DNA compaction by azobenzene-containing surfactant

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We report on the interaction of cationic azobenzene-containing surfactant with DNA investigated by absorption and fluorescence spectroscopy, dynamic light scattering, and atomic force microscopy. The properties of the surfactant can be controlled with light by reversible switching of the azobenzene unit, incorporated into the surfactant tail, between a hydrophobic *trans* (visible irradiation) and a hydrophilic *cis* (UV irradiation) configuration. The influence of the *trans-cis* isomerization of the azobenzene on the compaction process of DNA molecules and the role of both isomers in the formation and colloidal stability of DNA-surfactant complexes is discussed. It is shown that the *trans* isomer plays a major role in the DNA compaction process. The influence of the *cis* isomer on the DNA coil configuration is rather small. The construction of a phase diagram of the DNA concentration versus surfactant/DNA charge ratio allows distancing between three major phases: colloidally stable and unstable compacted globules, and extended coil conformation. There is a critical concentration of DNA above which the compacted globules can be hindered from aggregation and precipitation by adding an appropriate amount of the surfactant in the *trans* configuration. This is because of the compensation of hydrophobicity of the globules with an increasing amount of the surfactant. Below the critical DNA concentration, the compacted globules are colloidally stable and can be reversibly transferred with light to an extended coil state.

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I. INTRODUCTION

The last two decades have shown remarkable progress in gene therapy [1]. Different vectors have been developed to deliver genetic material into cells, tissues, or whole organs. Viral vector approaches [2] possess the highest transfection efficiency as a result of two primary factors: (i) targeted delivery, because viruses interact with specific receptors on cell surfaces, and (ii) delivery of DNA in an uncomplexed state readily accessible to expression. Although these vectors largely remain the approaches of choice, immunological risks associated with the usage of viruses still motivate the development of nonviral alternatives [3].

Nonviral vectors must be complexed with DNA, increasing cellular uptake as a result of DNA neutralization and compaction. Compaction has been achieved *in vitro* by complexation of nucleic acids with cationic and nonionic surfactants, lipids, polyelectrolytes, and neutral polymers [4], as well as multivalent ions [5]. However, the tight binding of these agents to DNA can significantly alter gene expression and the resulting nuclear uptake. To address this problem, several strategies of DNA decompaction have been proposed using anionic surfactants [6] and polyelectrolytes [7], as well as pH- [8] and redox-active [9] surfactants. These approaches are quite effective *in vitro* but very difficult to realize *in vivo*. Using an external stimulus to trigger DNA decompaction within a cell-sized compartment has thus become an important challenge. A promising solution is to use light as an external stimulus—the key tool in optogenetics [10]. This strategy is particularly appealing because nowadays, with optical fibers, one can address specifically very small parts of tissue [11] where control of DNA conformation is required.

Recently, Ny and Lee proposed a system where DNA conformation can be controlled by light in a reversible manner [12]. This was achieved by adding cationic surfactant containing azobenzene (AzoTAB) to a DNA solution. By switching the azobenzene molecule between a hydrophobic *trans* isomer and a hydrophilic *cis* isomer, the reversible transition of DNA between a compacted globular and an extended coil state was observed. Shortly afterwards, a very similar system was investigated with respect to changes in the conformation of individual DNA molecules upon the addition of the surfactant [13], and the kinetics of compaction and decompaction under isomerization of the surfactant [14] using fluorescence microscopy.

Although the approach of using photosensitive surfactants is very promising, its application *in vivo* is still challenging to realize. The main difficulty with such systems is a very low affinity of the surfactant with DNA. The affinity, which is qualitatively described by the critical association concentration (CAC), can be tuned by changing the hydrophobic part of a surfactant [15]. It was recently shown that the increase of an alkyl spacer between the azobenzene unit and the head group of a photosensitive surfactant (like AzoTAB) leads to a considerable reduction of the surfactant amount needed to trigger the compaction [16]. In the present work, the hydrophobic part of a photosensitive surfactant is increased by placing a longer alkyl spacer between the cationic charged group and the azobenzene unit, as well as by increasing the alkyl tail at the other end of the azobenzene. The binding efficiency between the surfactant and DNA is considerably improved. We have obtained the

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smallest value, to our knowledge, of a photosensitive surfactant concentration needed to initiate DNA compaction, reaching the limit (1:1) of the charge ratio. Notably, the limit has been already demonstrated by applying a photosensitive gemini surfactant [17]. However, the photosensitive unit did not trigger the changes in the hydrophobic tails of the surfactant and the reversible decompaction was not realized.

