A Synchrotron Radiation Electric Field X-ray Solution Scattering Study of DNA at Very Low Ionic Strength

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ABSTRACT: Small-angle X-ray scattering (SAXS) measurements were made on low ionic strength NaCl solutions containing 2–10 mg/mL short (150 base pairs) or long (2–22 kilobase pairs) DNA fragments in the semidilute regime. Whereas current theories of the scattering of polyelectrolytes predict a single peak in the scattering pattern in the absence of salt there is a clear interference corresponding essentially to the 20–30 nm center-to-center separation between overall isotropically distributed rigid DNA segments. Electric field X-ray scattering measurements indicate that these segments orient parallel to the electric field and that the relaxation times are identical to those of the birefringence signal.

Introduction

The existence of ordered phases in polyelectrolyte solutions at very low ionic strength was predicted at least 40 years ago.[1] Since, the structural properties of solutions of polyelectrolytes such as DNA have been extensively studied in particular by dynamic light scattering. The appearance of a slow mode in DNA solutions at very low ionic strength was attributed to the formation of an extraordinary phase[2] or of loose aggregates,[3] but these systems were not further characterized. Similarly, long relaxation times attributed to aggregation were observed in electric birefringence measurements on DNA solutions at very low ionic strength.[4]

The aim of the present work is to relate the structural properties of solutions of DNA as observed by X-ray scattering at very low ionic strength with their optical properties.

Theories of the scattering of polyelectrolyte solution[5,6] predict a single peak with a position depending as $C^{1/3}$ on the concentration (C) in the dilute regime and a $C^{1/2}$ dependence in the semidilute regime, where the separation between the molecules is on the order of their dimension. For rods of length L the critical concentration marking the transition between the dilute and semidilute regime is $C^* = 1$ rod/L^3. Wang and Bloomfield[7] have interpreted their X-ray scattering observations on 160 base pair DNA fragments within the frame of these theories. Monte Carlo calculations for independent rodlike particles,[8] however, also predict a similar concentration dependence but for the position of the main maximum of the structure factor which may have several side maxima. Hence, the validity of the different theoretical approaches in the case of DNA cannot unequivocally be decided on this basis.

Below, experimental evidence is given for clear interferences in the X-ray scattering patterns of semidilute solutions of DNA at millimolar salt concentrations resulting from an overall isotropic distribution of rods. Further, in order to correlate the structural and optical properties of these solutions, X-ray electric scattering and birefringence measurements were performed under identical conditions.

Experimental Methods

DNA Samples. Core particle DNA from chicken erythrocyte nucleosomes was prepared as described[9] but with a digestion time of 30 min. After ethanol precipitation, the DNA was dissolved in 1 mM EDTA (pH 7.5) and dialyzed against the same solvent. The samples were lyophilized and kept at −20 °C. Electrophoresis on 1% agarose gels indicated that they were essentially monodisperse (150–160 base pairs) with a minor contamination by di- and trinucleosomal fragments. For the experiments with long DNA, calf thymus, or Escherichia coli, DNA (Sigma–Aldrich, Deisenhofen, FRG) was resuspended in 1 mM EDTA (pH 7.5) and dialyzed against the same solvent.

Electrophoretic analysis indicated a length distribution between 2 and 22 kilobase pairs. The salt concentrations were adjusted as required several hours before the measurements, and the samples were kept in the cold. A concentration of 1 mg of DNA/mL corresponds to approximately 1.5 mM base pairs.

