Migration Effects for Small Phosphate-Labeled Single-Stranded DNA Fragments in Gels: Prediction and Experiment

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Polyacrylamide gel electrophoresis (PAGE) is used widely in applications that range from the analysis of conformational changes in nucleic acid fragments to the evaluation of binding constants between proteins and DNA.[1,2] The molecular weight of the nucleic acid fragments and the properties of the buffer solution usually govern the resolution characteristics of (non-denaturing) PAGE. However, anomalous migration effects for DNA sequences have been observed due to protein-induced bends as well as sequence-dependent curvature (relative to that of random sequences). Very short sequences of DNA or RNA also exhibit anomalous migration when analyzed under denaturing PAGE conditions.[2−6] In gel retardation experiments the DNA fragments are labeled at one end with a phosphomonoester typically using [γ^{32}-P] ATP and T_4 polynucleotide kinase to visualize the DNA. The prevalence of such experiments leads us to ask about the classical explanation of electrophoretic migration of DNA fragments in gels, and in free solution, when one terminus of a short oligonucleotide carries an additional small charged entity such as a phosphomonoester.

Qualitative and/or quantitative interpretations of electrophoretic mobilities of high molecular weight and low molecular weight DNA fragments are usually based on reptation and Ogston models, respectively.[7−11] The migration of DNA in free solution is governed by two factors. The first is that which results from the force exerted on DNA by an external electric field. In the absence of the external electric field, there is an additional force present that results when the DNA moves with a given velocity through a stationary fluid. Balancing these two forces suggests that the electrophoretic mobility of DNA fragments in free solution is given by $\mu_o = Q/\xi$, where μ_o is, by definition, the velocity of the DNA divided by the external electric field, Q is the effective charge of the DNA, and ξ is the frictional coefficient retarding the DNA motion. The free solution electrophoretic mobility of a polyion is independent of molecular weight since the total charge and the friction scales proportionally with the length of the molecule.

The relationship described above loses its validity for a composite oligomeric molecule containing additional charge, such as that resulting from a terminal phosphomonoester. The free solution electrophoretic mobility of such a composite (oligonucleotide + terminal charge) is not governed by its total charge. To demonstrate this point, we exploit the hydrodynamic-electric equivalence between the deformation of an end-anchored polyion in a hydrodynamic flow and in an external electric field.[12,13] Let μ_d and μ_p be the electrophoretic mobility of an oligodeoxynucleotide (d) and a phosphate anion (P), respectively, in an external electric field E. The velocity of the DNA is thus $\mu_d E$, while that of the anion P is $\mu_P E$. Let V_{dP} be the velocity of the molecule composed of both entities. Assume that the electrohydrodynamic equations can be linearized and that the hydrodynamic and electrostatic interactions between P and the DNA can be neglected. If the anion P alone is moving with velocity V_{dP}, then the flow field around it is a superposition of two fields.[12,13] One is due to P moving at velocity $\mu_d E$, while the other is due to P moving at velocity $V_{dP} - \mu_P E$ in the absence of an external field. The force acting on P due to the former is zero from the definition of electrophoretic mobility, while the force due to the later flow field is $\xi_P(V_{dP} - \mu_P E)$, where ξ_P is the frictional coefficient of P. Thus, the force balance on P needed to maintain it at velocity V_{dP} in electric field E is $F_P - \xi_P(V_{dP} - \mu_P E) = 0$.[13] A similar expression is obtained for the forces acting on the DNA if it is moving alone at velocity V_{dP}. Since the total force $F_d + F_P$ on the complex vanishes, the electrophoretic mobility of the complex is:

$$\mu = (\xi_d \mu_d + \xi_p \mu_p)/(\xi_d + \xi_p) \qquad (1)$$

The mobility is no longer proportional to the total charge of the complex.[13]

An important consequence of the above observation is that the gel retardation characteristics of small oligonucleotides with a terminal phosphomonoester in a polyacrylamide gel cannot be described by the Ogstron pore distribution model or by reptation models. Instead, we propose that the electrophoretic mobility of the end-labeled DNA in polyacrylamide gel be given by:[14−19]

$$\mu = \mu_c/[1 + (R/\zeta_{mesh})^{\alpha}] \qquad (2)$$

where μ_c is the free solution mobility of the complex given by eq 1, the exponent α depends on the gel concentration and hence the size of the pore (ζ_{mesh}), and R is the probe radius (i.e., the effective radius of the polyion).

