Screening and Fundamental Length Scales in Semidilute Na-DNA Aqueous Solutions

S. Tomić,* T. Vuletić, and S. Dolanski Babić†
Institut za fiziku, 10000 Zagreb, Croatia

S. Krča and D. Ivanković
Institute Rudjer Bošković, 10000 Zagreb, Croatia

L. Griparić
Department of Biological Chemistry, David Geffen School of Medicine, UCLA, Los Angeles, California 90095, USA

R. Podgornik
Department of Physics, University of Ljubljana and J. Stefan Institute, 1000 Ljubljana, Slovenia
and Laboratory of Physical and Structural Biology NICHD, National Institutes of Health, Bethesda, Maryland 20892, USA
(Received 10 February 2006; published 1 September 2006)

The fundamental length scales in semidilute Na-DNA aqueous solutions have been investigated by dielectric spectroscopy. The low- and the high-frequency relaxation modes are studied in detail. The length scale of the high-frequency relaxation mode at high DNA concentrations can be identified with the de Gennes–Pfeuty–Dobrynin correlation length of polyelectrolytes in semidilute solution, whereas at low DNA concentrations and in the low added salt limit the length scale shows an unusual exponent reminiscent of semidilute polyelectrolyte chains with hydrophobic backbone. The length scale of the low-frequency relaxation mode corresponds to a Gaussian chain composed of correlation blobs in the low added salt limit, and to the Odijk-Skolnick-Fixman value of the single chain persistence length in the high added salt limit.

DOI: 10.1103/PhysRevLett.97.098303 PACS numbers: 77.22.Gm, 82.39.Pj, 87.15.He

Charged polymers are ubiquitous in colloidal systems and soft matter in general [1] and play a fundamental role in determining the structure of various (macro)molecular assemblies. Their most basic role is, however, played in the biological context where they are an essential and fundamental component of the cellular environment and make their mark in its every structural and functional aspect. Because of their connectivity, stiffness, and strong electrostatic interactions, polyelectrolytes show a wide range of complex behaviors, depending on concentration, added salt, and valency of the counterions [2]. Deoxyribonucleic acid (DNA) is, in many respects, a paradigm of a stiff, highly charged polymer [3] where this complex behavior has been studied in great detail.

Electrostatic interactions for a single stiff polyelectrolyte chain such as DNA with monovalent counterions and added salt in aqueous solutions are reasonably well understood and are standardly approached *via* the Poisson-Boltzmann (PB) theory that combines electrostatics with statistical mechanics of mobile ionic species on a simplified mean-field level. The coupling between structure and interactions in semidilute polyelectrolyte solutions composed of many chains, their respective counterions, and added salt is a more complicated matter and a theoretical consensus is much less well established than in the case of a single polyelectrolyte chain or solutions of neutral polymers [2]. There are several fundamental concepts in the theoretical understanding of either a single charged polymer chain or semidilute solutions of many charged polymer chains that will shed some light on the data that we present in this contribution: the Manning-Oosawa (MO) counterion condensation, the Odijk-Skolnick-Fixman (OSF) electrostatic persistence length, and the de Gennes–Pfeuty–Dobrynin (dGPD) correlation length of a polyelectrolyte solution.

In the framework of the MO counterion condensation theory, as well as on the level of the PB equation [4] the counterions accumulate in the condensed layer exactly to such an extent that the effective charge density parameter, $\eta = zl_B/b > 1$, is reduced to 1 [3], i.e., the effective separation between charges is increased from b to l_B. Here z is the valency of the counterion, b is the linear charge spacing, and l_B is the Bjerrum length. If salt is added to the system, the mobile salt ions screen electrostatic interactions between fixed charges along DNA. For small enough fixed charge density, this screening is accurately described by the linearized form of the PB equation and is quantified by a screening or Debye length κ^{-1} defined as $\kappa^2 = 8\pi l_B n$, where n is the density of added salt. Indeed, for monovalent salts both effects, ionic screening as well as counterion condensation, coexist.

