Dynamics and Structure of Biopolyelectrolytes Characterized by Dielectric Spectroscopy

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Summary: Structural properties of Na-DNA and Na-HA aqueous solutions can be quantified using dielectric spectroscopy in the frequency range 100 Hz–100 MHz. Two relaxation modes are typically detected that can be attributed to diffusive motion of polyion counterions. The overall study as a function of polyion length, concentration and added salt concentration demonstrates that the motion of polyion counterions detected at MHz frequencies probes collective properties, whereas the motion at kHz range probes single-chain properties of polyelectrolytes. Fundamental length scales found to characterize the polyelectrolyte structure differ for the dilute and semidilute regime and also depend on the strength of electrostatic interactions and the flexibility. Characteristic length scales detected in the dielectric spectroscopy measurements compare well with the fundamental length scales predicted by theory and comply with those extracted from small-angle X-ray scattering.

Keywords: biomaterials; dielectric properties; dynamics and conformational changes; polyelectrolytes; small-angle X-ray scattering (SAXS)

Introduction

Polyelectrolytes play a major role in biology and materials science and are of considerable interest for soft matter physics from a basic scientific point of view.[1,2] In biology, it is widely accepted that the conformational properties of cellular components such as nucleic acids, polysaccharides, actin filaments, microtubules and proteins play a key role in determination of their functional behavior. Thus, over the years much effort has been invested to develop more advanced tools for structural determination, most of them being single-molecule techniques. Nevertheless, since the extraordinary conformational and dynamical properties of polyelectrolytes are tightly related, another route can be taken which involves measurement of dynamical properties of many polyelectrolyte chains in solution (*s.c.* tube experiment). The question which immediately arises is: can the spectroscopic tools applied in case of the tube experiment provide information about the single-chain structure? The purpose of this paper is to demonstrate the *dielectric spectroscopy (DS) technique* as a powerful tool, which is concomitantly able to detect and discern structural organization of the solution as an ensemble composed of many chains, as well as structural properties of a single chain. To this end, we overview in this paper our previously published DS study of DNA and HA aqueous solutions[3–6] and complement these results with the new results obtained by small-angle X-ray scattering (SAXS) measurements.

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Materials and Methods

What does DS measure? DS probes the dynamics of counterion atmosphere which surrounds each biopolymer in a solution. The DS has been known for more than 50 years and has been successfully applied in the study of dielectric properties of biological systems and synthetic polymers in solution.[7–9] Biopolymers like deoxyribonucleic acid (DNA) and hyaluronic acid (HA) standardly come as highly asymmetric salts with positive counterions such as sodium (Na) counterions. In aqueous solutions phosphate groups on DNA and carboxyl groups on HA are completely dissociated, making them charged polyelectrolytes. Intrinsic sodium counterions form the counterion atmosphere, and the dynamics of this fluctuating charge cloud, being sensitive to applied electric fields, can be studied by the DS. For the strongly charged polyelectrolytes such as DNA two distinct types of counterions can be distinguished. The first type are condensed counterions which are tightly bound to polyions, and the second type are free counterions distributed in a larger volume around the polyions. These counterion types should be considered transient, meaning that there is a constant dynamic exchange between them. On the other hand, in the case of weakly charged polyions such as HA the thermal energy is much stronger than electrostatic interactions and consequently only free counterions form the counterion atmosphere.

Schematically, we can represent the biopolymer as a negatively charged chain

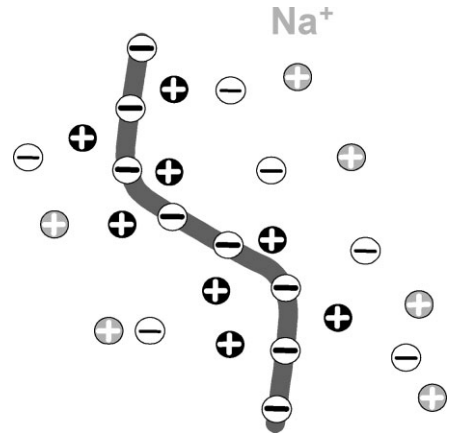

Figure 1.
Schematic representation of a (bio)polyelectrolyte: a negatively charged chain is surrounded by a cloud of positive sodium counterions (full symbols). White and gray circles stand for the electrolyte ions in which the biopolymer is dissolved.

in a cloud of positive sodium counterions (Figure 1). In a tube experiment there are many chains in a solution. Two physically distinctive cases are of note. In the semidilute regime the chains are long enough to be entangled with each other, whereas in the dilute regime each segment is well-separated from the others (Figure 2(a), (b)).

