Do Free DNA Counterions Control the Osmotic Pressure?

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The contribution of counterions to macroscopic properties of isotropic DNA solutions has been studied using osmotic pressure measurements in low added salt condition. In the high DNA concentration range, the counterion contribution prevails and the associated osmotic coefficient is equal to 0.245 ± 0.020. In the lower concentration range, the osmotic pressure may be exerted either by polymers or by ions, or due to a combination of both effects, depending on the added salt and DNA concentrations.

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Properties of polyelectrolyte have been highly studied by physicists for over fifty years. A revival of interest occurred recently, motivated by their implication in multiple biological systems (DNA-protein or DNA-lipid complexes, for instance). Surprisingly, even the simplest case of DNA surrounded by monovalent counterions still raises unsolved problems: For example, the contribution of counterions to macroscopic properties is still poorly understood. To address this question, simple osmotic methods [1] may be powerful in allowing us to measure directly their contribution. Indeed in salt-free conditions, the osmotic pressure π is given by

$$\pi = \phi C_c RT\,, \tag{1}$$

with C_c the counterion concentration and RT the thermal energy. Because of the counterion condensation along the polyelectrolyte, only a fraction of counterions participate in the pressure and one expects the osmotic coefficient ϕ to be lower than one. A specific property of the DNA molecule is to require the presence of a small amount of added salt to prevent its denaturation (i.e., dissociation of the two strands). This is *a priori* in contradiction with the salt-free condition required to apply Eq. (1). In fact, the presence of the added salt (C_s) cannot be neglected when $C_c < C_s$ and the osmotic pressure differs from the relation (1). On the other hand, for concentrated DNA solutions, the osmotic coefficient may be measured according to Eq. (1) since $C_c \gg C_s$. These two cases have been considered in this paper.

We recall that, for double-stranded DNA in B form, monomers correspond to base pairs of molecular weight 660 g/mol, spaced by $b = 3.4$ Å, where each base pair carries two phosphate charges. The linear charge density ξ, which is defined as the ratio of the Bjerrum length to the monomer size [2], is equal to $\xi = 4.2$. Here, we used nucleosomal DNA (of the order of 150 base pairs) prepared as described in [3]. Because the contour length L of our DNA fragments is of the order of the DNA intrinsic persistence length (50 nm), these fragments have a rodlike conformation and their overlap concentration value C^* ($\approx M/L^3$ with M their molecular weight) is estimated to be 2 g/l. Their isotropic-anisotropic transition concentration is of the order of 130 g/l [4]. The solutions were dialyzed against water containing either 2 mM NaCl or 2 mM Tris-EDTA buffer (TE) (2 mM Tris-HCl + 0.2 mM EDTA pH 7.6), or 10 mM TE (10 mM Tris-HCl + 1 mM EDTA pH 7.6) (where EDTA denotes ethylenediamine tetra-acetic acid). We have verified that these ionic conditions prevent DNA denaturation and that the DNA solutions are isotropic in the range of investigated concentrations. DNA solutions were set in dialysis bags (Spektrapor cellulose ester 10 000, Spektrum) and immersed into stressing polymer solutions for at least three days (usually one week) at room temperature (about 25 °C) or at 2 °C [5]. The measurements have been performed using two stressing polymers [PEG 20 000 and DEXTRAN 110 000 (Fluka)]. The investigated pressure ranges of PEG and DEXTRAN solutions were 5.6×10^4–1.6×10^6 dyn/cm^2 and 7.5×10^3–10^6 dyn/cm^2, respectively [6]. At equilibrium, the DNA concentrations C_{DNA} were measured, after dilution of an aliquot, from the absorbance at 260 nm ($A_{260} = 1$ corresponds to $C_{\mathrm{DNA}} = 50\ \mu$g/ml). To complete measurements at lower pressures (10^2–1.3×10^4 dyn/cm^2), we used a membrane-osmometer KNAUER. The reproducibility of measurements and the overlap of the data obtained with the osmometer and two different stressing polymers ensure the validity of the results.

