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Analysis of the electrophoretic migration of DNA frayed wires

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Abstract

We analyzed the electrophoretic behaviour of the unusual multi-stranded DNA complexes, frayed wires, in polyacrylamide gels under non-denaturing conditions. Frayed wires arise from the association of several strands of a parent oligonucleotide that possesses long terminal runs of consecutive guanines. According to the structural model proposed for frayed wires, there are two distinct conformational domains, a guanine stem and single stranded arms displaced from the stem. The presence of the two domains affects the electrophoretic migration of the frayed wires, resulting in a greater retardation compared to that of double stranded DNA of the same molecular weight. The degree of retardation is determined by the relative length of the stem and the arms; the complexes with longer arms display a stronger dependence on the total molecular weight. Reptation plots (mobility × molecular weight vs. molecular weight) were used to study the electrophoretic behaviour of frayed wires that arise from different parent oligonucleotides. The plots are unique for each type of frayed wire. The characteristic parameter, the position of the maximum of the reptation plot, depends on the type of the frayed wire as well as the total gel concentration. The plots become similar when we replot the mobility data taking into account only the single stranded arms of the frayed wires. The positions of the maximum and the overall shape are very close for the four types of frayed wires studied.

Keywords: Multistranded DNA; Frayed wires; Gel electrophoresis; Electrophoretic mobility; Reptation plot

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Abbreviations: bp, base pair; DTT, dithiothreitol; nt, nucleotide; TEMED, *N,N,N′,N′*-tetramethylethylenediamine; Tris, tris(hydroxymethyl)aminomethane

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1. Introduction

Gel electrophoresis is a powerful technique routinely used to separate biological macromolecules. Most often, it is currently exploited as a method to separate molecules on the basis of differences in their molecular weights, but it is also utilized to probe the conformational, topological and dynamic characteristics of the molecules. Generally, structural (shape and charge) and dynamic (flexibility and stability) aspects of the molecule determine its behaviour during gel electrophoresis. For example, double stranded DNA possessing phased tracts of adenine residues displays anomalously slow mobility in polyacrylamide gels when compared to the mobility of DNA fragments of the same molecular weight but with a random sequence [1,2]. The higher degree of retardation of adenine-rich sequences is attributed to the bending of the DNA double helix which leads to an increase in the effective size of the molecule. Resolution of supercoiled DNA according to the linking number is an example of an electrophoretic separation of DNA with identical molecular weight but different topology. For low superhelical density, the mobility of DNA increases with the absolute value of linking number [3]. Local conformational changes, such as the B-Z transition within negatively supercoiled DNA minicircles containing $d(GC)_n$ tracts, lead to changes in writhe and greater retardation of the species on the gel [4]. The presence of denatured regions in linear double stranded DNA results in lower mobilities relative to native DNA; if the unwound portion is sufficiently long the molecules are essentially precluded from entering the gel [5].

A number of theoretical descriptions of the electrophoretic migration of DNA and other flexible polyelectrolytes have emerged. Experimentally, electrophoretic migration of a polyelectrolyte, such as DNA, through the network of gel fibres is mainly determined by the size of the molecule relative to the mean pore radius of the gel. For small molecules, for which the radius of gyration (R_g) is smaller than the characteristic pore size, the electrophoretic mobility is adequately described by the Ogston sieving mechanism. The Ogston model treats the molecules as solid spheres whose mobility is determined by the volume fraction accessible to the spherical object of size R_g in the random polymeric network [6,7].

The electrophoretic motion of longer molecules that extend over several gel pores is not described by the Ogston theory. Their movement is strongly restrained by the gel fibres and the molecule is forced to move along its backbone. This end-on migration is successfully described by the reptation model [8–10]. Under certain conditions, the motion of polyelectrolytes of intermediate sizes are thought to involve yet another mechanism, namely entropic trapping [11,12]. In the entropic trapping regime, the flexible molecule partitions preferentially in large cavities. Small pores between such cavities represent entropic barriers to the electrophoretic movement. In this regime, the electrophoretic migration obeys a power-law relationship between the mobility (μ) and the molecular weight (N), $\mu \sim N^{-\beta}$, $\beta > 1$.