Under the applied small ac electric field the counterions oscillate and, since their displacements take place by diffusive motion, the dielectric response is basically characterized by the mean relaxation time $\tau_0 \propto L^2/D_{\text{in}}$, where L is the associated length scale, and D_{in} is the diffusion constant of counterions.[10] Experimental data[11] and

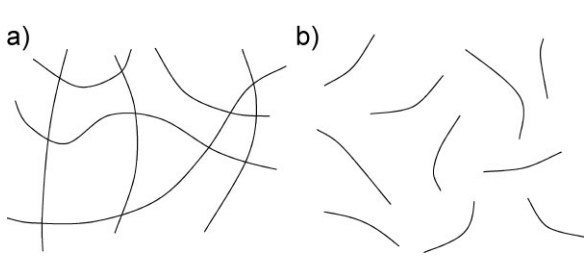

Figure 2.
Long overlapping chains form a semidilute solution (a), whereas short chains or chains at much smaller concentrations are well separated from each other forming a dilute solution (b).

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theoretical estimates[12] show that the renormalization of the diffusion constant of bulk ions due to the presence of polyions is negligible, leading to a value of $D_{in} = 1.33 \cdot 10^{-9} m^2/s$. In other words, if one can measure the relaxation time τ_0, then one can easily calculate corresponding length scales involved in counterion oscillations. As will become clearer in what follows, these length scales are defined by conformational features of either a single polyion chain or the structure of a many chain ensemble in solution.

Parameters expected to be relevant for counterion dynamics in polyelectrolyte solutions include valency, chain length, concentration of polyions and added salt ions. Our study of long (salmon testes, polydisperse, average fragments 4 μm long) and short (calf thymus, monodisperse nucleosomal fragments, 146 bp) DNA chains indeed confirmed this expectation.[3–5] Here we note that DNA dielectric properties have been studied by a number of authors since the early 1960 s;[13–17] however, no previous experimental work has been done in such a broad range of DNA concentration and added salt and no work has been able to detect concomitantly two dispersions and associate them with the solution and single-chain structural properties. Low protein content of samples was verified by UV spectrophotometry measurements. Specially designed DS measurements were used to study DNA behavior before and after thermally induced denaturation in order to check the stability and integrity of the double helix in pure water DNA solutions. These studies indicated that ds-DNA was never denatured into two spatially distinguishable and well-separated single strands. An additional study of long (polydisperse, average fragments 4 μm long) HA chains with much weaker electrostatic interactions and much higher chain flexibility as compared to DNA clearly showed the relevance of these two parameters.[6] What follows is a description of how the parameters characterizing the counterion dynamics from DS measurements are extracted and how they are connected with polyelectrolyte structural properties predicted by theoretical models.

DS measurements are performed at room temperature. Dry DNA samples were dissolved in either pure water or NaCl aqueous solution, and thus mother-solutions were prepared, with usual concentration of 5–25 mg/mL, depending on the experiment. Subsequent dilution by either pure water or appropriate NaCl solution was used to prepare lower DNA concentration solutions down to 0.01 mg/mL and with varying added salt content. The details of DNA (HA) solution preparation can be found in our precedent publications.[4–6] The DNA (HA) solution droplet of 100 μL is applied between platinum electrodes of a home-made capacitive chamber. The chamber is closed and connected to the temperature control unit and the Agilent 4294A precision impedance analyzer which operates in the 40 Hz–110 MHz frequency range. The properties measured are the conductance $G^{sample}(\omega)$ and the capacitance in parallel $C_p^{sample}(\omega)$, where $\omega = 2\pi\nu$ is the frequency. In addition, reference samples are measured in order to minimize stray impedances, including the free ion contribution and electrode polarization effects. Assuming that the conductivity contribution of each entity in the solution is additive,[1] the polyelectrolyte response is given by $G(\omega) = G^{sample}(\omega) - G^{ref}(\omega)$ and $C_p(\omega) = C_p^{sample}(\omega) - C_p^{ref}(\omega)$, where $G^{ref}(\omega)$ and $C_p^{ref}(\omega)$ is the response of reference samples. Finally, the real and imaginary parts of dieletric function are extracted using relations $\varepsilon'(\omega) = (l/S)(C_p(\omega)/\varepsilon_0)$ and $\varepsilon''(\omega) = (l/S)(G(\omega)/\omega\varepsilon_0)$, where $l/S = 0.1042 \pm 0.0008\ cm^{-1}$ is the chamber constant: $S = 0.98\ cm^2$ is the effective electrode cross-section corresponding to the sample of 100 μL and $l = 0.1021 \pm 0.0001$ cm is the distance between the electrodes. A detailed description of the measurements can be found in our precedent publications.[4,6]

In addition, we supplement the review of our DS results with Small Angle X-ray Scattering (SAXS) experiments as a complementary method for quantifying the

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polyelectrolyte solution structure. SAXS experiments were performed on a compact Kratky camera (System 3, Hecus X-ray Systems, Graz, Austria). The camera was mounted on a sealed-tube generator (GE-Seifert, Ahrensburg, Germany), which was operated at 2 kW. CuK_α radiation ($\lambda = 1.542$ Å) was selected using a Ni filter in combination with a pulse height discriminator. The X-ray beam size was set to 0.5 mm × 3.5 mm (V x H). The SAXS patterns were recorded using a linear, one-dimensional, position-sensitive detector (PSD 50, Hecus X-ray Systems, Graz, Austria). Samples were filled into 1 mm-thin-walled quartz-glass capillaries and vacuum sealed with wax. Experiments were performed at room temperature with exposure times of 3600 s. Angular calibration was performed with silver-stearate ($d = 48.68$ Å).

