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Characteristics of Migration Patterns of DNA Oligomers in Gels and the Relationship to the Question of Intrinsic DNA Bending

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Abstract: We have developed a methodology that is capable of quantitatively describing the electrophoretic mobility patterns of oligomeric B-DNA through polyacrylamide gels (PAG) in the presence of varying concentration of the organic solvent 2-methyl-2,4-pentanediol (MPD), used routinely to induce DNA crystallization. The model includes the ion atmosphere and its polarization, electrostatic excluded volume, hydrodynamic interactions, and fluctuation effects that characterize the overall size of the migrating polyion. Using this model, and by critically examining the mobility patterns of linear random-sequence B-DNA molecules in PAG as a function of MPD, we address the question of the discrepancy between current models used to explain the molecular origins of A-tract-induced DNA bending. Direct analysis of the mobility of B-DNA oligomers on PAG, and comparison to the mobility of A-tract-containing oligomers, shows a significant apparent effect of MPD on the mobility of generic B-DNA sequences, which is larger than the effect on A-tract-containing oligomers. The effect is chain-length dependent, especially at lower MPD concentration. Thus, the apparent reduction in gel mobility, as a function of MPD, is not unique to A-tract regions or A-tract-containing molecules. However, our analysis suggests that MPD molecules are probably excluded from the surface of both B-DNA and A-tract molecules. This is supported by circular dichroism studies on A-tract and B-DNA molecules in solutions containing various MPD concentrations.

Introduction

Sequence-dependent DNA structure has an active role in DNA recognition by drugs and proteins. A particular aspect of sequence-dependent DNA structure is that of sequence-dependent DNA bending, observed when the sequence contains phased repeats of A_4-A_6-tracts, called "A-tracts".[1,2] The molecular origins of A-tract-induced DNA bending are still debated. Solution studies point to the A-tract region as the source of global curvature.[1,3,4] According to this model, called the junction model, A-tracts are bent toward the minor groove by means of negative inclination of the bases relative to the helix axis, whereas the base pairs in the flanking B-DNA regions are essentially perpendicular to the helix axis. Therefore, to preserve continuous base stacking along the DNA, there is a change in the trajectory of the helical axis at the junction between the two regions. This results in global curvature which points into the minor groove at the center of each A-tract. Crystallographic studies on A-tract-containing DNA oligomers show that the base pairs in the A-tract region are invariably perpendicular to the helix axis.[2] These results are in conflict with the solution model. The model proposed from these studies[5,6] holds that A-tracts are straight (i.e., the base pairs are perpendicular to the helix axis), whereas the flanking B-DNA regions are gently writhed. The insertion of a straight helical segment within a writhed helix causes the global bending phenomena, with a net curvature pointing into the major groove within the B-DNA segments. Recently, Hizver et al.[7] have solved the crystal structure of an oligomer containing an A_2T_2-tract, in which both the magnitude and the direction of bending is in agreement with the findings of solution studies. In this structure, the A-tract is intrinsically bent toward the minor groove at the center of the A-tract. Both the A-tract and the non-A-tract regions contribute to the global curvature. A similar result was obtained from a recent NMR study, using residual dipolar coupling.[8] This structure, of an A_6-tract, showed increasingly negative base inclination within

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the A-tract, as one moves toward the 3′-end of the A-tracts, an inequality of the 5′ and 3′ junction bending, and an overall bend direction and magnitude compatible with results from gel electrophoresis studies. The curvature in this structure is divided 3:1 into the non-A-tracts versus the A-tract regions.

