DOI: 10.1002/masy.200551103

Dynamics of Biological Polyelectrolytes

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Summary: Biological polymers and structures, including proteins and DNAs, can be made in essentially monodisperse form. Proteins usually have well-defined shapes. Duplex oligonucleotides are rigid and rodlike, and longer DNAs are semiflexible coils. The DNAs also constitute a homologous series. The dynamics of both proteins and DNAs can be studied by readily available techniques such as dynamic light scattering (DLS) and fluorescence correlation spectroscopy (FCS). These systems can thus be used as model systems to elucidate elusive charge effects on the dynamics of macromolecules in solution (polyelectrolyte effects) for both rigid and semiflexible polymers. We present here as examples the results of measurements of mutual and self-diffusion coefficients dynamics of a rodlike oligonucleotide as functions of polymer concentration and the concentration of added salt (which screens the charges).

Keywords: diffusion; DNA; dynamic light scattering; fluorescence correlation spectroscopy

Introduction

The diffusion of macromolecules in dilute solution has been studied for many years and has reached a high level of sophistication. Most theories of the diffusion coefficient in these dilute solutions relate the macromolecular diffusion coefficient to quantities associated with the size and shape of the molecule and the viscosity of the suspending medium. Einstein derived the most important relation of this type in his celebrated papers of 1905 and 1906.[1] Einstein showed that the translational self-diffusion coefficient of a sphere immersed in a medium of viscosity η is related to its radius R by

$$D = \frac{k_B T}{6\pi\eta R} \qquad (1)$$

where k_B is Boltzmann's constant and T the absolute temperature. Einstein made one of the first realistic estimates of the size of a molecule (sucrose) using this relation. It is, in fact, one of the most important relations in physics because of both its historical significance and its current importance in sizing particles. Modifications of Equation (1) have been made for ellipsoids of revolution[2] and for rods.[3–5] In fact, there are now algorithms available to calculate the diffusion coefficient for molecules of arbitrary shape.[6–11] The only ambiguity in such calculations arises from solvation of the molecule. There may be a layer of solvent attached to the molecule and moving with it, so that its ''hydrodynamic dimensions'' may be somewhat larger than the dimensions obtained from static measurements (e.g., small-angle X-ray scattering, small-angle neutron scattering or, for large particles, static light scattering, direct imaging). For rigid polyelectrolyte particles in the very dilute particle region there is an ''electrolyte friction'' effect.[12] That is usually not important at high ionic strengths and is likely only a few percent at low ionic strengths. In fact, one application is to measure the self-diffusion coefficient of a molecule of complex shape, and then calculate various possible structures and their corresponding diffusion coefficients. Often this method allows one to

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determine which of the possible solution structures is correct. Thus, at least for rigid particles, the relations between particle size and shape and self-diffusion coefficients are well established.

The concentration and added salt dependencies of the diffusion coefficients of rigid polyelectrolytes, especially non-spherical polyelectrolytes, have, however, not yet been extensively investigated. The most studied systems to date have been spherical latex particles.[13] Systems containing relatively monodisperse rodlike and more complex shaped particles have been little studied and the degree of theoretical sophistication that is manifested in the infinitely dilute regime theories has not yet appeared in theories for the concentration dependencies.

In order to elucidate the principles of diffusion in non-dilute systems and the effects of the Coulomb forces in charged systems, it is important to perform experiments on systems that are monodisperse in size, shape, and charge. It is very difficult to achieve these conditions with systems of synthetic polymers. Biological polyelectrolytes are, however, ideal for his purpose. They also have the advantage of being rather soluble in water, allowing study of a wide range of concentrations in a benign solvent. Globular proteins, rodlike proteins, oligonucleotides, RNAs and DNAs, and even viruses are all ideal for this purpose. Most can be readily prepared. Some of them, e.g., the DNAs, allow the study of homologous series so that the effect of increasing the molecular size can be elucidated.[14] The shapes also vary. Short oligonucleotides as well as proteins like collagen resemble rigid rods; some proteins are spherical (e.g., ferritin) and others resemble ellipsoids of revolution. Eventually it is expected that the actual particle shape at the group or atomic level can be considered in the theories, just as in the dilute solution case, although we are still far from this objective. It should be noted that the results of such studies at non-dilute concentrations are especially relevant to *in vivo* biological systems where many components are present at non-dilute concentrations.

We should also emphasize that virus particles are now important model systems. The most important of these is perhaps tobacco mosaic virus, a rigid rodlike particle that is particularly useful for, among other things, studying transitions between isotropic and liquid crystalline phases.

As an example of the use of biological polyelectrolytes as model systems, we review here some studies on the dynamics of a rodlike oligonucleotide in isotropic solutions.

