Interaction between cationic agents and small interfering RNA and DNA molecules

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Abstract.
Azobenzene containing surfactant AzoTAB was used for investigation of binding in cationic-agent + nucleic acid in NaCl salt aqueous solutions. Two nucleic acids, macromolecular DNA and small interfering RNA, were examined upon the interaction with the surfactant. For DNA the interaction was studied using spectral methods and the methods of viscometry and flow birefringence measurement. For siRNA the possibility of surfactant-based delivery was checked in vitro.

1. Introduction
The interaction between cationic agents and nucleic acids DNA and RNA has been studied extensively for two main reasons. Firstly, the condensation that occurs for the solutions of DNA molecules in presence of cations has appealed to scientific interest since the 1970s [1, 2]. Being a transition from the rigid and charged molecule to the compact form, this process presents a change in conformational parameters of DNA and was studied for a variety of cationic-agent + nucleic acid in NaCl salt aqueous solutions. Two nucleic acids, macromolecular DNA and small interfering RNA, were examined upon the interaction with the surfactant. For DNA the interaction was studied using spectral methods and the methods of viscometry and flow birefringence measurement. For siRNA the possibility of surfactant-based delivery was checked in vitro.
therapeutic activity, especially for short and unstable RNAs. For siRNA delivery cationic lipids are widely used [8, 9], but some of them demonstrate high toxicity [9]. Surfactants, whose amphiphilic molecules lower surface tension and form micelles in solution, also have been used for the creation of nanocarriers [10], and have been employed for enhancing modification of delivery agents [11]. The interaction of surfactants with nucleic acids in solution has been scrupulously studied for DNA-surfactant systems [12, 13, 14, 15]. Addition of nucleic acids to the solution induces micellization of surfactant at concentrations lower than critical micelle concentration of surfactant, and micellar aggregates neutralize charges of phosphates, inducing intramolecular contacts. For DNA of high molecular weight it was shown that the addition of cationic surfactant leads to conformational changes of DNA in solution, and compact nanoparticles, which are promising for the delivery, were observed [12]. These conformational transitions can be controlled using photosensitive surfactants, but their toxicity should be taken into consideration [16].

The goal of our study is to obtain novel data on binding of azobenzene containing cationic surfactant AzoTAB to DNA and siRNA in vitro. For siRNA the conditions of the formation of nanostructures in solution are determined; we examined the efficiency of cell delivery for these systems.

2. Materials and methods
We used Sigma Aldrich calf thymus (CT) DNA and Dharmacon GE siRNA. For delivery study negative control siRNA-AF546 (with AlexaFlour-546 fluorescent label) was used. Molar concentrations of nucleic acids were calculated from average molecular weight per phosphate of DNA 330 g/mol and of siRNA 321 g/mol. Nucleic acids were dissolved in Milli-Q Type I ultrapure water or in Sigma ultrapure water. Surfactant samples (figure 1) were provided to us by Prof. S. Santer (University of Potsdam, Germany). We used C4-Azo-C6-TMAB (trimethylammonium bromide) for experiments with CT-DNA, and C4-Azo-C6-TEAB (triethylammonium bromide) for experiments with siRNA (transfection studies). Azobenzene containing surfactants undergo trans- to cis- isomerisation under UV irradiation (353 nm). Reverse cis-trans transition is induced by visible light (453 nm) or can be realized in darkness. Delivery of siRNA + surfactant systems was tested on MDCK cell line.

For spectral studies we used SF-56 spectrophotometer (OKB-Spectr, Russia). A low-gradient viscometer of Zimm-Crothers type [17] was used for viscosity measurements, and atomic force microscopy (AFM) studies were carried out using NanoScope IVa (Veeco) in tapping mode in air. Samples were prepared by placing solutions mixed with MgCl₂ on freshly cleaved mica surface for 2 min followed by washing the samples with 0.5 – 1 ml of distilled water and drying them in vacuum.

