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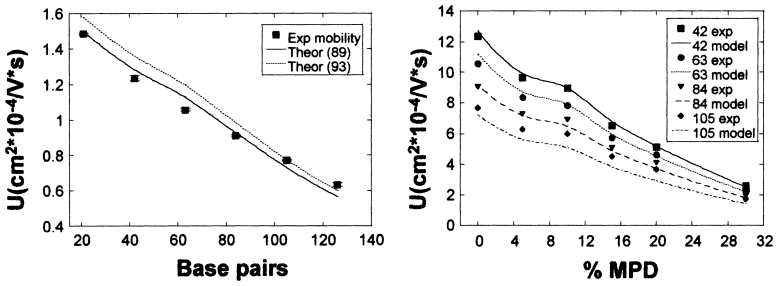

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Dynamics of Curved DNA Molecules: Prediction and Experiment

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The physical basis for the dynamics of migration of DNA oligomers through polyacrylamide gels is a long-standing and open question in physical and biophysical chemistry. In particular, no analytically tractable model exists that can describe quantitatively the migration patterns of intrinsically curved DNA sequences in gels. To advance our understanding of the physical basis of the dynamics of gel migration of DNA oligomers on the one hand and DNA bending on the other hand, we studied the gel migration of a DNA ladder built from phased repeats of the adenine-tract (A-tract) containing sequence Ast[1] and the effect of the organic solvent 2-methyl-2,4-pentanediol (MPD) on its migration. Previous studies observed that MPD reduced the anomaly in gel migration of A-tracts, interpreted as indicating a straightening of the helix by MPD.[2,3]

We have constructed a quantitative predictive model for the electrophoretic migration of intrinsically curved DNA fragments through polyacrylamide gels. Imagine a polyelectrolyte chain such as DNA threading its way through the pores of the gel. As the DNA threads its way through the pores of the gel, the tangential force on the macromolecule due to the external electric field balances the drag forces under the steady-state condition. The tangential force on a DNA of contour length L due to a constant external electric field E is proportional to the effective charge of the DNA and the end-to-end distance along the direction of the external field.[4] By Stokes law, the drag force on the DNA is the product of the mean velocity of the DNA and the translation frictional coefficient. Under steady-state conditions, the forces balance and one finds that the gel electrophoretic mobility is the product of the free solution mobility, μ_o, of the polyion and the ratio of the end-to-end distance of the molecule divided by its contour length.

In a remarkable and insightful work, Olson and co-workers have argued that the retardation characteristics of intrinsically curved DNA fragments in a gel is governed not only by its average size or special configuration, due to sequence-dependent effects, but also by the amplitude of local structural fluctuations.[5] On the basis of this work, we assume that an additional source of friction experienced by the macromolecule through the pores of the gel is due to fluctuations in its structure and that these fluctuations are a consequence of the rearrangements of the polyacrylamide segmental density in a region (termed the depletion layer) around the DNA. Furthermore, fluctuations of the segment density lead to an inhomogeneity in the polymer network. Let λ be the correlation length that is a measure of the inhomogeneity. We assume that λ is much larger than the size of the DNA. In the nondraining approximation, the time scale for the decay of inhomogeneities in the gel is much larger than the time scale τ_e for reorganization of the depletion layer.[6,7] What this means is that for time scales much larger than τ_e, the DNA migrates with the depletion layer as a "dressed" macromolecule through a fluctuating inhomogeneous network. By computing the fraction of volume accessible to the DNA and relating this to the work w_d of inserting the DNA into the entangled matrix, one finds that the extra contribution to the friction is given by $\exp(-w_d/k_BT)$, where k_B is the Boltzmann constant and T is the absolute temperature. The work of depletion can be expressed in terms of the dimensionless ratio d/λ, where d is the diameter of the DNA.[6,7]

To obtain the free solution mobility of the DNA, one needs to account for several forces that act on the macromolecule.[8,9] First, there are forces acting on the monomer unit of the polyelectrolyte chain. The sum of the forces due to chain connectivity acting on the monomer unit plus that due to electrostatic interactions between the monomer unit and all other monomers balance the forces due to friction on a monomer unit and that due to electric force acting on the monomer unit. Second, there are the forces that are acting on the solvent molecules. Here, the frictional forces acting on the solvent plus that due to the pressure acting on the solvent balance the force due to the counterions in solution. Finally, the DNA motion is coupled to that of the solvent by employing the appropriate boundary condition. An approximate solution to these equations leads to a relation for the free solution mobility that includes asymmetric field effects. This relation for the free solution mobility has been tested for oligomeric DNA.[8,10,11]

Assume that the intrinsically curved polyelectrolyte chain has n coplanar bends that separate $n+1$ segments, each of length s. By a synthesis of the steps discussed above, we find that the gel electrophoretic mobility of the DNA is approximately given by

$$U = C(\epsilon/\eta)e^{(-d/\lambda)}\sum_{i=1}^{N-1} e^{-\kappa bi}(i^{-1} - N^{-1})[(2P_o/L\{1 - (P_o/L)\{\sum_{m=1}^{n+1}(1 - e^{-s/P_o}) - \sum_{m=1}^{n+1}\cos(\beta)(1 - e^{-s/P_o})^2 + \sum_{m=1}^{n-1}\sum_{p=m+2}^{n+1}\cos(\sum_{i=m}^{p-1}\beta)(\prod_{j=m+1}^{p-1} e^{(-s/P_o)})(1 - e^{-s/P_o})^2\}\})^{1/2}] \quad (1)$$

where C is a constant; ϵ and η are respectively the dielectric constant and viscosity of the solvent; d and P_o are respectively the superhelical diameter and the persistence length of the DNA; β is the bend angle; b is the charge spacing; κ is the Debye screening parameter; and N is the length of the DNA fragment in base pairs. The quantity within the square brackets in eq 1 is the end-to-end distance of the DNA and was obtained as follows. Let a single intrinsic bend θ be located at a distance s from one end of the DNA. If the backbone is viewed as a wormlike chain, then the mean-squared end-to-end distance has contributions from orientation correlation between unit tangents from segments located on the same side and from opposite sides of the bends[12]

