Multimolecular Aggregation in Concentrated Isotropic Solutions of Mononucleosomal DNA in 1 M Sodium Chloride

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Introduction. From a physical point of view, semiflexible polymer chains are interesting because the persistence segments have a large aspect ratio. For this reason, the interaction between two segments may be sensitive to molecular detail and the orientational and translational degrees of freedom are strongly coupled especially at high concentrations. Double-stranded DNA is an important prototype. In addition, one hopes that physicochemical insight into the behavior of concentrated solutions of DNA will lead, in the long run, to some understanding of complex biophysical phenomena.[1]

We had several straightforward reasons for starting a comprehensive light scattering investigation of concentrated aqueous solutions of mononucleosomal DNA (i.e., concentrations of about 50 g/L up to the onset of the isotropic-to-cholesteric transition). Several earlier studies simply ended at lower concentrations[2-4] although other techniques have been employed in the isotropic concentrated regime.[5,6] Next, we wanted to test theoretical predictions for the statics and dynamics of charged rods[7,8] and to uncover the import of pretransitional phenomena, if any. A final reason was to discover whether attractive forces may be discernible at high ionic strengths, as is the case for xanthan solutions.[9] However, in the course of our studies, the scattering curves turned out to deviate so spectacularly from what we expected that we switched our focus to a possible aggregation phenomenon not discussed before. This paper highlights several preliminary investigations of this effect.

Experimental Section. Substantial quantities of mononucleosomal DNA were isolated from nucleosome core particles by digestion of calf thymus chromatin with micrococcal nuclease according to the method described by Wang et al.[10] Characterization by a combination of gel permeation chromatography and low-angle laser light scattering showed that the DNA had a molecular weight of about 1.1×10^5. The UV absorbance ratio A_{260}/A_{280} of the sample was 1.91, indicating that the sample was free from protein. The final pellet was resuspended in a buffer containing 50 mM NaCl and 24 mM EDTA. Salt-free DNA was obtained by prolonged dialysis of the resulting suspension against water of millipore quality until the conductance of the outer solution equaled that of pure water. During the dialysis, which was performed at 4 °C, the DNA concentration was never below 22 g/L in order to avoid possible denaturation of the DNA. Melting experiments showed a hypochromic effect of 30.2%, indicating that the DNA was double-stranded. Atomic absorption spectroscopy showed an upper bound of one Ca^{2+} ion per 10 DNA molecules, i.e., less than 0.04% of the Na^+ counterion concentration. The solution was further concentrated and freeze-dried.

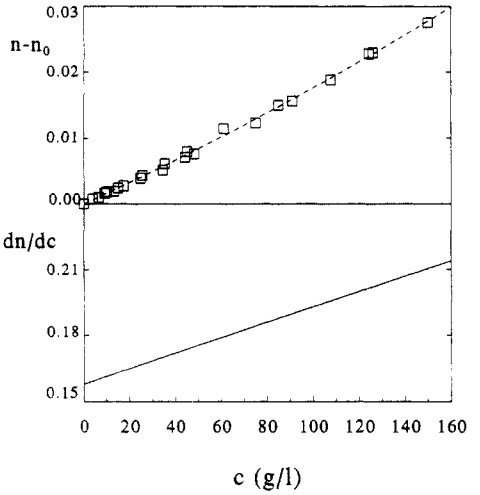

Figure 1. (Upper diagram) Increase of the refractive index of DNA solutions with respect to that of a 1.0 M NaCl solution ($n - n_0$), as a function of the DNA concentration, at 25 °C. The dashed line is a second-order polynomial fit. (Lower diagram) Calculated refractive index increment (dn/dc) which depends linearly on the DNA concentration.

For the light scattering experiments we prepared the most concentrated solution (150 g/L) first by dissolving salt-free DNA in 1.0 M NaCl. After an equilibration time of 24 h, we started to carry out our scattering experiments. Just after these measurements the concentration of the solution was determined more precisely from the UV absorbance at 260 nm using an extinction coefficient of 20 mg mL^{-1} cm^{-1}. Errors in the DNA concentration are estimated to be less than 2%. Subsequently we diluted the solution and performed a new scattering experiment. By repeating these actions, a series of solutions of concentrations descending from 150 to 0.8 g/L was investigated. All solutions remained isotropic.