It was proposed that the organic solvent 2-methyl-2,4-pentanediol (MPD), used routinely to induce DNA crystallization, is the reason behind the discrepancy between the solution and crystallographic results. The hallmark of curved DNA is its anomalous migration on polyacrylamide gels (PAG). DNA oligomers containing A-tracts migrate through PAG as if they are 2−3 times larger than their actual length.[3] MPD was shown to reduce the anomaly in gel migration of A-tract-containing DNA molecules, relative to a random-sequence size marker.[9] The authors interpreted the results as indicating a partial straightening of the helix by MPD. Subsequent studies showed results that were consistent with a specific effect of MPD on the A-tract region exclusively.[10,11] Hence, understanding the effects of MPD on general sequence B-DNA molecules versus A-tracts-containing sequences can help us resolve the conflict between solution and crystallographic results and thus contribute to our understanding of the molecular origin of sequence-directed curvature, as well as to our understanding of the migration of DNA molecules through gels.

We have been studying the electrophoretic mobility on PAG of linear random-sequence B-DNA molecules as well as A-tracts as a function of MPD concentration. We have analyzed the results for absolute gel mobility of all DNA sequences, instead of analyzing the mobility of the A-tract-containing molecules relative to the mobility of the random-sequence B-DNA size marker. We show that MPD has a larger apparent effect on the gel mobility of generic B-DNA molecules than on A-tracts-containing molecules. Moreover, we have developed a methodology that is capable of quantitatively describing the electrophoretic mobility of oligomeric B-DNA molecules through PAG in the presence of varying concentrations of organic solvents such as MPD. The model captures the essential features of the experimental data.

Experimental Methods

The oligodeoxynucleotides used in this study were synthesized on an automated DNA synthesizer. The deprotected oligodeoxynucleotides were purified on denaturing PAG using standard procedures.[12] A ligation ladder was constructed from [γ^{32}ATP]-labeled double-stranded monomeric 21-bp fragments using protocols established in our previous studies on DNA bending.[13,14] To separate the various multimers of the oligonucleotides, the products of the ligation ladders were analyzed on native 8% (w/v) PAG (29:1 acrylamide/bis) in 0.09 M Tris−borate buffer at 20 °C in a Hoffer thermostated gel box. MPD was included in both the gel matrix and the running buffer at varying concentrations from 0% to 30% (v/v). All gels were run until the BPB dye migrated 24 cm in a 20 cm × 32 cm × 0.75 cm gel. Hence, different voltages and run times were used for the different % MPD as follows: 0% MPD, 300 V, 325 min; 5−30% MPD were run at 380 V for 5%, 360 min; 10%, 385 min; 15%, 525 min; 20%, 660 min; and 30%, 1020 min.

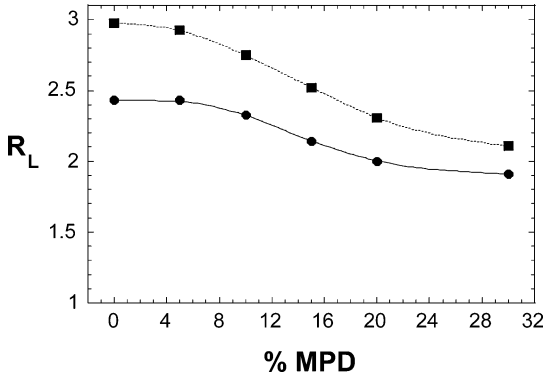

Figure 1. R_L values as a function of % MPD for the 147 bp (●) and 168 bp (■) multimers of the sequence Ast.[4] R_L is the ratio of apparent length (in bp), determined from comparison with a size marker sequence, to real chain length (in bp). The results correspond to one of three independent experiments.

The ratio of apparent length to real chain length (both in base-pairs), R_L, was calculated relative to the random B-DNA size marker, VW, used in our previous studies.[4,13−15] The absolute mobility (expressed in units of cm^2/V s) of each multimer in each sequence was calculated by dividing the distance from the well by the velocity multiplied by the run time.