![Figure 1. Structure of C4-Azo-C6-TMAB (AzoTMAB). AzoTEAB has ethyl groups instead of methyls on the polar ‘head’ of the surfactant.](image)

3. Results and discussion
3.1. Spectral studies
UV-Spectra of the surfactant (figure 2a and 2b) show the small shift in absorption maximum (importantly, nucleic acids have no absorption on these wavelengths) upon the addition of nucleic acid, which induces the formation of micelles for the concentrations lower than critical micelle concentration (CMC), and the shift in the same direction (to higher wavelength for cis- isomers and to lower wavelength for trans-isomers) occurs for the concentrations of surfactants higher than CMC in absence of nucleic acids. The addition of La^{3+} ions (figure 2b) to the solution interferes in binding of
surfactant to nucleic acid, and the shift is insufficient in this case (figure 2b line c). The addition of La$^{3+}$ to the surfactant solution without DNA induces no shift in surfactant absorption maximum (control, figure 2b line d).

**Figure 2a.** Normalized spectra: a – cis- surfactant AzoTEAB in 5 mM NaCl solution, C < CMC; b – the same upon addition of siRNA, C(siRNA) = 16 μM; c – cis-surfactant, C > CMC; d – trans-surfactant in 5 mM NaCl solution, C < CMC; e – the same upon addition of siRNA, f – trans-surfactant, C > CMC.

**Figure 2b.** Normalized spectra: a – trans- surfactant AzoTMAB in 5 mM NaCl solution, C < CMC; b – the same upon addition of CT-DNA, C(DNA) = 60 μM; c – DNA + trans-surfactant upon the addition of LaCl$_3$; d – trans-surfactant upon the addition of LaCl$_3$ (control). a and d are normalized to 0.9 to distinguish them from a and b.

Furthermore, the shift in surfactant absorption maximum is also observed upon addition of denatured DNA (figure 3a) to the surfactant solution, indicating that inner sites of the molecule, which are not exposed in the native DNA structure, play no role in inducing the surfactants binding, and the destruction of DNA secondary structure seems not to prevent it. This adds the data we obtained from spectra of circular dichroism (CD) of DNA (figure 3b), which show no sufficient changes upon the binding for the studied values of $z = C$(surfactant) / C(DNA), and also indicate external binding of the surfactant (to the phosphates of nucleic acid).

**Figure 3a.** Normalised spectra: a – trans- surfactant (AzoTMAB) in 5 mM NaCl solution, C < CMC; b – trans- surfactant + native DNA, C(DNA) = 125 μM; c – trans- surfactant + denatured DNA.

**Figure 3b.** CD spectra for DNA + surfactant (AzoTMAB) in 5 mM NaCl, C(DNA) = 242 μM, $z = C$(surf.) / C(DNA): a – DNA without surfactant ($z = 0$), b – 0.1 DNA + cis- surf.; c – 0.3 DNA + trans- surf.; d – 0.3 DNA + cis- surf.; e – 0.4 DNA + cis- surf.
3.2. DNA-surfactant interaction

We estimated the change in reduced viscosity \((\eta_r - 1)/C(DNA)\), where \(\eta_r\) – relative viscosity (solution viscosity / solvent viscosity), of the DNA + surfactant solutions for increasing concentration of the surfactant (ratio \(z = C(\text{surf.})/C(DNA)\) is increasing, \(C(DNA)\) remains constant). The measurement of flow birefringence provides us with the anisotropy of optical polarizability of DNA coil in the solution \((\gamma_1 - \gamma_2)\), where 1 and 2 are two molecular axis. From the values of reduced viscosity and \((\gamma_1 - \gamma_2)\) of the molecule, we calculated optical anisotropy of the DNA molecule statistical segment \((\alpha_1 - \alpha_2)\) (detailed information in [12]). We observed the decrease in relative \((\alpha_1 - \alpha_2)\) with the growing C(surfactant) in the DNA solution in low salt concentration 5 mM NaCl (figure 4a). Importantly, it was followed by the decreasing viscosity (figure 4b). The results allowed us to demonstrate and estimate the conformational changes of DNA upon the interaction with the surfactant (trans- and cis- isomers). We also tested the DNA + surfactant solutions in high salt concentration 1 M NaCl. These systems do not demonstrate similar decrease in viscosity and optical anisotropy. However, these novel results for the complex two-component systems require further investigation both for lower and higher salt concentrations.

