Tracer Diffusion of Proteins in DNA Solutions

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ABSTRACT: We have used fluorescence photobleaching recovery to measure the translational diffusion of bovine serum albumin (BSA) in DNA solutions as a function of DNA concentration (from 2 to 36 mg/mL) and added salt concentration (0.1 and 0.01 M NaCl). The BSA was labeled with fluorescein isothiocyanate; the DNA was 160 base pair mononucleosomal material, about 1 persistence length. Lowering the salt concentration decreases the BSA tracer diffusion coefficient, probably due to the expanded counterion atmosphere and consequently greater effective excluded volume of the polyions. The DNA-concentration dependence is fit well by an accessible volume fraction model and also by a stretched exponential function $D/D_0 = \exp(-ac^{\nu})$. The data follow a $\nu = 1$ dependence, rather than the $\nu = {}^1/_2$ seen in some other recent studies of rigid rods.

Introduction

The diffusion of macromolecules in polymer solutions is an important component of many different industrial and biological problems such as transport across membranes, chromatography, flow through porous media, and intracellular protein diffusion. Probe diffusion in dilute and semidilute polymer solutions has been studied as a function of probe size, polymer molecular weight and polymer concentration with quasielastic light scattering (QELS),[1] pulsed field gradient NMR (PFG-NMR),[2] and fluorescence photobleaching recovery (FPR).[3] Several different theories have been proposed to relate the diffusion coefficient to the properties of the matrix and probe.[4-7] While each is derived from a different physical model, a common feature is an exponential decay of the diffusion coefficient with polymer matrix concentration. However, the physics involved in probe diffusion is still not well understood, and a universal theory has not been developed.

Our aim in this paper is to ascertain the extent which these probe diffusion concepts can be applied to a biologically important system. The diffusion of proteins in DNA solutions plays a large role in biological processes such as gene regulation. The effects of nonspecific and specific interactions, ionic strength, macromolecule volume fraction, and other solution variables have been investigated.[8] In order to observe the role of electrostatics, we have chosen to use 160 base pair DNA for the polymer matrix and bovine serum albumin labeled with fluorescein isothiocyanate (BSA-FITC) as the probe. The length of the DNA is approximately 560 Å, about 1 persistence length. BSA was chosen as the probe molecule since it has been well-characterized in terms of size, shape, charge, and diffusion coefficient.[9] It is a globular protein with an ellipsoidal shape of a 3.5 to 1 axial ratio and hydrodynamic radius approximately 34.5 Å. Inasmuch as BSA is not very asymmetrical and rapid rotation will effectively average over orientations, the system may be modeled as a sphere diffusing through a solution of rods.

Experimental Section

Sample Preparation. The DNA was kindly provided by John Duguid, University of Minnesota. It was a monodisperse preparation of 160 base pair mononucleosomal DNA from calf thymus with an estimated average molecular weight of $(1.15 \pm 0.05) \times 10^5$ a diffusion coefficient of $(2.99 \pm 0.05) \times 10^{-7}$ cm^2/s.[10a] The BSA-FITC was obtained from Sigma Chemical Co. (St. Louis, MO). The BSA was dialyzed for 2 days at 4 °C with three changes of buffer (pH 7.5, 10 mM Tris–1 mM EDTA with either 0.1 M NaCl or 0.01 M NaCl) in order to remove any free FITC from the solution. The DNA was dialyzed against purified water extensively to remove any excess salt. Assuming that 76% of the Na^+ required to balance the DNA charge was bound by counterion condensation[10b] and 24% was free in solution, the free Na^+ in solution ranged from 0.023 M for 30 mg/mL of DNA to 0.002 M for 2 mg/mL of DNA. The ionic strength for the 0.1 M NaCl samples will not be affected significantly by the Na^+ from DNA, but for the 0.01 M NaCl samples the ionic strength varies from 0.022 at 30 mg/mL of DNA to 0.011 at 2 mg/mL of DNA. DNA–BSA samples of 200–250 μL of the total volume were prepared, and the concentration was measured by absorbance at 260 nm (A_{260}). The DNA concentration was varied between 0 and 40 mg/mL, and the BSA concentration was kept constant at 0.3 mg/mL. The samples were prepared at the University of Minnesota and sent to Louisiana State University for FPR analysis.