Results and Discussion

Different Analyses of Gel Mobility Lead to Different Results Regarding the Influence of MPD on DNA Molecules. We have critically studied the electrophoretic mobility of linear random-sequence B-DNA molecules in PAG, versus that of the intrinsically bend sequence, Ast,[4] as a function of MPD concentration. The molecules are head to tail multimers produced by ligation of monomeric 21-bp DNA fragments. The sequence of the B-DNA building block corresponds to the B-DNA size marker used in all of our previous studies, "VW".[4,13−15] Figure 1 shows the conventional analysis, in which the anomaly in gel mobility of the test sequence is assessed by calculating its R_L values, which is defined as the ratio between the apparent length of the test sequence and its real length in base pairs. The apparent length of the test sequence is determined relative to a size marker, which is taken to be of generic B-DNA conformation. When analyzed in this manner, the A-tract sequence, Ast, seems to lose about 45% of its anomaly in gel migration, as observed before.[9] However, such an analysis does not take into account possible effects of MPD on either the B-DNA size marker or the gel matrix itself.

To explore the possibility that MPD has an effect on the mobility of the B-DNA size marker, we have compared the mobility of the two sequences directly. Figure 2 shows the absolute mobility of each DNA multimer as a function of MPD concentration. Each line in the graphs represents the distance from the well of one multimer, as a function of the MPD concentration in the PAG and in the running buffer. Figure 2 clearly shows that MPD has a more significant effect on the mobility of the B-DNA size marker (Figure 2A) than on the mobility of the A-tract-containing molecules (Figure 2B),

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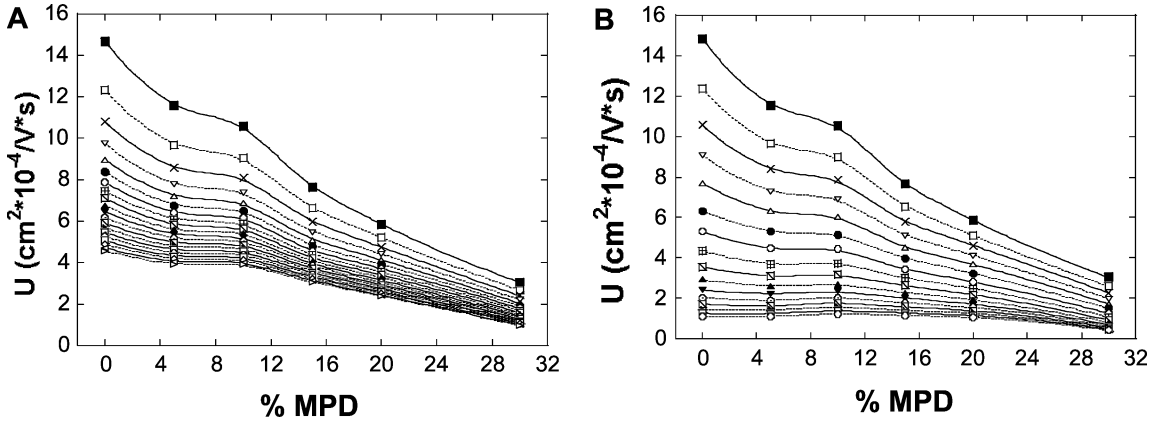

Figure 2. Absolute electrophoretic mobility of multimers of the VW size marker (A) and Ast (B) as a function of %MPD. Graphs show increasing chain lengths, in increments of 21 bp, from 21 to 420, going down from the top of each plot. Absolute mobility (expressed in units of cm^2/V s) was calculated by dividing the distance from the well by the velocity multiplied by the run time.