SAXS Measurements. The measurements were performed on the double-focusing monochromator–mirror camera X33[10] in HASYLAB on the storage ring DORIS of the Deutsches Elektronen Synchrotron (DESY) using quadrant or linear delay line readout detectors[11] and the standard data acquisition and evaluation systems.[12,13] The observation range was $0.01 < s < 0.2$ nm^{-1}, where $s = 2 \sin \theta/\lambda$, 2θ is the scattering angle and λ the wavelength (0.15 nm). The measurement cells were thermostatized at 7 or 20 °C. The patterns of the solutions and the corresponding solvents were measured in six separate 1 min time frames to monitor radiation damage and beam stability. As DNA is quite radiation sensitive, usually less than four frames could be averaged. Data reduction, background subtraction, and correction for detector response were done following standard procedures[14] using the program SAPOKO (Svergun and Koch, unpublished). Radii of gyration and distance distributions were calculated using the indirect transform method based on the regularization technique as implemented in the program GNOM.[15,16] The pair distribution functions were calculated using the program SPHEROKO (Svergun, unpublished).

Electric Field SAXS. The experimental setup for electric field X-ray scattering has been described earlier.[17,18] Samples containing 2 mg/mL of calf thymus DNA were dialyzed overnight against dichroism buffer (0.3 mM NaCl, 0.2 mM Tris·HCl (pH 7.5), 3 μM EDTA, 0.5 mM PMSF). Cells with electrode spacings of 2 or 4 mm were used, applying electric field pulses of 1 or 2 ms at 0.5–5 kV/cm. The cells had 20 μm thick mica windows for the X-ray and 1 mm quartz for the birefringence measurements. The latter were carried out with

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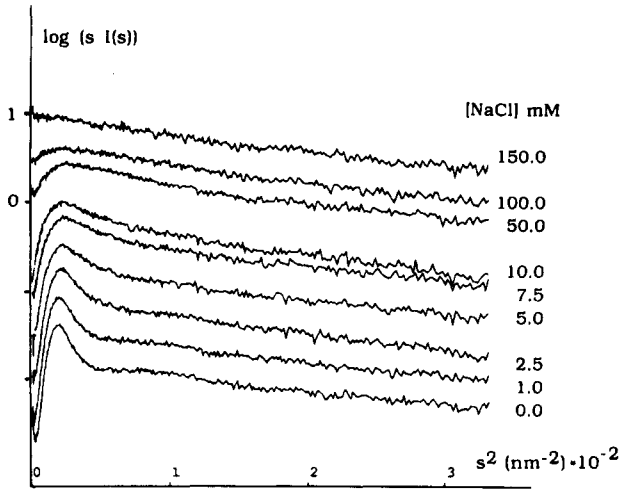

Figure 1. Plot of $\log(s(I(s))$ vs s^2 for solutions of calf thymus DNA (5 mg of DNA/mL) at different salt concentrations. The curves have been displaced along the ordinate for better visualization.

an He/Ne laser ($\lambda = 625$ nm) using a time resolution of 1 ms. To minimize electrophoretic effects, the polarity was inverted after each pulse.

Results and Discussion

Of all polyelectrolytes DNA is probably the one that is most closely matched by the ideal persistence chain. It has indeed been shown by Oberthuer[19,20] that the scattering of solutions of high molecular weight DNA in 0.2 M NaCl can be described as that from a chain with a statistical element length of about 100 nm. In the range $0.01 < s < 0.3$ nm^{-1} the scattering corresponds to that of infinite rods with a diameter around 2.8 nm. This must also be true at lower ionic strengths where the persistence length increases. Long thin rods only contribute to the scattering when their long axis is nearly perpendicular to the scattering vector,[21] and their scattering is thus essentially that of the cross section. Actually, calculations[22] and experimental results on short (150 base pairs) DNA fragments indicate that the radius of gyration of the cross section (R_c) increases by less than 5% between 0 and 10 mM NaCl as a result of the decrease in persistence length. For such a system a plot of $\log(s(I(s))$ vs s^2 gives a straight line with a slope $-R_c^2/2$ and an intercept proportional to the mass per unit length of the rod. Thus, as illustrated in Figure 1 for calf thymus DNA in 150 mM NaCl, a value of $R_c = 1.0 \pm 0.05$ nm corresponding to a fiber diameter of 2.8 nm is obtained. In contrast, the scattering curves at lower salt concentrations in Figure 1 display increasingly large interferences which can be interpreted as follows.