Here we explore the electrophoretic behaviour of an unusual multistranded DNA conformation, frayed wires, under native conditions. Frayed wires have two conformational domains, an extremely stable and presumably rather rigid stem comprised of guanines, and single stranded arms, projecting from the stem [13,14]. The two conformational domains in frayed wires are structurally and thermodynamically independent. Frayed wires migrate more slowly than double stranded DNA of the same molecular weight under the same electrophoretic conditions. In addition, retardation coefficients derived from the Ogston description of electrophoretic migration are larger and display stronger dependence on the molecular weight of the aggregates than duplex DNA. We show that the reptation plots (μN vs. N) for frayed wires are smooth curves with a maximum, consistent with the existence of an entropic trapping regime. The reptation plots for the frayed wires depend on the length and composition of the parent oligonucleotide. We also show that the length of the single stranded arms, rather than the total number of bases in the complex, determines the electrophoretic diffusion of the frayed wires through the gel.

2. Materials and methods

Synthetic oligodeoxyribonucleotides $d(A_5G_{15})$ — *5–15*, $d(A_{10}G_{15})$ — *10–15*, $d(A_{15}G_{15})$ — *15–15*, $d(A_{30}G_{15})$ — *30–15*, and $d(A_{20}G_{10})$ — *20–10* were purchased from the HSC Biotechnology Service Centre at the University of Toronto. Their concentrations were determined spectrophotometrically using extinction coefficients at 260 nm calculated from the nearest-neighbour model [15,16].

To label the 5′ end of an oligonucleotide, 100 pmol of strands were reacted with T4 polynucleotide kinase and [γ-^{32}P]ATP in 70 mM Tris–HCl (pH 7.6) containing 10 mM $MgCl_2$ and 5 mM DTT. Unincorporated ^{32}P was removed from the solution by gel filtration chromatography using spin columns (BioRad) prewashed with deionized water according to the buffer exchange protocol outlined in the manual.

Samples of frayed wires with a total volume of 10 μl were prepared by mixing an aliquot of the stock solution of oligonucleotide and $\sim$5 pmol of the corresponding ^{32}P-labelled strand in 90 mM Tris borate, pH 8.3, 5 mM $MgCl_2$ (TBM). The samples were 2 μM in strands of the unlabelled parent oligonucleotide. After heating the solution to 100°C, it was allowed to cool slowly to room temperature followed by incubation at 10°C for 10–15 h. The frayed wire ladder was resolved on non-denaturing polyacrylamide gels with TBM as a running buffer. The gels were run for about 15 h at low electric fields ranging from 3 to 6 V/cm. Temperature was controlled by a circulating water bath set at 7.5°C. Gels were dried under vacuum at 80°C. Band patterns were visualized using an Ambis model 4000 radioanalytic imaging system (Scanalytics, Inc., Bellerica, MA).

Extra attention was given to the gel preparation procedure to ensure a reproducible extent of polymerization. For each gel, a freshly prepared stock solution of acrylamide:bis-acrylamide (T = 20%, C = 3%) in water was mixed with appropriate amounts of TBM and water, and degassed under vacuum for 20 s. The amounts of TEMED and ammonium persulfate were adjusted for different total gel concentrations (T = 8–14%) so that polymerization took from 15 to 20 min. The gels were prerun for 30 min or until the electric current stabilized.

Positions of the bands were recorded using the digitized image of the ladder pattern of the radioactively labelled frayed wires. A 50-bp ladder (Pharmacia) was used as a standard and was visualized by ethidium bromide staining. After the completion of the gel electrophoresis, the gels were soaked in buffer containing 10 μg/ml ethidium bromide for 30 min and the positions of the bands were recorded by measuring the distance migrated from the origin. The electrophoretic mobility (μ) of the species comprising each band was calculated as $\mu = Lt^{-1}E^{-1}$, where L, t and E are the distance from the origin, time of the gel electrophoresis, and electric field, respectively.