FPR Technique. Details of the FPR apparatus will be described elsewhere.[11] In brief, our previously described system[12] has been updated to include a modulation detector, similar in principle to the one invented by Lanni and Ware.[13] A striped pattern of period L is bleached into the sample by intense laser illumination (at 488 nm) of a coarse diffraction grating placed in the rear focal plane of an objective mounted on a standard epifluorescence microscope. Following the bleaching pulse, the laser intensity is greatly reduced so that virtually no further photobleaching occurs, and the Ronchi ruling is translated at a steady speed in a direction perpendicular to the stripes. Thus, the permanent, mobile incident illumination pattern falls alternatively into and out of phase with the transient, stationary photobleached pattern in the sample. Initially, the fluorescence intensity detected by a photomultiplier tube approximates a sawtooth wave. The ac modulation at the fundamental frequency is extracted with a tuned amplifier and peak detection circuit. As diffusion erases the striped pattern in the sample, the ac signal decays at rate $\Gamma = DK^2$, where D is the tracer diffusion coefficient and $K = 2\pi/L$. ac and dc light levels are digitized and recorded by a personal computer. The decay rate is extracted by a nonlinear least-squares algorithm using a floating base line which, in the present case, always closely approximated the prebleach, noise-limited value.

Samples were drawn into rectangular capillary cells (Vitrodynamics) of path length 200 μm. In order to prevent binding

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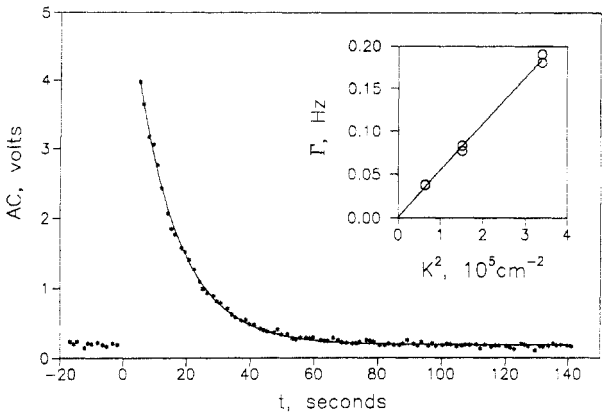

Figure 1. Peak ac voltage vs time for a typical measurement. The solid line is a fit to a single exponential with a floating base line. Inset: Decay rate vs squared spatial frequency, showing a linear dependence and absence of nondiffusive recovery modes.

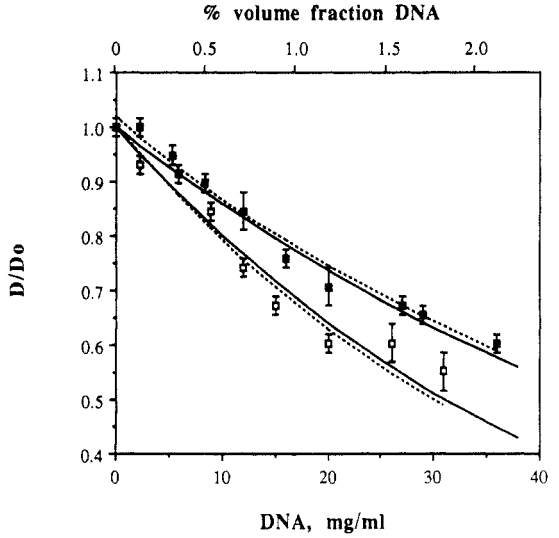

Figure 2. FPR diffusion coefficient data for BSA-FITC in DNA solution and model predictions: (■) 0.1 M NaCl, (□) 0.01 M NaCl. The dashed curves are nonlinear least-squares fits according to stretched exponential eq 3. The solid curves are best fits according to the accessible volume model (eq 2), and R = 41 Å (0.1 M NaCl) and R = 49 Å (0.01 M NaCl).