Static light scattering experiments at zero scattering angle were performed with a Chromatix KMX-6 low-angle laser light scattering photometer whose light source is a He–Ne laser at a wavelength of 633 nm. The sample cell consisted of two thick silica windows separated by a Teflon spacer. The samples were filtered through 0.22-μm Millipore (Millex-GV) filters and brought into the measuring cell of the KMX-6 by continuous pumping. We started our measurements after the flow had stopped. The scattering intensity of the concentrated solutions at first increased with time after filtration; it was measured after it had shown no significant change for several hours. The intensity of the solutions which were more dilute remained constant from the start and was measured within 1 h. The quantity determined was the ratio of the scattered to the transmitted radiant power from which the Rayleigh factor (R_0) was computed with the help of the geometric parameters of the instrument. No reference to external standards or arbitrary calibration factors was required. We expressed the scattered intensities in terms of the quantity Kc/R_0, in which c is the polymer concentration (in g/mL) but where K does not have exactly the same meaning as usual. The refractive index was determined as a function of concentration with a Pulfrich refractometer modified for use with a 633-nm He–Ne laser source. At higher concentrations the increase in the refractive index (n) of the solution compared with n_0 of a 1.0 M NaCl solution was found to deviate slightly but significantly from a linear dependence with DNA concentraiton, as shown in Figure 1. Accordingly, we determined the refractive index increment (dn/dc) as a function of the

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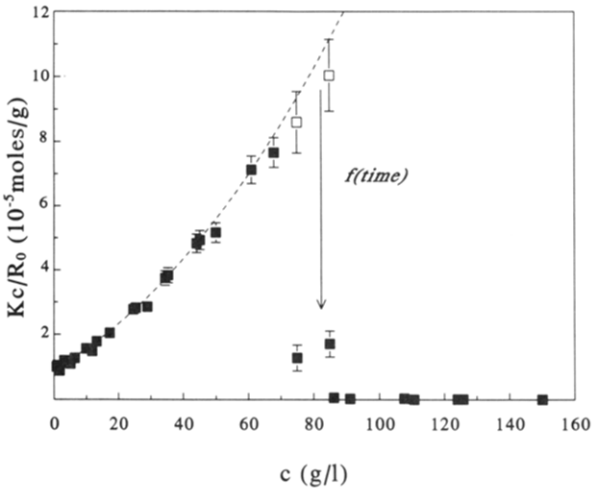

Figure 2. Inverse specific intensity Kc/R_0 at zero scattering angle as a function of the DNA concentration in 1.0 M NaCl, at 25 °C: equilibrium values (■); values just after filtration (□). The dashed line is a second-order polynomial fit for concentrations up to 68 g/L.

concentration from the slope of Figure 1. Next, a concentration dependent K was calculated from the experimentally determined n and dn/dc, in accordance with a recent scattering theory of nondilute solutions.[11] All scattering experiments were performed at 25 °C.

Freeze transmission electron microscopy was performed on samples of 144 and 37.3 g/L in order to investigate possible aggregation phenomena. The solutions were prepared by dissolving salt-free DNA in 1.0 M NaCl and then filtered through 0.22-μm Millipore filters (Millex-GV) so as to mimic the same conditions as in the scattering experiments. They were allowed to reach equilibrium before freezing. The DNA suspensions were applied to copper platelets and cryo-fixed by rapid freezing (10^5 K/s) in liquid propane, using the plunging method. Next, we put the frozen samples in a Baltzers BAF400D freeze etching device (Baltzers, Liechtenstein) at a sample temperature of −150 °C and a pressure of 2.5×10^{-5} Pa. The surface of the frozen sample was shadowed by platinum/carbon evaporation (2.5 nm of Pt) at an angle of 45°. In order to strengthen the replicas, a 30-nm carbon layer was evaporated onto the surface. After thawing, the replicas were cleaned overnight in water or a 30% sodium hypochlorite solution. The replicas were examined by means of a transmission electron microscope (Philips EM410, Eindhoven, The Netherlands), the images being recorded at a magnification of 31 000× and photographically enlarged.