Results and Discussion

The dielectric function spectra of long DNA and HA chains (referred to as DNA and HA), as well as of short (referred to as 146 bp) DNA fragments, exhibit two prominent broad modes in MHz and kHz frequency range (see Figure 3). We denote these modes as the high-frequency (HF) and the low-frequency (LF) modes, respectively. The data can only be successfully fitted to a sum of two Cole-Cole forms

$$\varepsilon(\omega)-\varepsilon_\infty = \frac{\Delta\varepsilon_{\mathrm{LF}}}{\left[1+\left(i\omega\tau_{0,\mathrm{LF}}\right)\right]^{1-\alpha_{\mathrm{LF}}}} + \frac{\Delta\varepsilon_{\mathrm{HF}}}{\left[1+\left(i\omega\tau_{0,\mathrm{HF}}\right)\right]^{1-\alpha_{\mathrm{HF}}}}$$

where ε_∞ is the high-frequency dielectric constant, $\Delta\varepsilon$ is the dielectric strength, τ_0 the mean relaxation time and 1-α the symmetric broadening of the relaxation time distribution function of the LF and HF dielectric mode. Measured data were analyzed by using the least squares method in the complex plane assuring that the same set of parameters fits both the real and imaginary parts of spectra. Besides improving our ability to resolve two modes, this also provides an important Kramers-Kronig consistency check for the data obtained by the reference subtraction procedure.

SAXS scattering intensity data obtained for pure water solutions of long DNA are shown in Figure 4. The intensity curve features a scattering peak whose maximum shifts toward smaller inverse wave vectors q_m^{-1} with increasing DNA concentration. Such a behavior is common for polyelectrolyte solutions at low salt concentrations and these observed peaks are known as the Coulomb peaks[18–20] corresponding to a spatial structuring of the polyelectrolyte due to long range Coulomb interactions. The peak scattering vector is associated with the characteristic length scale in the SAXS experiment as $q_m \propto L^{-1}$, where L is the size of the exclusion volume around a polyion in solution determined mostly by electrostatic excluded-volume interactions.

First, results are described which demonstrate that the HF mode in MHz range probes the collective properties of the polyelectrolyte solution. In the case of short 146 bp DNA fragments in dilute regime[5] the characteristic length scale decreases with increasing concentration following power law $c_{\mathrm{DNA}}^{-0.33}$ as expected for the average distance between chains in this regime (Figure 5). On the other hand, the size of the observed characteristic length scale is smaller than the chain contour length of 50 nm (not expected in dilute regime) and can thus be ascribed to the reduced average distance between chains. It is noteworthy that these results are in nice agreement with the predictions of the theoretical model by Deshkovski *et al.* [21] and Dobrynin *et al.* [22] developed for polyelectrolytes in dilute regime where chains are as far away from one another as possible, due to the long-range repulsive interactions between them. In this model, the polymer is placed at the center of a cell that is subdivided into two zones: a smaller cylindrical one, inside which the electrostatic interaction energy is large, and a larger spherical zone where the electrostatic interactions are described by the low-

