Persistence Length of Single-Stranded DNA

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ABSTRACT: The self-diffusion coefficient of a series of DNA fragments ranging from 280 to 5386 bases has been measured by fluorescence recovery after photobleaching after thermal denaturation in 8 M urea. The total persistence length p of single-stranded DNAs and its variation in ionic strength down to 10^{-3} M has been deduced. The importance of the value of p versus the pore size a and contour length L of the DNA in the optimization of sequencing by gel electrophoresis is emphasized.

Introduction

Gel electrophoresis of single-stranded fragments of DNA in polyacrylamide gels is a key process in sequencing DNA fragments. Separation of fragments differing by one nucleotide is presently limited to a few hundred base fragments. Separation of large double-stranded DNA in agarose gels has been improved from a few thousand to a few hundred thousand base pairs using pulsed field gel electrophoresis in conditions of reptation where the dsDNA persistence length p, contour length L, and the gel pore size a obey the relation $p \ll a \ll L$. The persistence length is an important parameter, which characterizes the flexibility of linear macromolecules and therefore their conformation in the absence of excluded volume interaction. Finding a similar regime could lead to an enhancement of the limit of sequencing. The knowledge of p, with the pore size a, will allow the quantitative test of the current models of gel electrophoresis, the biased reptation model (BRM) of Lumpkin et al.[1] and Slater et al.,[2] and the biased reptation model with fluctuations (BRF) proposed by Duke et al.[3]

Not much is known however on the persistence length of single-stranded DNA. Available data on the intrinsic viscosity and diffusion constants, as well as radii of gyration, are limited to little data on very large fragments where it is almost impossible to evaluate the respective role of rigidity, as measured by the persistence length, and the excluded volume. Moreover none have been measured in the 8 M urea denaturing medium used in sequencing gel electrophoresis. It is the purpose of this paper to present data on the diffusion constants of a series of small fragments of ssDNA in 8 M urea, to show that excluded volume effects can be neglected and to deduce useful values of ssDNA persistence length.

Direct measurement of the radius of gyration of ssDNA fragments of this size would require rather high quantities and be nevertheless very difficult in such high density mediums where density fluctuations due to convection dominate over concentration fluctuations. Measurement of diffusion constants using fluorescence recovery after photobleaching (FRAP) has the advantage to only require small quantities of DNA and to perform the measurement over the distance of a few optical interfringes.

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Table 1. Influence of the Dye on the D_s Values of pKS

DNA−dye	pKS−fluorescein	pKS−YOYO
D_{ds} 10^{-8} (cm^2 s^{-1})	3.4 ± 0.6	2.85 ± 0.4
D_{ss} 10^{-8} (cm^2 s^{-1})	6.5 ± 0.5	6.8 ± 0.3

Experimental Section

Materials. Plasmid DNAs were linearized with different restriction enzymes: EcoR1 for pKS (2961 bp) and pBR322 (4363 bp), Pst I for ΦX 174. The 763 bp chain is obtained by cutting pBR322 with BsmAI. The 407 base sample was obtained by maximum sonication of calf thymus DNA. Its molecular length was determined by GPC using light scattering on line.

To decide whether the labeling of dsDNA by an intercalating fluorescent cationic dye such as YOYO (Molecular Probes, Oregon) still applies to ssDNA, without contribution of the free dye to the diffusion coefficient by a rapid exchange, we have carried out a parallel measurement of the diffusion coefficient of ss pKS DNA labeled with non-covalently-bound YOYO and by covalently-bound fluorescein. The delicate and time-consuming covalent binding of fluorescein-12-dUTP (fluorescein high prime, Boehringer Mannheim, Germany) was achieved by a Klenow reaction[4] using the Boehringer operating mode. The highest molecular ratio obtained at the end of the reaction was one modified nucleotide per 20−25 nucleotides.[5] Purification of labeled DNAs was achieved by two precipitations in ethanol 100% and rinsing in ethanol 80%. However a large amount of unreacted fluoresceinated nucleotide remained trapped in the precipitate. To remove it, a filtration of the solution by an electrophoretic separation was performed: after centrifugation, samples were dissolved in Tris borate EDTA buffer (TBE) 10^{-2} M; samples were introduced into a well in a 10%T 3%C polyacrylamide gel (pore size ≈ 55 Å,[6] *i.e.* ≪persistence length of double-stranded (ds) DNA ≈ 500 Å), and a weak electric field was applied (1 V/cm). After 3 h, the unreacted fluorescein-12-dUTP was eliminated, and the solution was extracted from the well.

The much simpler labeling with YOYO was performed by the simple mixing of the DNA solution (60 μg/mL) with the fluorophore solution in proportion calculated to achieve a nucleotide to dye ratio $r \cong 50$ and incubation for 1 h. In both cases the ssDNA solutions were obtained by denaturing the dsDNA solutions in boiling water for 8 min in the presence of 8 M urea.