Results. The plot of the inverse scattering intensity versus the concentration is not unusual at low concentrations (Figure 2), the slope at zero concentration corresponding to a second virial coefficient of 2.9×10^{-4} mol mL g^{-2} in agreement with the value of Ferrari and Bloomfield.[4] At moderate concentrations the influence of the third virial coefficient causes the slope of the scattering curve to increase monotonically (Figure 2).

However, strong anomalies occur at a critical concentration of 75 g/L and beyond: the intensity of the scattered light is enhanced immediately after filtration. Initially, the increase is rapid, but the intensity reaches a plateau slowly after 1 or 2 days, the ultimate equilibrium value exceeding the initial value by more than 2 orders of magnitude (Figure 2). The time needed to reach equilibrium increases with increasing DNA concentration. Only in the transition region (75–85 g/L) is it possible to measure both the initial and the equilibrium values (Figure 2). At higher concentrations the intensity simply increases too fast.

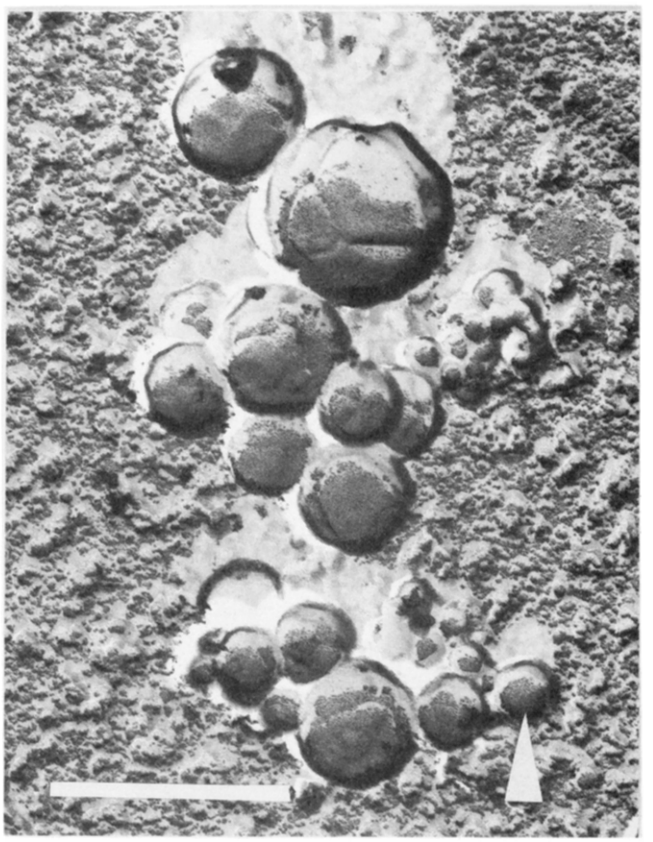

Figure 3. Electron micrograph of 144 g/L of DNA in 1.0 M NaCl. The bar corresponds to 500 nm; the arrow represents the direction of Pt evaporation.

Figure 2 is so remarkable that it does not seem explainable by an appeal to molecular forces alone, nor have we discerned any signs of phase separation: the DNA solutions remain clear and isotropic at all times. The enormous increase in scattering intensities at high concentrations may be rationalized by postulating a considerable increase in the molar mass of the scattering particles, as the intensity scales with the number of scattering particles times their molar mass squared. It must be kept in mind that in our scattering experiment the concentration series is obtained by repeatedly diluting one sample. We thus start at a high concentration where presumably particles of very high molar mass are present and reach, merely by dilution, the regime in which the DNA solutions behave in the usual thermodynamic fashion. This demonstrates that the large particles must be formed by the DNA itself.