3. Results and discussion

3.1. Frayed wires

A typical gel electrophoresis ladder pattern of DNA frayed wires is shown in Fig. 1a. The fastest migrating band in each lane corresponds to the single stranded oligonucleotide, bands with lower mobility represent the species formed via association of different numbers of parent strands. A schematic of the structure we have proposed for these aggregates, is presented in Fig. 1b [13]. We have shown that the high molecular weight complexes arise from an interaction between the guanines of the parent strands, forming a guanine ‘stem’. The 5′-end adenines, or ‘arms’, are displaced from the stem, and, presumably, are not engaged in any stable interaction. The length of the 3′-end guanine run determines the number of stable aggregates, i.e. the degree of polymerization of the parent oligonucleotide into high molecular weight complexes, and the relative abundance of different complexes in the frayed wire sample [17]. Therefore, some of the bands are missing or are less pronounced in the pattern of frayed wires arising from different parent strands.

3.2. Absolute electrophoretic mobility of frayed wires

The log–log plots of the absolute mobility ver-

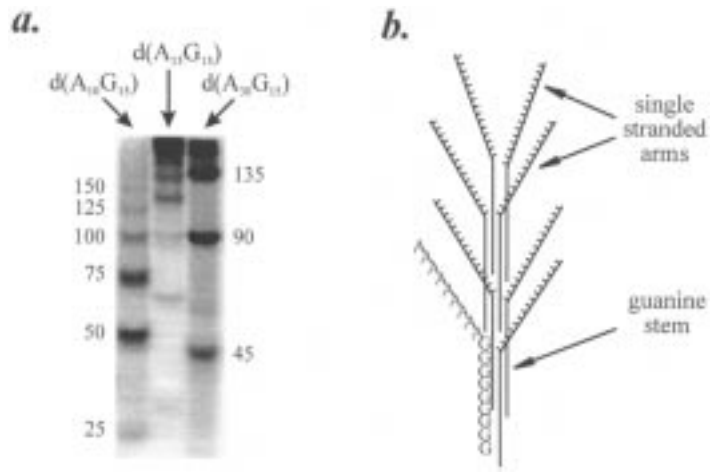

Fig. 1. (a) Typical band pattern of *10–15*, *15–15*, and *30–15* frayed wires (see Section 2 for nomenclature) resolved under non-denaturing conditions. The band with the highest mobility in each lane corresponds to the monomer, slower migrating complexes result from the association of the increasing numbers of parent strands. Molecular weights (in bases) of monomers and multistranded complexes are indicated beside the corresponding bands. (b) A diagram of the proposed structure of DNA frayed wires. The complex arises from the interaction of an integer number of parent strands. The strands are held together via formation of a stem between the terminal runs of consecutive guanines while the non-guanine portion of parent oligonucleotides remain single stranded and are displaced from the stem of the complex.

sus molecular weight for frayed wires arising from three oligonucleotides with 15 guanines and different arm lengths, *30–15*, *15–15*, and *10–15*, are compared in Fig. 2. As the number of aggregated strands increases, the mobilities of the complexes follow a smooth, decreasing curve with negative curvature. There is a substantial difference in the mobilities in the high molecular weight range of the frayed wires comprised of different parent oligonucleotides. The frayed wires with longer arms (*30–15*) display lower mobilities than the complexes of *10–15* in the same molecular weight range. The difference is more pronounced for the complexes composed of larger number of strands. The same trend is observed in both total gel concentrations, 8 and 12%, shown in Fig. 2.