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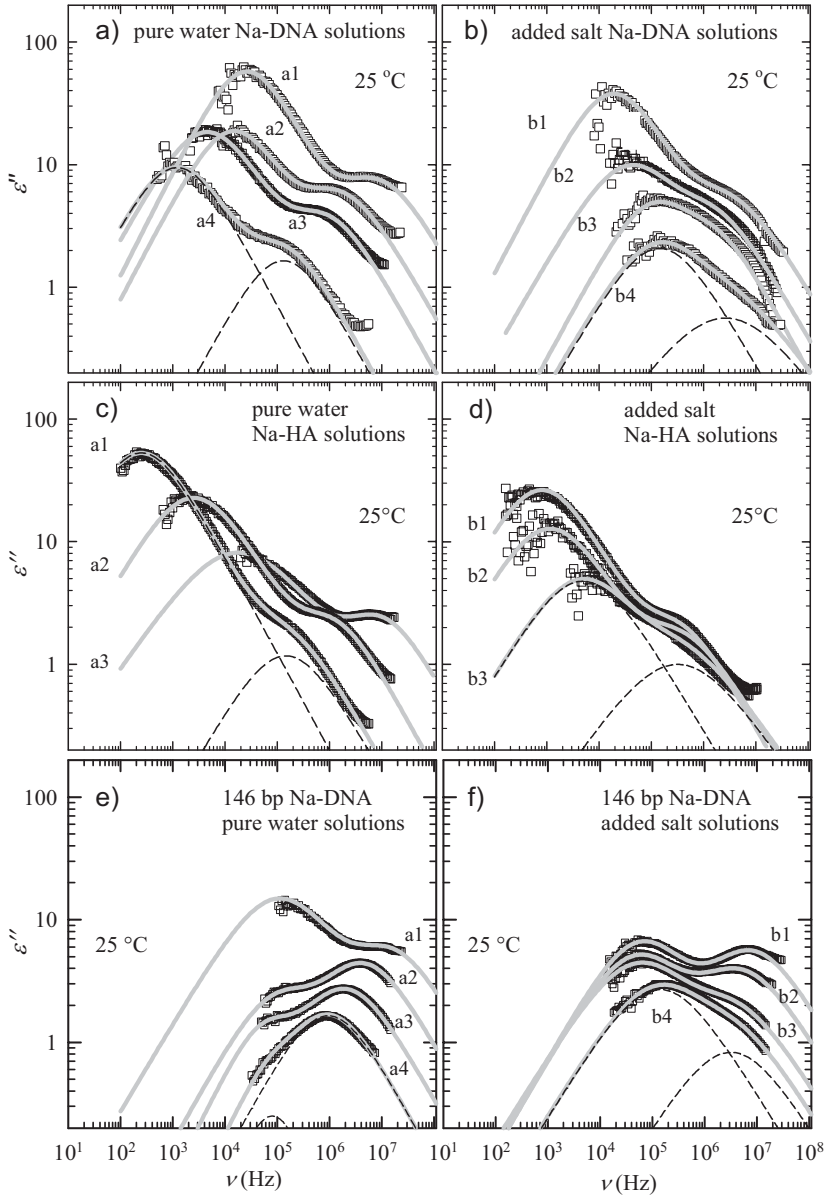

Figure 3.

Imaginary part of the dielectric function ($\varepsilon''(\omega)$) versus frequency at $T = 25\ ^{\circ}C$ of (a) pure water DNA solutions (for DNA concentrations a1–a4 (2.5, 0.4, 0.1, 0.0125 mg/mL)); (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)); (c) pure water HA solutions for representative a1–a3 (1, 0.1, 0.0125 mg/mL) HA concentrations; (d) HA solutions of concentration $c_{HA} = 0.03$mg/mL for three representative b1–b3 (0, 0.12, 0.25 mM) added salt concentrations; (e) pure water 146 bp DNA solutions (for DNA concentrations a1–a4 (5, 0.5, 0.15, 0.05 mg/mL)) and (f) 146 bp DNA solutions with added salt $I_s = 1$ mM (for DNA concentrations b1–b4 (1.5, 0.8, 0.4, 0.3 mg/mL)). The full lines are fits to the sum of the two Cole-Cole forms, while the dashed lines represent a single form. (Adapted from refs. [4,5,6]).

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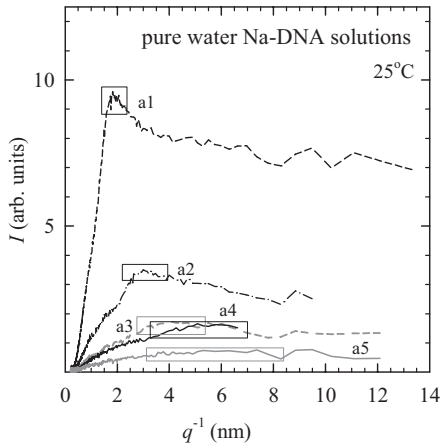

Figure 4.
SAXS scattering intensity (I) versus inverse wave vector (q^{-1}) at $T = 25\,^{\circ}C$ of pure water DNA solutions (for DNA concentrations a1–a5 (25, 12.5, 6, 3, 1.6 mg/mL)). Rectangles denote positions and error bars of the peak wave vectors q_m.

coupling Debye-Hückel approximation. In the latter zone the counterions are distributed almost uniformly due to rather small variations of the electrostatic potential there. The response of DNA counterions to an applied ac field is mostly confined to the smaller cylindrical volume, thus their oscillation naturally reflects the cylinder size (as found in DS experiments) instead of the size of the sphere which corresponds to the average distance between chains.

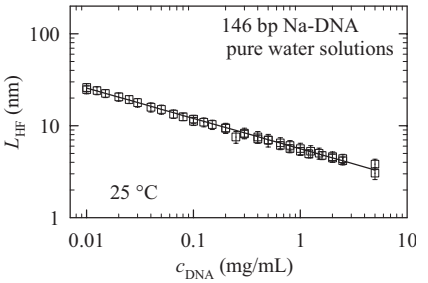

Figure 5.
Characteristic length of the HF mode (L_{HF}) for pure water 146 bp DNA solutions in dilute regime as a function of DNA concentration (c_{DNA}). The full line is a fit to the power law $L_{HF} \propto c_{DNA}^{-0.33}$. The observed fundamental length scale corresponds to the reduced average distance between chains in dilute regime. (Adapted from ref. [5]).