The scattering $I(s)$ of a solution of identical particles can be expressed as the product of the structure factor of the solution $S(s)$ and of the form factor of the isolated particles $F^2(s)$:

$$I(s) = F^2(s)\, S(s) \qquad (1)$$

$S(s)$ contains information about the distance between particles, their shape, and their relative orientation. As the scattering curves $I(s)/C$ of a concentration series (1–5 mg of DNA/mL) in 150 mM NaCl indicate that they are independent of the concentration, it can be safely assumed that they also represent the form factor of DNA in the experimental s-range at infinite dilution. Furthermore, since the persistence length increases at lower ionic strength, this form factor, which as already mentioned depends on the cross section of the molecules, should also be valid in this s-range for the lower salt concentrations.

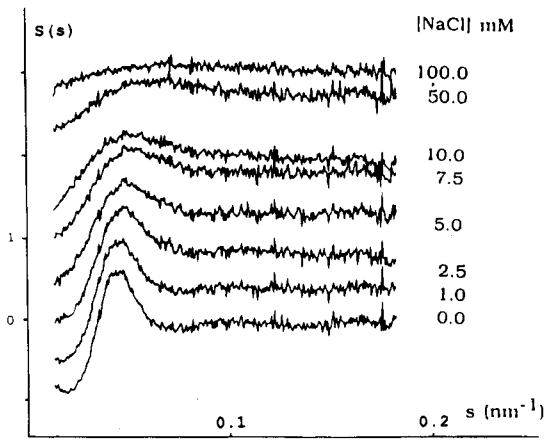

Figure 2. Structure factors $S(s)$ for solutions of calf thymus DNA (5 mg of DNA/mL) at different salt concentrations. The curves have been displaced along the ordinate for better visualization.

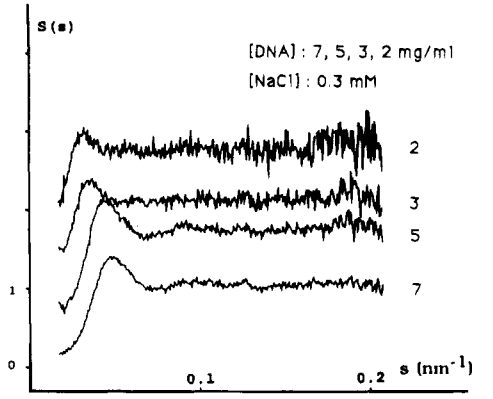

Figure 3. Structure factors $S(s)$ for solutions with different concentrations of 150 base pair DNA fragments from chicken erythrocyte nucleosomes in dichroism buffer (0.3 mM NaCl, 0.2 mM Tris·HCl (pH 7.5), 3 μM EDTA, 0.5 mM PMSF). The curves have been displaced along the ordinate for better visualization.

The structure factor $S(s)$ can then be obtained in a straightforward manner by dividing the scattering patterns at low salt concentration by the pattern at 150 mM NaCl. Alternatively, linear extrapolation of the higher angle part of a $\log(sI(s))$ vs s^2 plot to the origin can be used to recover the structure factor. The results are illustrated in Figure 2 for different salt concentrations. Similar results were obtained with the 150 base pairs DNA fragments from chicken erythrocyte nucleosomes as shown in Figure 3 for solutions of different DNA concentrations in dichroism buffer. Clearly, the shape of $S(s)$ at lowest ionic strengths is that of a classical interference function with a series of subsidiary maxima. The reason why these maxima have not been detected previously[7] is probably that the statistical precision of the experimental data obtained on a conventional X-ray generator with slit collimation was insufficient and that the data had to be desmeared.