The absolute mobilities of the frayed wires are much lower than the values for double stranded DNA resolved under the same experimental conditions. For example, a 100-bp fragment ($N = 200$ bases) has a mobility of 2.77×10^{-5} cm^2 V^{-1} s^{-1} in an 8% gel, while the frayed wires in the same molecular weight range display mobilities of ca. 2.0×10^{-5} and 1.5×10^{-5} cm^2 V^{-1} s^{-1} for *15–15* and *30–15*, respectively. The deviation between the mobilities of duplex DNA and frayed wires becomes larger with increasing molecular weight.

This discrepancy in the absolute electrophoretic mobility of frayed wires and double stranded DNA results from the differences between these two DNA conformations. The influence of the structural characteristics of DNA on its electrophoretic behaviour is further supported by the difference in the parameters derived from the treatment of the mobility data in the framework of the Ogston approach. We have previously reported that the retardation coefficients for frayed wires are consistently higher than those of duplex DNA [17], and that they display a stronger dependence on the molecular weight than those of double stranded DNA.

The slopes of the individual curves on the double logarithmic plots give the phenomenological scaling relationship between the mobility of the species and molecular weight, $\mu \sim N^{-\beta}$. For double stranded and single stranded DNA the value of β is expected to equal 1 in the high molecular weight range, this corresponds to end-on motion, or reptation, of the linear polyelectrolyte in the gel (see for example, [2,18]).

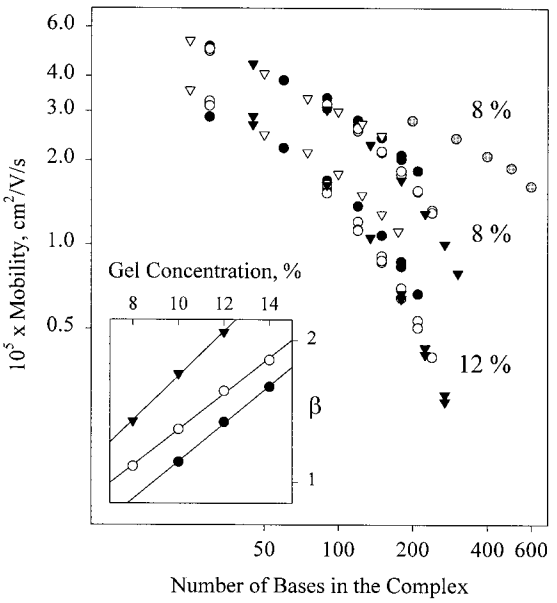

Fig. 2. Plots of absolute mobilities of the frayed wires of (▼) *30–15*, (○) *20–10*, (●) *15–15*, (▽) *10–15* vs. the total number of bases in the complex. The plots for two gel concentrations, indicated on the right, are presented. Absolute mobilities of the 50-bp double stranded DNA ladder resolved under the same conditions are shown for comparison (grey circles). The insert shows the limiting slopes (β) of the plots for the three types of frayed wires.

The value of the exponent may be more negative for single stranded DNA under conditions where entropy driven motion predominates [19]. Because the molecules preferentially partition into large cavities, where the number of allowable states is higher, their motion is retarded to a greater extent. The exponent β is a measure of the strength of the entropic effects in the electrophoretic migration of the flexible polyelectrolytes of sizes comparable to the mean pore size of the gel network. The values of β, ranging from ca. 1.5 for 10% and decreasing to 1 with the dilution of the gel, correspond to the exponent observed for single stranded DNA migration in the entropic trapping mode [19].

In the case of frayed wires, the limiting slopes for individual curves are much steeper than -1 (insert in Fig. 2), and there is a clear correlation between the limiting slope and the type of frayed wire. The frayed wires with longer arms display a larger dependence on the molecular weight, i.e. higher β values, than the complexes with shorter arms. In the 12% gel, β ranges from 1.4 for *15–15* to 2.0 for *30–15*. In addition, the limiting slopes for any given type of frayed wire display the expected dependence on the total gel concentration, or mean pore size of the gel. Higher values of β found for denser gels are consistent with the enhancement of the effects of the gel matrix on the anomalous migration of frayed wires.