The motivation for the denominator in eq 2 is 2-fold. First, various experimental studies indicate that the ratio of the coil size R to the mesh spacing (ζ_{mesh}) dictate the diffusion of the polyion in polyacrylamide gels.[14,15] Second, a number of theoretical studies of probe molecule diffusion in semidilute and in concentrated polymer solutions based on screening of hydrodynamic interactions suggest the plausibility of the terms in the denominator of eq 2.[16−19]

To evaluate the frictional coefficient of translation (ξ_d) of the polyion we make use of a model described by Manning that takes into account hydrodynamic screening and counterion condensation:[20,21]

$$\xi_d \approx N\{6\pi\eta/[\sum_{i=1}^{N}\sum_{j=1;i\neq j}^{N} \langle r_{ij}^{-1} \exp(-\kappa r_{ij})\rangle]\} \qquad (3)$$

where κ^{-1} is the Debye screening length, r_{ij} is the distance between

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the phosphate atoms *i* and *j*, η is the viscosity of water, and the number of phosphate atoms is denoted by *N*. The double sum in eq 3 is explicitly evaluated based on the assumption that the chain is stiff on the length scale κ^{-1}.[21] In this way end effects[22−24] have been accounted for in the model.

An external electric field distorts the ion atmosphere that surrounds the DNA fragment. This distortion during migration is such that the charge density ahead of the DNA decreases, while that behind the polyion increases.[20] Consequently, an internal electric field is created that acts in a direction which is opposite to that of the external field.[20] This phenomenon is the relaxation effect and its presence requires the addition of an extra frictional term, proportional to *N* (to be added to the right-hand side of eq 3). In this analysis, we have ignored the relaxation field contribution to the frictional coefficient since its contribution to the electrophoretic mobility is small.[20,21]

The frictional coefficient of the phosphate residue (P) of radius R_P in a solvent is described by Stokes law: $\xi_P \approx 6\pi\eta R_P$. The radius and the friction coefficient of the residue P is taken to be 4 Å and 0.8×10^{-4} cm^2/(V·s), respectively.

We now estimate the effective radius *R* for eq 2 for the various phosphate-labeled DNA fragments. The retardation coefficient for small DNA fragments is proportional to the geometric mean radius (i.e., the radius of a sphere whose volume is equal to that of the volume of the DNA).[25] In this view, the DNA is roughly a cylinder of appropriate radius and length; the latter is equal to the charge spacing multiplied by the number of residues. In the present study, we have taken the effective radius of the polyion [p(Np)$_n$] to be one-half of its length, *Nb*/2, where *N* is the number of residues and *b* is the charge spacing. One could instead use the geometric mean radius for the probe molecule, but this would not change the qualitative characteristics of our results.[25]

The single-stranded DNA fragments used in the experimental portion of this study are based upon the 20-mer 3′-d(GCTATG-GTGACTGGTAGTCG)-5′. The fragments (pN)$_n$ were prepared to correspond with the 20-mer sequence with "*n*" beginning at the 3′-terminus. The phosphomonoester was then added to each 5′-terminus by an enzymatic phosphorylation.