All results are summarized in Fig. 1, where the pressure π (dyn/cm^2) is plotted versus DNA concentration C_{DNA}(g/l). At low DNA concentrations, the pressure increases with C_{DNA} in two distinct ways, depending on salt concentration C_s. In the high DNA concentration range, the pressure becomes independent of C_s and proportional to the DNA concentration (solid line in Fig. 1). This regime exists for $C_{\mathrm{DNA}} \geq 27$ g/l at $C_s = 2$ mM and for $C_{\mathrm{DNA}} \geq 48$ g/l at $C_s = 10$ mM. From all of the data obtained in this regime, the values of the osmotic coefficient $\phi = \pi/(RT \times C_c)$ can be determined. These values are plotted as a function of the counterion concentration C_c in Fig. 2. C_c is equal to the DNA phosphate concentration $C_{\mathrm{phosphate}}$ with $C_{\mathrm{phosphate}}(\mathrm{M}) = C_{\mathrm{DNA}}(\mathrm{g/l})/330$. We find a constant value $\phi = 0.245 \pm 0.020$ as though $24.5 \pm 2.0\%$ of the counterions were free to create the observed osmotic pressure. Similar ϕ values can also

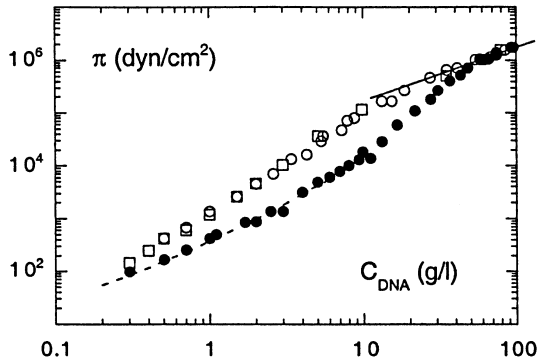

FIG. 1. Log-log plot of the pressure π as a function of DNA concentration C_{DNA}. Two concentrations C_s of added monovalent salt have been considered: 2 mM NaCl (○) or 2 mM TE (□), and 10 mM TE (●). The solid line indicates that π is proportional to C_{DNA} over the higher C_{DNA} range, as expected for a gas of counterions. The dashed line corresponds to a virial development, as expected for a gas of DNA fragments.

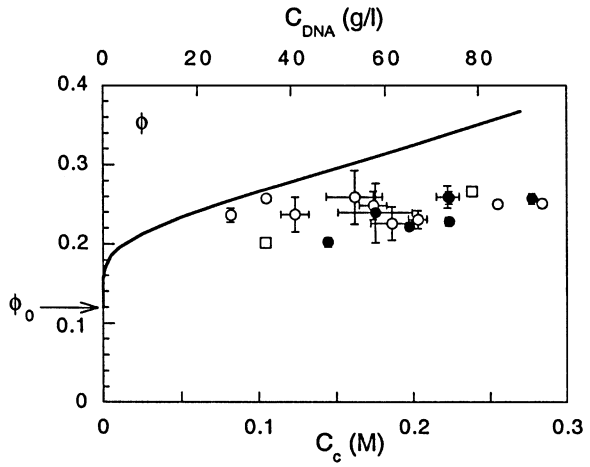

FIG. 2. The osmotic coefficient $\phi = \pi/(RT \times C_c)$ as a function of the counterions concentration C_c for the highest DNA concentration solutions. Symbols are the same as in Fig. 1. The experimental data are found lower than the ϕ values predicted by the Poisson-Boltzmann cell model (solid line). Theoretically the osmotic coefficient depends on the concentration and reaches Manning's limit value $\phi_0 = 1/(2\xi)$ when $C_c \to 0$.

be extracted from bibliographical data in the lower and in the higher concentration ranges, either from the Donnan salt-exclusion factors (for the lowest added salt quantity) [7] or from the osmotic pressure experiments in the anisotropic phase [1]. Therefore we suspect this ϕ value to remain constant over the whole DNA concentration range.

This constant experimental value is not predicted by the Poisson-Boltzmann theory using the cell model [8]. In this model, the solution is considered as a close packing of independent cylindrical (or spherical) cells, each of them containing one polyelectrolyte with its own counterions. Only the counterions located on the surface of the cell are assumed to contribute to the osmotic pressure: $\pi = RT \times C_c(R)$ with $C_c(R)$ their concentration and R the cell radius [9–11]. For infinitely long rods, one may write the osmotic coefficient as $\phi = C_c(R)/C_c = (1 + \lambda^2)/(2\xi)$ [10] with ξ the linear charge density. The numerical variable λ is computed from the condition $\lambda \ln(a/R) = \arctan[(1 - \xi)/\lambda] - \arctan(1/\lambda)$ with a the radius of the rod. When the ratio a/R is close to zero, i.e., for a highly diluted solution or for infinitely thin rods, λ becomes negligible and the osmotic coefficient reaches the Manning limit $\phi_0 = 1/(2\xi)$ [2,11]. The prefactor $\frac{1}{2}$ comes from the screening effects of the interactions by the free counterions, and a fraction $1/\xi = 2\phi_0$ of counterions is expected to be free [2]. The predicted values of ϕ are given in Fig. 2 for DNA ($a = 10$ Å, $\xi = 4.2$, and $\phi_0 = 0.12$) and do not describe the constant measured values. Our experimental values are found close to, but lower than, the predicted ones in the investigated concentration range and twice the Manning limit ϕ_0.

