z}^{-1} \mathbf{Q} \tag{17}
\]

Note that the “0” subscript has been removed from \( \mathbf{M} \) since it is origin-independent in the absence of translation−rotation coupling.

Results

A. 20 bp Duplex DNA (pd(A)\textsubscript{20}•pd(T)\textsubscript{20}) in KCl. Laue and co-workers\textsuperscript{3} have carried out membrane-confined analytical electrophoresis (MCAE) of pd(A)\textsubscript{20}•pd(T)\textsubscript{20} in a buffer solution containing 0.1 M KCl plus 0.02 M Tris HCl at pH 8.0 and 20 °C. At low field strength, they measured an electrophoretic mobility, \( \mu \), of \(-3.1 \pm 0.1 \times 10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1} \). In all subsequent discussion, \( \mu \) shall be reported in units of \(-10^{-4} \text{ cm}^2 \text{ V}^{-1} \text{ s}^{-1} \). Although numerous mobility measurements of DNA have been reported over the years, the Laue measurements were carried out under conditions that make it ideal for making a comparison with model studies. These include the absence of a gel support medium (which is an added complication in modeling), the use of a small and well-characterized polyion, which is nonetheless sufficiently charged to make ion relaxation effects significant, and a relatively simple buffer solution. Past modeling of this system using caged cylinder models and extrapolating to the limit of \( 1/N \to 0 \) gave mobilities in fairly good agreement with experiment.\textsuperscript{19} The CC’s are referred to a specific point, the center of diffusion, \( \mathbf{d} \), of duplex DNA modeled as a circular cylinder of radius \( R = 10 \text{ Å} \) and length \( L \) (in Å) = 3.4\( n_b \) where \( n_b \) is the number of base pairs.\textsuperscript{46,47} For this model, \( D_t \) is given by\textsuperscript{48}

\[
D_t = \frac{k_B T}{3 \pi \eta L} (\ln b + \gamma) \tag{18}
\]

where \( \eta \) is the solvent viscosity at temperature \( T \), \( b = L/2R \), and

\[
\gamma = 0.312 + 0.565/b + 0.010/b^2 \tag{19}
\]

For 20 bp DNA in an aqueous medium at 20 °C and \( \eta = 0.01 \) poise, eqs 18 and 19 give \( D_t = 10.80 \times 10^{-7} \text{ cm}^2/\text{s} \). In the present work, we shall extend previous work to also include a detailed surface model made up of 352 plates.

At pH 8.0, half of the Tris is protonated, so the ambient counterion distribution consists of 0.10 M K\textsuperscript{+} and 0.01 M Tris\textsuperscript{+} (Tris\textsuperscript{+} = C(CH\(_2\)OH\(_2\)H\(_3\)N\(_3\)+), while the coion distribution is 0.11 M Cl\textsuperscript{−}. In modeling, we need the diffusion constants of the small ions (the \( D_a \) in eq 4b) or, equivalently, the hydrodynamic ion radii, \( r_a \), which is related to \( D_a \) by the Stokes−Einstein relationship \( r_a = k_B T / 6 \pi \eta D_a \). These radii are estimated from limiting molar conductivities, \( \lambda_m \), and the Nernst−Einstein relation.\textsuperscript{49} In an aqueous media, \( r_a \) (in Å) is related to \( \lambda_m \) (in \( 10^{-4} \text{ S m}^2/\text{mol} \)) at 25 °C and the valence of the ion, \( z_a \) by the relation

\[
\lambda_m = \frac{2}{\lambda_a} \tag{20}
\]

The \( \lambda_m \)'s of K\textsuperscript{+} and Cl\textsuperscript{−} are similar,\textsuperscript{29,49} and if they are averaged, \( r_a = 1.242 \text{ Å} \). For Tris\textsuperscript{+} and acetate anion,\textsuperscript{28} \( r_a = 3.136 \) and 2.278 Å, respectively. (Although acetate is not present here, it appears in the 18 bp DNA system considered in a later subsection and is included here for completeness.) It should be emphasized that in this work, the ions are treated as point charges and the ion size enters only through the small ion mobilities (the \( r_a \) or \( D_a \) in eq 4b). Thus, the equilibrium electrostatic potential of a “three-ion” buffer solution containing 0.10 M K\textsuperscript{+} + 0.01 M Tris\textsuperscript{+} + 0.11 M Cl\textsuperscript{−}, would be identical to that of a “two-ion” buffer solution containing 0.11 M K\textsuperscript{+} + 0.11 M Cl\textsuperscript{−} at the approximate level considered in this paper. However, since the \( D_a \)’s for K\textsuperscript{+} and Tris\textsuperscript{+} are different, the nonequilibrium charge distributions will be different, and so will the polyion mobilities.