Apart from being a charged polymer, DNA is also molecularly rather stiff. The stiffness of a polymer chain is usually described *via* its persistence length L_p, which, according to the OSF theory [5], can be decomposed into a structural (L_0) and electrostatic (L_e) contribution as $L_p = L_0 + L_e = L_0 + l_B/(2b\kappa)^2$. The usually accepted value of L_0 for DNA is about 500 Å [3]. Assuming the MO con-

densation one gets $L_p = L_0 + 0.324 I_s^{-1}$ (in Å), where I_s is the ionic strength of the added salt (in M). The OSF result, though it can be applied only at restrictive conditions, appears to work well for monovalent counterions when compared to experiments [6]. Conversely, in the regime of no added salt and weak electrostatic screening, a semiflexible charged chain in a semidilute polyelectrolyte solution behaves like a random walk of correlation blobs with chain average size $R \propto c^{-0.25}$ [2].

The third important concept is the dGPD correlation length ξ or the mesh size of the semidilute polyelectrolyte solution [7]. Above the semidilute overlap concentration (c^* [2]) the polyelectrolyte chains maintain their highly extended conformation up to the scale of ξ, while on larger scales the chains are random walks [7]. In semidilute polyelectrolyte solutions ξ scales as $c^{-0.5}$ and is expected to be proportional to the screening length due to both free DNA counterions and added salt ions [2]. This correlation length divides the semidilute polyelectrolyte solution into decorrelated volumes of size ξ^3.

In an attempt to test the fundamental concepts describing the behavior of semidilute polyelectrolyte solutions we have undertaken an investigation of dielectric relaxation properties of Na-DNA aqueous solutions [8] that covers a broad range of DNA and added salt concentrations. A detailed account of the experiment will be published elsewhere [9].

Figure 1 shows the frequency dependent imaginary part of the dielectric function for solutions with selected DNA concentrations. The results for pure water [10] DNA solutions are shown in panel (a), while results for DNA solutions with added salt of ionic strength $I_s = 1$ mM are shown in panel (b). The observed dielectric response is complex [11] and the data can only be successfully fitted to a formula representing the sum of two Havriliak-Negami (HN) [12] functions, $\varepsilon(\omega) - \varepsilon_{\mathrm{HF}} = (\varepsilon_0 - \varepsilon_{\mathrm{HF}})/(1 + (i\omega\tau_0)^{1-\alpha})$. The fit to the HN functions allows us to extract the corresponding mean relaxation time τ_0 for each of the relaxation curves. The main features of this response, for pure water DNA solutions, as well as for DNA solutions with added salt, are two broad modes, whose amplitude and position in frequency depend on the DNA concentration. In the remainder of this Letter, we will refer to these modes as the high-frequency (HF), within frequency range 0.1 MHz $< \tau_0^{-1} <$ 15 MHz, and low-frequency (LF) mode, within frequency range 0.5 kHz $< \tau_0^{-1} <$ 70 kHz, respectively. The parameter $1 - \alpha$, which describes the symmetrical broadening of the relaxation time distribution function, is concentration independent and similar for both modes $1 - \alpha \approx 0.8$.

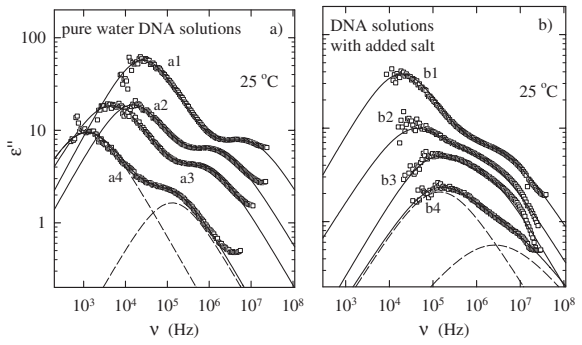

FIG. 1. Double logarithmic plot of the frequency dependence of the imaginary part of the dielectric function (ε'') at $T =$ 25 °C of (a) pure water [10] DNA solutions (for DNA concentrations a1–a4: 2.5, 0.4, 0.1, 0.0125 mg/mL) and (b) DNA solutions with added salt $I_s = 1$ mM (for DNA concentrations b1–b4: 0.83, 0.5, 0.31, 0.125 mg/mL). The full lines are fits to the sum of two HN (see main text) forms; the dashed lines represent the single HN form.

