

Structural investigation of complexes formed by DNA+CTAB and DNA+DDAB and Designing a method to increase salt ions between DNA and the Surfactant rods.

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ABSTRACT

The internal structure of DNA-CTAB and DNA-DDAB is investigated by a Small Angle X-ray Scattering (SAXS) instrument. Hexagonal packing of DNA was observed for DNA complex with CTAB, and for DDAB complex is observed lamellar structure. Variations in the internal spacing and degree of long-range ordering are dependent on both surfactant type and concentrations of added salt. When we increased the amount of salt into our complex we observed that the d spacing ($d = 2\pi/q$) are increased.

Keywords: DNA; X-ray; Complex; Salt

INTRODUCTION

The DNA double helix is highly negatively charged and it interacts strongly with oppositely charged species. In the intracellular environment, the DNA is associated with cationic histone proteins resulting in the formation of compact chromatin aggregates. Clearly, attractive electrostatic forces are important in promoting this compact structure. In vitro, an analogous compaction of the DNA can occur on association with cationic amphiphiles [2-4]. Interestingly, studies of such systems show that hydrophobic forces are also involved and can be decisive in determining the physicochemical properties and structure of such complexes [1, 4, 5]. Currently there is a considerable interest in DNA-amphiphile complexes due to the possibility of applying such nonviral assemblies as vehicles for delivery of

foreign DNA into cells [6,7]. To control the delivery process, it is essential to understand how the amphiphile self-assembles in the presence of DNA and its molecular properties in the complexes. These contain substantial amounts of water, and the hydration of the charged DNA and lipid groups is an important factor for determining the molecular organization in the complexes [1]. Depending on the molecular character of the amphiphile, both lamellar and hexagonal structures have been found for charge-compensated DNA/amphiphile aggregates [1-3]. Lamellar DNA-lipid systems have been extensively studied with respect to both molecular organization and thermodynamic properties [8-11]. It is found that the lipid properties are qualitatively similar to those of pure lipid lamellar systems, but the presence of the DNA results in substantial quantitative changes.

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Single-chain amphiphile, which by themselves would typically form micelles and normal hexagonal structures can be used more readily than the lamellar-forming lipids to produce complex particles containing only a single compacted DNA molecule. The packing in such particles is expected to resemble the structure of a normal hexagonal phase, and for a system formed by precipitating such particles one observes a small angle X-ray pattern typical for 2-D hexagonal structure [5].

Recently, thermodynamic hydration properties of such precipitated complexes were studied by Leal et al. they concluded that as the water content decreases there is a gradual change in the properties of the hydration chain and that in the dry state the surfactant aggregate is highly deformed to accomplish local charge neutralization with respect to the anionic phosphate groups on the DNA [1].

In the present paper we investigate the influence of salt content on the swelling of DNA complex with different surfactants such as:

- 1) Hexadecyltrimethylammonium bromide (CTAB)
- 2) Didodecyltrimethylammonium bromide (DDAB)

In the electroneutral DNA-CTAB and DNA DDAB aggregates, the space between DNA and surfactant is filled with water if we include salt ions in this water reservoir the electrostatics precipitate in the systems will be perturbed. The plan is to start with the electroneutral precipitate and designing a method to increase salt ions between DNA and the surfactant rods. Then we will try to calculate how much water the aggregate takes at different salt concentration.

MATERIALS

We have used sodium salt DNA: Calf thymus (fibrous type 1 "highly polymerized"). It was obtained from Sigma Company as received. Hexadecyltrimethylammoniumbromide (CTAB), Didodecyltrimethylammonium bromide (DDAB), sodium chloride and potassium carbonate (Merck p.a. quality) and sodium bromide (Riedel-dellaen extrapure quality) was used as received. The water used was from a Milli-Q filtration system (Millipore).

SAMPLE PREPARATION

DNA solutions were prepared by weighting the desired amount and dissolving it in 5 mM NaBr in a cold room during 1-2 days. DNA-Surfactants stoichiometric aggregates were prepared by mixing CTAB & DDAB (5 mM) and DNA under stirring. The number of surfactants equals the number of phosphate groups on the DNA. The precipitates formed were equilibrated in solutions for 48h. They were then separated from the aqueous phase by filtration at reduced pressure, and washed extensively with Millipore water and were dried in high vacuum during 3 days at room temperature. These solutions NaCl 0.14 M and 1.46 M and 3.08 M, saturated NaCl and K_2CO_3 were

prepared and placed in desiccators at 25 °C allowed for relative humidity of 99.5%, 95%, 90%, 75%, 43.2% respectively. The macromolecular complexes salt (DNACTAB and DNADDAB) were stored inside a desiccator.

Studied Systems:

Surfactants are amphiphilic molecules containing a hydrophilic head group that can be charged and a hydrophobic tail. In aqueous solution surfactants assemble in various structures.

