DNA Condensation Induced by Cationic Surfactant: A Viscosimetry and Dynamic Light Scattering Study

S. Marchetti and G. Onori

Dipartimento di Fisica, Universitá di Perugia and CEMIN (Centro Eccellenza Materiali Innovativi Nanostrutturati), Via A. Pascoli, I-06123, Perugia (Italy) and INFM-CRS SOFT, Unitá di Perugia

C. Cametti*

Dipartimento di Fisica, Universitá di Roma "La Sapienza", Piazzale A. Moro 5, I-00185- Roma (Italy) and INFM-CRS SOFT, Unitá di Roma 1

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The compaction of DNA induced by two simple amphiphiles, cetyltrimethylammonium bromide [CTAB] and dodecyldimethylamine oxide [DDAO], has been investigated by means of combined viscosity and dynamic light scattering measurements, to demonstrate the formation of soluble DNA/surfactant complexes, undergoing a coil−globule transition, upon the increase of the amphiphile concentration. In both of the two systems investigated, the complexation process reaches a maximum for a value of the surfactant to DNA phosphate groups molar ratio of about $X = 1$. Below this critical concentration, the coil and the globule state coexist in the solution, as clearly shown by the bimodal size distribution obtained from the light scattering intensity correlation functions. Some suggestions are given to support a molecular mechanism responsible for the complex formation, both in the case of a cationic surfactant (CTAB) and of a pH-dependent neutral or cationic amphiphile (DDAO), where the hydrophobic interactions play an important role.

1. Introduction

Compaction of DNA induced by cationic surfactants have attracted in the recent years a large amount of interest due to its importance both in technological[1] and biomedical applications, particularly for the potential use of these systems as vehicles for gene delivery and gene transfection.[2−4] One of limiting factors for gene therapy is the DNA transport, since, under normal physiological conditions, DNA is a highly charged polyion that is repelled by the similarly negative cell membrane. In the complexation between DNA and cationic species, the effective negative charge of DNA is lowered, allowing the complex to approach the charged cell membrane. In addition, it has been shown that cationic surfactants collapse individual DNA molecules and lead to small particles, allowing an efficient internalization of these complexes into the cells. A wide variety of physical methods have been applied to the study of DNA−surfactant interactions and recently these interactions have been also studied at the single-molecule level, with the use of a fluorescent microscopy technique.[5,6] It has been found that isolated DNA chains undergo a discrete coil−globule transition upon the increase of the concentration of amphiphiles in the solution. The molecular mechanism leading to this conformational change has been described as follows. Cationic surfactants interact with DNA by a combination of initial electrostatic interaction followed by a cooperative binding of surfactant ligands to the same DNA molecule, driven by hydrophobic forces.[5] This means that coil and globule forms coexist in the surfactant solution at a concentration interval in which the cooperative continuous transition is observed in the macroscopic ensemble of DNA chains. The existence of a bimodal distribution between elongated coil and compact globule states has been also pointed out by Mel'nikov et al.[7] in a study of interaction between the cationic surfactant cetyltrimethylammonium bromide [CTAB] and DNA. In that work,[7] results of direct observation by fluorescence microscopy were compared to those of a potentiometric study.

A more direct observation of the surfactant-induced conformational changes of a single DNA chain and of the size distribution of DNA/surfactant complexes could be obtained using dynamic light scattering (DLS) technique which probes the changes in the translational diffusion coefficient, from the elongated coil state to the compacted globule state, induced by the addition of the surfactant.

Whereas interaction between DNA and cationic surfactants has been widely investigated,[5,8−12] less attention has been addressed to the DNA behavior in the presence of neutral surfactants, which should not affect its conformational state until at very high concentrations,[13] giving rise to a compaction mechanism that could be, in principle, basically different from the one occurring in the presence of cationic surfactants.

In the present work, viscosity and DLS techniques have been used to study the compaction of DNA induced by two simple model amphiphiles, cetyltrimethylammonium bromode [CTAB], a single chain cationic surfactant, and dodecyldimethylamine oxide [DDAO], which can exist in either nonionic or cationic (protonated) form, depending on pH. Recently, Mel'nikova and Lindman[6] have studied the behavior of DNA/DDAO system as a function of pH using fluorescence microscopy. A collapse from coil to globule was observed as DDAO molecules are protonated, by decreasing the pH of the aqueous solution.