Theoretical Background

There are two diffusion coefficients of interest. The self-diffusion coefficient (sometimes called the ‘‘interdiffusion coefficient’’) is a measure of the mean-squared distance traveled by a particle in a time t:

$$D_{\mathrm{self}} = \frac{\langle r^2(t) \rangle}{6t} \tag{2}$$

Einstein showed that the self-diffusion coefficient may be expressed in terms of a ‘‘friction coefficient’’ ζ_{self}:

$$D_{\mathrm{self}} = \frac{k_{\mathrm{B}} T}{\zeta_{\mathrm{self}}} \tag{3}$$

The friction coefficient may often be calculated from macroscopic hydrodynamics. When the Stokes relation for the friction of a sphere in infinitely dilute solution is used in Equation (2), the Stokes-Einstein relation [Equation (1)] for a sphere is obtained.[1]

The other diffusion coefficient of interest is the mutual diffusion coefficient, which gives the rate at which a concentration gradient relaxes. This diffusion coefficient involves motion of both the large molecule and the solvent and is not purely a dynamic or ‘‘frictional’’ effect. The mutual diffusion coefficient also involves a ‘‘thermodynamic driving force.’’ The extent of the deviation of the local concentration from the average concentration affects the rate of the return to the average value. The mutual diffusion coefficient may be written as a product of the frictional term ζ_{mutual} and the thermodynamic term:

$$D_{\text{mutual}} = \frac{(1-\varphi)}{\zeta_{\text{mutual}}}\left(\frac{\partial \Pi}{\partial c}\right) \tag{4}$$

where Π is the osmotic pressure of the solution and c is the number concentration of solute.[15] The factor containing the volume fraction of solute (ϕ) arises from the particular choice of the frame of reference for the diffusion coefficient (volume-fixed frame of reference).

It should be noted that the frictional coefficient for mutual diffusion is, in general, different from that for self-diffusion. The differences between the two have been measured for some dispersions of spherical colloidal particles.[16,17] There is some experimental evidence that for globular protein solutions they are approximately equal.[18,19] Of course, at infinite dilution, the friction coefficients (as well as mutual and self-diffusion coefficients) are equal. There have been few investigations of the comparison between the two friction coefficients for non-spherical macromolecules or particles. Comparisons between the diffusion coefficients have been made for a few systems, but a detailed comparison of the friction coefficients, which must include a measurement of the thermodynamic factor, has been done only in a few cases, most notably, for a series of α-helical polypeptides in an organic solvent.[20] This latter case does not, of course, exhibit any polyelectrolyte effects and has the complications of sample polydispersity and molecular flexibility.[20]

It should also be noted that the diffusion coefficients for interacting systems usually depend on the time scale on which they are measured.[13] We are here discussing what are normally referred to as the ‘‘long time diffusion coefficients.’’

Experimental Techniques

One reason for the revived interest in the field of polyelectrolytes among polymer scientists is the recent availability of sophisticated and complementary experimental techniques. Several of these techniques are older ones that are now available in commercial instruments that can be used even by non-specialists in the technique. The most notable of these for our purposes are dynamic light scattering (DLS) for measuring the mutual diffusion coefficient[15] and fluorescence correlation spectroscopy (FCS) for measuring the self-diffusion coefficient.[21–27] DLS measures the time correlation function of the fluctuations in the intensity of light scattered from a system while FCS measures that of the fluctuations of the fluorescent intensity.

Dynamic light scattering was developed in the 1960s, but remained a tool for experts until recently when compact easily aligned equipment became available. Because of the use of avalanche photodiodes as detectors, new equipment does not require high-power lasers (5 W was typical) that needed special plumbing for cooling water and high-amperage power lines. In addition, the high-power laser tubes would frequently break down and had to be replaced at high cost. FCS was first developed in the early 1970s, but was utilized by only a few experts for many years. The development of the confocal microscope that greatly reduced stray light was largely responsible for the revival of the technique. The use of FCS is now spreading to a wide range of applications. It is sensitive enough to be used with single molecules.

Example—DNA

DNA is a biological polyelectrolyte that has been used as a model polyelectrolyte for many years.[14] When its protons are fully dissociated, it has a very high negative charge density. It can be made in essentially monodisperse form in a wide range of contour lengths. Short pieces of DNA are rodlike and stiff. As the chain length increases to the persistence length (about 50 nm) and beyond, the molecule exhibits flexibility for motions involving large parts of the chain. Thus, DNAs can be used to model rigid rods and semistiff chains; very large DNAs (e.g., lambda phage DNA) may even be used as models of flexible coils.[28] DNA may be studied in water solution, and the DNA and added salt concentrations may be varied over wide ranges, allowing systematic study of polyelectrolyte effects. DNA is indeed a very rich system for the polymer physicist.