![Figure 4a](image1)

**Figure 4a.** Changes in relative segmental optical anisotropy \((\alpha_1 - \alpha_2)\) is for DNA solution with the increase of \(z\) value: a – in 1 M NaCl for trans- surfactant; b – in 1 M for cis- surfactant; c – in 5 mM for trans-; d – in 5 mM for cis-

![Figure 4b](image2)

**Figure 4b** Changes in relative reduced viscosity: a – in 5 mM for trans- surfactant; b – in 5 mM for cis- surfactant; c – in 1 M for trans-

3.3. Delivery studies

For the transfection test we prepared siRNA + surfactant (AzoTEAB) solutions. Starting this experiment, we tested solutions in 0.15 M NaCl and in 5 mM NaCl. However, for higher salt concentration 0.15 M NaCl we observed no delivery in studied conditions. This indicates that due to neutralizing of nucleic acid’s phosphates by Na+ ions higher salt concentration (higher ionic strength) is less favorable for the binding and, consequently, for the formation of nanocarriers. These systems were containing two concentrations of siRNA 25 \(\mu\)M and 10 \(\mu\)M, and the concentration of the surfactant (AzoTEAB) in its trans- form was varied \((z = C(\text{surf.})/C(DNA) = 0.8, 3.25, 5, 12)\).

After incubating the similar volume \((V = 0.175 \text{ ml})\) of samples with cells in plates (for samples with 25 \(\mu\)M of siRNA we had 1500 ng of siRNA in each well, and for samples with 10 \(\mu\)M of siRNA we had 600 ng in each well), they were washed with PBS, and the delivery was observed (figure 5, 6).

Since the final quantities of siRNA per each well in fig. 5a and 5b were different, the intensity of fluorescence cannot be compared, but the result shows that for both concentrations of nucleic acid
complexes are formed, and the delivery occurs. An increasing quantity of delivered siRNA is demonstrated for the growing surfactant concentration (growing $z$), together with the increase in toxicity. Importantly, we observed no delivery for $z = C(\text{surf.}) / C(\text{DNA}) = 0.8$, that confirms the absence of formed nanocarriers for the smaller ratios.

Figure 5. Images of cells (left – fluorescence, right – visible light) after treatment with fluorescently labeled siRNA ($C(\text{siRNA}) = 25 \mu\text{M}$) + surfactant: a – $z = C(\text{surf.}) / C(\text{NA}) = 3$; b – $z = 5$; c – $z = 12$

Figure 6. The same for $C(\text{siRNA}) = 10 \mu\text{M}$: a – $z = C(\text{surf.}) / C(\text{NA}) = 5$; b – $z = 6.5$.

The formation of nanostructures (figure 7) is also indicated by AFM images obtained for siRNA + surfactant in 5 mM NaCl, $C(\text{siRNA}) = 1 \text{mM}$ with the addition of MgCl$_2$ ($C(\text{MgCl}_2) = 0.5 \text{mM}$) on mica surface. However, possible interaction between cationic surfactant and mica (see figure 7b) does not allow regarding the observed structures as fully equivalent to those formed in solution.

Figure 7. AFM images of siRNA + surfactant: a – without surfactant; b – $z=0.6$; c – $z=1.6$; d – $z=8$

4. Conclusion

We examined binding of cationic agent (azobenzene containing surfactant AzoTAB) to the molecules of nucleic acids siRNA and DNA using UV-Vis spectroscopy. For DNA macromolecules of high molecular weight we also carried out a combined viscometry/flow birefringence study to examine DNA conformational changes upon binding with trans- and cis- isomers of the surfactant. For siRNA + surfactant systems we aimed to determine the conditions of gene vectors’ creation by AFM observation and by testing the delivery on MDCK cell line.
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