Since the counterion displacement is controlled by diffusion, the dielectric response is basically characterized by the mean relaxation time $\tau_0 \propto L^2/D_{\mathrm{in}}$, where L is the associated length scale, and D_{in} is the diffusion constant of counterions which is sufficiently well approximated by the diffusion constant of bulk ions [11,13]. We discuss possible assignments for these relaxation modes inside the framework of the theoretical approaches for polarization response of charged biopolymers in solution [11].

Our first important result concerns the characteristic length of the HF mode. For pure water DNA solutions the characteristic length L_{HF} increases with decreasing DNA concentration following the power law $L_{\mathrm{HF}} \propto c_{\mathrm{DNA}}^{-0.5}$ down to a crossover concentration $c_{\mathrm{co}} \sim 0.6$ mg/mL, at which point it gives way to a less rapid concentration dependence characterized by $L_{\mathrm{HF}} \propto c_{\mathrm{DNA}}^{-0.33}$ (main panel of Fig. 2). The power law behavior $L_{\mathrm{HF}} \propto c_{\mathrm{DNA}}^{-0.5}$ above c_{co} suggests that the dGPD semidilute solution correlation length ξ [2] can be identified as the characteristic length scale of the HF mode at no salt conditions. On the other hand, in the regime below c_{co}, L_{HF} displays a behavior that is quite unusual for semidilute solutions. It appears as though at low DNA concentrations local conformational fluctuations partially expose the hydrophobic core of DNA so that the correlation length scales as $c_{\mathrm{DNA}}^{-0.33}$, as is the case for a solution of charged chains with hydrophobic cores [2]. On the other hand this scaling form is also typical for the average distance between chains, but in the dilute regime [2].

In the case of 1 mM added salt the dGPD behavior of L_{HF} remains unchanged as long as the concentration of intrinsic counterions c_{in} (proportional to c_{DNA}) is larger than the concentration of bulk added salt ions $2I_s$ (main panel of Fig. 2). Data at lower DNA concentrations could point to a leveling off of L_{HF} close to the value of the Debye length appropriate for this added salt concentration. This statement should be made cautiously since the data are unfortunately much less reliable exactly at low DNA concentrations. (see error bars in Fig. 2). Two sets of additional data (inset of Fig. 2) for two representative DNA concentrations with varying added salt also reveal that L_{HF} does not vary with I_s in most of the *measured*

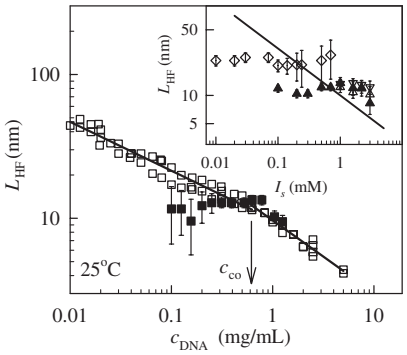

FIG. 2. Main panel: Characteristic length of the HF mode (L_{HF}) for pure water DNA solutions (open squares) and for DNA solutions with added salt $I_s = 1$ mM (full squares) as a function of DNA concentration (c_{DNA}). The full line is a fit to the power law $L_{HF} \propto c_{DNA}^{-0.33}$ and $\propto c_{DNA}^{-0.5}$ for c_{DNA} smaller and larger than $c_{co} \sim 0.6$ mg/mL, respectively. Inset: L_{HF} for DNA solutions with added salt (I_s) for two representative DNA concentrations: $c_{DNA} = 0.05$ mg/mL (diamonds) and $c_{DNA} = 0.5$ mg/mL (full triangles, open and open inverse triangles). Different symbols refer to different protocols of sample preparation [9]. The full line denotes the Debye screening length κ^{-1} for the studied range of added salt.