Apparently, the difference between the electrophoretic motion of a linear polymer, such as double stranded DNA, and frayed wires originates from their divergent structural and dynamic characteristics. Given the structure proposed for frayed wires, they might be expected to exhibit electrophoretic behaviour distinct from duplex DNA. In general, molecules migrate through a pathway of percolating pores large enough for the molecule. The persistence length of double stranded DNA is about 50 nm, or approximately 150 base pairs, thus the molecules in the lower molecular weight range are stiff and asymmetric, with a relatively small cross-section for end-on migration. By means of occasional end-on motion, the molecule can fit through the pores which would be inaccessible to molecules with larger cross-sections, for example frayed wires. Frayed wires presumably not only have a stem that is thicker than double stranded DNA, but the single stranded arms further increase their cross-section. Given the Ogston approach, based upon the notion that the mobility is proportional to the volume fraction accessible to the molecule, the mobility of frayed wires is expected to be less than that of the duplex DNA.

Another factor, important for the understanding of the electrophoretic motion of frayed wires, is the presence of the conformational flexibility of the arms. The flexibility should affect the mobility, especially in dense gels with mean pore sizes close to the effective size of the frayed wires. In this circumstance, the entropy contribution might affect the mobility of the complexes; a flexible molecule may squeeze through a small pore, but

would be preferentially located in a larger pore that allows more degrees of freedom (greater entropy).

3.3. Reptation plots for the frayed wires

Reptation plots (mobility × molecular weight vs. molecular weight) were originally introduced to distinguish the onset of reptation of the linear molecule, i.e. the transition between the Ogston sieving and reptation regimes [20–22]. Recently, reptation plots were used to reveal the existence of a crossover regime, entropic trapping, in the migration of single stranded DNA [19]. Reptation plots are based on the relationship between the mobility of molecules and their molecular weights under a given set of conditions as put forth in the theoretical description of the migration regime. The relation between μN and molecular weight displays several regimes. At low molecular weights, μN is an increasing function, this is the Ogston sieving regime. At higher molecular weights, in the entropic trapping regime, μN decreases. This is followed at still higher molecular weights by the reptation regime in which μN increases linearly with N. The position of the maximum on the reptation plot corresponds to the transition between the sieving and entropic trapping regimes and gives an estimate of the effective size of the molecule relative to the mean pore size of the gel.

Reptation plots for the bands arising from the *20–10* frayed wire resolved in four gel concentrations are presented in Fig. 3a. The plots are smooth curves with a maximum. The position of the maximum shifts towards higher molecular weights with the dilution of the gel, due to an increase in the gel mean pore size. For example, if the position of the maximum in an 8% gel is about 150 nt, the plot for the 14% gel reaches its maximum at less than 90 nt. A reptation plot for double stranded DNA, presented along with the frayed wire ladder in Fig. 3b, is a monotonically increasing curve. As expected, extrapolation of the data to the low molecular weight range gives the same origin as the plot for the frayed wire at a given gel concentration.

Reptation plots for frayed wires with a 15-guanine stem and arm lengths ranging from 5 to 30 bases are shown in Fig. 4a. From these data, it is apparent that the parent strand determines the shape of the curve and the position of the maximum. There is a correlation between the position of the maximum and the arm length. For all three gel concentrations, the data for *30–15* reach extremes at lower values of N, i.e. at lower molecular weights, than the data for frayed wires with shorter arms. As expected, the position of the maximum shifts towards higher molecular weights