CTAB: Hexadecyltrimethylammonium bromide is a cationic surfactant with a sixteen carbon hydrophobic tail that can assemble into rod-like micelles.

DDAB: Didodecyltrimethylammonium bromide is a double chain cationic surfactant contains twelve carbon atoms in its hydrophobic regions and predominantly form lamellar phases in aqueous solution.

METHODS

1. Desiccator method

18 samples with 50 mg of dry precipitate in a weighting container were allowed to equilibrate for 4 days in a desiccator with a salt solution of relative humidity of 99.5%. They were weighted before and after equilibration and calculated how much water the precipitate sorbed.

To the first 3 samples 0 mg NaCl and the second 3 samples 1 mg NaCl and the third 3 samples 1.5 mg NaCl and the fourth 3 samples 2 mg NaCl and the fifth 3 samples 2.5 mg NaCl and the last 3 samples 4 mg NaCl were added and allowed them to equilibrate for 3 days outside the desiccator at 25 °C and were weighted before and after equilibration. The samples were placed in vacuum for 3 days and placed them back in the same desiccator, let them equilibrate for 3 days and weighted them before and after equilibration and were measured how much water the precipitates were sorbed after inclusion of salt.

Those above samples were dried in vacuum for 3 days and placed in desiccator with a salt solution of relative humidity of 75% and let them to equilibrate for 3 days. They were weighted before and after the equilibration and calculated how much water sorbed by the precipitations.

Those samples were dried again in vacuum for 3 days and placed in desiccator with a salt solution of relative humidity of 43.2% and let them to equilibrate for 3 days. They were weighted before and after the equilibration and calculated how much water sorbed by the precipitations.

The results of our experiments were shown in table 1, 2.

Table 1.The amount of water sorbed by the DNA+DDAB complex in different Relative Humidity

RH%	99.5%	75%	43.2%
Salt (mg)			
0 mg NaCl	0.452	0.142	0.0125
1 mg NaCl	0.475	0.146	0.028
1.5 mg NaCl	0.549	0.160	0.0312
2 mg NaCl	0.668	0.185	0.0328
2.5 mg NaCl	0.741	0.198	0.0522
4 mg NaCl	0.999	0.209	0.0654

Table 2.The amount of water sorbed by the DNA+CTAB complex in different Relative Humidity

RH%	99.5%	75%
Salt (mg)		
0 mg NaCl	0.529	0.128
1 mg NaCl	0.648	0.146
1.5 mg NaCl	0.708	0.170
2 mg NaCl	0.978	0.210
2.5 mg NaCl	1.08	0.234
4 mg NaCl	1.126	0.271

2. Sorption Microcalorimeter:

A double-twin isothermal microcalorimeter was used to study the hydration of DNA and DNaCTAB and

DNADDAB complex. A detailed description of the instrument is given elsewhere [12-14]. Several applications of this new technique have been carried out, such as studies of phospholipids hydration [15, 16] and hydration of single-chain alkylglucosides [17]. The instrument consists of a calorimetric cell constituted by two vessels connected by a stainless steel tube and an isothermal double twin microcalorimeter placed in the top calorimeter and the bottom vessel is in the bottom calorimeter.

At the start of the measurement, the bottom vessel contains 20-100 mg of a dry sample and the top vessel about 100 μ l of water. During the measurement, water is vaporized and diffuses through the steel tube (the maximum diffusional flow is about 0.4 μ g of H₂O per s) down to the bottom vessel where is taken up by the sample. An experiment can be seen as a continuous titration of dry sample with water vapor, and a measurement is stopped after 3-7 days when the water activity of the sample has reached 0.90-0.95. With this method, one can get both sorption isotherms (water content as a function of water activity) and the differential enthalpy of sorption. A sorption calorimetry was used to measure the swelling isotherm of precipitates (DNaCTAB & DNADDAB).

In this research, we present a calorimetric study of the hydration of the stoichiometric complex between DNA and those above cationic surfactants. We used exactly those desiccator samples with different amount of salt in calorimetry measurement. And the isotherms of both desiccator and calorimetry were shown in Fig 1, 2.

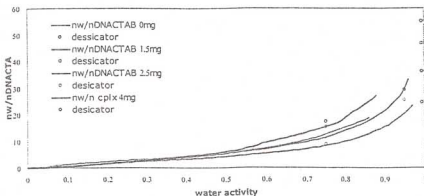


Fig.1. Sorption isotherm at 25 °C for DNaCTAB (Water molecules/ DNaCTAB) vs. Water activity at different amount of added NaCl.

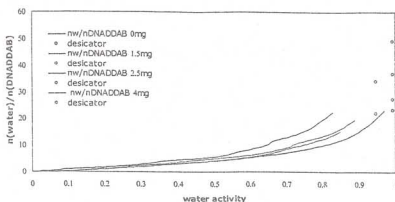


Fig.2. Sorption isotherm at 25 °C for DNADDAB (Water molecules/ DNADDAB) vs. Water activity at different amount of added NaCl.