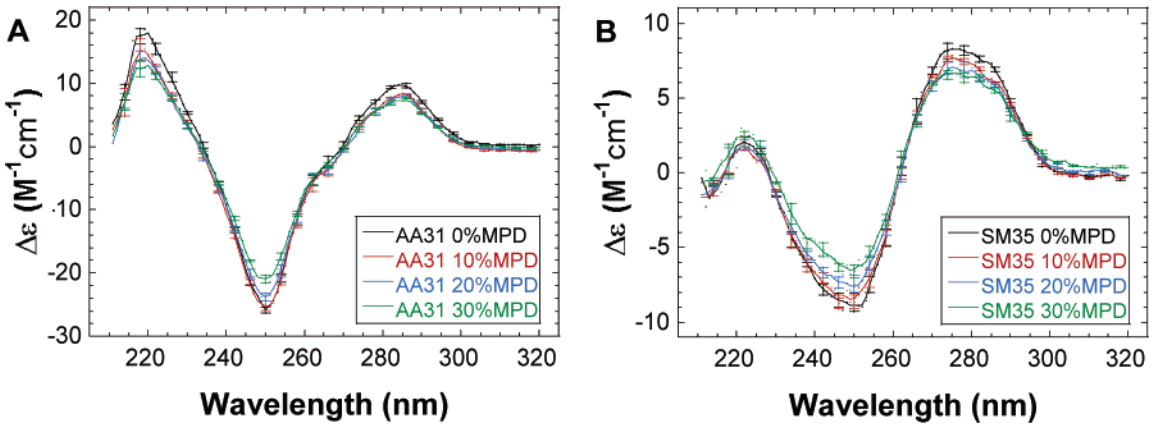

Figure 3. CD spectra as a function of %MPD. (A) A 31-bp oligomer containing phased A-tracts. (B) A 35-bp oligomer containing the VW sequence. The graphs represent the average of two independent experiments. For clarity, the error bar of only every fourth data point is shown.

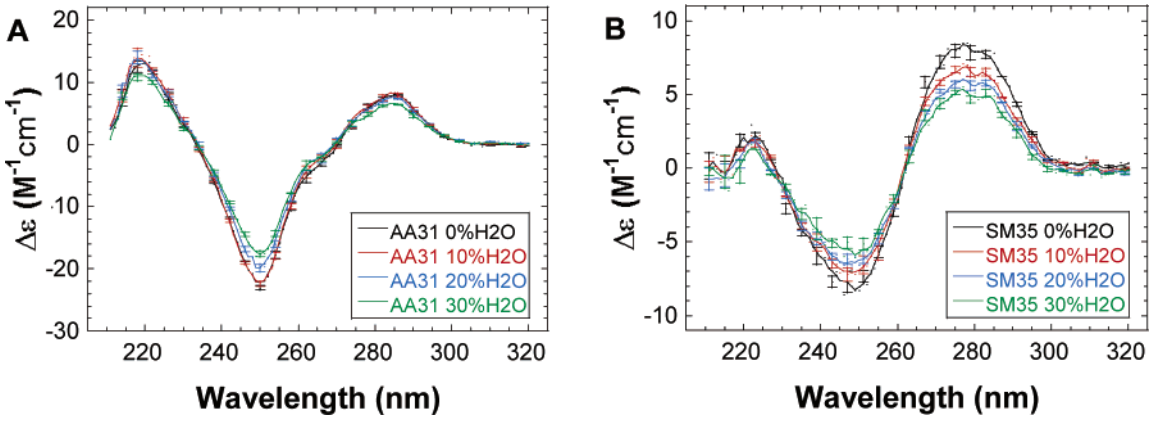

Figure 4. CD spectra as a function of H_2O dilution. Samples were diluted in H_2O, exactly as for the MPD addition experiment. (A) A 31-bp oligomer containing phased A-tracts. (B) A 35-bp oligomer containing the VW sequence. For details, see Figure 3.

especially at longer DNA fragments. Note that at higher chain length (i.e., lower in the graph) a negative slope remains for panel A, whereas the slope is close to zero in panel B of Figure 2. This chain-length dependence is especially pronounced at lower MPD concentration. This establishes that the effects of MPD on A-tracts cannot be invoked as the reason for the discrepancy between the solution and crystal structure of A-tract-containing DNA molecules, because the effect of MPD on B-DNA molecules is apparently larger than its effect on A-tract-containing oligomers.