First, consider a capped cylinder (CC) model made up of 96 plates, \( \varepsilon_i = 4 \), with a double helical charge distribution within and a total polymer charge of −40. For the three-ion buffer solution discussed previously, the calculated mobility is \( \mu = \text{Tr}(\mathbf{M})/3 = 3.53 \) where \( \mathbf{M} \) is the electrophoretic mobility tensor ( eq 13 or 17). Since the Tris\textsuperscript{+} concentration is only one-tenth that of K\textsuperscript{+}, it can be expected that a two-ion buffer (0.11 M K\textsuperscript{+} + 0.11 M Cl\textsuperscript{−}) model should give a similar mobility, and that is the case. The same 96 plate CC model considered above, but with the two-ion buffer gives a calculated mobility of 3.54. Although we expect more ion relaxation and, hence a lower mobility, in the three-ion buffer since it contains a small amount of the bulky Tris\textsuperscript{+} cation, the overall effect on polyion mobility is seen to be very small. In addition to the 96 plate, two-ion CC model, additional CC models consisting of 72, 144, and 288 plates were also examined and the extrapolated shell\textsuperscript{19,22} limit yields \( \mu = 3.27 \). The CC model extrapolated shell mobility is larger than the experimental value by 5.5%.

Next, we consider a detailed model derived from the secondary structure of pd(A)\textsubscript{20}•pd(T)\textsubscript{20}. First of all, SYBYL is used to generate the atomic coordinates, as discussed previously. To generate the surface, \( S_b \), we simply set the atom radii and...
also the probe radius to 1.4 Å. As mentioned previously, this choice is consistent with the distance of closest approach of the oxygens in water, which is 2.8 Å.29 The resulting surface is the lower object in Figure 1. Following the procedure discussed previously, the \( \varepsilon_r \) and \( \xi_{nc} \) are determined, and then eqs 15a, 15b, and 16 yield a mobility. To within 0.1%, all of these are equal 10.88 Å/s cm²/volt. This shows that translation–rotation coupling is negligible and that our choice of origin is near the center of diffusion. This \( D_t \) is very close to the value of 10.80 × 10⁻⁷ cm²/s expected for DNA modeled as a right-circular cylinder discussed previously. On this basis, we conclude that it is not necessary to include a Stern layer in modeling DNA. This conclusion is consistent with the conclusions of Stigter with regards to other systems.23 However, because of the large charge on the DNA, we can also expect electrolyte friction31 to reduce the \( D_t \) of our model by a few percent. When a highly charged macromolecule such as DNA translates through a solution, there is a tendency of its electric dipole moment. This, in turn, produces an additional electric dipole moment. The resulting surface is also the probe radius to 1.4 Å. The resulting surface is also the probe radius to 1.4 Å.

### TABLE 1: Extrapolated Shell CC Model for 18 bp DNA (25 °C in 0.02 M Tris⁺Ac⁻⁻)

<table>
<thead>
<tr>
<th>( Z )</th>
<th>( \mu(\text{nr}) )</th>
<th>( \mu(\text{r}) )</th>
<th>( \mu(\text{t}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>1.198</td>
<td>1.187</td>
<td>0.966</td>
</tr>
<tr>
<td>0.2</td>
<td>2.346</td>
<td>2.263</td>
<td>1.844</td>
</tr>
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<td>0.3</td>
<td>3.399</td>
<td>3.082</td>
<td>2.521</td>
</tr>
<tr>
<td>0.4</td>
<td>4.323</td>
<td>3.696</td>
<td>3.036</td>
</tr>
<tr>
<td>0.5</td>
<td>5.111</td>
<td>4.122</td>
<td>3.401</td>
</tr>
<tr>
<td>0.65</td>
<td>6.080</td>
<td>4.524</td>
<td>3.757</td>
</tr>
<tr>
<td>0.80</td>
<td>6.857</td>
<td>4.767</td>
<td>3.986</td>
</tr>
<tr>
<td>1.0</td>
<td>7.674</td>
<td>4.995</td>
<td>4.204</td>
</tr>
</tbody>
</table>