Next, we review results in the MHz range for the semidilute regime observed in long DNA[3,4] and HA chains.[6] In pure water polyelectrolyte solutions, the characteristic length L_{HF} extracted from DS and SAXS measurements decreases with increasing DNA and HA concentration (Figure 6). At high DNA concentrations, as well as at all measured HA concentrations L_{HF} follows the power law $c_{DNA}^{-0.5}$. SAXS measurements standardly require much higher concentrations of DNA than DS measurements since their sensitivity is much smaller. Nevertheless, we have succeeded to cover an intermediate DNA concentration range 1.5-5 mg/mL by both techniques despite rather large uncertainties in q_m. It is gratifying that in these as well as for all studied DNA concentrations (above 0.6 mg/mL) the same scaling behavior for the DS and SAXS data is observed.

This dependence corresponds to the solution mesh size or the de Gennes-Pfeuty-Dobrynin (dGPD) correlation length ξ, as theoretically expected for salt-free semidilute polyelectrolyte solutions.[23–26] The correlation length was first introduced by de Gennes as the length scale delimiting the space around each polyion in the solution from which other polyions are strongly expelled. In one of our previous publication[4] we have discussed at length this assignment of the characteristic length scale in DS experiments. Here we note that the same arguments based on the scaling theory as introduced by de Gennes[24] can be applied to the characteristic length scale in SAXS experiments. Moreover, Odijk[27] proposed that the solution correlation length gives physical meaning to Mandel's statistically independent chain segments formed due to potential barriers along the polyion.[14] This idea can also be applied to the characteristic length scale for the exclusion volume probed in scattering experiments as postulated by Koyama.[18] Thus the exclusion volume determined by the segment size of a polyion, inside which the probability of finding segments from other polyions is negligible, can naturally be associated with the volume delimited by

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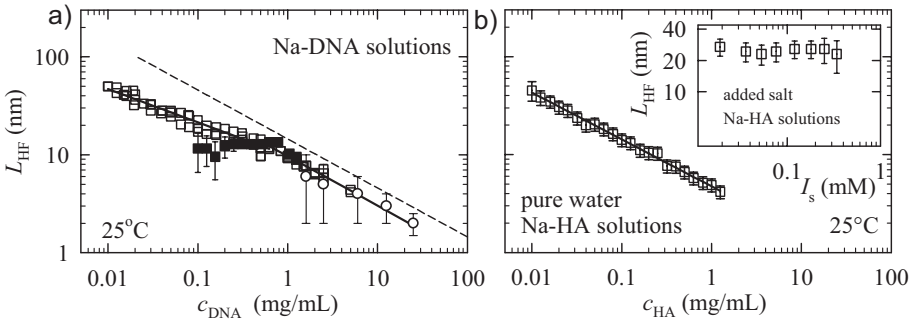

Figure 6.

(a) 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) in semidilute regime as a function of DNA concentration (c_{DNA}). Open circles denote the length scale $L \propto q_m^{-1}$ derived from the SAXS measurements. The full line is a fit to power laws $L_{HF} \propto c_{DNA}^{-0.33}$ and $\propto c_{DNA}^{-0.5}$ at low and high DNA concentrations. The fundamental length scale observed for $c_{DNA} > 0.6$ mg/mL corresponds to the semidilute correlation length. The dashed line denotes $q_m^{-1} = 14.6 \cdot c^{-1/2}$ corresponding to the theoretical Koyama prediction for the wormlike chain model. (b) Characteristic length of the HF mode L_{HF} for pure water HA solutions in semidilute regime as a function of HA concentration (c_{HA}). The full line is a fit to the power law $L_{HF} \propto c_{HA}^{-0.5}$. The observed fundamental length scale corresponds to the semidilute solution correlation length. Inset: L_{HF} for HA solutions as a function of added salt I_s for a representative HA concentration $c_{HA} = 0.03$ mg/mL. (Adapted from ref. [4,6]).

Mandel's segment length. Finally, the scaling of the characteristic lengths in DS and SAXS experiments conforms nicely with theoretically predicted solution correlation length. However, a question remains open whether the size of the correlation volume corresponds in absolute value to the those probed by measurements. In other words, does the region around the polyion segment in which intrinsic counterions respond to an applied ac electric field in the DS experiment correspond exactly to the one detected in the SAXS experiment and how do these two compare to the theoretically expected solution correlation volume? It is rather surprising that similar values for L from DS ($L = (\tau_0\, D_{in})^{1/2}$) and SAXS ($L = q_m^{-1}$) measurements are obtained (open squares and open circles in Figure 6(a), respectively).

