Anomalous Migration of Short Sequences of Nucleic Acids in Polyacrylamide Gels: Prediction and Experiment

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Received May 19, 1998

Polyacrylamide gel electrophoresis (PAGE) is employed nearly universally in the analysis of nucleic acid fragments, whether it be for sequencing, the determination of kinetic parameters, conformational analyses, or a wide variety of other applications. Resolution of DNA fragments by PAGE generally depends on a length or molecular weight criterion, but sequence dependent curvature[1,2] as well as protein-induced bends[3] can result in anomalous migration of sequences analyzed with nondenaturing gels. Curvature or bending effects tend to retard DNA sequences,[4,5] relative to corresponding random sequence fragments lacking those effects. In some experiments involving the analysis of short enzymatically synthesized sequences under denaturing conditions, the relative mobility of the shorter DNA/RNA fragments appears to be reversed,[6−8] with shorter sequences apparently retarded during gel migration, in a stepwise fashion, such that very short sequences migrate anomalously.

Our current understanding of how DNA migrates through a gel matrix is based either on the Ogston model[9−11] or on "tube" reptation ideas,[12−14] both of which are dependent upon the inherent "entanglement properties" of the gel network. In the former model, the gel fibers are viewed as a random meshwork with a characteristic distribution of pore sizes.[9−11] The electrophoretic mobility of the DNA (μ) is then a product of the free-solution mobility (μ_o) and an exponential function of cross-sectional area of the polyion divided by the mesh size. The free solution electrophoretic mobility μ_o of a polyion, however, depends on the ratio of the magnitude of the total charge Q to the translational frictional coefficient f.

In the reptation model, the gel fibers impose a sideways constraint on the DNA and result in tube-like regions.[12−14] The motion of the polyion is snake-like along the tube axis. By projecting the motion of the DNA along the field direction, an expression is obtained for the electrophoretic mobility of the polyion in terms of the total charge Q, the contour length L, the translational frictional coefficient, and the mean square end-to-end distance $\langle h_x^2 \rangle$ along the direction of the field.[12,13]

The model proposed here is based on the following observations: (i) From a novel capillary electrophoresis technique which substantially reduces electroendosmotic flow, Stellwagen and coworkers have observed, in agreement with theoretical predictions,[15] that the free solution mobility of DNA *decreases* with a *decrease* in molecular weight.[16] (ii) Both simulations and experiments indicate that there are fewer condensed counterions near the ends of a short oligonucleotide in comparison to a longer DNA fragment.[17,18] (iii) By approximating a structural unit of the polyion as a point source of friction and by considering the screening of the hydrodynamic interactions between the structural units, Manning showed[19] that the translational friction coefficient f is given by

$$f \approx N \frac{6\pi\eta}{\sum_{i=1}^{N}\sum_{i=1;i\neq j}^{N} \langle r_{ij}^{-1} \exp(-\kappa r_{ij}) \rangle} \qquad (1)$$

where is η the viscosity of the solvent, r_{ij} is the distance between monomer units i and j, N is the number of phosphates, the angular brackets denote an average over the conformations of the polyion, and κ is the Debye screening parameter. Although DNA is generally viewed as a free-draining molecule in solution,[19] it is certainly not so in the range of base pairs considered in the experiment. (iv) For a univalent salt, the number of condensed counterions per polyion charge for an oligomer[20] is given by

$$\theta = 1 + \frac{\ln(\kappa b)}{\xi \ln(L/b)} \qquad (2)$$

where ξ is the reduced linear charge density, L is the length of the polyion, and $b = L/N$ is the spacing between the phosphate charges. (v) Both the Ogston[9−11] and reptation models[12,13] are unable to explain the qualitative features of the observed experimental data (see Figure 2). (vi) There are experimental observations[21,22] indicating that the ratio of the coil size R to the

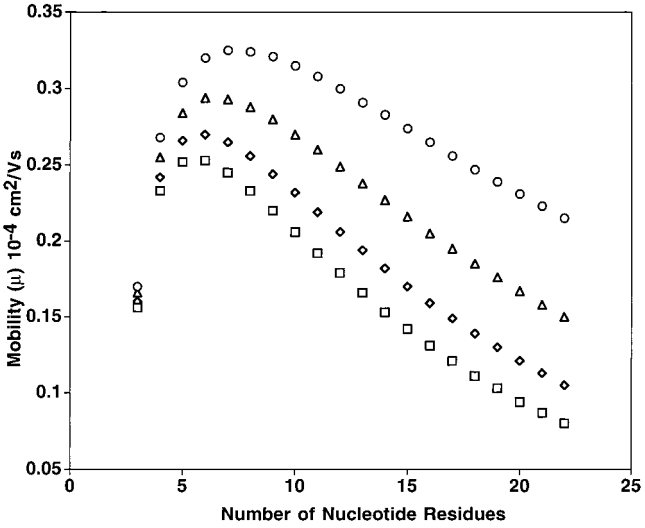

Figure 1. Plots of calculated mobility (μ) vs length of the DNA fragment (no. of nucleotide residues), based on eq 3, for gels with pore sizes of 25 Å (□), 30 Å (◇), 40 Å (△) and 60 Å (○).