The viscosity measurement is a simple method to obtain information on the macroscopic properties of a heterogeneous medium, such as an aggregate suspension. In this paper, the effect of the two CTAB and DDAO amphiphiles on the

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compaction of DNA has been monitored by measuring the viscosity of the system and the hydrodynamic size of the dispersed aggregates at very low concentrations, to avoid precipitation of complexes.[14,15] The surfactant concentrations employed are much below the critical micelle concentrations (for CTAB about 0.92 mM and for DDAO about 0.7 mM, as inferred from surface tension measurements (data not shown)), and hence binding of surfactants to DNA occurs, under these conditions, predominantly in monomeric form and not in micellar form. The observed decrease of viscosity suggests the formation of soluble DNA/surfactant complexes with a more compact structure. The behavior of the hydrodynamic radius and the size distribution of these complexes have been studied directly by DLS, evidencing a bimodal distribution with the simultaneous presence of coil and compact globule states, whose relative concentration changes with the surfactant concentration. The presence of these globular structures with both the two amphiphiles investigated gives further support to the hypothesis that hydrophobic interactions are involved in the aggregation mechanism.

2. Experimental Section

2.1. Materials. Calf thymus NaDNA (ctDNA), purchased from Sigma Chem. Co., was diluted in deionized water and fragmented by sonic vibration with a Vibra Cell sonifier by Sonic and Materials Inc. (5 mg/mL concentration, 1 min sonication time) in order to obtain DNA fragments shorter and with a narrower size distribution than those of the commercial sample. The final concentration employed was 0.1 mM in terms of phosphates for both viscosity and light scattering measurements. Surfactants cetyltrimethylammonium bromide [CTAB] and dodecyldimethylamine oxide [DDAO] were purchased from Fluka and used without further purification. The aqueous solutions were prepared by weighing. In all the experiments, the DNA concentration was kept constant while the concentration of CTAB or DDAO was appropriately varied, changing the surfactant to DNA molar ratio [Surfactant]/[PO_4^-] from 0 to about 4. The pH of the solutions was measured by means of a Crison micropH model 2000. The values of the pH refer to DDAO, CTAB and DNA solutions respectively, before mixing[6] and were adjusted by adding a negligible volume of HCl or NaOH at an appropriate concentration. No appreciable changes in the pH values were observed in time after mixing.

2.2. Viscosity Measurements. Viscosity measurements were carried out in a themostated bath at a temperature of 25 °C by means of a viscosity measuring system Schott Gerate mod. AVS 400, equipped with an Ubbelhode viscometer. A reproducibility of ±0.1% was obtained. Viscosity measurements, if properly interpreted, are able to provide information about the shape of the dissolved particle. Viscosity depends on a variety of parameters, such as the shape of dissolved particles, their rigidity and the state of aggregation, the amount of hydration, and the forces acting between the particles. We characterize the viscosity behavior of the DNA−surfactant solutions through the relative viscosity η_r, defined as the ratio $\eta_r = \eta/\eta_0$ between the viscosity of the suspension and the viscosity of the pure solvent. The relative variation of this quantity is directly related to the viscosity of the DNA solution, η_{DNA}, and to the viscosity of the DNA−surfactant solution, $\eta_{complex}$, by means of the relationship

$$\frac{\Delta\eta_r}{\eta_r} = 1 - \frac{\eta_{DNA}}{\eta_{complex}} \qquad (1)$$

2.3. Dynamic Light-Scattering Measurements. The size and size distribution of DNA and DNA−surfactant complexes have been characterized by means of dynamic light scattering measurements.[16] The apparatus used was a homemade spectrometer equipped with a Brookhaven BI9000AT logarithmic correlator and a 10 mW HeNe laser source of wavelength 632.8 nm. Light scattered by the sample, placed in a thermostated bath maintained at a temperature of 25 °C, was detected at an angle of 90°. In dynamic light scattering experiment, we measure the normalized time autocorrelation function of the intensity of the scattered light $g^{(2)}(q,\tau)$ that can be expressed as

$$g^{(2)}(q,\tau) = \frac{\langle I(q,t)I(q,0)\rangle}{I(q,0)I(q,0)} = A[1 + \beta|g^{(1)}(q,\tau)|^2] \qquad (2)$$

where A is the measured baseline, β the spatial coherence factor, and $g^{(1)}(q,\tau)$ the normalized electric field correlation function.