of labeled BSA to the glass walls, the cells were passivated by rinsing in 0.2 mg/mL of unlabeled BSA solution, followed by vacuum drying at room temperature. The cells were held at 25 ± 0.2 °C during measurement. All samples were measured repetitively at each of several K values. D was determined as slope of Γ vs K^2 plots by a linear least-squares fit, which also provided the uncertainty in D. Bleaching was difficult compared to most other aqueous samples (e.g., FITC-labeled dextrans). Bleach times were up to 2 times longer than optimum (ideally, bleach time < $0.1\Gamma^{-1}$). The typical bleach depth was <10% of the total signal. Figure 1 shows a typical single measurement. The inset shows a typical Γ vs K^2 plot; the zero intercept implies the absence of nondiffusive recovery modes (e.g., convection or chemical recovery).

Results and Discussion

The BSA tracer diffusion coefficients measured by FPR as a function of DNA concentration (c) appear in Figure 2. The upper and lower points are for the 0.1 M NaCl and 0.01 M NaCl buffer samples, respectively. The BSA diffusion coefficient in the absence of DNA was (5.8 ± 0.1) × 10^{-7} cm^2/s for both NaCl solutions, which agrees with previously published literature values.[9b-d] The larger decrease in the diffusion coefficient with increasing DNA concentration in the 0.01 M NaCl buffer than in the 0.1 M NaCl buffer may be explained by a greater increase in the effective volume of the charged molecules due to electrostatic repulsion. No calculations of the effective volume for BSA in DNA solutions have been done to our knowledge, but some work on the effective volume of DNA in NaCl solutions has appeared.[14] The effective radius of DNA was calculated to be 28 and 79 Å in 0.1 and 0.01 M NaCl, respectively. The BSA surface charge is a function of pH and NaCl concentration so the electrostatics are more complicated. Decreasing the NaCl concentration reduces the number of bound Cl^- and reduces the charge but increases the Debye–Hückel length. The BSA surface charge was calculated for each salt concentration from BSA pH titration[15] and Cl^- binding data[16] and numerical solution of the Poisson–Boltzmann equation.[17] A procedure for estimating the effective radius of BSA in concentrated BSA solutions has been proposed by Minton and Edelhoch.[18] From this method, the effective BSA radii are 38 Å in 0.1 M NaCl and 63 Å in 0.01 M NaCl at pH 7.5. While neither of these methods provides a good model for our system, the dimensions indicate the general range of values. The Debye–Hückel lengths and surface charges are listed in Table I for comparison.

Table I
DNA and BSA Charge and Electrostatic Parameters, pH = 7.5

NaCl (M)	DNA effective radius (Å)[a]	BSA charge[b]	Debye–Hückel length (Å)[b]	accessible volume model R (Å)
0.1	28	−16	10	41
0.01	79	−10	33	49

[a] From ref 11. [b] From Poisson–Boltzmann calculation.

The simplest model which explains the change in the diffusion coefficient is an accessible volume model. D is assumed to be proportional to the unoccupied volume fraction $1 - \phi$:

$$D/D_0 = 1 - \phi \tag{1}$$

where D_0 is the diffusion coefficient of BSA in an infinitely dilute solution. The statistical theory developed by Ogston was used to calculate the probability distribution for the radius of space between randomly oriented rigid fibers of negligible thickness.[19] The probability for finding space with a minimum radius of R is equivalent to the fraction of the total volume that can accommodate a particle of radius R

$$1 - \phi = \exp[-(\pi n F R^2 + {}^4/_3 \pi n R^3)] \tag{2}$$

where n = number density of fibers and F = fiber length. A DNA persistence length of 500 Å was used in the calculation for the fiber length. Accessible volume model curves for D/D_0 were generated by calculating the accessible volume fraction $(1 - \phi)$ for a range of DNA concentrations and various values of R. The curve with the R value which best fits the experimental data was chosen as the appropriate model.