In our DS experiments[3–6] various length scales are found to correspond to the theoretically expected values both qualitatively and quantitatively indicating that $L = (\tau_0\, D_{in})^{1/2}$ holds without rescaling and with prefactors roughly of the order of one.[28] Thus it is tempting to conclude that $L = q_m^{-1}$ also holds, implying concomitantly that the characteristic length scales probed by the DS and SAXS experiments coincide. Theoretical worm-like chain model calculations for SAXS intensity[18] predict $q_m^{-1} = 14.6\,\text{nm}\ (\text{mg/mL})^{1/2} \cdot c^{-1/2}$ for the position of the structural peak in polyelectrolytes at vanishing salt concentration. The plot q_m^{-1} vs c_{DNA} in Figure 6(a) (dashed line) reveals that this theoretical model calculations apply in as far as the scaling behavior is concerned, while the numerical values are larger by a prefactor of 1.5 as compared to the experimentally detected ones. It is not surprising that this numerical prefactor, depending on the details of the assumed interaction potential, deviates from the experimental value.[29,30]

Theoretically, the scaling dependence of the correlation length on the polyelectrolyte density is obtained by Dobrynin *et al.* by minimization of the total interaction energy which consists of the electrostatic part and the elastic contribution due to the stretching of the chain.[26] The interpretation of this scaling result is that for volumes smaller than ξ^3 the polyelectrolyte chain is stiffened by electrostatic interactions, whereas for scales larger than ξ it behaves as a free-flight chain (random walk). In other words, the chain is a random walk of

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correlation blobs. Earlier, de Gennes approach[24] was based on the argument that for long chains local properties should be independent of the degree of polimerization (N) implying that the correlation length is independent of N. Taking into account that at the crossover concentration, where dilute and semidilute regimes meet $c^* \propto 1/L_c^2$, the correlation length is comparable to the contour length L_c, and assuming the scaling form $\xi \propto L_c \cdot (c^*/c)^m$ one gets $\xi \propto c^{-0.5}$.

Coming back to the DS results in semidilute regime of long DNA, it is noteworthy that at low DNA concentrations the correlation length scales as $c^{-0.33}$ (see Figure 6(a)) which is typical for semidilute solutions of charged chains with hydrophobic cores. The interpretation of this result is that at these low DNA concentrations and at the time scales of DS experiment local conformational fluctuations (DNA denaturation bubbles[31,32]) partially expose the hydrophobic core of DNA. We speculate that DNA denaturation bubbles with their exposed nitrogen bases could play the roles of hydrophobic cores as in the model of weakly charged polyelectrolytes.[26] Finally, upon adding salt the dGPD correlation length gives way to the Debye screening length as a new relevant length scale for HF mode (full squares in Figure 6(a)). This occurs exactly when sufficient salt is added so that the corresponding Debye screening length becomes comparable to and eventually smaller than the dGPD correlation length. On the other hand, this event cannot be observed in our experimental window for the case of weakly charged HA (Inset in Figure 6(b)) since the screening length and the correlation length would become comparable only at very high added salt concentrations.

In what follows, results are shown which demonstrate that the LF mode in kHz range probes single-chain properties of the polyelectrolyte solution. The dilute regime was studied for the case of short 146 bp DNA fragments.[5] Theoretical models describe them as nonuniformly stretched chains due to the fact that interchain interactions are negligible compared to intrachain interactions (in contrast to the semidilute regime). Indeed, our observations confirm this expectation and reveal the concentration-independent contour length of 50 nm as the fundamental length scale (see Figure 7). This result holds as long as DNA concentration is larger than the added salt concentration. Once the added salt prevails, an unexpected behavior emerges: the single-chain length scale shrinks in size, becoming two times smaller than the nominal contour length of the chain. This effect cannot be due to a decrease of rigidity as quantified by the persistance length, since structural persistance length is 50 nm for ds-DNA. The interpretation of this result is that it is due to incipient dynamic dissociation which induces short bubbles of separated strands. Model calculations on short DNA fragments indeed showed [31,32] that these local denaturations can occur at physiological temperatures and despite being rare and transient lead to a lower value for the persistence length of short DNA segments. Independent Fluorescence Energy Transfer (FRET) and SAXS experiments performed on a set of short DNA fragments between 10 bp to 89 bp in size (3–30 nm) confirm this result.[33] Moreover, these experiments also reveal that DNA appears softer as its length

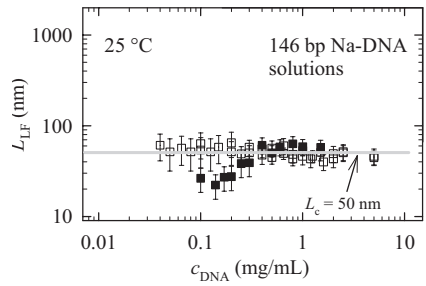

Figure 7.
Characteristic length of the LF mode (L_{HF}) for pure water 146 bp DNA solutions (open squares) and for DNA solutions with added salt $I_s = 1$ mM (full squares) in dilute regime as a function of DNA concentration (c_{DNA}). The observed fundamental length scale for pure water DNA solutions in dilute regime corresponds to the contour length. In the high salt limit the single-chain length scale reduces in size due to the incipient dynamic dissociation which induces short bubbles of separated strands. (Adapted from ref. [8]).