For a dilute suspension of monodisperse particles, $g^{(1)}(q,\tau)$ decays exponentially with a decay rate $\Gamma = Dq^2$, where q is the magnitude of the scattering wave vector and D is the translational diffusion coefficient, which is related to the hydrodynamic diameter $2R_H$ through the Stokes−Einstein relationship $D = k_BT/6\pi\eta R_H$, with k_BT the thermal energy and η the viscosity of the aqueous phase. For a dilute suspension of polydisperse particles, the correlation function $g^{(1)}(\tau)$ has no longer a single-exponential decay and can be written as the Laplace transform of a continuous distribution $G(\Gamma)$ of decay times:

$$g^{(1)}(\tau) = \int_0^\infty G(\Gamma)\exp(-\Gamma\tau)\,d\Gamma \qquad (3)$$

The analysis of the decay time distribution has been carried out by the inverse Laplace transformation by means of a fit routine CONTIN,[17] which employs the constrained regularization method. A typical correlation function for surfactant-free DNA aqueous solution is shown in Figure 1. The inset shows the relaxation time distribution rising from the superposition of a mode associated with the translational diffusion of the chain and a mode associated with internal motion of the chain. These spectra are in agreement with the results given by Cardenas et al.[12]

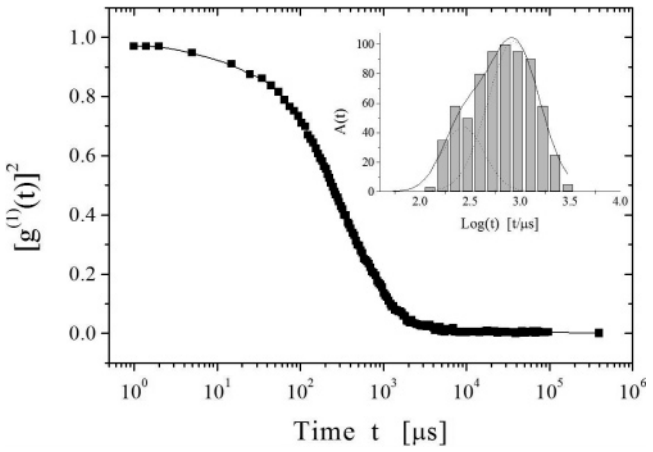

Figure 1. Normalized scattered intensity correlation function $|g^1(t)|^2$ for surfactant-free DNA aqueous solution (0.1 mM phosphate groups concentration). The corresponding time relaxation distribution, obtained from the inverse Laplace transformation algorithm CONTIN is shown in the inset. The distribution can be deconvoluted in a shorter mode associated with the translational motion and a faster mode associated with the internal motion of the coil.

3. Results and Discussion

The relative viscosity η_r of an aqueous solution of ctDNA chains in elongated coil state at a concentration of 0.1 mM in

terms of phosphate groups is around 1.1, which is considerably higher than the expected value for the same solution of ctDNA molecule in a more compacted globule state. On the other hand, the relative viscosity of surfactant solution, either CTAB or DDAO, when the molar concentration is equal to DNA nucleotide, is practically very close to 1. Hence, it must be expected that the coil−globule transition of DNA, induced by aggregation with oppositely charged surfactant molecules, may be accompanied by significant decrease of the relative viscosity value. A direct observation of the surfactant induced conformational changes of a single DNA chain, resulting in a decrease of its apparent hydrodynamic radius, can be obtained using dynamic light scattering techniques which probe the changes in the translational diffusion coefficient, from the elongated coil state to the compacted globule state, induced by the addition of the surfactant.

To verify if conformational changes occur upon binding of CTAB or DDAO to the ctDNA, viscosity and dynamic light scattering measurements were performed on solutions of ctDNA at a fixed nucleotide concentration 0.1 mM, in the presence of an increasing concentration of CTAB or DDAO. In the following, we will describe in details the behavior of the two systems, separately.