Cationic azobenzene-containing surfactants are ideal for proof-of-concept investigations. Among all surfactant types, the cationic ones are the best choice to realize DNA compaction [18]. The azobenzene unit is preferred because of its exceptional photoreaction (robust, clean, and reversible), thereby providing a reliable model system for the investigation of light-controlled effects [19]. On the other hand, the toxicity of such surfactants is one of the main obstacles on the way to applications *in vivo*. One can address this problem in two ways: (i) by reducing the amount of the toxic compound, that is, decreasing the CAC discussed above, or (ii) by switching from the cationic head group to another type (e.g., zwitterionic) and using another photosensitive unit (e.g., spiropyran), which is less toxic. As the model surfactant is believed to be the most efficient and its interaction with DNA is still not understood, the success of the latter approach—involving many trial-and-error experiments—is questionable. Therefore, attention is primarily focused on an understanding of the complexes, optimization of the cationic azobenzene-containing surfactant, and the realization of an efficient and completely reversible light-induced DNA compaction process.

In this paper we take further steps toward understanding the interactions between photosensitive surfactant and DNA molecules. We investigate the role of both *trans* and *cis* isomers in the DNA compaction process. As the azobenzene unit possesses characteristic absorption lines in the UV-visible spectrum, a major part of the present investigation is devoted to the analysis of changes in UV-visible spectra under isomerization of the surfactant. The study is supplemented by dynamic light scattering, fluorescence spectroscopy, and atomic force microscopy measurements.

II. EXPERIMENT

Materials. Azobenzene containing trimethylammonium bromide surfactant (C_4-Azo-OC_6TMAB) was synthesized, with slight modifications, according to the procedure described in Ref. [20]. The chemical structure of C_4-Azo-OC_6TMAB and its isomerization spectra are shown in Fig. 1. The spectra do not differ much in water and in a 5-mM NaCl water solution, and peak positions with the corresponding extinction coefficients are very close to those for similar surfactants [21]. The presented spectra under UV and visible irradiation correspond to saturation, or the so-called steady state, where the rates of *trans-cis* and *cis-trans* isomerizations are the same. The surfactant is characterized by a very long lifetime of the *cis* isomer (48 hours); therefore, the spectra under irradiation do not change considerably after the irradiation is turned off. To estimate the amount of *trans* isomers in solution under the isomerization process, we utilize the absorption at 376 nm, where the influence of the *cis* isomer is minimal. Using the extinction coefficient at this wavelength, the amount of *trans*

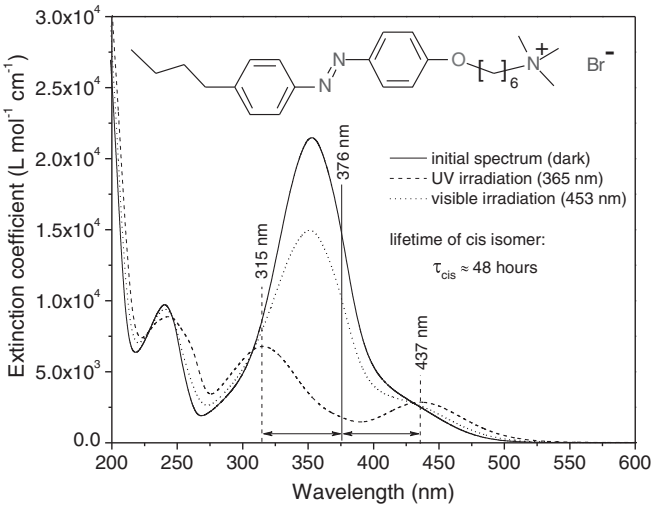

FIG. 1. Chemical structure and extinction spectra of azobenzene-containing surfactant (C_4-Azo-OC_6TMAB). The spectra under irradiation correspond to saturation, or the so-called steady state.

isomer relative to the dark relaxed state was assessed. Notably, the surfactant in the dark state should not be considered to be in a pure *trans* configuration. There is always some amount of *cis* isomer dictated by thermodynamic equilibrium. There is also some residual amount of the *trans* isomer after UV irradiation. As can be seen from the spectra, the irradiation with visible light does not result in complete transition to the initial dark state.

The critical micelle concentration (CMC) of the surfactant in the dark state solved in pure water was determined, using a VP-ITC Isothermal Titration Calorimeter (GE Healthcare Europe GmbH, Germany), to be ~0.5 mM. All experiments were carried out with concentrations of the surfactant below the CMC.