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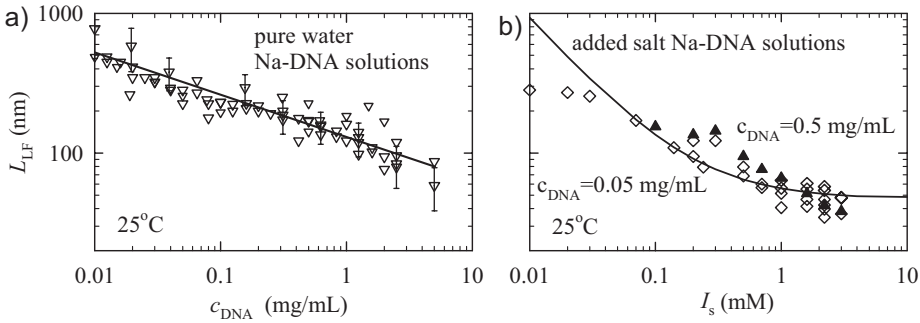

Figure 8.
(a) Characteristic length of the LF mode (L_{LF}) for pure water DNA solutions as a function of DNA concentration (c_{DNA}). The full line is a fit to the power law $L_{LF} \propto c_{DNA}^{-0.25}$. The observed single-chain fundamental length scale corresponds to the averaged size of the chain. (b) 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 (triangles). The full line is a fit to the the power law $L_{LF} \propto I_s^{-1}$ and corresponds to the Odijk-Skolnick-Fixman persistence length expected for rigid chains. (Adapted from ref. [7]).

decreases and that this base-pair breathing happens at millisecond time scales.

Next, we address the LF mode in the semidilute regime. In the case of long DNA chains which are strongly charged and semiflexible,[4] the characteristic length L_{LF} decreases with increasing DNA concentration following the power law $L_{LF} \propto c_{DNA}^{-0.25}$ (Figure 8(a)). This dependence corresponds to the average size of the chain R, as theoretically expected for the salt-free semidilute polyelectrolytes solutions.[22,24] In this low added salt limit, DNA acts as its own salt. We note that in this regime the polyelectrolyte is theoretically expected to behave as a random walk of correlation blobs. In our case, at $c_{DNA} = 1$ mg/mL the correlation length L_{HF} is about 10 nm (Figure 6a), while the chain size L_{LF} is about 100–150 nm (Figure 8a). One easily calculates that a chain behaving as a random walk of correlation blobs of the size $\xi = 10$ nm and of about $n = 250$ steps would have size $R = \sqrt{n} \cdot \xi = 150$ nm – very close to L_{LF} that we found. Further, this chain would have a contour length somewhat larger than $n \cdot \xi = 2500$ nm – and we remind that the contour length of long Na-DNA that we studied was 4 micrometers in average. Thus, the relationship between the correlation length and the chain size holds quantitatively within our data-set.

On the other hand, in the limit of high added salt the characteristic length L_{LF} behaves as the persistence length $L_p = L_0 + aI_s^{-1}$ (Figure 8(b)) where $L_0 = 50$ nm is the structural persistence length and aI_s^{-1} is Odijk-Skolnick-Fixman electrostatic persistence length contribution, proportional to the inverse of the added salt ionic strength I_s, a quadratic function of the Debye length.[34] The persistence length is the correlation length of the polymer's molecular axis; in other words, this is the length over which the original directional correlation is lost. It is a measure of the chain flexibility and can be understood as the boundary between rigid behavior over short distances and flexible over large distances.

While DNA maintains essentially the same direction over its relatively long structural persistence length of about 50 nm, HA is much more flexible and its structural persistence length is about 9 nm. Therefore, the case of weakly charged and flexible long chains was studied in solutions of HA polyelectrolyte.[6] For pure water HA solutions, the characteristic length L_{LF} decreases with increasing HA concentration following the power law $L_{LF} \propto c_{HA}^{-0.5}$ (Figure 9(a)). This scaling behavior differs from the one observed for long DNA chains and corresponds to the de Gennes-Dobrynin (dGD) renormalized Debye screening