3.1. DNA—CTAB Complexes in Aqueous Solution. It is well-known that cationic surfactants, even at very low concentration, well below the cmc, force a conformational change in the DNA molecules, from an elongated coil state to a more compacted globule one. Mel'nikov et at.[5] have recently reported a coil−globule transition of high-molecular weight DNA induced by CTAB, using fluorescence microscopy measurements. These authors observe that the long axis length of the DNA chain varies from about 3 μm (coil state) to about 0.7−0.8 μm (globule state), as the surfactant concentration is increased from 2×10^{-6} to 6×10^{-4} mol/L. These values were also rather well confirmed by the behavior of the translational diffusion coefficient D obtained from the measured time dependence of the mean square displacement of the center of mass of the DNA molecules, by means of fluorescence microscopy methods.[5]

The complex formation, resulting in the globule DNA conformation, involves both attractive Coulombic interactions, between positively charged headgroups of surfactant molecules and negative charges of the phosphate groups, and steric hindrance interactions. Since the CTAB headgroup size (about 6.9 Å[19]) is larger than the charge−charge distance (about 5 Å[20]) along the DNA chain, the surfactant molecules collapse only partially on the DNA and a complete binding of CTAB with ionized phosphate groups of DNA does not occur. At this stage, the DNA "condensation" proceeds, driven by hydrophobic interactions between the alkyl moieties in the surfactant molecules, whose cooperative reorganization may stabilize the complexes and favor the DNA compaction, giving rise to the conformational transition from coil to globule state.[5]

In the present work, the above stated phenomenology has been observed by means of the combined use of viscosity and dynamic light scattering measurements, studying the relative viscosity η_r and the average hydrodynamic radius $\langle R_H \rangle$ of the surfactant−DNA aggregates.

Figure 2 shows the relative variation of the relative viscosity η_r, i.e., $\Delta\eta_r/\eta_r = 1 - \eta_{DNA}/\eta_{complex}$ as a function of the surfactant to phosphate group molar ratio $X = [CTAB]/[PO_4^-]$. As can be seen, this quantity decreases rather steeply as X increases, reaching a saturation value at approximately $X \approx 1$.

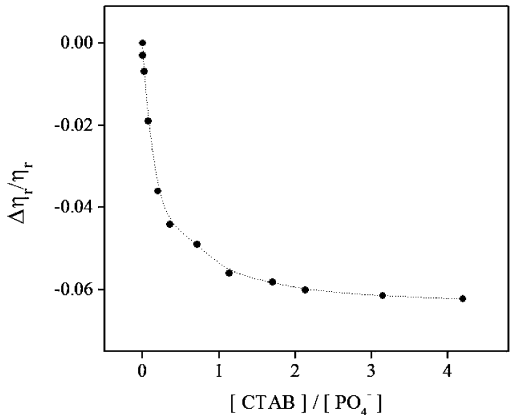

Figure 2. Dependence of the ratio $\Delta\eta_r/\eta_r$ on the surfactant to phosphate groups molar ratio $X = [CTAB]/[PO_4^-]$ for DNA−CTAB aqueous solution. The concentration of the phosphate groups is maintained constant to the value of 1 mM.

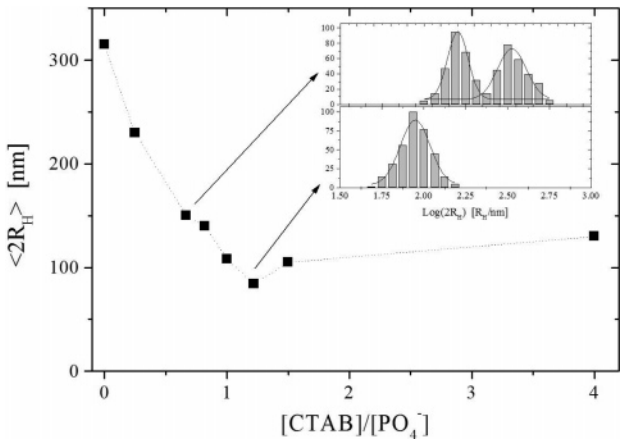

Figure 3. Average hydrodynamic diameter $2R_H$ of DNA−CTAB complexes as a function of the surfactant to DNA−phosphate molar charge ratio X. The inset shows the size distribution at two different values of X, before and close to the neutralization condition, $X = 0.55$, where there is a bimodal distribution and $X = 1.22$, where only a monomodal distribution appears.