Calf thymus DNA, type I, containing 6% sodium salt, was purchased from Sigma (no. D1501). The molecular weight was determined, using intrinsic viscosity measurements, to be 10.8×10^6 Da (16.4 kbp). Aqueous solutions of the DNA and surfactant were prepared in 5-mM NaCl using deionized water (MilliQ). The estimated value of the DNA radius of gyration at these conditions is about 310 nm, which corresponds to the overlap concentration of $(\mathrm{DNA})^{\mathrm{overlap}}_{\mathrm{nucleotides}} \approx 3.3 \times 10^{-4}$ M; this value was not exceeded in all experiments.

Both C_4-Azo-OC_6TMAB and DNA solutions were diluted with 5-mM NaCl prior to mixing to get a desired concentration of DNA and a molar ratio of charges of C_4-Azo-OC_6TMAB and DNA defined as $Z = (C_4\text{-Azo-}OC_6\text{TMAB})/(\mathrm{DNA})_{\mathrm{nucleotides}}$. To obtain good absorption signals for complexes of different surfactant-to-DNA ratios, most of the experiments were carried out with a DNA concentration of 5×10^{-5} M (M = moles of nucleotides per liter). Other concentrations in the range from 10^{-7} to 10^{-3} M were investigated only to build a phase diagram of the DNA compaction.

Ethidium bromide (EtBr) was purchased from Sigma (no. 46065). An aqueous solution of EtBr was prepared in 5-mM NaCl using deionized water (MilliQ). The concentration of EtBr in the solution was determined spectroscopically

using the molar extinction coefficient 5600 L mol^{-1} cm^{-1} at 480 nm [22]. The DNA solution was mixed with EtBr directly in the fluorescence cell.

Methods. Irradiation of C_4-Azo-OC_6TMAB–DNA complexes was done by UV light (365-nm UV lamp: VL-4L, Fisher Scientific SAS, France) and visible light (453-nm LED Spot Luxeon Royal Blue, P453E-PR09, Conrad, Germany). All samples were irradiated at a distance of 10 cm from the light source. The UV-visible spectra of complexes were measured with a Cary 5000 UV-Vis-NIR spectrophotometer (Varian, Inc., USA). Fluorescence spectra measurements were carried out with a Fluorolog FL3-22 fluorescence spectrometer (HORIBA JobinYvon GmbH, Germany). Characterization of the DNA globules (and their aggregates) size distribution was done with a Zetasizer Nano ZS (Malvern Instruments Ltd., United Kingdom) using a spherical nanoparticle model. DNA-surfactant complexes were studied using a tapping mode atomic force microscope (AFM) (Nanoscope V, Veeco). The DNA-surfactant complexes were deposited on a mica surface from a water solution of 5-mM NaCl by spin casting at 2000 rpm. The commercial tips (Nanosencers) with a spring constant of about 50 N m^{-1} and an average apex radius of 5 nm were utilized.

III. RESULTS AND DISCUSSION

A. DNA compaction and precipitation

In this section, we discuss the behavior of the DNA-surfactant complexes without exposure to light. Therefore, prior to the mixing with DNA, the surfactant C_4-Azo-OC_6TMAB was kept in the dark for several days to ensure complete relaxation to the *trans* configuration. All experiments were carried out in a room with yellow light to avoid any isomerization of the surfactant.

Complexes of the azobenzene-containing surfactant (azo-surfactant) and DNA of different charge ratios, $Z = (C_4\text{-Azo-}OC_6\text{TMAB})/(\text{DNA})_{\text{nucleotides}}$, were prepared and left to equilibrate at room temperature for at least 24 hours. The concentration of DNA was kept constant at 5×10^{-5} M and the concentrations of azo-surfactant were varied. Figure 2 shows a series of flasks with azo-surfactant–DNA solutions of different Z ratios. Three different regimes are visible: (i) $Z < 1.2$, (ii) $1.2 < Z < 2.4$, and (iii) $Z > 2.4$. In the first region, one can see the increase of absorption (yellow color) in the complexes with an increase of the charge-mixing ratio Z. There is no visible formation of precipitate. Within several minutes after mixing of the components, strong scattering and formation of the precipitate was observed for $1.2 < Z < 2.4$ (second region). When the complexes were left to equilibrate for a day, the precipitate settled to the bottom of the samples (see Fig. 2). Starting from $Z > 1.2$, there is a considerable drop of the absorption indicated by the white color of the solutions. This was an initial qualitative hint that most of the surfactant was precipitating. In the third region, starting from $Z = 2.4$, a stable solution without visible scattering and precipitate formation can be observed. The solution became yellowish with an increasing concentration of azo-surfactant.