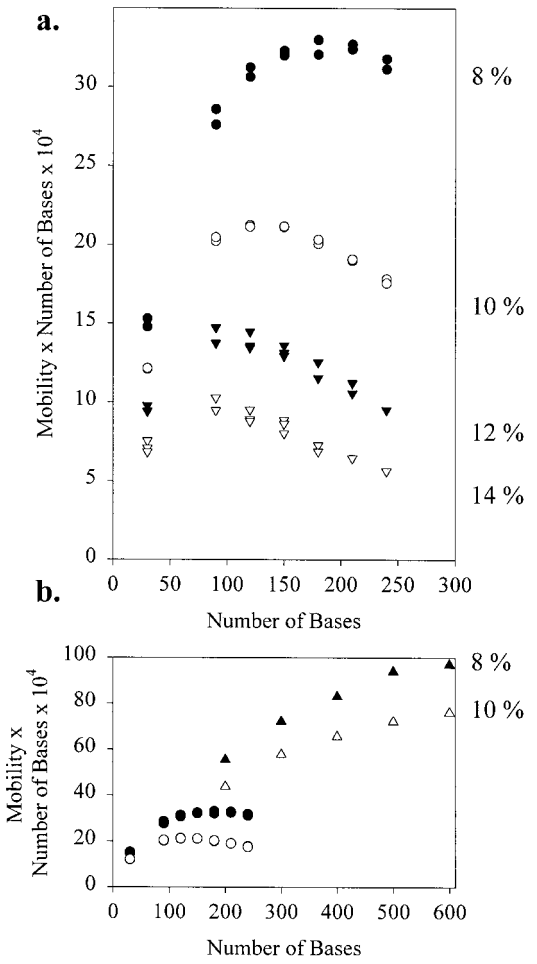

Fig. 3. (a) Reptation plots for the frayed wires of *20–10* resolved in four different gel concentrations. (b) Reptation plots for the *20–10* frayed wires (circles) are compared with the plots for the 50-bp DNA ladder (triangles) resolved under the same conditions. Gel concentrations are indicated on the right.

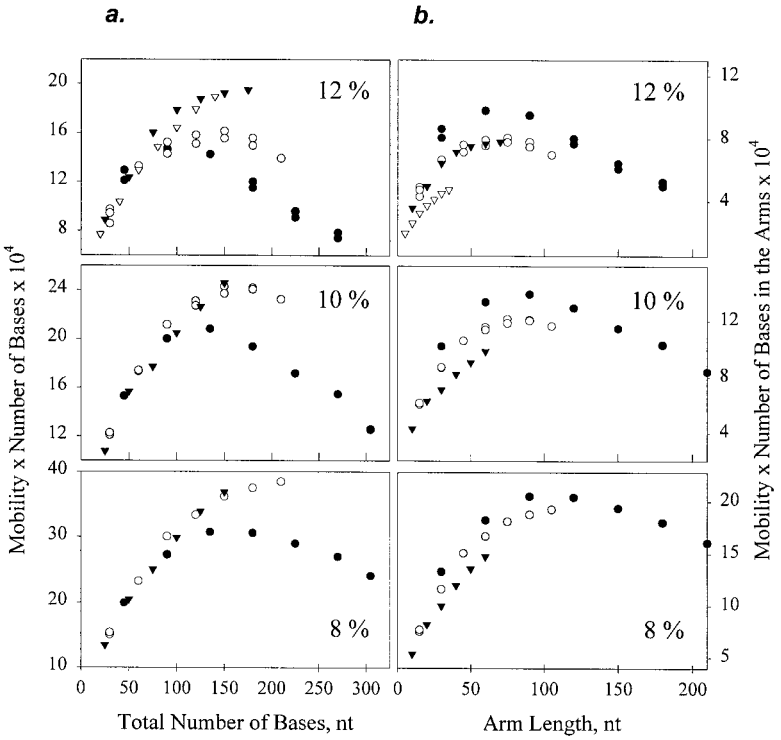

Fig. 4. Reptation plots for frayed wires of (●) *30–15*, (○) *15–15*, (▼) *10–15*, and (▽) *5–15* for three gel concentrations. Panel (a) shows the plots vs. total number of bases in the complex. Panel (b) presents the data plotted vs. the number of bases in the single stranded arms.

with the dilution of the gel for all the oligonucleotides shown. For example, the plot for the frayed wire arising from *15–15* in a 12% gel displays a maximum at about 150 nt, while in an 8% gel no maximum was observed within the range of molecular weights studied.