The observed decrease of the viscosity is in agreement with the formation of DNA−CTAB complexes with a compact globular structure such as those observed in a coil−globule transition. This result underlines the crucial role played by electrostatic interactions in the complex formation, being in good agreement with previously reported data about the interaction between cationic surfactants and DNA. For example, Mel'nikov et al.[7] have examined binding isotherms of CTAB with DNA and have founded that the slope of the isotherms decreases to zero at a ratio X close to 0.8.

The average hydrodynamic radius of the complexes formed by DNA and CTAB as a function of the surfactant to DNA−phosphate molar ratio $X = [CTAB]/[PO_4^-]$ in the same concentration range is shown in Figure 3. As can be seen, with the increase of the surfactant content, a progressive decrease of the hydrodynamic diameter $2R_H$ appears, from the value of about 300 nm for surfactant-free DNA to about 80 nm for DNA−CTAB complexes at the charge ratio $X \approx 1$. As the DNA charge neutralization proceeds, the chain undergoes a shape transition from a semiflexible coil state to a more compact globular conformation.

The inset of Figure 3 shows the size distribution of the complexes at two different charge ratios, before the neutralization condition $X = 1$. For example, at $X = 0.55$, a bimodal distribution appears, the two populations corresponding to the

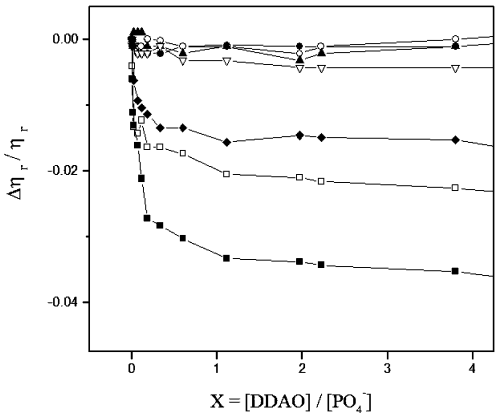

Figure 4. Relative viscosity variation of DNA−DDAO complexes as a function of the surfactant to DNA−phosphate molar ratio X at different values of pH: (●) pH = 8.0; (○) pH = 7.54; (▲) pH = 7.43; (▽) pH = 7.35; (◆) pH = 7.26; (□) pH = 7.12; (■) pH = 7.08. Dotted lines are drawn to guide the eye only.

free DNA and the DNA−CTAB complexes, being characterized by different average hydrodynamic diameters (315 and 150 nm, respectively). The percentage size reduction is in agreement with the values observed by Mel'nikova et al.[5] In these intermediate concentration ranges, extended DNA chains coexist with more compacted ones.

As X is further increased, above the nominal electroneutralization condition $X \approx 1$, the distribution tend to be unimodal, the free DNA peak disappears and only DNA−CTAB complexes (with a more compact globular shape of about 84 nm in diameter) are present.

3.2. DNA—DDAO Complexes in Aqueous Solution. DDAO is an amphiphile that exists either in a neutral or cationic protonated form, depending on the pH of aqueous solutions. DDAO becomes ionized at pH lower than 7.0 (the pK value is around 5.0[21,22]) and is fully positively ionized at pH close to 2.0.[23] This peculiar feature reflects its capability to interact with DNA. To understand this mechanism, we have investigated by means of viscosity measurements the condensation of DNA in the presence of this pH-sensitive amphiphile.

In Figure 4, the data on the relative viscosity $\Delta\eta_r/\eta_r = 1 - \eta_{DNA}/\eta_{complex}$ for the DNA−DDAO systems are reported. As can be seen, in the pH range from 8 to 7.43, no changes of viscosity of DNA−DDAO solution are observed as a function of the ratio X. This suggests that no formation of DNA−DDAO complex is occurred, the interaction between DNA and nonionic DDAO being very weak, as expected for a polyion−neutral surfactant system. In fact, at these pH values, the protonation of HO−N group of DDAO is negligible, and the surfactant can be considered to be practically in a neutral state.