The pair distribution function of a system of interacting rodlike particles depends, in general, on their shape and average relative orientation.[23] The system under investigation is, however, overall isotropic, and the distances between rods are an order of magnitude larger than their diameter. At the DNA concentrations used

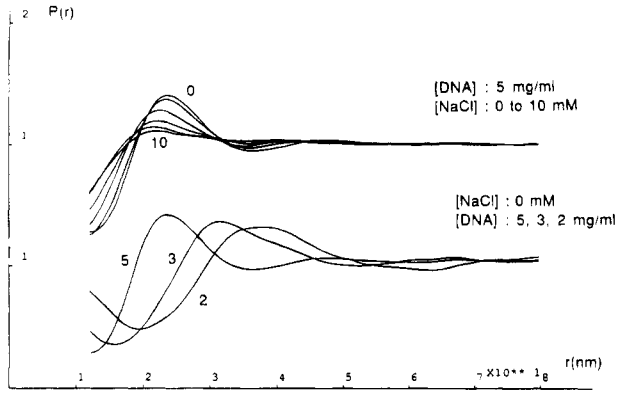

Figure 4. Pair distribution functions calculated from the structure factors of solutions of calf thymus DNA at different DNA concentrations in the absence of salt and at different salt concentrations and fixed DNA concentration.

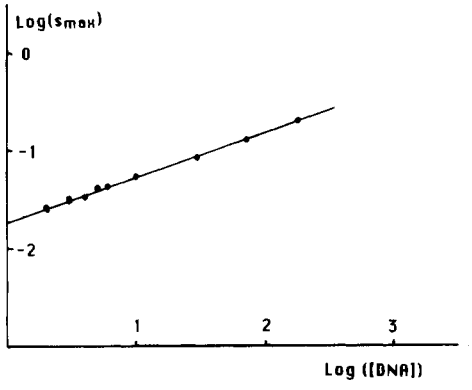

Figure 5. Dependence of the position of the maximum of the structure factor on DNA concentration for calf thymus DNA and 150 base pair fragments from chicken erythrocytes. The values above 10 mg of DNA/mL are taken from Wang and Bloomfield.[7]

the pair distribution function of the rods is dominated by their center-to-center distance distribution.[8] The latter is well approximated by the classical Zernike–Prins equation:

$$P(r) = 1 + (4\pi s^2/C)\int_0^\infty (S(s) - 1)[\sin(2\pi sr)/(2\pi sr)]\, ds \quad (2)$$

where C is the concentration of the particles.

The effect of DNA concentration in the absence of salt on the pair disribution function is illustrated in Figure 4.

There is, as expected, an approximate $C^{1/2}$ dependence of the position of the maximum of the structure factor, which corresponds to the inverse of the position of the maximum of the pair distribution function, on the DNA concentration as shown in Figure 5. The graph presents results of the measurements on calf thymus DNA and on 150 base pair fragments. For concentrations above 10 mg of DNA/mL the data are taken from Wang and Bloomfield.[7] Note that the results do not seem to depend on the length of the DNA fragments used, suggesting that the position of the main maximum of the interference function is independent of the molecular weight (or contour length) of the DNA fragments. This is also in agreement with the fact that the position of the main maximum of the pair distribution function mainly reflects the center-to-center distance between rigid DNA segments, whereas the detailed shape of the distribution is influenced by the shape and relative orientation.