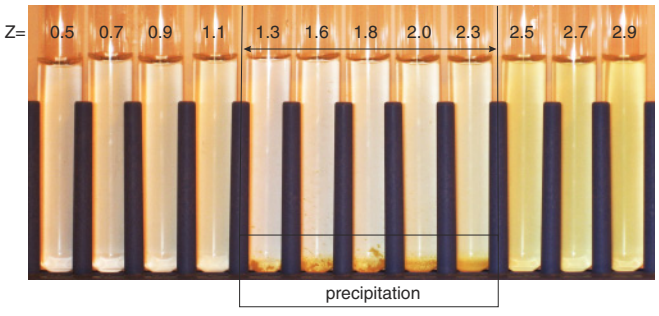

FIG. 2. (Color online) Photo of C_4-Azo-OC_6TMAB–DNA complexes of different mixing ratios Z the next day after mixing. A clear precipitate formation and reduction of extinction for $1.2 < Z < 2.4$ can be noticed.

For qualitative analysis, the UV-visible spectra of samples of different charge ratios in the range of $0.0 \leqslant Z \leqslant 5.6$ were measured. Only the top two-thirds of the solution from the precipitated samples was carefully taken to ensure the absence of the precipitate. The results are presented in Fig. 3. To simplify the analysis of the spectra for solution makeups, the absorptions at 376 nm (absorption of the surfactant) and at 260 nm (absorption of the DNA + surfactant) were plotted as a function of Z (see inset in Fig. 3). In all complexes, the concentration of DNA was the same; therefore, in the bottom inset, the absorption coefficient of the DNA is indicated by a horizontal gray line. For comparison, the spectrum of a pure DNA solution with the same DNA concentration is also presented. The presence of the DNA in the samples can be noticed in the spectra as a shoulder in the range $270 < \lambda < 290$ nm.

We analyze the data in three ranges of the charge-mixing ratio Z as follows:

Region (i). $Z < 1.2$: The DNA shoulder can be easily detected in the presented spectrum for $Z = 1.1$. In the inset graphs, it is seen that both absorptions increase linearly with Z, indicating that both the DNA and C_4-Azo-OC_6TMAB are in the solution. The absorption spectra of the complexes here

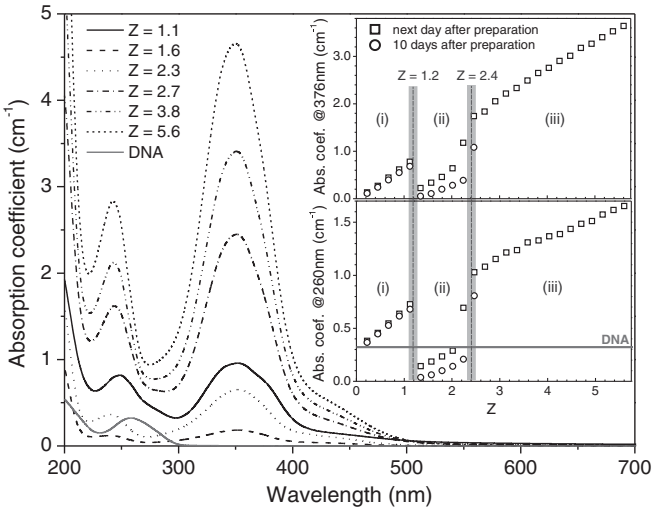

FIG. 3. UV-visible spectra of DNA and its complexes with C_4-Azo-OC_6TMAB at different ratios Z. Inset: Absorption coefficient at 376 nm (surfactant) and 260 nm (DNA + surfactant) vs. Z. A considerable drop of absorption in the range of precipitate formation $1.2 < Z < 2.4$ can be observed.

is not quite a sum of the spectra of both components, although the differences are minor, as one can expect due to the DNA azo-surfactant interactions.

Region (ii). $1.2 < Z < 2.4$: The absorption is much smaller than that expected as a sum of the DNA and surfactant. In addition to the decrease of absorption of the surfactant, the DNA band is practically absent in the spectra, indicating that most of the DNA was precipitated. A comparison of these spectra and the spectrum of pure surfactant revealed the practically complete absence of DNA in the solutions and the surfactant concentration gradually increasing with Z. This behavior can be clearly seen from the inset graphs in Fig. 3, where a considerable drop in the absorption of both components is observed. Extrapolation to zero of the absorption components of the solutions after complete settling of the precipitate gives the composition of the precipitate, $Z_{\text{precipitate}} \approx 1.2$. Notably, the complete settling of the precipitate took several days and the presented results, which represent the measurements carried out in ten days after preparation of the samples. The same value of $Z_{\text{precipitate}}$ was obtained from a comparison of the solution spectra with the extinction spectrum of the surfactant. The surfactant quota in the precipitate slightly increased with Z.