On the contrary, as the pH is lower than 7.43, where the degree of ionization of DDAO becomes appreciable, complex formation begins to occur, with an upper limit, as it previously happened for DNA−CTAB complex, at approximately $X \approx 1$. In correspondence of this value, when the pH decrease from 7.43 to 7.03, a progressively increasing variation of the relative viscosity occurs and, moreover, a reversible DNA−DDAO complex formation can be observed, in a very narrow pH range close to physiological values (7.08 < pH < 7.43) (Figure 5).

This result shows that compaction degree of DNA to a spherical shape enhances by increasing the protonation state of DDAO, as, on the other hand, it should be reasonably expected, since the negatively charged phosphate groups of DNA exhibit higher shielding effects.

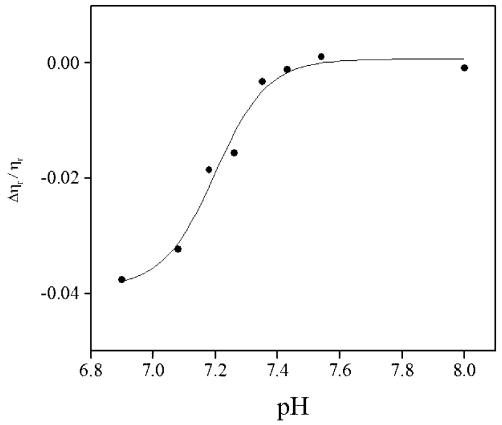

Figure 5. Relative variation of the relative viscosity η_r of DNA−DDAO aqueous solution as a function of pH, at the surfactant to DNA−phosphate molar ratio $X = [DDAO]/[PO_4^-] \approx 1$.

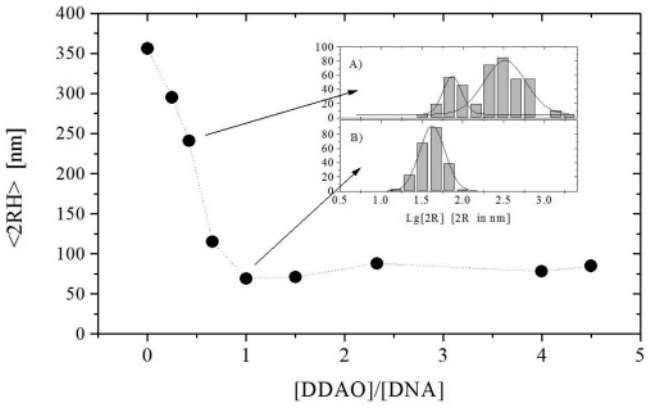

Figure 6. Average hydrodynamic diameter $\langle 2R_H \rangle$ of DNA−DDAO complexes as a function of the surfactant to DNA−phosphate molar charge ratio X. The pH of the solution is pH = 7.10. The inset shows the size distributions at two different values of X: (A) $X = 0.47$; (B) $X = 1.0$.

However, it must be noted that DNA−DDAO complexes are formed, although the DDAO ionized molecules percentage is extremely low (≈3% at pH = 7.08), whereas DNA is fully charged in the pH interval examined (the pK of the phosphate group is about 1.5)

In relation to this, it is clear that interactions between the alkyl moieties in amphiphilic surfactant molecules, like dispersion forces and hydrophobic interactions, must play a crucial role for ensuring the stability of the complex.

Moreover, for both the two systems investigated, DNA−CTAB and DNA−DDAO, the corresponding saturation binding value, $X \approx 1$, indicates that geometric restrictions arising from the steric size of the polar head of amphiphilic molecules more than the charge ratio surfactant/DNA presumably regulate the complex stoichiometry.

The same scenario comes from dynamic light scattering measurements. The overall behavior of the hydrodynamic radius of the aggregates is shown in Figure 6, as a function of the DDAO to DNA nucleotide molar fraction $X = [DDAO]/[PO_4^-]$, at a pH value close to 7, where the amphiphile is very weekly protonated. Also in this case, the bimodal distribution of the aggregates, indicating the simultaneous presence of coil and globular structures, evolves toward a single monomodal distribution, as the molar fraction X tends to unity.