The values of the exponent α are approximately in the range between one-half and two. For intermediate ranges of gel concentrations the exponent α in eq 2 is taken to be unity, while for lower gel concentrations the exponent α is taken to be 2. In the later case, the cross-sectional area of the probe relative to that of an average pore in the gel plays an important role in the transport mobility.[25]

The ionic strength of the TBE buffer consisting of 1 mM EDTA and 45 mM Tris borate, pH 8.4, is 0.0256, while the Debye screening parameter is 0.0523 in units of inverse angstroms.[19,26] Polyacrylamide gel pore size diameters vary between 20 and 200 Å at 6−15% (w/v) monomer, as determined from a variety of experimental techniques and corresponding fits to models.[25,27]

The theoretical predictions for the electrophoretic migration of very short (compared to its persistence length) end-labeled ssDNA in polyacrylamide gels of various pore sizes is illustrated in Figure 1. It is predicted that for gels of pore sizes in the approximate range 25−80 Å slight anomalous behaviors occur at short sequence lengths. The anomalous behavior disappears at higher gel concentrations.

Experimental gels (Figure 2) have substantiated the features predicted by the model. Observe that at 13% (w/v) monomer a slight anomalous migration effect is observed since the mobility

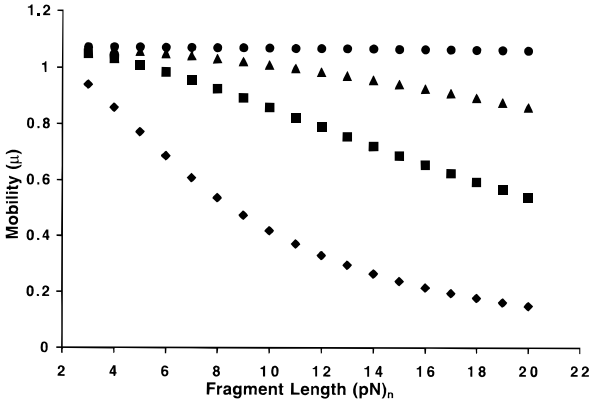

Figure 1. Predicted electrophoretic mobility (μ) in units of 10^{-4} cm^2/(V·s) versus the length of DNA fragments (number of phosphates) based on eq 2 for gels of various pore sizes: 180 (●), 80 (▲), 40 (■), and 16 Å (◆).

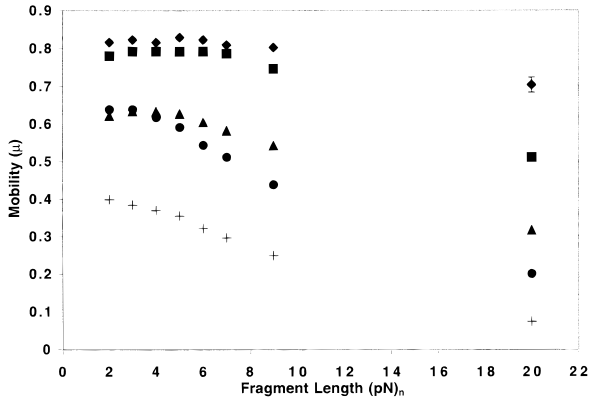

Figure 2. Experimental electrophoretic mobility (μ) in units of 10^{-4} cm^2/(V·s) versus the length of DNA fragments (number of phosphates) for gels of various polyacrylamide gel concentrations: 20% (+), 16.5% (●), 13% (▲), 9.5% (■), and 6% (◆). Standard deviations have been determined to be ±0.01−0.03. The error bar represents a standard deviation of ±0.02.

of a 2-residue fragment is comparable to a 5-residue fragment. At higher gel concentration, corresponding to 16.5% (w/v) monomer, the anomalous behavior is eliminated.

As Figure 2 illustrates, at low gel concentration [6% (w/v) monomer] the electrophoretic mobility for very small fragments is virtually independent of oligonucleotide sequence length. Such behavior is contrary to that observed in free solution studies of DNA.[22,28] The proposed model can explain this phenomenon: At low concentrations of gel the mesh size is large with respect to the size of the polyion and consequently the frictional effects on the DNA fragment due to the presence of the gel matrix are expected to be small. From eq 1, one observes that the electrophoretic mobility of very small phosphate-labeled sequences in free solution is governed by the mobility of the phosphomonoester anion. In these cases, eq 1 predicts that the electrophoretic mobility will be defined by $\mu \sim \mu_P$, independent of the length of the polyion.

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Supporting Information Available: Experimental procedures (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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