Region (iii). $Z > 2.4$: The absorptions of both the DNA and surfactant are again present in the spectra. The absorption of the surfactant dominates and the shoulder of DNA can hardly be resolved. The absorption components (in the inset graphs) gradually increase with the increase of Z; there is some nonlinearity, which can be attributed to some kind of interaction between the surfactant and compacted DNA globules.

The onset of precipitation upon the increase of the surfactant/DNA ratio was also observed by Ny and Lee for $Z \approx 3.5$ and a comparable DNA concentration [12]. Smaller values of Z in our case can be attributed to the differences in structure of the used surfactant. As the DNA compaction process is strongly influenced by the hydrophobic part of a surfactant, a longer alkyl tail and longer spacer between the azobenzene unit and the ammonium group of C_4-Azo-OC_6TMAB result in a decrease of Z required to trigger the formation of compacted globules. A similar effect is well investigated for nonphotosensitive [15] and photosensitive (such as AzoTAB) [16] surfactants. The ratio of a surfactant to DNA corresponding to the onset of DNA compaction cannot be reduced infinitesimally. The lower limit for Z is close to unity, which is connected to the facts that (i) at least 90% of the DNA charges should be neutralized for condensation to occur [5], and (ii) there are no counterions present in the compacted DNA-surfactant complexes [23].

B. Behavior of azo-surfactant–DNA complexes under irradiation

In this section, we describe the changes in the DNA-photosensitive surfactant complexes during the isomerization reaction of the surfactant. For a fixed value of Z, the surfactant in the complex was reversibly switched between a *trans* and *cis* configuration by UV and visible light irradiation, respectively. The amount of *trans* isomers after irradiation was calculated using an extinction coefficient at 376 nm

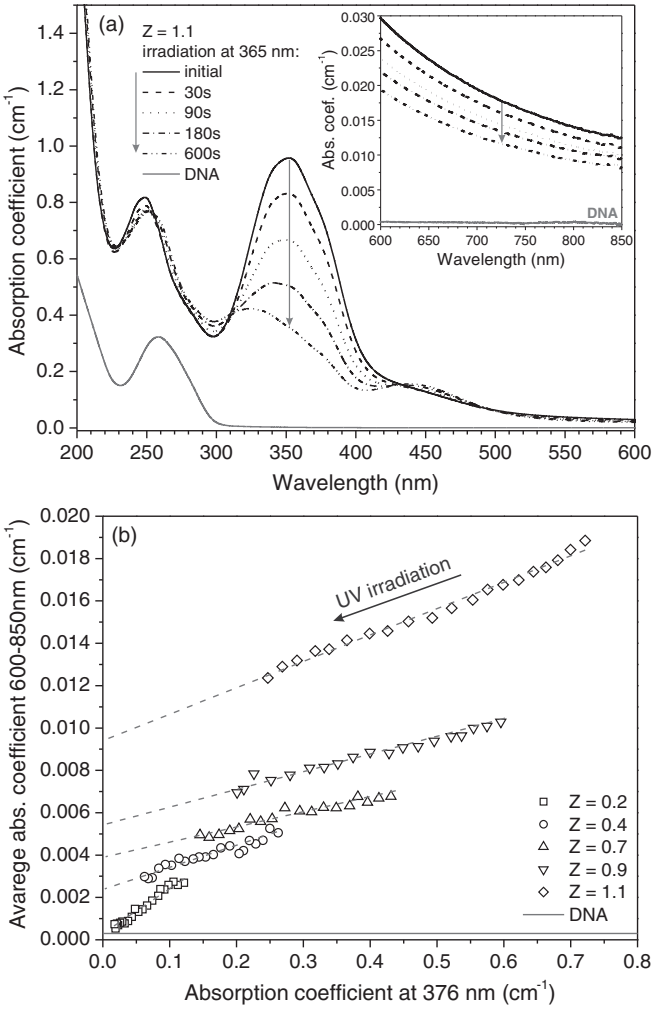

FIG. 4. (a) Changes in UV-visible spectra of the C_4-Azo-OC_6TMAB–DNA complex ($Z = 1.1$) in response to *trans-cis* isomerization of azobenzene (UV irradiation). Inset: The corresponding changes of light scattering in the long-wavelength range. (b) The dependence of the average absorption coefficient (scattering) in the long-wavelength range vs. the amount of *trans* isomer (absorption at 376 nm) under irradiation of the complex with UV light (*trans-cis* isomerization) for different values of Z.