The model curves which best fit the data are plotted in Figure 2. The R values which best fit the data are 41 and 49 Å for 0.1 and 0.01 M NaCl, respectively. The accessible volume model gives a reasonable fit to the data, but the R value is probably underestimated. Since the Ogston calculation assumes the rods to have negligible thickness, R represents the sum of the BSA radius and the DNA radius for the analysis of the experimental data. The BSA hydrodynamic radius is 34.5 Å and the semimajor and semiminor axes of BSA for a prolate ellipsoid shape are 70 and 20 Å.[9a] Excluding electrostatic effects and assuming a minimum radius of 10 Å for the DNA helix and a BSA

Table II
Nonlinear Regression Parameters in Equation 3

	K_0	α	ν
	Experimental Data		
0.1 M NaCl	1.02 ● 0.02	0.018 ● 0.006	0.95 ± 0.09
0.01 M NaCl	1.00 ± 0.02	0.024 ± 0.007	0.99 ± 0.09
	Accessible Volume Model		
R = 41 Å	1.0002 ± 0.0002	0.0153 ± 0.0001	1.001 ± 0.002
R = 49 Å	1.0000 ± 0.0003	0.0224 ± 0.0001	0.9995 ± 0.001

radius of 34.5 Å, the minimum sum of the two radii is 44.5 Å. The assumption that the fibers are stationary may be responsible for the low estimate of R from eq 2. The electrostatic repulsion between the DNA and BSA, both negatively charged, may increase the distance between the DNA molecules as the BSA diffuses through the matrix.

The experimental data and accessible volume model curves were also fit by a nonlinear regression routine to an exponential equation in order to determine the coefficients for the stretched exponential equation

$$D/D_0 = K_0 \exp(-\alpha c^{\nu}) \quad (3)$$

where c is in units of mg/mL. The fitting parameters are shown in Table IIand a plot of the experimental data with the exponential fit appears in Figure 2. The accessible volume model and experimental data are both well fit by the exponential equation, and the parameters agree within the standard deviation of the data. The ν exponent is approximately 1 for both salt concentrations, but α is 0.018 ± 0.006 $(mL/mg)^{1/0.95}$ and 0.024 ± 0.007 $(mL/mg)^{1/0.99}$ for the 0.1 and 0.001 M NaCl buffers, respectively. While the difference between α's may not be statistically significant, there is a distinct difference between the two sets of data as plotted in Figure 2. Further work will be necessary to obtain more precise estimates of α. The ν and α parameters fall in the ranges previously reported for probe diffusion in polymer matrices[6] and agree with parameters found for probe diffusion in dextran matrices[3] for a variety of probe and polymer matrix dimensions. However, other experimental work on a 1000-Å-radius latex probe diffusing in xanthan (15 000 Å long by 20 Å in diameter) found ν = $^1/_2$.[20] A hydrodynamic model for transport among rigid rods developed by Ogston, that assumed transport was determined by the frequency of probe collisions with rods, predicted ν = $^1/_2$ also.[4] Recently, data reported for the diffusion of coated silica spheres (60.4-nm radius) in poly(γ-benzyl α-L-glutamate) rods (70 nm long) gave stretched exponential parameters of α = 0.16 ± 0.02 $(mL/mg)^{1/0.81}$ and ν = 0.81 ± 0.07.[21]

In summary, values between 0.5 and 1.0 have been reported for ν. Strong hydrodynamic interactions and high concentrations have been suggested to give rise to the larger values of ν.[21] The electrostatic forces present in our experiment and the DNA mobility may be responsible for the ν = 1 behavior. The increase in α as the salt concentration is lowered suggests that it may be a useful parameter for characterizing the electrostatic interactions involved in probe diffusion.

Summary

We have measured the diffusion coefficient of BSA in a matrix of DNA rods at two different salt concentrations with FPR. Lowering the salt concentration causes a decrease in the BSA diffusion coefficient. The data follow a stretched exponential curve, and a model for the accessible volume fraction gives a reasonable representation of the data. The data follow a ν = 1 rather than ν = $^1/_2$ dependence as seen for some other cases of rigid rods.

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