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S0002-7863(98)01745-4 CCC: $15.00
Published on Web 08/05/1998

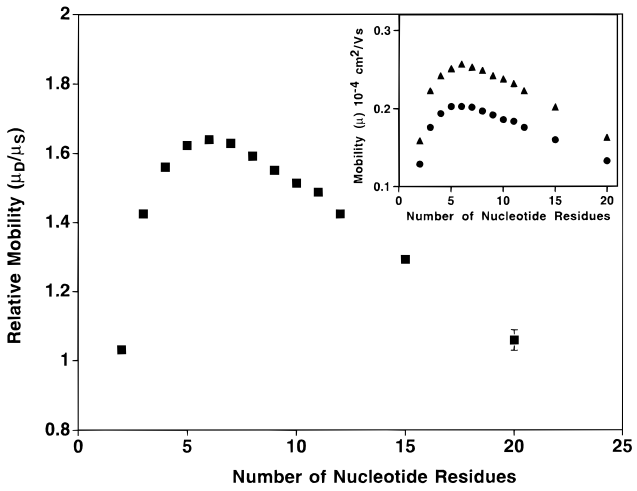

Figure 2. Measurement of relative gel mobility, μ_D/μ_S (where μ_D = mobility of the DNA fragment, and μ_S = mobility of the bromphenol blue marker) for DNA fragments [$(N_p)_nN$] containing from 2 to 20 nucleotides. Standard deviations varied from ±0.01 to ±0.03, the maximum value is illustrated. Inset: raw data, before correction with respect to the tracking dye, showing absolute mobilities for two independent experiments.

mesh spacing ζ_{mesh} may govern the mode of transport in gels for a certain range of *N*. Probe diffusion studies in polyacrylamide gels indicate that the diffusion coefficient in a gel is accurately represented by the free diffusion coefficient in pure solvent times an exponential function of $-(R/\zeta_{mesh})^{\alpha}$, where the exponent α is of an order of unity.[22] On the basis of the above observations, it is proposed that the electrophoretic mobility of short ssDNA is described by

$$\mu = \mu_o \exp(-R/\zeta_{mesh}) \quad (3)$$

Here, the free solution mobility (μ_o) is the ratio of the magnitude of the total charge *Q* to the frictional coefficient of a polyion of *finite size*, with account taken for Manning's[20] counterion condensation and end effects through eq 2.

To evaluate the frictional coefficient for a finite polyion, we assume that parts of the chain are locally stiff on the length scale comparable to the Debye length κ^{-1}. Then eq 1 can be numerically evaluated by first converting the double sum into a single sum and then evaluating the single sum by the Euler−Maclaurin formula.[23] For ssDNA, the charge spacing $b \approx 4$ Å, while at 25 °C, the viscosity of water and the dielectric constant are respectively 0.891×10^{-3} kg/m·s and 78.3. From geometrical considerations, the radius *R* of the probe is equal to *Nb*/2 [while other choices of *R* exist,[24,25] they would not alter the qualitative features of our predictions (Figure 1)].

In polyacrylamide gels, the pore size can vary between 22 and 70 Å for gels prepared from solutions of 8.0% (w/v) monomer.[24−27] The TBE buffer consists of 1 mM EDTA and 45 mM Tris borate with pH = 8.4. The pK_a values for boric acid and Tris are 9.24 and 8.07, respectively. After Debye−Huckel activity coefficient corrections, the pH for the boric acid component is 9.16, while that for the Tris component is 8.15. At pH = 8.4, the $EDTA^{3-}$ is the predominant EDTA species. To obtain the ionic strength for this buffer, the Davies and the Henderson−Hasselbach equations are solved iteratively with the solution of $I = 0.0256$ M.[28,29] The inverse of the Debye screening length follows from the relation $\kappa = 0.327 \times I^{1/2}$(1/Å).

The theoretical predictions for the migration of short DNA fragments in polyacrylamide gels indicate a linear relationship between the electrophoretic mobility (μ, in units of cm^2/Vs) and the fragment length (number of nucleoside residues, where *N* = number of internucleotide phosphate residues), except at very short sequence lengths (Figure 1). At short sequence lengths, significant anomalous migration is predicted to begin with sequences of ∼7 residues and continuing such that the mobility for the shortest fragment in the series, the *N*p*N*p*N* trimer,[30] is comparable to a 14-residue fragment (Figure 1). The onset of the anomalous migration phenomenon is dependent upon the pore size of the gel as determined for 25-, 30-, 40-, and 60-Å pore sizes (Figure 1). The position in the series at which the anomalous migration begins (transition point) also varies with pore size. These transition points occur with sequence lengths of 7−8 for the 60-Å pore size and decrease to sequence lengths of 3−4 for pore sizes of 25 Å. The free solution mobility of poly(styrene sulfonate) polyelectrolytes exhibits a similar anomalous effect at short polymer lengths.[31]

Experimental gels have substantiated this phenomenon (Figure 2). In the experimental case, data for the NpN sequence could also be obtained, and this dimer sequence exhibited the largest anomalous migration effect (migrating with an apparent sequence length of >20). The transition point in the experimental gels occurred at sequence lengths of 5−6. On the basis of theoretical predictions, this transition point would correspond to a pore size in the gel averaging 30−40 Å, consistent with previous predictions.[24−27]

It should be noted that in biochemical assays generating a series of small nucleic acid fragments, usually with some premature termination products (primase, RNA polymerase assays),[6−8] reduced processivity by the enzyme could result in the presence of small DNA/RNA fragments whose position in the gel would overlap larger fragments and confuse the interpretation of the assay.

Acknowledgment. This work was supported by a grant from the NSF (MCB-9723844) and by a research grant from Boston College. We thank Prof. Joe Billo, Department of Chemistry, Boston College, for assistance in characterizing the ionic strength of the TBE buffer

Supporting Information Available: Sample preparation procedures, conditions of gel electrophoresis, a sample gel for the resolution of N(pN)$_n$ series of fragments from which Figure 2 was derived are all available (4 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

JA981745W

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