CD Spectra Support the Results from PAG Electrophoresis. The results from the analysis of electrophoretic migration of ligation ladders on PAG are in agreement with circular dichroism (CD) spectra of sequences, 31-bp and 35-bp long, containing phased A-tracts and the VW sequence, respectively (Figure 3). For comparison, the changes in the CD spectra when the same amount of water, instead of MPD, is added to these sequences are depicted in Figure 4. Note the remarkable similarity between the CD spectra for phased A-tracts depicted in Figures 4A and 5A. The same is true between the spectra

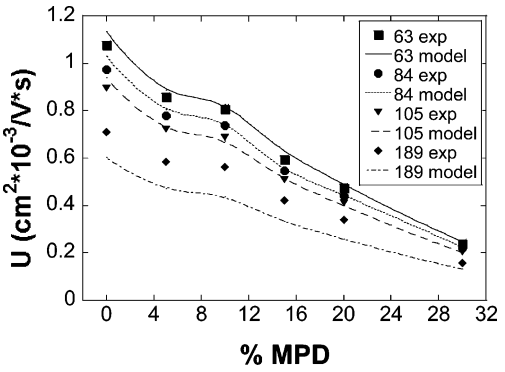

Figure 5. Theoretical predictions for the gel electrophoretic mobility of VW63 (i.e., three ligated VW monomers, of total length 63 bp)−VW105 as well as VW189 are compared to experimental data.

shown for the VW sequence in Figures 4B and 5B. Consequently, the spectra of phased A-tracts and the VW sequence exhibit less than significant changes in the presence of MPD, in the range of concentration studied here.

Dynamics of Oligomeric B-DNA Molecules in PAG in the Presence of MPD. Qualitative Considerations. To qualitatively investigate the effects of MPD on the electrophoretic mobility of B-DNA, let us consider the case in which the contour length L of the DNA is less than the persistence length P. Assume that the mechanism of migration of the DNA through PAG can be described by a suitable generalization of the classical Ogstron pore size distribution model[16,17]

$$\mu = \mu_o \exp(-f) \tag{1}$$

where the free solution mobility is denoted by μ_o. The function $f = \upsilon K_r T^\alpha$, where $\%T$ is the gel concentration, α is an exponent, υ is a constant, and K_r is the retardation coefficient

$$\sqrt{K_r} = a_o + a_1(r_o + R) \tag{2}$$

Here, r_o is the effective radius of the gel fiber, R is the effective radius of the migrating DNA, and a_o and a_1 are constants.

The Ogstron model ignores the polyelectrolyte features of DNA and the ionic strength of the buffer, and treats the free solution mobility as a parameter. The proposed generalization of the Ogstron model consists of replacing the free solution mobility μ_o by $\mu_o(Q,\kappa,\eta,L)$, where $1/\kappa$ is the Debye screening length, η is the viscosity of the solvent, and Q is the effective charge of the solvent. Thus, the polyelectrolyte features of the DNA and the ionic strength of the buffer have been included in a description of electrophoretic migration.

By incorporating hydrodynamic interactions and screening of electrostatic interactions, a qualitative model for the free solution mobility can be obtained[18]

$$\mu_o(L,\kappa,\eta,Q) = \frac{Q}{6\pi\eta R_g} Y(\kappa R_g) \tag{3}$$

where $Y = H(\nu)/(\kappa R_g)^{(1/\nu)-1}$, $H(\nu)$ is a function of the exponent ν, and R_g is the radius of gyration of the macromolecule. Because, by assumption, the polyion is stiff, the radius of gyration is proportional to its length. The effective charge of the polyion $Q = Nq/\xi$, where ξ is the Manning linear charge density of the polyion, N is the number of phosphates, each of charge q. For oligomeric DNA, Coulombic end-effects modify the characteristics of counterion condensation near the ends of the polyion.[19,20]

Let us denote the dielectric constant of the solvent in the presence and in the absence of MPD by ϵ_o and $\sqrt{\epsilon}$, respectively. Note that in the presence of MPD the linear charge density and the Debye screening length scale with the dielectric constant as ϵ and $\sqrt{\epsilon}$, respectively. From eqs 1−3, we can extract the dependence of the ratio of the electrophoretic mobility of the DNA in the presence and in the absence of MPD solvent, on the dielectric constant of the solvent

$$\left(\frac{\epsilon}{\epsilon_o}\right)^{1/2\nu+1/2} \left|1 + \frac{\ln\sqrt{\epsilon/\epsilon_o}}{\ln(\vartheta b/\epsilon_o)}\right| \exp(-\Psi) \tag{4}$$