As an example, we review here some results on a 20-base pair duplex oligonucleotide with the base pair sequence 5′-CGT ACT AGT TAA CTA GTA CG-3′. Models of this DNA, of course, show it to be a double helix with major and minor grooves. Hydrodynamic studies using both polarized and depolarized light scattering on a series of dilute oligonucleotides solutions studied at high added salt concentrations (to minimize intermolecular interactions) have been performed by Eimer and Pecora.[29] Both the translational and rotational diffusion coefficients were measured and the results were fit with the hydrodynamic model for short rigid rods of Tirado and colleagues.[4,5] The fits give the same diameter for each of the fragments (2 nm) and the lengths expected from the helical models. It was concluded from these studies that the rigid rod model is adequate to describe the hydrodynamics of short oligonucleotides. Since the diameter obtained from these studies is the same as the outer distance between the phosphate groups obtained from solid-state studies, it was concluded that water of hydration largely fills the grooves of the double helix, giving rodlike hydrodynamic behavior. Thus, we model the 20-mer in solution as a rigid rod with length 6.8 nm and diameter 2 nm. In addition, we note that its negative ion has a charge of 42 electron charges and its molecular weight is 13 kDa.

The mutual diffusion coefficients as a function of DNA concentration and added salt (NaCl) concentrations have been measured by Liu et al. using DLS.[30,31] The results are shown in Figure 1.

Note that the mutual diffusion coefficients increase as the 20-mer concentration is increased. This increase is faster as the added salt concentration is lower, showing the influence of the polyelectrolyte effects.

Measurements of the self-diffusion coefficients of the same 20-base pair oligonucleotide labeled with a fluorescent dye have been performed by Wilk et al.[32] The results are summarized in Figure 2. Because of the sensitivity of the FCS method, measurements could be made to much lower DNA concentrations than by DLS. Note that the ordinate is logarithmic to

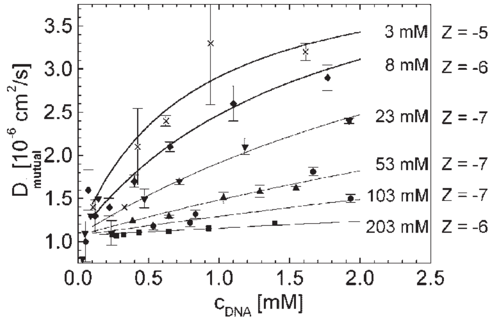

Figure 1.

Mutual diffusion coefficients of the 20-base pair oligonucleotide measured by DLS versus oligonucleotide concentration for various salt concentrations. The lines are best fits of the data points to the coupled mode theory. *Z* is the effective charge of the 20-mer obtained from the best fit. The data are from ref.[30]

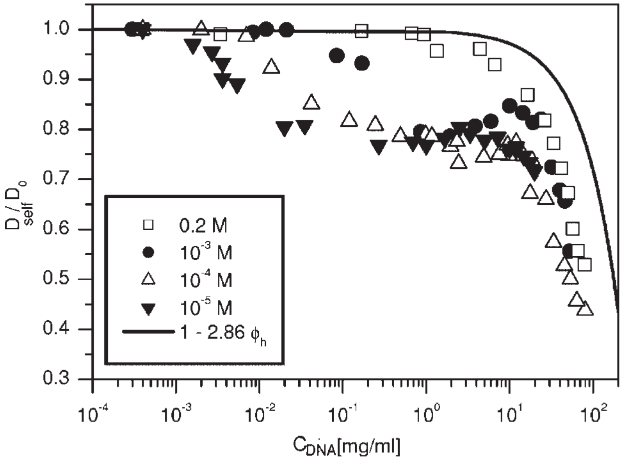

Figure 2.
Self-diffusion coefficients of the 20-base pair oligonucleotide as a function of the 20-mer concentration at different added salt (NaCl) concentrations (inset). The diffusion coefficients are divided by the value at zero concentration. Note that both the DNA and salt concentrations go to much lower values than for the mutual diffusion coefficient data shown in Figure 1. The solid line is a fit to the hard rod theory of Dhont et al.[33] The data are from ref.[32]

allow data over such a wide concentration range to be clearly displayed.

It should be noted from Figure 2 that the self-diffusion coefficients decrease as the 20-mer concentration increases, but that at low added salt the decrease is not monotonic.

The DLS results in Figure 1 are well fit by the "simple coupled mode theory."[30] Coupled mode theory includes the coupling of the motion of the small ions to that of the macromolecules through the Coulomb forces. It includes a simple form of the solution structure factor and does not include any hydrodynamic interactions. It is likely that the simple coupled mode theory would break down at lower added salt concentrations than those shown in Figure 1.

More sophisticated forms of this theory have been developed (generalized coupled mode theory), but have so far been applied only to spherical (or nearly spherical) particles.[34] The most extensive application of this theory has been to analyze the mutual diffusion coefficients of a spherical protein (apoferritin).[35,36]

The self-diffusion data still await a theoretical interpretation. It is clear from the studies of Wilk et al. that the Coulomb forces are important and that simple attempts to "renormalize" hard rod theories of the self-diffusion coefficient do not work. In these theories the effects of the Coulomb forces are treated in an approximate way by considering the diffusing particle to be a hard rod, but with larger dimensions than its actual geometrical dimensions.[32]

This example illustrates the quality of the data and the depth of the information that may be obtained using biological polyelectrolytes as model systems.

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