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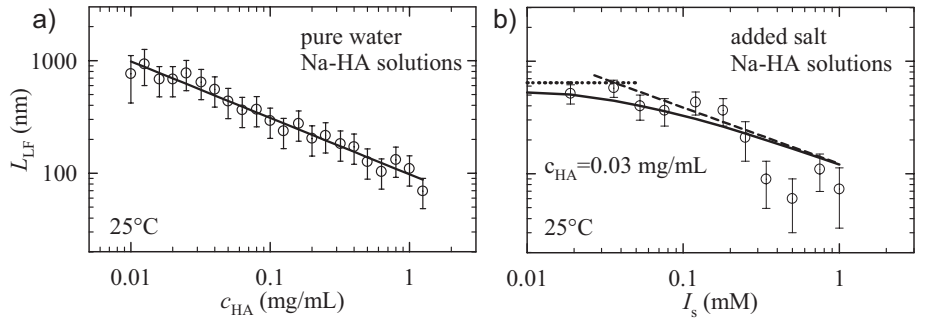

Figure 9.
(a) Characteristic length of the LF mode (L_{LF}) for pure water HA solutions as a function of HA concentration (c_{HA}). The full line is a fit to the power law $L_p \propto c_{HA}^{-0.5}$. The observed single-chain fundamental length scale corresponds to the renormalized Debye screening length. (b) L_{LF} for HA solutions with varying ionic strength of added salt (I_s). The full line is a fit to the the power law $L_{LF} \propto I_s^{-0.5}$ and corresponds to the de Gennes-Dobrynin electrostatic persistence length expected for the flexible chains. (Adapted from ref. [9]).

length.[22] This length scale is larger than the Debye screening length since renormalization takes into account the polyion chain properties. For HA, as in the DNA case, the screening is due to polyions and counterions. Why is there a difference between the DNA and HA as far as the pertinent length scale for kHz relaxation in salt-free regime is concerned? A plausible origin for this difference is the absence of Manning-condensed counterions due to weaker electrostatic interactions in the case of HA. For the strongly charged polyelectrolytes condensed counterions are those which oscillate in the kHz range and reveal single-chain properties. Since in the case of weakly charged HA all counterions are free, they participate in the LF relaxation and their oscillation in the volume around the chain naturally brings in the renormalized Debye screening length, which has a stronger concentration dependence and therefore appears as a more pertinent length scale than the average size of the chain.

Upon adding salt L_{LF} follows a similar power law behavior $I_s^{-0.5}$ (Figure 9(b)). This length scale can be identified, by analogy with DNA, as the de Gennes-Dobrynin electrostatic persistence length. It differs from the OSF electrostatic persistence length observed for DNA which scales as $L_p \propto I_s^{-1}$. Again in the case of HA, just as for DNA, the observed behavior of L_{LF} can be decoupled into high- and low-salt regimes. In the former, L_{LF} is dominated by the influence of the added salt ions, whereas the low salt L_{LF} levels off with the limiting value corresponding to the salt-free solutions for this HA concentration. This observation indicates how added salt ions and HA counterions compete for the dominant role in the screening of HA polyions which at the same time reveals a competition between two fundamental length scales describing screening due to two different origins. Finally, the question arises why does HA with finite added salt feature the electrostatic persistence length which scales as $L_p \propto I_s^{-0.5}$, as opposed to DNA which shows the OSF behavior. A plausible explanation might be a much higher flexibility of HA, as compared to DNA, so that the OSF approach in which the chain is modelled as a rigid rod would not be valid. Instead, one should invoke theoretical approaches based on the variational calculation for a Flory-type flexible chain which predict the persistence length behavior as observed for HA.[35–38]

Summary

In summary, our results obtained in the tube experiments demonstrate that dielectric spectroscopy is a powerful tool which reliably reveals the structural features of a

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single polyelectrolyte chain as well as structural organization of the polyelectrolyte solution composed of many chains. Since the DS technique can be applied at low polyelectrolyte concentrations below 10 mg/mL, it stands out as an important experimental method which complements scattering techniques like SAXS and SANS limited to highly concentrated polyelectrolyte solutions. The experiments are done in the repulsive regime (univalent counterions) and in both the dilute and the semidilute limit. The question however remains, how specific is the observed behavior of DNA and HA and whether some of these results can be taken as generic properties of biopolyelectrolytes?

Obviously, de Gennes-Pfeuty-Dobrynin solution correlation length, which describes collective properties of semidilute solutions, stands out as a generic property of diverse polyelectrolytes. On the other hand, some features like extremely high flexibility of short ds-DNA and locally fluctuating regions of exposed hydrophobic cores of long DNA are certainly very specific. Furthermore, the chain flexibility is revealed to be the key parameter which determines scaling of the electrostatic persistence length: the scaling is a quadratic function of the Debye length as rationalized by the OSF formula for the rigid and semi-flexible chains, whereas it becomes linear for the flexible ones. An intriguing route to be taken in further research will focus on polyelectrolytes, specifically DNA, with polyvalent counterions in order to study the behavior in the vicinity of the attractive (correlation) regime of electrostatic interactions.[39] A standing challenge is to compare the behavior of polyelectrolytes in repulsive and attractive regimes with results of single-molecule experiments in solutions.

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