In addition, mobilities for the model with the external field oriented parallel and perpendicular to the helix axis will be reported, and these are indicated by \( "\|" \) and \( "\perp" \) subscripts. In terms of the electrophoretic mobility tensor defined by eq 13, \( \mu_\| = (\mu_{xx}, \mu_{yx}, \mu_{yx}) \), and the orientationally averaged mobilities are computed from \( \mu = \mu_\| + 2 \mu_\perp \). The extrapolated shell CC mobility for this model is found to be 4.20, which is about 28% larger than the experimental mobility. The relative discrepancy between model (CC, extrapolated shell) and experimental mobilities is seen to be substantially larger for the 18-mer in Tris acetate than the 20-mer in KCl. It is readily verified by modeling that this discrepancy is not due to the difference in temperature, salt concentration, or fragment length but is most likely due to the different buffer ions present. A possible explanation is that there is an association between the Tris⁺ counterions and the DNA over and above the electrostatic interaction that is incorporated into the model via the Poisson equation. A simple way of including this association in the framework of the CC model is to reduce the polyion charge by uniformly reducing (by the fractional amount \( \chi \)) the helical charges of the polyion. Summarized in Table 1 are CC-extrapolated shell results for a range of polyion charges with \( \chi \) ranging from 0.1 to 1.0 (fully charged). Included in the table are reduced averaged surface potentials, \( y_s \), defined by

\[
y_s = \frac{q\langle \Lambda_\| \rangle S}{k_BT}
\]

where \( q \) is the protonic charge and \( \langle \Lambda_\| \rangle S \) is the equilibrium electrostatic potential averaged over the polyion surface. Reported are both “unrelaxed” (\( \text{nr} \)) and “relaxed” (\( \text{r} \)) mobilities for cylinders oriented parallel and perpendicular to the applied field. In addition, dimensionless reduced mobilities, \( C \), are reported which are defined

\[
C = \left( \frac{4\pi\eta q}{\varepsilon_r k_BT} \right) \frac{\mu}{y_s}
\]

The \( C \)'s are useful in helping us to understand and disentangle how factors such as polyion charge (or electrostatic potential), geometry/orientation, and ion relaxation influence mobility. We will discuss this more later. In the meantime, let us return to the question of what the charge reduction model gives us—a mobility which matches the experimental mobility. From the Table, \( \chi \) between 0.4 and 0.5 gives a \( \mu(\text{r}) \) consistent with that from the experiment. On this basis, we conclude that a CC model with the DNA phosphate charges reduced from \( -1 \) to about \( -0.45 \) gives a mobility in the best agreement with the experiment.
One factor that might account, in part, for the discrepancy is our modeling of Tris acetate as a strong electrolyte with an effective ionic strength equal to half of the actual Tris acetate concentration. It would be better to model the buffer as a weakly dissociating electrolyte. Although such a model has not yet been applied to general model polynions, it has been formulated and applied to small spherical model particles and fluid droplets. However, Figure 10 of ref 52 shows that the electrophoretic mobility of a “nonconducting drop”, which is closest to the model polynions considered in our work, is not very sensitive to the degree of dissociation of the buffer. On this basis, we conclude that incomplete dissociation of Tris acetate is not a significant factor in influencing the electrophoretic mobility. We shall return to this point later.