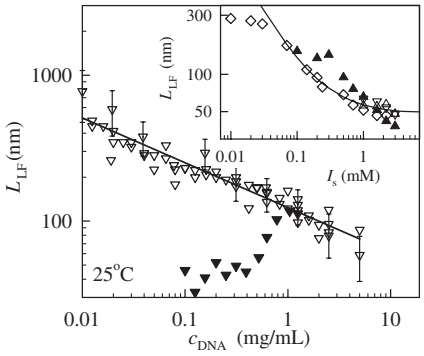

FIG. 3. Main panel: Characteristic length of the LF mode (L_{LF}) for pure water DNA solutions (open inverse triangles) and for DNA solutions with added salt $I_s = 1$ mM (full inverse triangles) as a function of DNA concentration (c_{DNA}). The full line is a fit to the power law $L_{LF} \propto c_{DNA}^{-0.29\pm0.04}$. Inset: L_{LF} for DNA solutions with varying ionic strength of added salt (I_s) for $c_{DNA} = 0.05$ mg/mL (diamonds) and for $c_{DNA} = 0.5$ mg/mL (full triangles, open, and open inverse triangles). Different symbols refer to different protocols of sample preparation [9]. The full line is a fit to the expression $L_p = L_0 + aI_s^{-1}$ with $L_0 = 480$ Å and $a = 0.09$ ÅM.

range of added salt. The accuracy of the data again becomes much less reliable due to the progressive merging of the HF and LF modes when one approaches the regime, where L_{HF} becomes apparently larger than the nominal Debye length at that salt concentration.

The second important result concerns the LF mode. For pure water DNA solutions the characteristic length L_{LF} increases with decreasing DNA concentration in almost three decades wide concentration range (open inverse triangles in the main panel of Fig. 3) following the power law $L_{LF} \propto c_{DNA}^{-0.29\pm0.04}$. This dependence is consistent with a scaling picture, where electrostatic interactions are screened by other chains and counterions to such extent, that the DNA polyelectrolyte chain behaves as a random walk of correlation blobs with a size $\propto c_{DNA}^{-0.25}$ [2]. For DNA solutions with added salt, $I_s = 1$ mM (full inverse triangles in Fig. 3), the measured L_{LF} coincides with the characteristic length found in the salt-free case only in the regime of DNA concentrations where the concentration of intrinsic counterions c_{in} (proportional to c_{DNA}) is larger than the concentration of bulk added salt ions $2I_s$. In the opposite case the measured L_{LF} starts to deviate from the line $L_{LF} \propto c_{DNA}^{-0.29}$ and decreases to attain a value of about 500 Å at small enough DNA concentrations at which it finally saturates.

The details of the dependence of L_{LF} on added salt ionic strength I_s is shown in the inset of Fig. 3 for two DNA concentrations. The observed data can be fit to the OSF behavior [5] which means that L_{LF} in the finite added salt regime could be interpreted as the DNA persistence length. From the fit we deduce that $L_0 = 480$ Å and $a = 0.09$ ÅM. While the value of L_0 close to 500 Å is in accordance with standard expectations for DNA structural persistence length [3], the value of the coefficient a differs from the OSF expectation $a = 0.324$ ÅM. A different value ($a = 0.8$ ÅM) is also found in measurements of DNA elastic properties [6]. In an attempt to reconcile these values, we have rescaled the characteristic length of the LF mode as $2.5\ L_{LF} \rightarrow L_{LF}$ in order to collapse the behavior from dielectric and elastic experiments onto a single curve (Fig. 4) [14]. The fit to the OSF expression of this new rescaled version of L_{LF} and of L_P from DNA pulling data gave $L_0 = 530$ Å and $a = 0.69$ Å. The difference in the measured and theoretically expected value of the coefficient a of the OSF form for the persistence length signals a different effective linear charge density and is consistent also with other experiments that provide a value for the effective charge density different from the MO counterion condensation theory prediction [15].