3. SAXS measurements:

SAXS measurements in this work were made with a Kratky camera in physical chemistry department in Lund University, Sweden. The X-ray beam is generated by accelerating electrons to a Cu anode. The Cu gives rise to a K_{α} line with a wavelength of 1.54 Å. There is also a K_{β} line, which is taken away by a Ni-filter. The X-ray beam is focused by a collimation system with long slit, geometry. Due to the asymmetry of the slit, the scattering becomes smeared. In the diffraction spectra this is seen as sharp end of the peak at low q and a long tail towards large q . [18] However, this does not impose a problem for the relative positions of the peaks in the spectra.

SAXS-measurements were also performed with a synchrotron X-ray beam (D43 at L.U.R.E., France). In a synchrotron, the electrons circulate with a high velocity in a large closed orbit. Due to the bended route, the electrons circulate with a high velocity in a large closed orbit. Due to the bended route, the electrons emit a well defined radiation with a very high intensity. In addition, pin-hole collimation of the beam was used; which gives rise to symmetric peaks. Hence, the X-ray beam is well suited for complicated structures. We used exactly the same samples that were used for Calorimetry.

The results of our experimental were shown in the fig 3, 4.

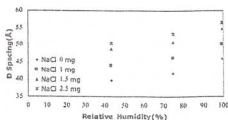


Fig. 3. D spacing dependence ($D=2l/q$) vs. Relative Humidity (%) at different amount of added salt in DNACTAB complex.

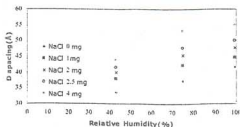


Fig. 4. D spacing dependence ($D=2l/q$) vs. Relative Humidity (%) at different amount of added salt in DNADDAB complex.

RESULTS AND DISCUSSION

The stoichiometric DNaCTAB (1:1) precipitate has previously been studied by X-ray small angle scattering [4], we repeated such experiments on samples prepared the same way as for the calorimetric measurements and found agreement with the results of Mel'nikov et al.

The small-angle region showed 2 diffraction peaks consistent with a 2D hexagonal lattice with a period of 49.4 Å for the DNaCTA complex prepared at water activity of 1.0. We performed ^1H NMR on dry and wet DNaCTA precipitates [19], and for the fully hydrated complex, several narrow peaks were observed, whereas for the dry complex there was one rather broad peak. The spectrum for the wet complex is similar to the one observed for CTAB rod like micelles arranged in an hexagonal structure [20]. This indicates that, in the most hydrate state of the DNaCTA complex, the amphiphile is arranged as in a liquid crystalline hexagonal structure.

When DNA interacts with double-chain lipids that by themselves form lamellar liquid crystals, the DNA- lipid complex is also lamellar [2]. Thus it is reasonable assumption that, since CTAB by itself easily forms a normal hexagonal phase, the structure of the hexagonal DNaCTA complex is also of the normal type. The fact that there are no extra counterions implies that the DNA and the CTA must pack in such a way that there is charge neutralization on as small a scale as possible. In the hexagonal phase of CTAB, there is an area per headgroup of $53\text{\AA}^2$ and a cylinder diameter of $43\text{\AA}$ [21]. This means that, projected on the cylinder axis, there is one charge per $0.36\text{\AA}$. Surfactant aggregates are formed by a self-assembly process so that under different conditions one can expect adjustments in these numbers, but there should not occur major changes; a change in headgroup area exceeding, for example, 20% appears unlikely. For DNA, there are more fixed atomic positions in the double helix

so the structure is locally stiffer. On the other hand, there are different conformations of the helix, and for the pure DNA in particular, we considered the A-B transition. The general observation is that A DNA is favored when the electrostatic repulsion along the helix is reduced either by lowering the vapor pressure or by the addition of salt [22]. Binding of the highly positive CTA aggregate is an extreme case of reducing the electrostatic repulsion, and on the basis of this argument, one should expect that in the DNaCTA system the A form is favored relative to the surfactant, there are spectroscopic CD observations that DNA remains in the B form on complexation [23, 24]. At present, this issue appears unresolved, but it has a significant bearing on the possible mutual arrangement of DNA helices and CTA cylinders in the complex.

CONCLUSION

In this paper we investigated the internal structure of DNA-CTAB and DNA-DDAB. We observed the hexagonally packed for DNA-CTAB complexes and lamellar packed for DNA-DDAB complexes. These structures were in agreement with all observed X-ray patterns, for both complexes, whereas the internal spacing displays a dependence on the chemical nature of the surfactant. With addition of salt the lamellar and hexagonal spacing were increased.

On the other hand we develop a consistent method to include salt ions with in the DNA- Surfactant aggregates.

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