(see Sec. II). Three different regions were investigated: (i) complexes before precipitation $Z < 1.2$, (iii) colloidally stable globules $Z > 2.4$, and (ii) precipitated complexes $1.2 < Z < 2.4$.

Region (i). $Z < 1.2$: Initial complexes were prepared in the same way as described in the previous section. The samples were irradiated with UV light to trigger *trans-cis* isomerization of the azobenzene unit. To track the changes in the spectra at different stages of isomerization, the irradiation was done with steps of small irradiation doses. After each irradiation step, an absorption spectrum was measured. An example of the changes in spectra during isomerization for $Z = 1.1$ is shown in Fig. 4(a). For comparison, the spectrum of pure DNA of the same concentration is presented. One can see a typical isomerization behavior of the surfactant. However, the isobestic points do not stay intact under isomerization, as in the case for the solutions of pure surfactant. This behavior was

attributed to the presence of scattering, which was characterized in the long-wavelength spectral range [see inset in Fig. 4(a)] and always accompanied the changes in isomerization spectra. The scattering of the complex is obviously larger than that of pure DNA of the same concentration; whereas the absorption of the pure surfactant is zero for $\lambda > 550$ nm (see Fig. 1). Analogous results were obtained for other mixing charge ratios for $Z < 1.2$. All changes in spectra were completely reversible on *cis-trans* isomerization triggered by visible light irradiation or thermal relaxation in the dark.

The appearance of scattering can be attributed to the formation of complexes between the DNA and the surfactant. To analyze this interaction, the average absorption coefficient in the range from 600 to 850 nm (scattering) versus the absorption at 376 nm (amount of *trans* isomer) was plotted [Fig. 4(b)]. The scattering decreases linearly with the reduction of the amount of the *trans* isomer. For the case of low azo-surfactant concentrations at $Z = 0.2$, the scattering returns to the level of pure DNA when approximating the linear dependence to zero absorption at 376 nm. At this point, complete conversion to the *cis* form occurs. Starting from $Z \approx 0.3 \pm 0.1$, the scattering does not return to the value of pure DNA when approximating the linear dependences. The approximated residual scattering is an indication of DNA-surfactant complexes resulting from the interaction between the DNA and *cis* isomer. From this behavior, we can deduce that not only *trans* isomers bind to DNA, but also *cis* isomers participate in the complex formation. The critical value of the charge ratio $Z \approx 0.3$, above which there is an interaction between the *cis* isomer and DNA, can be classified as a critical association concentration of the *cis* isomer ($CAC_{cis} \approx 1.5 \pm 0.5 \times 10^{-6}$ M). The results in Fig. 4(b) can serve as a qualitative comparison of the interaction strengths for *cis* and *trans* isomers. Thus, if all surfactant molecules were switched from *trans* to *cis* form, the scattering of the complex would drop only to half of the initial value. In this case, we can make a cautious conclusion that either two *cis* isomers or one *trans* isomer might have a comparable effect on the DNA conformation.

Region (iii). $Z > 2.4$: As already noted, no precipitation was observed for these mixing ratios, and both the surfactant and DNA are present in the solution. The stabilization of the complexes with a further increase of the surfactant concentration is an unusual behavior because DNA-surfactant complexes do not redissolve with an excess of surfactant [15]. There should be some other process besides simple redissolving of the complex, which we supposed to be the colloidal stabilization of DNA globules. Therefore, prior to the discussion of the behavior of these complexes under irradiation with UV light, it is worthwhile to consider the experimental evidence supporting the existence of DNA globules in solution. This will simplify the understanding of changes in the UV-visible spectra under the *trans-cis* isomerization of the surfactant presented below.

To investigate how surfactant quantity influences the colloidal stability of compacted complexes, the distribution of their effective hydrodynamic diameter was determined using dynamic light scattering. The spherical particle model used for the data processing was not exact for the description of the globules and their aggregates; nevertheless, it was sufficient for qualitative estimations. The results are presented in Fig. 5(a).