However, since at this pH values the degree of ionization is small, the binding of DDAO to DNA chain driven by electrostatic interaction must involves only few amphiphile molecules

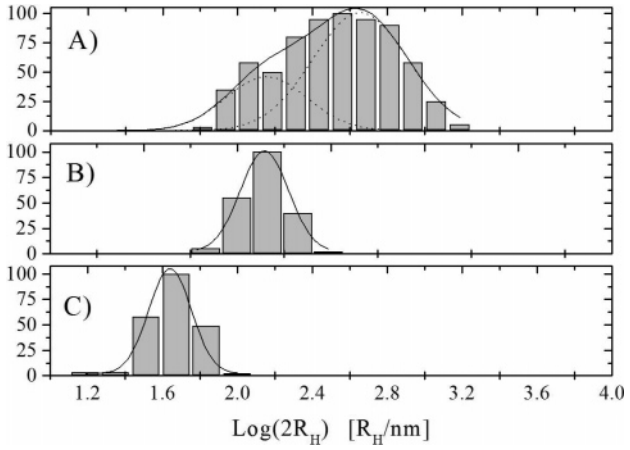

Figure 7. Average hydrodynamic diameter $\langle 2R_H \rangle$ of DNA−DDAO complexes as a function of pH at the surfactant to DNA−phosphate molar ratio $X = [\text{DDAO}]/[\text{PO}_4^-] = 1$. The inset shows the size distribution of the aggregates at two different values of pH, (B) pH = 7.30 and (C) pH = 6.90, compared to (A) the distribution of the amphiphile-free DNA solution.

which give rise to a highly cooperative binding by means of hydrophobic effect, resulting in the transition between elongated coil to collapsed globule.

In Figure 7, we show the effect of pH on the aggregation process, for a DDAO−DNA system at a molar fraction $X \approx 1$. As can be seen, at pH $\approx$ 8, where the molecule is in a neutral state, no formation of DNA/DDAO complex is observed. On the contrary, at pH 7, where the molecule becomes to be charged, even if the degree of ionization is very low, complex formation occurs. It must be noted that, in the experimental condition investigated, only a single population is present in the solution, whose average size progressively decreases as pH changes from 7.5 to 4.0. Under more alkaline conditions, at pH higher than pH = 7.5, interaction between DNA and nonionic DDAO is very weak and, as expected for polyelectrolyte-neutral surfactant systems, the polyion chain remains substantially unchanged. Similar findings were observed by Mel'nikova and Lindman[6] for t4DNA and DDAO system, where changes from unfolded DNA chains to compact globules were seen with fluorescence microscopy methods. It must be noted, however, that these measurements were carried out in the presence of added salt (0.05 M NaCl) and the DNA concentration employed (0.5 mM) is relatively higher than the one used in this investigation. This means that the surfactant concentration necessary to reach the complete DNA condensation exceeds, in some cases, the cmc and consequently DNA interactions may occur in the presence of micelles. These DNA−micelle interactions may differ in principle from those occurring with a single surfactant molecule. These authors[6] pointed out and our results confirm that one can effectively control the process of DNA condensation by regulating the degree of protonation of DDAO.

4. Conclusions

At submicellar concentration, cationic surfactants interact with DNA resulting in a continuous transition between random coil to compact globule states, which coexist until a surfactant to DNA phosphate groups molar ratio close to unity is reached. The relative viscosity of the solution and the average hydrodynamic radius of the aggregates vary accordingly, indicating a progressive increase of the globule state concentration. As pointed out by Mel'nikov,[5] at a level of single chain, the transition is discrete, even if the coexistence of coil and globule states imparts a whole feature typical of a cooperative transition. Moreover, DNA globules are stable in the solution and no further aggregation was observed, at least in the surfactant concentration range investigated. The interaction is initiated by electrostatic attraction between the positively charged headgroups of the surfactant molecules and the negative charge of the phosphate groups, followed by a cooperative accumulation of surfactant molecules driven by hydrophobic interactions, where the steric hindrance must play an important role. The overall phenomenology observed for CTAB and DDAO surfactants is quite similar, although, in the latter one, the pH-induced degree of protonation is small. This finding gives support to the idea that, as pointed out by Mel'nikov et al.,[5] a cooperative effect in the aggregation of the surfactant molecules caused by hydrophobic interaction must be invoked. Further studies on the coil−globule transition of a single DNA molecule should be performed to clarify the formation of polyion-colloid complexes partially stabilized by the hydrophobic moieties of the surfactant molecules.

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