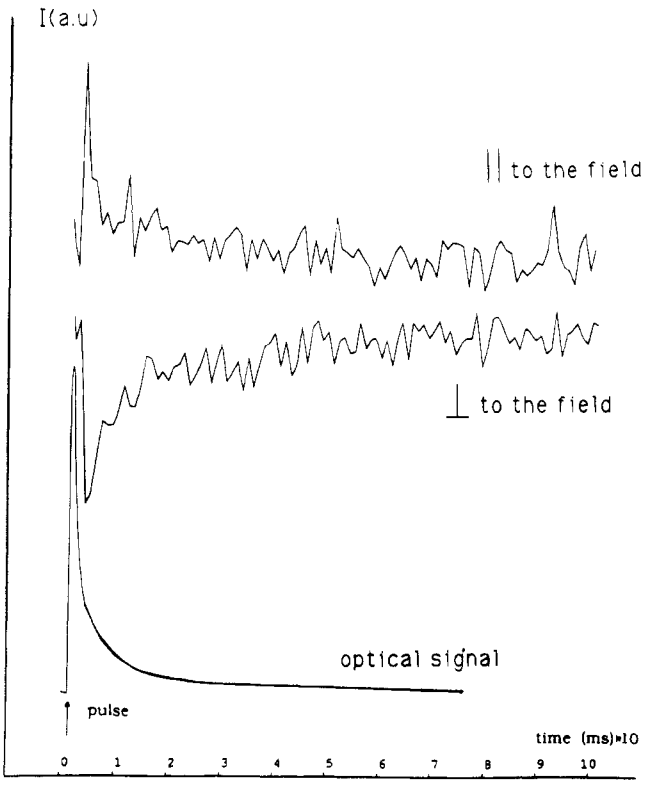

Figure 6. Time course of the X-ray scattering intensity in the directions parallel (||) and perpendicular (⊥) to the electric field and of the birefringence signal measured at 625 nm for a solution of 2 mg/mL of calf thymus DNA during and after an electric field pulse of 5 kV/cm. The ⊥-direction corresponds to the meridian and the || one to the equator of the DNA fiber diffraction pattern.

With the data in Figure 5 the actual exponent of the concentration dependence is 0.444 (9).

The evolution of the pair distribution function with increasing salt concentration at a fixed DNA concentration is also illustrated in Figure 4. There is relatively little change in the position of the maximum of the scattering factor with salt concentration as already observed by others.[7] Similar results (not shown) were obtained with 150 base pair DNA fragments in the presence of spermidine, a polyamine frequently used in biochemical experiments, in the concentration range of $0 <$ [spermidine] < 0.15/phosphate.

The results of electric field X-ray solution scattering and birefringence measurements on solutions of calf thymus DNA are illustrated in Figure 6. The accessible field strengths and time resolutions were insufficient to observe the orientation of the 150 base pair DNA fragments. The X-ray traces in Figure 6 represent the average of 50 pulses, whereas the optical signal could be obtained with one pulse. Within experimental error the relaxation times of the X-ray and birefringence signals are identical ($t_{1/2} \approx 3 \pm 1$ ms).

These results confirm that the DNA segments orient with their axis parallel to the field, leading to a reduction of the scattered intensity along the meridional (field) direction and in a concomitant increase along the equator, perpendicular to the field.

The view often presented in theoretical papers[5,6] that the scattering of polyelectrolytes in the absence of salt in the semidilute regime should be characterized by a single maximum is thus an incomplete description of the situation in DNA solutions. The main maximum of the interference function is located as foreseen by all

theories at a value of the scattering vector equal to the inverse correlation length for segmental interaction which in the case of dilute long rod segments corresponds to their average center-to-center distance. The present data do not suffice for a complete quantitative analysis of the shape and amplitude of the structure factor, but the curves are qualitatively in good agreement with those of more recent Monte Carlo calculations for rodlike particles in solution above the critical concentration.[23]

Given the large center-to-center distances between DNA segments, the term aggregates suggested by the long correlation times in light scattering seems to be somewhat of a misnomer. Although the solutions are overall isotropic, it cannot be excluded on the basis of the present data that small domains of oriented rods would exist which could cause, or contribute to, the long relaxation times observed by different methods. There is clearly a need for more accurate experimental data to establish the merit of the different theories[5,6] describing the properties of polyelectrolytes in the semidilute regime, especially as the nature of the attractive forces that balance the more obvious repulsive interactions remains a matter of debate.[24]

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