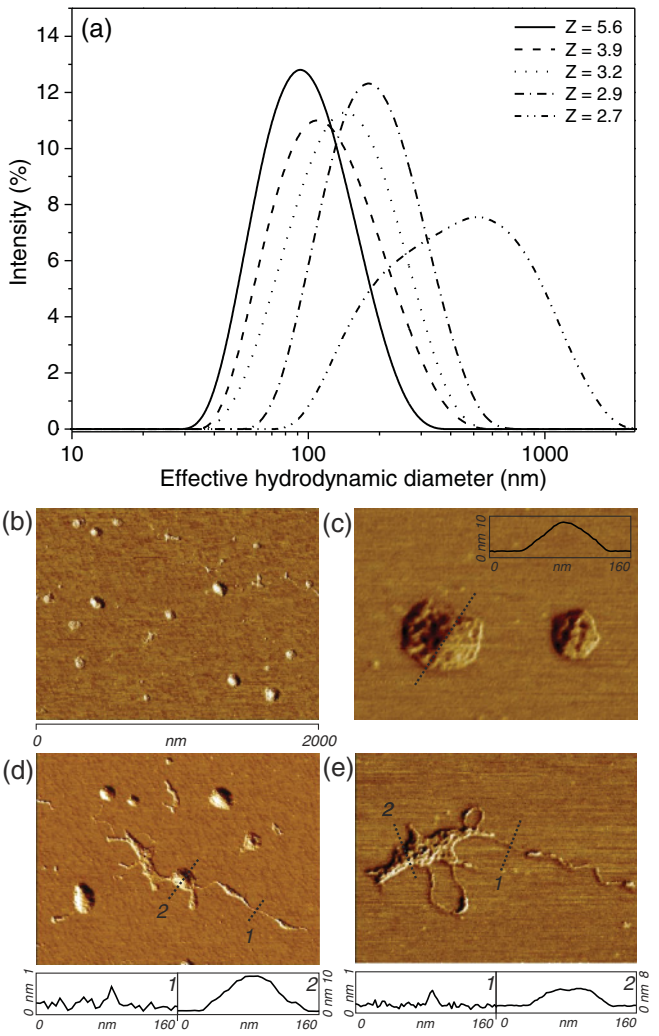

FIG. 5. (Color online) (a) Intensity distribution of the effective hydrodynamic diameter of aggregates of azo-surfactant–DNA complexes at different Z ratios determined with dynamic light scattering. With the increasing of surfactant concentration, the average value of the effective hydrodynamic diameter decreases, approaching 90 nm for $Z = 5.6$. (b)–(e) AFM micrographs of azo-surfactant–DNA complexes at $Z = 5$, spin coated on mica.

One can see that for large Z values, the DNA complexes are colloidally stable with an average size of about 90 nm. With a decrease of Z, the globules start to aggregate. For $Z = 2.7$, the size of the aggregates reaches 1 μm and is followed by precipitation for smaller Z.

The presence of azo-surfactant–DNA globules was also proved with atomic force microscopy (AFM) measurements [Figs. 5(b)–5(e)]. Figures 5(b) and 5(c) show an ensemble of DNA globules on mica. The height of the objects is 7 ± 2 nm, and the diameter is 180 ± 40 nm. Note that the actual size distribution is not Gaussian, but bears small tails, evidenced by some objects in the AFM micrograph much smaller than sizes close to the average. For the following discussion, we neglect this contribution. The objects have a "pancake" shape, probably resulting from a deformation of soft globules due to strong adsorption forces. In Fig. 5(c), one sees a magnified image of two globules. Some globules were

partially decompacted containing a bare DNA of about 1 nm in height connected to compacted regions [Figs. 5(d) and 5(e)]. The presence of decompacted globules can be explained by the interaction of globules with a negatively charged mica surface. The mica surface acts in a similar way to an anionic surfactant, which is a well-known decompacting agent [4].

Estimating the volume of the globules observed by AFM using an ellipsoid model reveals that this volume is similar to the globular volume determined by dynamic light scattering (DLS). By knowing the molecular weight of the azo-surfactant and assuming the density of the globular to be 1 g cm^{-3}, one can estimate the Z value of the globule. This rough estimate yields a value of $Z = 5.5$, which is close to the value in the solution.

All colloidally stable complexes were irradiated with UV light to induce *trans-cis* isomerization of the surfactant. After each irradiation step, a UV-visible spectrum was collected. During irradiation, typical *trans-cis* isomerization changes in the UV-visible spectra up to some irradiation dose were observed [55 seconds in Fig. 6(a)]. Starting from this point, the next irradiation step resulted in a pronounced increase of the scattering and precipitation observed even visually. Further irradiation after the precipitation onset led to a decrease of scattering and absorbance; this can be explained by the formation of large aggregates, which settle on the bottom of a cuvette and do not scatter and absorb light. This behavior can be tracked by a decrease of the peak at 245 nm (absorption of benzene rings in the azobenzene unit of the surfactant + absorption of DNA), which is insensitive to the isomerization process.