The quantity ϑ has units of inverse length and $\Psi = 2T^\alpha a_1(a_o + a_1 r_o)[R_g(\epsilon) - R_g(\epsilon_o)] + a_1^2 T^\alpha [R_g(\epsilon) - R_g(\epsilon_o)][R_g(\epsilon) + R_g(\epsilon_o)]$. In deriving the expression for Ψ, we assumed that the effective radius or size of the macromolecule is given by the root-mean-square radius of gyration.

Several comments are in order. First, as the concentration of MPD increases, $\epsilon < \epsilon_o$. Second, if Coulombic end-effects are not included in the model, eq 4 predicts that an oligo DNA will migrate slower in a solvent consisting of MPD molecules than in a pure solvent. Third, with the inclusion of end-effects, there is a range of MPD concentration for which DNA will migrate faster in MPD than in pure solvent. Fourth, if the concentration of MPD is larger than a threshold value, the DNA will migrate slower in MPD solvent. Fifth, these predictions are in qualitative accord with experimental data. Finally, the above arguments can be generalized to describe the electrophoretic mobility when the persistence length is less than the contour length or when the reptation mode of migration is valid.

Quantitative Model. We next explore a quantitative model for the electrophoretic patterns of random-sequence B-DNA molecules in MPD solvent. The model is a synthesis of several key ideas in the polyelectrolyte and dynamic behavior of nucleic acids. First, the forces exerted on a monomer of the macromolecule in free solution are due to Brownian motions, relaxation field, polarization and frictional effects, and external electric field. Furthermore, a charged macromolecule that is in motion in an aqueous medium will produce an electric field, which in turn will perturb the polarization cloud; this is the so-called asymmetric field effect. The total electric field has contributions from the moving charges as well as ionic charge densities.

The frictional forces acting on a monomer j at $\vec{r}_j$ are

$$\Gamma(\vec{v}_j - \vec{u}(\vec{r}_j)) \tag{5}$$

where Γ is the solvent−monomer friction coefficient, $\vec{u}(\vec{r}_j)$ is the velocity field of the solvent at the location of monomer, and $\vec{v}_j$ denotes the velocity of the monomer. The velocity field of the solvent can be expressed as[19,21]

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$$\vec{u}(\vec{r}_j) = \sum_k \overleftrightarrow{O}(r_j - r_k)\exp(-\kappa(r_j - r_k))\cdot\vec{F}_{\text{ext}} + \overleftrightarrow{O}(r_j - r_k)\cdot[\vec{F}_{k;\text{rand}} + \vec{F}_{k;\text{pol}}] + \sum_k \overleftrightarrow{T}_{\text{sc}}(r_j - r_k)\cdot q\vec{E}_{\text{relax}} \quad (6)$$

where $\overleftrightarrow{O}$ and $\overleftrightarrow{T}_{\text{sc}}$ denote, respectively, the Oseen and the screened Oseen tensors. On balancing the frictional force with the sum of the forces due to Brownian motion $\vec{F}_{j;\text{rand}}$, external force $\vec{F}_{\text{ext}}$, polarization force $\vec{F}_{j;\text{pol}}$, and that due to relaxation field $q\vec{E}_{\text{relax}}$, where $\vec{E}_{\text{relax}}$ is the electric field generated due to moving charges, an equation of motion is obtained for the monomer under question. Summing this force balance equation over all monomers, making use of eq 5 and the definition of the center of mass velocity, and ignoring terms higher than quadratic in the velocity field, one obtains an expression for the free solution mobility in terms of the structural parameters of the DNA and the buffer.[19,21]