In duplicate measurements, in which freshly isolated DNA is used, the anomalous scattering disappears at exactly the same concentration; all curves are repeatable. This suggests that the particles of high molar mass are apparently stable at high DNA concentrations.

If they exist, the large particles ought to be seen by electron microscopy. In Figure 3 we show a region typical of those we find in a carbon replica of the surface of a frozen sample (c = 144 g/L). It is clear that the sample abounds with spherical globules. By contrast, in solutions of concentrations below 75 g/L, only a small fraction—less than a few percent—of a carbon replica shows similar particles of a smaller size.

Discussion. In the concentrated isotropic regime at concentrations of 75–150 g/L in 1 M NaCl, mononucleo-

somal DNA does not at all behave in a way one would expect. Both the scattering at zero angle and the electron micrographs indicate that a substantial fraction of DNA molecules must be packed into globules that are more or less spherical. Either of these techniques by itself would yield an interpretation subject to some ambiguity, but the consistency between both experiments allows us to draw a firm conclusion. Actually, there is indirect corroborative evidence from another source. In DNA solutions containing 1 M NaCl, Strzelecka and Rill[6] established that the ^{23}Na NMR relaxation as a function of DNA concentration consists of several regions, a crossover at about 76 g/L separating two of these which are definitely isotropic (see leftmost arrow in Figure 1A of ref 6). However, the theoretical interpretation of this effect is still obscure.

By analyzing the micrographs, we have determined the size distribution of the DNA globules at a concentration of 144 g/L, which is very broad with a maximum at around 100 nm (detailed results to be published). Judging from the level of scattering, we estimate the globules to consist of several hundred DNA molecules. If we naively disregard the respective mechanisms for globule formation, we are tempted to compare our distribution with that found recently for dilute DNA condensed into tori (and sometimes other types of globules) by trivalent ions.[12,13] The latter distribution for the outer diameter is also quite broad and centered around 80 nm. Moreover, the amount of DNA contained in a typical torus (10^4–10^5 basepairs) agrees with that within one of our globules.

Now, a vexing problem, is, of course, how the aggregation of DNA into globules comes about spontaneously, well before the onset of the cholesteric phase.[14] The packing of DNA into globular states induced by external agents is well established (e.g., into phage heads,[15] in chromatin,[1] by trivalent ions,[12,13] by polymer and salt),[16] although certainly not quantitatively understood. In the present case we are not aware of having overlooked possible contaminants (for instance, calcium ions which are present in the preparatory stage have been effectively ruled out in the final DNA). Therefore, we are forced to conclude that the DNA must aggregate because of interactions involving the DNA molecules themselves.

The usual double-layer interaction[17–19] on its own appears to be unable to explain the formation of a globular state. The transitions to either the cholesteric[14] or hexagonal[20,21] phases are described fairly well by current statistical theories of charged worms.[21–25] According to these conceptions, the osmotic pressure of a 1 M saline/DNA suspension of concentration 75 g/L (the onset of aggregation) seems way too low to induce any kind of order. We are then led to surmise the existence of attractive forces of as yet unknown origin. Indeed, Figure 2 does show that the scattering by the *unstable* solutions of concentrations 75 and 85 g/L, i.e., as measured immediately after they have been filtered, is greater than expected on the basis of an extrapolation of the virial series representing the stable solutions (0–65 g/L). It appears that in the former case the virial coefficients higher than the second have changed sign. The instability we find is at least consistent with theoretical arguments,[26] suggesting that weak attractive forces may cause a breakdown of the virial series for a solution of rods, signaling a transition to either an aggregated state or a new phase; note that the second virial coefficient is predicted to be strongly positive at the transition.[26] Apparently, filtration of the suspension causes flow gradients that are strong enough to break the aggregates into individual DNA rods, at least temporarily.

These theoretical considerations must be regarded as tentative for now. We are currently investigating the aggregation phenomenon in more detail, in particular with regard to the dynamics and its dependence on ionic strength.

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