Method. Diffusion coefficients were mesured with a fluorescence recovery after photobleaching setup similar to that described by Davoust et al.[7] A fringe pattern is created inside the solution by an intense bleach pulse of light (1 W for 1 s). This pattern tends to disappear because of the diffusion of the molecules between bleached and unbleached regions. The loss of contrast with time is observed with an identical fringe pattern of low intensity, moving periodically, and therefore modulating the fluorescence of the sample. The phase-detected fluorescence signal is a monoexponential decay with a characteristic time τ. The self-diffusion coefficient is given

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Table 2. Self-Diffusion Coefficients and Deduced Hydrodynamic Radius for Different Lengths of ssDNA

number of bases	5386	2961	1916	763	407	280
D_{ss} 10^{-8} (cm^2 s^{-1})	4.4 ± 0.9	6.7 ± 0.3	7.1 ± 0.4	12 ± 3	16 ± 2	19 ± 3
R_h (Å)	321.5	211.2	199.3	117.9	88.4	74.5

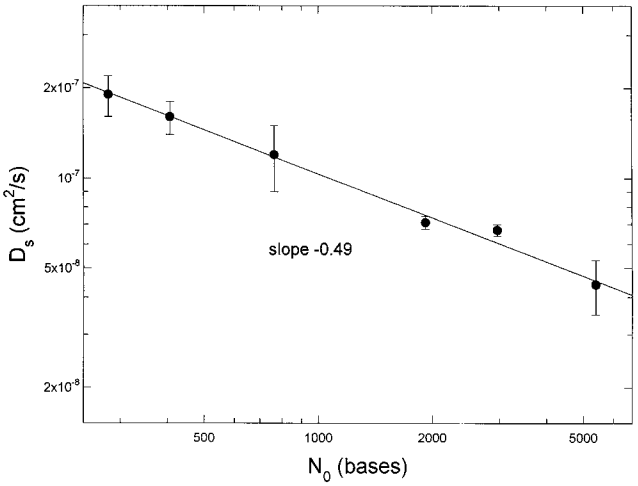

Figure 1. Variation of D_s with the number of bases of single-stranded DNA.

by $D_s = 1/\tau\mathbf{q}^2$, where $\mathbf{q}$ is the scattering vector related to the interfringe i of the pattern by $\mathbf{q} = 2\pi/i$.

FRAP experiments were carried out in TBE 10^{-2} M/urea 8 M at DNA concentrations below the overlap concentration in order to remain in the dilute regime and to prevent any renaturation of the single strands. A kinetic survey during 24 h after denaturation showed reproductible values of the diffusion coefficient, indicating that no re-association is occurring. Such observation have been done on solutions without urea 8 M and have shown a clear decrease of the diffusion coefficient (roughly by a factor 2) within 30 min, indicating in this case strong and wild reassociations.

Results and Discussion

The values of the diffusion coefficients of pKS (2961 bp) at 21 °C obtained with the two types of labeling before (D_{ds}) and after (D_{ss}) denaturation are given in Table 1. The results do not depend on the type of labeling within experimental error.

The diffusion coefficients of denatured solutions of six DNA of varying number of base N_0 have consequently been measured on YOYO-labeled samples at 21 °C. The results are given in Table 2 and plotted in Figure 1, where it is seen that the diffusion coefficient scales as $N_0^{-0.49}$, indicating that, in this range of N_0, ssDNA behaves in TBE 10^{-2} M, 8 M urea as a random non-free-draining coil with no excluded volume.

We tried to compare the diffusion coefficient calculated from the results of Rosenberg and Studier.[8] These authors determined the sedimentation coefficient S on ssDNA T7 in various conditions. Taking $S = 24.1$ in neutral conditions 10^{-2} M, correcting with the viscosity of urea 8 M, we found $D_s = 6.7\ 10^{-9}$ cm^2/s. Extrapolating our D_s values to $N_0 = 38\ 200$ (number of bases in T7), we found $1.7\ 10^{-8}$ cm^2/s. Our value is 2.6 times higher. This discrepancy can be attributed to excluded volume effects which should exist for this molecular length.

D_s is therefore related to the coil hydrodynamic radius R_h by the Stokes−Einstein relation

$$D_s = kT/6\pi\eta R_h$$

where R_h is proportional to the radius of gyration R_g which is itself given by

$$R_g = (Lp/3)^{1/2}$$

Extracting p from D_s requires therefore the knowledge of the easy to measure solvent viscosity η (1.5 cP at 21 °C) but principally of two less directly available quantities:

(1) The proportionality constant b_0 between the contour length L and N_0. A reliable value of the change of b_0 from 3.4 Å in ds DNA to 4.3 Å in ssDNA has been derived in the literature from the slope of the melting temperature of the dsDNA−ssDNA transition as a function of ionic strength, on the basis of the change in mean charge per unit length controlling the condensation of counterions.[9]

(2) The proportionality constant ζ between R_h and R_g. For the random coil model in the non-free-draining regime of the Kirkwood−Riesemann approximation, one has $R_h/R_g = (3\pi/128)^{1/2} \cong 0.664$.[10] This value has never been totally confirmed by experiments. Comparison of R_g from static light scattering and R_h from diffusion measurements derived from quasi elastic light scattering on θ solutions of uncharged polystyrene of small polymolecularity suggests $\zeta \sim 0.8$.[11] From measurements of D_s on long monodisperse dsDNA of well-known L and p, correcting for excluded volume contribution using the Stockmayer−Fixman procedure, a value $\zeta \sim 0.50$ has been derived.[12]