3.4. The electrophoretic motion of the frayed wires is primarily determined by the arm length

We replotted the data presented in Fig. 4a taking into account only the number of bases in the arms of frayed wires (Fig. 4b). It is clear that frayed wires with the same stem length but different arms display similar curves. The curves reach maxima at approximately the same positions and the overall shape of the plots for the four types of frayed wires is similar, the same tendency is observed for all three gel concentrations. Again, the position of the common maximum shifts to higher molecular weight with an increase in the mean pore size of the gel.

3.5. Electrophoretic diffusion of the frayed wires through the gel

To our knowledge, there is no theoretical description of the motion of molecules like frayed wires through the gel network; however, here we can speculate about their migration in the gel. As implied in some of our arguments given above, the Ogston sieving model adequately describes the migration of the smallest aggregates and/or the behaviour in the gels with large pores. The Ogston approach was developed to describe the behaviour of solid spherical molecules and later was extended, although with substantial assumptions, to the description of flexible polyelectrolytes. It is applicable to frayed wires with sizes smaller than the mean pore size. The discrepancy

between the electrophoretic mobility of double stranded DNA and frayed wires most likely arises from the hydrodynamic factors, such as cross-section and shape, which are important for the migration in an inhomogeneous medium such as a gel.

However, as the radius of gyration increases the molecules can no longer be considered inert spherical objects and the structural features of frayed wires become important. An essential feature of frayed wires is the presence of single stranded arms, that possess substantial conformational flexibility. Spectroscopically, the arms of frayed wires behave identically to the corresponding adenine oligonucleotides, e.g. dA_{15}, indicating the independence of the arms from the rest of the molecule [23]. The lack of significant interaction between the arms and the stem is further supported by melting experiments. The midpoint of thermal denaturation transition of the duplex formed between the adenine arms of $d(A_{15}G_{15})$ frayed wires and dT_{15} is the same as that of dA_{15}:dT_{15}. In addition, the arms of the frayed wire are not conformationally constrained within the complex. Short oligonucleotides, like dT_{10}, can form base-pairs with the adenine arms of the frayed wire even during gel electrophoresis (unpublished results). The complexes are rather unstable, and the short duplexes subsequently denature during the course of migration, giving a characteristic band pattern. This observation is further evidence that the flexible arms contribute to the behaviour of the complex in the gel.

In situations where the radius of gyration is comparable to the mean pore size, the electrophoretic migration of flexible polyelectrolytes, such as single stranded DNA, is thought to be governed by entropic effects. Within the polymeric gel network, the molecules move from one large pore to another allowing them to maximize their conformational entropy. What happens when the radius of gyration of the frayed wire is close to the size of the pore? It seems reasonable to assume that the entropic trapping model describes the electrophoretic migration of frayed wires with the sizes on the order of the characteristic gel pore size. Within the entropic trapping regime, there is greater retardation of the molecular motion through the gel. This effect has also been observed in the migration of single strands in the polyacrylamide gels and double stranded DNA in agarose gels [19,24].

Electrophoretic migration of a flexible polyelectrolyte in the entropic-trapping mode is expected to be determined by the molecular weight but independent of the topology of the molecule [25]. Plotting μN vs. N for our data shows that the sieving-entropic trapping transition is strongly dependent on the length of the arms of the frayed wire. Since the single stranded arms presumably account for the majority of the total conformational entropy of the complex, the electrophoretic motion in the entropic-trapping regime should be governed by the single stranded portions of the frayed wires. This is supported by the similarity of the plots for different types of frayed wires when the data are plotted taking into account only the number of nucleotides in the arms.

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