Second, a fundamental assumption in the model is that the amplitude of structural fluctuations in aqueous solution determines the shape and the overall size of a nucleic acid during electrophoretic migration.[22] These structural fluctuations will result in crucial rearrangements of the polyacrylamide chain density in the proximity of the DNA undergoing electrophoresis. Let τ_s be the average time for reorganization of the polymer chains in the dynamic depletion layer. For a time scale much larger than τ_s, the DNA together with the depletion layer migrates as a "dressed" macromolecule through the gel matrix.[23] The volume accessible to the DNA is obtained as follows. The volume V occupied by the polyacrylamide chains is partitioned into cubic blocks, such that within a block the DNA sees a homogeneous background of the polymer network. Let C_i denote the excluded volume between the DNA and the polymer chains within block i. The total volume accessible to the DNA is therefore $\langle\prod_i(1 - C_i/V)\rangle$, where the angular brackets denote an ensemble of polymer configurations.[23] To order $(C_i/V)^2$, and in the thermodynamic limit, the accessible volume is $\langle\exp(-\sum_i C_i/V)\rangle$.

The excluded volume C_i in block i can be evaluated in terms of the work of depletion in that block. If W_d is the work of inserting the polyion into the entangled matrix, then in addition to the Ogston factor,[17,19] $\exp(-R/\chi)$, where R is the size of the DNA and χ is the mesh spacing, there is an additional contribution to the friction given by $\exp(-W_d/k_BT)$. The work of depletion is expressible in terms of the dimensionless ratio d/λ, where d is the diameter of the DNA and λ is the correlation length that is a measure of the inhomogeneity in the gel matrix.[22]

Third, any changes in the Bjerrum length of the ion pair in water would significantly affect the linear charge density parameter, which exquisitely governs the counterion condensation and hence the electrostatic stability characteristics of the DNA.[24,25] Consequently, an important ingredient in the model is the inclusion of the variation of the dielectric constant ϵ and the viscosity η of the solvent with MPD. The former is obtained from the literature, whereas the later was measured experimentally. We find that the dielectric constant of water decreases with increasing MPD.[26] In contrast, the viscosity increases and affects the translation friction of the DNA undergoing electrophoresis.

Finally, the effective charge of the DNA fragment is obtained from the Manning counterion condensation formalism.[24] The concentrations of the various species in the TBE buffer are 0.09 M Tris, 0.09 M borate, and 2 mM EDTA. The ionic strength of the buffer is 0.032 M. This is obtained by a self-consistent solution of the Henderson−Hasselbach and the Davies equations.[27] The Debye screening parameter in the absence of MPD is $\kappa = 0.327\sqrt{I}(1/\text{Å})$, where I is the ionic strength. The diameter of B-DNA is around 18 Å, whereas the charge spacing is 3.4 Å. For the cross-link ratio and gel concentration used in our gel electrophoresis experiment, the apparent pore size varies in the range between 60 and 200 Å.[17,28]

An integration of the ideas discussed above leads to an approximate expression for the electrophoretic mobility U of B-DNA in MPD

$$U = \Delta\left(\frac{A_o\zeta\sigma}{1 - T_o} + A_o\right)\exp(-R/\chi)\exp(-d/\lambda) \quad (7)$$

In deriving eq 7, we have assumed that the polyelectrolyte chain of contour length L has N phosphate charges, the distance between the phosphate charge is b, and the polyion is essentially stiff on the length scale of the Debye screening length. The mesh spacing χ is obtained from experimental data,[17,28] whereas from geometrical considerations the size of the chain R can be approximated by $bN/2$.[27] In eq 7, Δ is inversely proportional to the viscosity of water in the presence of MPD. The quantity ζ can be expressed as

$$\zeta = \sum_{i=1}^{N-1}\exp(-\kappa bi\sqrt{B})\left(\frac{1}{i} - \frac{1}{N}\right) \quad (8)$$

where B accounts for the electrostatic excluded volume[29] and is given by

$$B = \frac{\kappa d}{2}((\ln(\kappa d)/4)\Omega + (2/\kappa d) + 0.0193\kappa d)$$