For lower DNA concentrations, the pressure variation with C_{DNA} strongly depends on C_s (see Fig. 1) which indicates that the effects of added salt become nonnegligible ($C_c < C_s$). For $C_s = 2$ mM NaCl and 2 mM TE, the data superimpose and the pressure may be fitted by a simple power law of exponent 1.90 ± 0.05. This behavior may be compared to the classical Donnan effect. As recalled in Refs. [11,12], the pressure is dominated by both free counterions and added ions, which are not equally distributed on both sides of the semipermeable membrane. For $C_c \ll C_s$, this ionic contribution may be written as $\pi/RT \approx (\phi C_c)^2/4C_s$ and depends on the polyelectrolyte only via the coefficient ϕ. In Fig. 3(a), we used the reduced variables suggested in Ref. [12] and the ratio $\pi/(RT \times \phi C_c)$ is plotted as a function of $\phi C_c/C_s$. Data collected on poly(styrene-sulfonate) [13] are also plotted for comparison. The good superimposition of the data confirms the relevance of the reduced variables. The data are also compared to the more general expressions given in Ref. [12] $[\pi/(RT \times \phi C_c) = 1/(1 + 4/X)$ with $X = \phi C_c/C_s]$ and in Ref. [11] $[\pi/(RT \times \phi C_c) = (1 + 4/X^2)^{1/2} - 2/X]$. These expressions reproduce correctly the variation of the experimental data, confirming the ionic contribution to the osmotic pressure. This agreement also suggests that, in these experimental conditions, the osmotic coefficient of DNA seems to be constant and independent of C_c (cf. the discussion above).

For 10 mM TE solutions, the variation of π with C_{DNA} cannot be fitted by a simple power law. At low C_{DNA} (<9 g/l), the variation of the data may be described by a virial development $\pi/RT = (C_{\mathrm{DNA}}/M) \times (1 + MA_2C_{\mathrm{DNA}} + \cdots)$, where M is the molecular weight of DNA fragments ($M = 9.6 \times 10^4$ g/mol) and A_2 is the second virial coefficient ($MA_2 = 0.45$ l/g)—see the dashed line in Fig. 1. This behavior is expected in the case of dilute macromolecular systems; i.e., the pressure is essentially due to the polymeric contribution, and the ionic pressure may be neglected (cf., for instance, Ref. [12]). At higher C_{DNA}, in the semidilute range $9 < C_{\mathrm{DNA}}(\mathrm{g/l}) < 50$, the osmotic pressure increases