Next, consider detailed models derived from the atomic coordinates of the 18 bp sequence, 5'-AGATCACCCTTGGCT-CAC-3', generated by SYBYL. As in the case of the 20 bp DNA fragment, we set $\sigma_i = \sigma_{probe} = 1.4$ Å and produce a 320 plate representation of $S_d$. In the absence of ion relaxation, this structure gives $D_i = 13.05$ (in units of $10^{-7}$ cm$^2$/s), and when ion relaxation is included, which is equivalent to accounting for electrolyte friction, $D_i = 11.51$ if the full phosphate charges are included ($\gamma = 1.0$), and $D_i = 12.22$ if we reduce the absolute phosphate charges by 50% ($\gamma = 0.5$). The corresponding $\mu(r)$'s are $4.17$ ($\gamma = 1.0$) and $3.31$ ($\gamma = 0.5$). If these values are compared with the corresponding CC-extrapolated shell mobilities listed in Table 1, we observe good agreement between the two models despite significant differences in the model shapes and the charge distributions within. What the CC and detailed models have in common are the same buffer/salt, similar $D_i$'s, and the same net charges within. These factors are evidently very important in determining electrophoretic mobility. A limited extrapolated shell calculation has also been carried out on the detailed 18 bp model with $\gamma = 0.5$. For 240, 320, and 440 plates, the corresponding $\mu(r)$'s are $3.46$, $3.31$, and $3.31$; respectively. If these values are compared with the corresponding CC-extrapolated shell mobilities listed in Table 1, we observe good agreement between the two models despite significant differences in the model shapes and the charge distributions within. What the CC and detailed models have in common are the same buffer/salt, similar $D_i$'s, and the same net charges within. These factors are evidently very important in determining electrophoretic mobility. A limited extrapolated shell calculation has also been carried out on the detailed 18 bp model with $\gamma = 0.5$. For 240, 320, and 440 plates, the corresponding $\mu(r)$'s are $3.46$, $3.31$, and $3.31$; respectively. Although some variation in mobility with number of plates is observed, that variation is seen to be very small presumably because we are dealing with large plate numbers to begin with.

The absolute electrophoretic mobilities of the models considered so far are significantly larger than those from the experiment unless the absolute charges of the polyanion are represented by the bead arrays. The absolute mobility of the explicit model is due to the lower absolute charge models as a careful comparison of cases B and C reveals. Even though both of these models have the same net charge, the absolute reduced surface potential, $\gamma_S$, is lower for the model with explicit Tris$^+$. Also, the “explicit” model has a lower absolute mobility. Since the reduced mobilities ($C'$s) are comparable for the two cases, we can conclude that the lower absolute mobility of the explicit model is due to the lower absolute surface potential. This example does demonstrate the subtle point that two structures with the same net charge do not necessarily have the same mobility. A possible explanation for the lower surface potential of the explicit model can be attributed to the concentration of positive charge (from the Tris$^+$) near $S_d$ while the dominant negative charge is generally positioned more in the polyanion interior. This interpretation, however, is not complete. If a CC model is examined with a charge distribution mimicking that of the explicit model, $\gamma_S$ is found to be about the same as that of a CC model with the “reduced charge” model. In addition to the actual charge

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{Tris}^+\text{Ac}^-]$</td>
<td>0.04</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>$\phi_{\text{buffer}}$</td>
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<td>-18</td>
<td>-18</td>
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<td>-22</td>
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<tr>
<td>$\phi_{\text{polyion}}$</td>
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<td>0.50</td>
<td>0.61</td>
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</tr>
<tr>
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<td>$# \text{ explicit Tris}^+$</td>
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<td>0</td>
<td>18</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

= 0.5). Entries A and B differ in the assumed ambient salt concentration, with B corresponding to the experimental salt conditions prevalent in the bulk solution in which half of the Tris is protonated (pH 8). However, it is possible that the protonated form of Tris may be stabilized near a highly charged polyanion and entry A corresponds to the extreme limiting case of all the Tris being protonated. From Table 2, $\mu(r)$ decreases from 3.31 to 3.06 when the Tris$^+\text{Ac}^-$ concentration increases from 0.02 to 0.04 M. It should be emphasized, however, that stabilization of the protonated form of Tris by the polyanion may only be significant near the DNA. On this basis, we expect B to model more accurately the actual experimental situation, but A is included to illustrate the effect total dissociation would have (lower the mobility by about 8%). The actual lowering due to incomplete dissociation would undoubtedly be less than 8%, which is consistent with prediction for spherical nonconducting polynomials.