$$\Omega = \frac{\kappa d}{4} + 0.0293(\kappa d)^3 \quad (9)$$

The quantity A_o is a function of the linear charge density parameter, the dielectric constant of MPD in water, the Debye screening length $1/\kappa$, and the quantity B. In the model, relaxation or the asymmetric field effect has been accounted for through the quantity T_o defined as

$$T_o = \frac{\kappa l_B\sqrt{B}}{3\delta}\sum_{i=1}^{N-1}\exp(-\kappa bi\sqrt{B})\left(1 - \frac{1}{N}\right) \quad (10)$$

δ is a numerical coefficient. In deriving eqs 8 and 10, we have assumed that the chain is locally stiff on the scale of the Debye screening length. Finally, the quantity σ is expressed in terms

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of the Debye screening parameter and the diameter of the DNA

$$\sigma = \exp(\kappa d\sqrt{B/4})/(1 + \kappa d\sqrt{B/4})$$

There is paucity of experimental data on the variation of the correlation length with %MPD.[30] Consequently, we varied the correlation length with %MPD so that the electrophoretic mobility characterizes the dynamics of the dimeric fragment VW42 (i.e., two ligated monomeric units of total length 42 bp). On the basis of this set of values for λ, we have predicted the gel electrophoretic mobility patterns of multimers of VW63−VW105, as well as VW189 as a function of MPD concentration (Figure 5). The results are in good agreement with experimental data. Our prediction for the VW189 fragment leads to the possibility that the inhomogeneity of the gel matrix in the presence of MPD may be chain-length dependent.

Conclusions

Our work has established that distinct analyses of gel mobility lead to different results regarding the influence of MPD on DNA. For example, when the gel data are examined in terms of the apparent length, the A-tract sequence, Ast, loses about 45% of its anomaly in gel migration, in agreement with earlier observation.[9] However, such an analysis is misleading because it does not take into account effects of MPD on either the B-DNA used as a size marker or the gel matrix itself.

Another conclusion is that MPD has a more consequential effect on the absolute electrophoretic mobility of B-DNA than on the mobility of A-tract-containing molecules, especially at longer DNA fragments. Furthermore, this dependence on chain length is pronounced at lower MPD concentration.

The CD spectra of sequences containing phased A-tracts or the VW sequence exhibit less than significant changes in the presence of MPD, in the range of concentration studied here, relative to the changes in spectra when the same amount of water, instead of MPD, is added. This suggests that there are no noticeable global changes in DNA as a result of its interaction with MPD. These results, together with our previous work,[31] imply that MPD cannot be invoked as the sole basis for the discrepancy between the solution and crystallographic studies on A-tract-containing sequences.

Our model predicts reasonably well the gel electrophoretic mobility patterns of multimers of VW63−VW189 as a function of MPD concentration. The model includes the ion atmosphere and its polarization, electrostatic excluded volume, hydrodynamic interactions, and fluctuation effects that characterize the overall size of the migrating polyion.

The model proposed here differs in two noticeable ways from that proposed for A-tracts.[31] First, there is a significant contribution to the electrophoretic mobility of A-tracts that comes from its end-to-end distance. To put it differently, the mechanisms of migration of A-tracts and oligomeric B-DNA in gels in the presence of MPD are different. Second, the analysis presented here takes into account excluded volume effects in an approximate way.

It has been proposed that cation localization in the grooves of DNA is responsible for the phenomenon of A-tract bending,[32,33] as well as bending by G-tracts (tracts of the form G_nC_m or C_nG_m, $n + m > 4$).[34] The gels in this study were run in 1XTBE buffer only (with and without MPD), without added divalent ions. However, we have data indicating that divalent ions effect a priori bent sequences, increasing bend magnitude to a similar extent in all sequence types.[35] Theoretical analysis on the effects of multivalent ions on the dynamics of curved DNA fragments in gels supports this assertion.[36]

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