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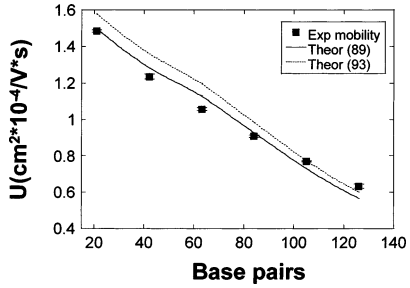

Figure 1. Electrophoretic mobility (U) of Ast as a function of DNA length in base pairs, in the absence of MPD. Experimental values (squares) are compared to predicted values at correlation lengths of 89 Å (solid line) and 93 Å (dotted line), respectively. Error bars represent the standard deviation of three independent experiments.

$$\langle h^2 \rangle = \int_0^l ds' \int_0^l ds \, \langle \hat{\mathbf{t}}(s) \cdot \hat{\mathbf{t}}(s') \rangle + 2 \int_0^l ds' \int_l^L ds \, \langle \hat{\mathbf{t}}(s) \cdot \hat{\mathbf{t}}(s') \rangle + \int_l^L ds' \int_l^L ds \, \langle \hat{\mathbf{t}}(s) \cdot \hat{\mathbf{t}}(s') \rangle \quad (2)$$

where $\hat{\mathbf{t}}(s)$ and $\hat{\mathbf{t}}(s')$ are respectively the unit tangent vector at s and s'. Since, segments of DNA on both sides of the intrinsic bend behave as straight polyions, the first and the third terms can be readily evaluated. The second term, however, depends on the magnitude of the bend angle θ and can be analytically carried out using the identity $\langle \hat{\mathbf{t}}(s) \cdot \hat{\mathbf{t}}(s') \rangle_\theta = \cos(\theta) \exp(-|s' - s|/P)$.[12]

The variation of viscosity of water with % MPD has been obtained experimentally. The dielectric constant of water decreases with increase of % MPD.[13] The ionic strength of TBE buffer is obtained from a self-consistent solution of the Hendeson−Hasselbach equation and the Davies equation and is found to be 0.0256 M. The Bjerrum length of water at 25 °C is 7.1 Å. The persistence length for A-tract DNA is taken to be 530 Å, whereas for the sequence investigated here, namely, Ast, the superhelical radius is around 61 Å.[14] The charge spacing is taken to be 3.4 Å. The effective charge of the DNA fragment is obtained from Manning's seminal work on counterion condensation.[15]

The predictions of the gel electrophoretic mobility of the Ast sequence as a function of DNA length in base pairs, in the absence of MPD, are shown in Figure 1. The predictions are in good agreement with the experimental data. Since the dependence of the correlation length with % MPD is not known, we varied λ with % MPD so that the predicted electrophoretic mobility is capable of describing the migration of the monomeric unit Ast21. On the basis of this set of values for the correlation length λ, which are consistent with that based on scattering data,[16] we have predicted the gel electrophoretic mobilities of the multimers Ast42 to Ast105 as a function of the MPD concentration (Figure 2).

To address the issue of whether MPD is excluded from the surface of DNA or not, we have determined the excluded volume parameter, w, as a function of the % MPD from a knowledge of the correlation length λ, the overlap concentration, the concentration of the gel, the Debye screening length, and the Bjerrum length.[17] Further, the excluded volume parameter is related to the Flory χ parameter through the second virial coefficient. The Mark−Houwinck equation for intrinsic viscosity of polyacrylamide−water system allows determination of the overlap concentration.[18] A striking result is that w is less than zero for the entire range of concentration of MPD. This in turn implies that the Flory χ parameter is positive and larger than 1/2. Thus, the solvent is a poor solvent for sufficiently high MPD. To put it differently, in this system, A-tracts−solvent contacts are less favored compared with A-tract−A-tract and solvent−solvent contacts.

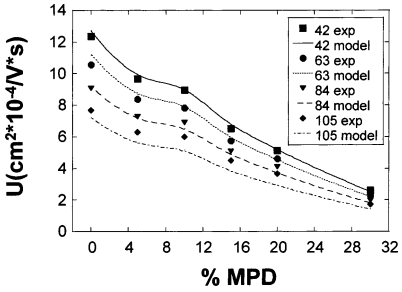

Figure 2. Electrophoretic mobility (U) of Ast21 multimers as a function of % MPD (symbols) is compared with the theoretical predictions (lines). See the inset for details.

In our model, several delicate factors govern the migration patterns of DNA fragments in the presence of MPD. First, MPD lowers the dielectric constant, which in turn affects the Bejrrum length, the Debye screening length, the screening of the hydrodynamic interactions, the electrostatic persistence length, the amplitude of thermal fluctuations, the counter condensation, and the electrostatic stability of DNA. Second, MPD increases the viscosity and, hence, the frictional coefficient. The discrepancy between experimental and theoretical prediction of Ast105 is less than significant if one notes that bending by different A-tract-containing sequences can change by 10%[1] and that the results can be accounted for in the model by a modest increase in the persistence length.

In summary, the longstanding problem of a quantitative predictive model for the absolute electrophoretic mobility of intrinsically curved DNA fragments in polyacrylamide gels has been essentially solved.

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