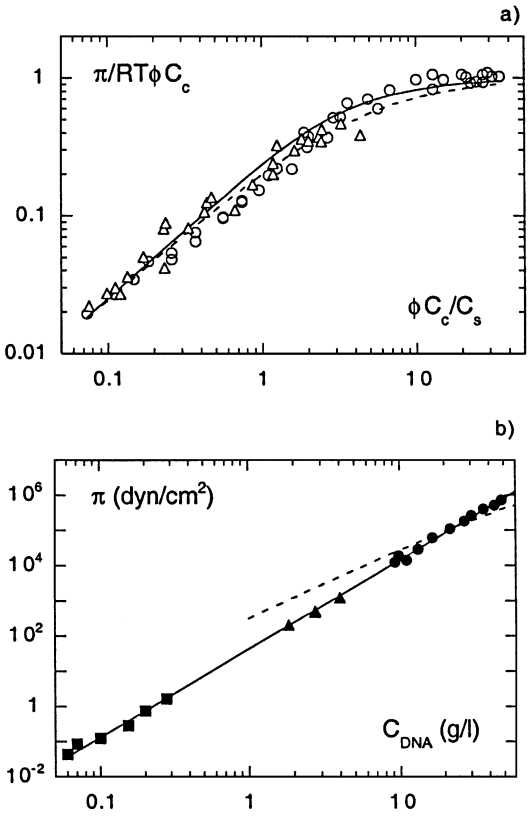

FIG. 3. (a) Comparison between theoretical curves and experimental data obtained on DNA and poly(styrene-sulfonate) solutions, using the reduced variables $\pi/(RT \times \phi C_c)$ and $\phi C_c/C_s$. C_c corresponds to the phosphate or to the sulfonate concentration. For DNA (○), only the measurements performed at $C_s = 2$ mM are plotted, and, for poly(styrene-sulfonate) (Δ), the data come from Ref. [13]. The theoretical curves represent the ionic contribution and are based on the expressions given in Ref. [11] (for the solid line) and in Ref. [12] (for the dashed line). (b) Variation of the osmotic pressure versus the DNA concentration, for 10 mM added salt. Our data obtained with fragments in the intermediate concentration range (●) are compared to the data obtained with λ DNA (■) [15] and Col $E1$ plasmid (▲) [14]. The solid line represents a power law fit of exponent 2.5 and the dashed line represents the expected ionic contribution to the osmotic pressure (taken from Ref. [12] with $\phi = 0.245$ and $C_s = 10$ mM).

more strongly with C_{DNA}. In Fig. 3(b), these data have been compared to results previously obtained with longer DNA chains in the semidilute range and in the presence of 10 mM added salt. In the log-log plot, our values align with the data measured on Col E1 plasmid (6600 base pairs) [14] and λ DNA (43 000 base pairs) [15] solutions. The whole set of data can be fitted by a power law of exponent 2.5 [$\pi(\mathrm{dyn/cm^2}) = 44 \times C_{\mathrm{DNA}}^{2.51}$ with C_{DNA} in (g/l)]. As the method used to investigate λ DNA solutions reveals a polymeric contribution ($\pi \sim C_{\mathrm{DNA}}^{2.2\pm0.2}$) [15], the alignment of the three series of points suggests that the polymeric contribution could also be predominate in the intermediate regime of our short fragments and the ionic contribution could be neglected. However, because of the large spacing between the three series of points, we cannot exclude a possible variation of the slope between the two extreme sets of values. This question is delicate, and intercalated data would be extremely useful. Anyway, in the semidilute regime of neutral rodlike polymers, the polymeric contribution is sensitive to the binary contacts between monomers, and the pressure is expected to be equal to $\pi/RT \approx A_2 C_{\mathrm{DNA}}^2$ [16], which does not explain the strong experimental variation. Neither the ionic part $\pi \sim (\phi C_{\mathrm{DNA}})^2/4C_s$ nor the polymeric part $\pi \sim A_2 C_{\mathrm{DNA}}^2$ explain this steep slope ($\pi \sim C_{\mathrm{DNA}}^{2.5}$). In fact, this intermediate concentration range is confined between the dilute regime, where the osmotic pressure is governed by the polymeric contribution, and the higher C_{DNA} range, where the counterion contribution prevails. One may then suspect that, in this intermediate range, the two species, ions and polymers, contribute to the strong increase of the osmotic pressure. How these two contributions interfere remains an open question.

In summary, we report the existence of a DNA and salt concentration range for which the osmotic pressure is proportional to the DNA concentration and independent of added salt. As a consequence, the pressure exerted by the initial DNA counterions predominates and prevents the separation of the strands of the double helix structure. The role of the added salt in the stabilization process becomes negligible. The isotropic-anisotropic transition is also expected to be independent of C_s.

We infer from this proportionality an osmotic coefficient ϕ equal to 0.245 ± 0.020 and a concentration of bulk counterions which contribute to the osmotic pressure, varying from 0.02M to 0.07M in our experimental conditions. The concentrations of both bulk counterions and DNA are close to the concentrations measured *in vivo*: of the order of 0.15M Na^+ or K^+ and higher than 10 g/l DNA whatever the biological cell type. We therefore suspect that such ion concentrations can be reached simply by the release of monovalent counterions from DNA or from other charged biological macromolecules. We may wonder whether the biological cell could not therefore be considered as a system without "added salt" in the polyelectrolyte sense. Theoretical and experimental work done in the absence of added salt but in concentrated polyelectrolyte regimes could then be relevant for biological systems.

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