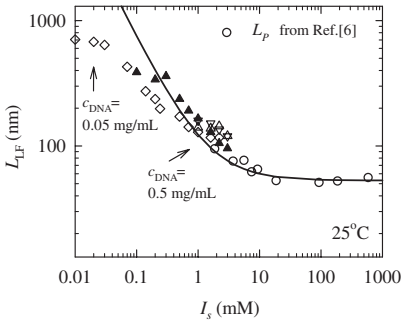

FIG. 4. Characteristic length of the LF mode, rescaled as $2.5\ L_{LF} \rightarrow L_{LF}$ (L_{LF} data are from Fig. 3) are shown together with data from pulling of DNA (open circles) taken from Ref. [6]. The full line is a fit to OSF theory with $L_0 = 530$ Å and $a = 0.69$ ÅM.

Our next important result concerns the issue of the respective roles of intrinsic DNA counterions and ions from the added salt in ionic screening. It is noteworthy that the OSF model applies as long as the ionic strength of added salt is larger than the concentration of the intrinsic counterions. The data for $c_{\mathrm{DNA}} = 0.05$ and 0.5 mg/mL deviate from the OSF behavior for $I_s < 0.03$ mM and for $I_s < 0.3$ mM, respectively. At these low added salt values ($2I_s < 0.4c_{\mathrm{in}}$), we expect that the intrinsic counterions become dominant in determining the behavior of L_{LF} and indeed it attains the same value as in pure water DNA solutions (see the main panel of Fig. 3). As already noted, in this limit DNA polyelectrolyte chain behaves as a random walk of correlation blobs with a chain size $\propto c_{\mathrm{DNA}}^{-0.25}$. Our data thus seem to indicate that for the LF mode the characteristic length in our system in the low added salt limit is given by the average size of the chain, i.e., DNA polyelectrolyte chain behaves as a Gaussian chain with a size $\propto c_{\mathrm{DNA}}^{-0.25}$ [2], and with added salt goes smoothly into the OSF form of the persistence length (see the inset of Fig. 3). This means that when salt is added to the system the role of screening is transferred from other chains and counterions, thus from DNA as its own salt, to the added salt ions and thus emerges as the OSF value of the persistence length. Very similar conclusions have been reached also by Record [16] while analyzing T4 and T7 phage DNA denaturation. He observed that in this system too the screening depends not only on added salt concentration, but also, and more importantly, on the concentration of intrinsic DNA counterions—on the DNA as its own salt.

In conclusion, our results demonstrate that there are three fundamental length scales that determine the dielectric response of a semidilute DNA solution [9]: the OSF salt-dependent persistence length of a single polyelectrolyte chain, the size of the chain in the salt-free polyelectrolyte solution and the dGPD semidilute solution correlation length. The first two length scales are important for the low-frequency response, while the last length scale is important for the high-frequency response. Our results for semidilute DNA solutions quantitatively coincide with the dGPD theoretical prediction describing the concentration dependence of the correlation length of semidilute solutions with flexible chains as $\propto c^{-0.5}$. Moreover, they confirm the prediction of Odijk [17] that the same scaling law should be valid for semiflexible DNA polymers in semidilute solutions as well. Our data also seem to indicate that once the added salt becomes larger than the concentration of DNA intrinsic counterions, the high-frequency characteristic length, rather than scaling as the semidilute solution correlation length, levels off at a value close to the corresponding Debye length. More systematic experiments are needed to assess the possible added salt dependence in this regime of salt concentrations. In the limit of low DNA concentrations and low added salt, the semidilute solution correlation length smoothly crosses over to a less rapid scaling as $\propto c^{-0.33}$ probably reflecting the appearance of locally fluctuating regions with exposed hydrophobic cores. The OSF prediction for the persistence length as a function of added salt ionic strength is also verified in the high added salt limit giving very good agreement with DNA pulling data. Finally, in the limit of low added salt the characteristic length scale smoothly merges with the average size of the polyelectrolyte chain where electrostatic interactions are screened by other chains and counterions, where thus DNA acts as its own salt.

*Electronic address: stomic@ifs.hr
†Permanent address: Department of Physics and Biophysics, Medical School, University of Zagreb, 10000 Zagreb, Croatia.

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