Considering the chemical similarity of the two systems, as polyelectrolytes in water, we have assumed in our calculation $0.5 \leq \zeta \leq 0.664$, and using the value L/N_0 of 4.3 Å, we obtain in 10^{-2} TBE/8 M urea

$$31 \pm 3\ \text{Å} \leq p \leq 52 \pm 5\ \text{Å}$$

Ionic Strength Dependence of *p*

To obtain a further indication, we have measured the ionic strength dependence of D_s and therefore of p. These experiments have been performed on pKS DNA labeled with covalently-bound fluorescein to eliminate uncertainties which could arise from a reduced binding at high ionic strength. The results are given in Table 3 and plotted in Figure 2

The persistence length can be described as the sum of two terms: a bare persistence length p_i which results from the intrinsic rigidity of the chain and an electrostatic contribution p_e which depends on the ionic strength:

$$p = p_i + p_e$$

The value obtained in 10^{-1} TBE demonstrates a very low value of p_i as compared to dsDNA ($p_i \approx 450$ Å), comparable to that of polystyrene sulfonate.[13] For this vinyl polymer, p has been derived from magnetic birefringence experiments, and the change in p between 10^{-1} and 10^{-3} M salt solutions is very close to that reported in Figure 2 for $\zeta = 0.50$.

There is much debate on the ionic strength dependence of p_e. Odijk's model—near the rod limit—predicts $p_e \sim I^{-1}$ [14] while the model of Barrat and Joanny[15] for weakly charged flexible polyelectrolyte predicts $p_e \sim I^{-0.5}$. As for polystyrene sulfonate, the change in p_i calculated with $\zeta = 0.50$ between 10^{-2} and 10^{-1} M salt is close to the absolute value calculated with Odijk's

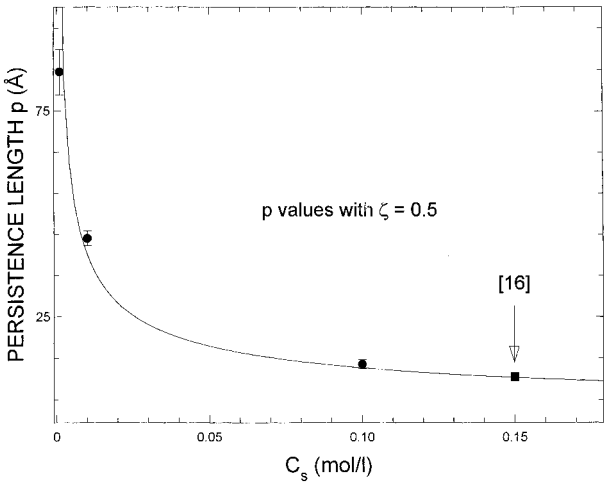

Figure 2. Variation of the persistence length p with the ionic strength I. The solid line is a guide for the eyes and corresponds to p- (Å) = $6.42.10^{-8} + 4C_s^{-1/2}$ (mol/L). The point at C_s = 0.15 M is derived from,[16] assuming b_0 = 4.3 Å for ssDNA.

Table 3. Ionic Strength Dependence of the Persistence Length of ss DNA

I (mol/L)	10^{-3}	10^{-2}	10^{-1}
D_s 10^{-8} (cm^2 s^{-1})	4.7 ± 0.6	6.5 ± 0.5	12 ± 0.2
p (Å) − ζ = 0.664	49.4 ± 15.5	25.8 ± 4.2	8 ± 2.5
p (Å) − ζ = 0.50	84.4 ± 21.5	44.1 ± 6.8	13.5 ± 4.5

model ($\Delta p_i \approx 27$ Å) while the change between 10^{-3}–10^{-2} is much lower than that predicted by a I^{-1} dependence, giving credit to the $I^{-1/2}$ dependence despite a strong charge per unit length.

At high salt concentrations, p goes down to an intrinsic persistence length p_i which lies between 8 and 13 Å (ζ = 0.5 or 0.664 respectively). From a direct measurement of the force versus extension of one single ss λDNA molecule in 0.15 M salt, Smith et al.[16] have derived b_0 = 5.6 Å and p = 7.5 Å. The model used to separate b_0 and p at high extensions is questionable, while the product b_0p, obtained from the low extension modulus is model independent. Therefore with b_0 = 4.3 Å, one gets p = 10.5 Å in fair agreement with our results for ζ = 0.50.

Conclusion

We therefore conclude that a total persistence length of ≈40 Å at 10^{-2} M should be used to model our measurements of ssDNA electrophoresis in sequencing gels. Since electrophoresis may be carried out in different ionic conditions, it is important to consider the change in persistence length with ionic strength as given in Figure 2 to optimize the conditions of electrophoresis in particular with respect to the sequencing gel mesh size (the double inequality $p \ll a \ll L$ must be verified).

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