Entries C, D, and E have Tris$^+$ cations explicitly included in the rigid structure. As expected, the more Tris$^+$ that is bound, the lower the absolute mobility and case D with 14 Tris$^+$ cations gives a mobility in excellent agreement with experiment. A ball-and-stick representation of this complex is shown in Figure 3, where the Tris$^+$ cations are represented by the bead arrays. The models with explicit Tris$^+$ are not entirely consistent with the reduced charge models as a careful comparison of cases B and C reveals. Even though both of these models have the same net charge, the absolute reduced surface potential, $\gamma_S$, is lower for the model with explicit Tris$^+$. Also, the “explicit” model has a lower absolute mobility. Since the reduced mobilities ($C'$s) are comparable for the two cases, we can conclude that the lower absolute mobility of the explicit model is due to the lower absolute surface potential. This example does demonstrate the subtle point that two structures with the same net charge do not necessarily have the same mobility. A possible explanation for the lower surface potential of the explicit model can be attributed to the concentration of positive charge (from the Tris$^+$) near $S_d$ while the dominant negative charge is generally positioned more in the polyanion interior. This interpretation, however, is not complete. If a CC model is examined with a charge distribution mimicking that of the explicit model, $\gamma_S$ is found to be about the same as that of a CC model with the “reduced charge” model. In addition to the actual charge
distribution within the polyion, the detailed nature of $S_d$ as well as the choice of $\varepsilon_i$ influence the average surface potential and also the mobility. Entries D and E show that altering $\varepsilon_i$ also affects the average surface potential and electrophoretic mobility in subtle ways.

Summary

In the present study, the electrophoretic mobility of short duplex DNAs has been modeled for the purpose of understanding the different association duplex DNA has with different buffers. On the basis of experiment, it has been recognized for some time that Tris acetate and Tris borate buffers interact quite differently with DNA. However, because the association of Tris borate with DNA is undoubtedly quite complex, we have focused in the present study on a simpler comparative study involving the counterions $K^+$ and $\text{Tris}^+$. Without modeling, differences in association are inferred on the basis of experimental observations such as differences in electrophoretic mobility, $\mu$, but those observations alone do not tell what those associations entail on a molecular level. First of all, BE modeling can be used to identify what factors influence $\mu$. In addition, since modeling provides an absolute number for $\mu$, it can be used to identify the nature of specific associations.

Our analysis of the 20 bp DNA fragment in KCl shows that simple continuum modeling explains reasonably well the observed electrophoretic behavior. Also, $\mu$ does not depend strongly on the details of the surface or charge distribution within the model polyion. Although translation—rotation coupling is expected to influence transport, the effect is seen to be very small in this case. In addition, the precise identification of the center of diffusion is not necessary to accurately determine $D_c$.

On the other hand, the calculated mobility of the 18 bp DNA fragment in Tris acetate is found to be substantially larger than that from the experiment unless either the charge on the DNA is reduced or $\text{Tris}^+$ counterions are specifically bound to the DNA. In the modeling, the mobile ions are treated as points, and it can be argued that this approximation would produce systematic errors in modeling, particularly for a large and bulky cation such as $\text{Tris}^+$. Accounting for the finite size of the counterion, however, would have to yield a higher absolute mobility rather than a lower one since a counterion with a large excluded volume would not be able to screen the polyion charge as effectively as one with a smaller excluded volume. A model in which about 40% of the DNA phosphates have $\text{Tris}^+$ counterions specifically bound is consistent with the experimental $\mu$. We are not claiming that 40% of the DNA phosphates are, in fact, permanently neutralized by $\text{Tris}^+$—only that such a model gives a mobility consistent with experiment. What this study does show is that in addition to the purely classical electrostatic interaction between $\text{Tris}^+$ and DNA which is accounted for in the modeling, there is an additional association present. This might be hydrogen bonding, but from the experimental mobilities and modeling methods employed in this study, we cannot say anything further about the nature of this association. In addition, there are differences in calculated electrophoretic mobilities for the detailed models even when the total polyanion charge is held constant, but these differences are small. Finally, binding of $\text{Tris}^+$ might also explain why the calculated mobility of the 20 bp DNA fragment in 0.10 M KCl plus 0.01 M Tris Cl is high. Although the counterion is primarily $K^+$, there is a small amount of $\text{Tris}^+$ present which associates more strongly with DNA than $K^+$.

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References and Notes

(3) Stellwagen, N. C.; Gelfi, C.; Righetti, P. G. *Biopolymers* 1997, 42, 687.