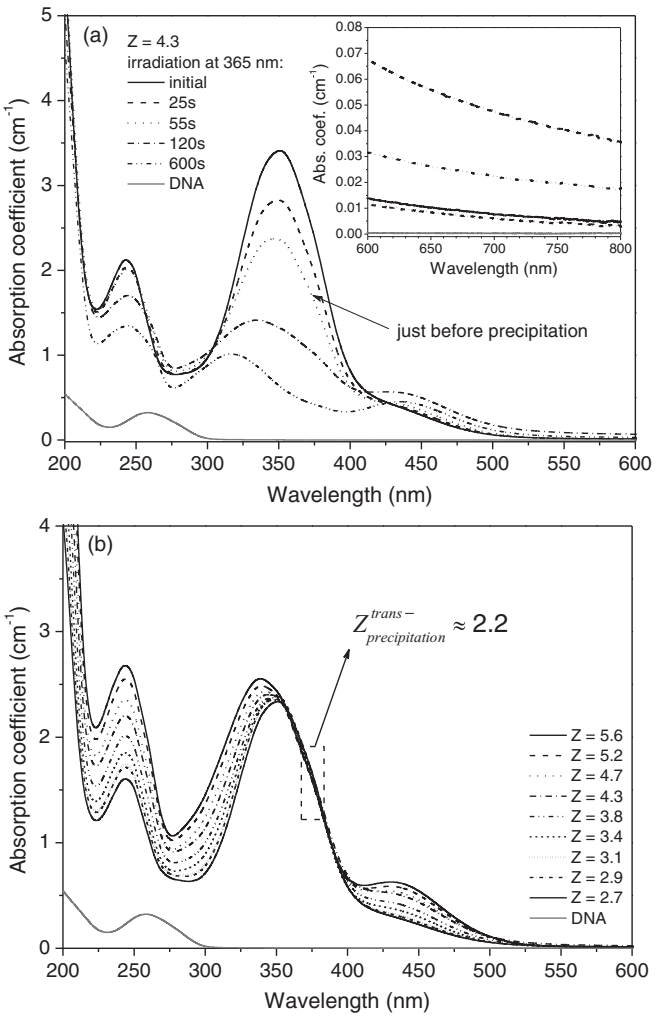

FIG. 6. (a) Changes in UV-visible spectra of C_4-Azo-OC_6TMAB –DNA complex ($Z = 4.3$) during UV irradiation. Inset: The corresponding changes of light scattering in the long-wavelength range. (b) Spectra of the complexes for $Z > 2.4$, irradiated with UV light measured just before the onset of precipitation.

The irradiation time before the precipitation increases with Z. This means that more surfactant molecules have to be converted to the *cis* state to reach the precipitation point. To understand this behavior, the spectra of all samples with $Z > 2.4$ just before the onset of precipitation were plotted together [Fig. 6(b)]. It is clearly seen that the absorption coefficient near 376 nm is the same for all spectra. As mentioned above, the absorption at this wavelength corresponds to the *trans* isomer. The extinction coefficient at 376 nm was used to assess the concentration of *trans* isomers of the surfactant, which is about 1.1×10^{-4} M. Division by the DNA concentration gave the corresponding charge ratio $Z^{trans-}_{precipitation} \approx 2.2$ between the *trans* isomer and DNA. The fact that this value is the same for all charge ratios indicates that only the *trans* isomer of the surfactant is responsible for the colloidal stability of globules. The precipitation will take place if the charge ratio of *trans* isomers to DNA is smaller than $Z^{trans-}_{precipitation}$. This is very close to the value $Z = 2.4$ found in the experiments without a sample irradiation (see above). The difference can be explained with kinetic reasons because the system is metastable in this range. One can see from the inset in Fig. 3 that on the next day after mixing, the precipitation took place at $Z \approx 2.2$, which is the edge of instability. With time, this value shifted to 2.4. Because the spectra were collected within several minutes after each irradiation step, which reduces the amount of the *trans* isomer, we were able to reach smaller Z ratios for the *trans* isomer.

Region (ii). $1.2 < Z < 2.4$: Irradiation of these complexes with UV light (*trans-cis* isomerization) led to dissolution of the precipitate. The process depended on the irradiation light intensity and on Z. For $Z = 1.3$, the complex was completely dissolved within an hour. With an increasing value of Z, the dissolution time also increased. After irradiation with visible light, the precipitate reappeared again. The process of precipitate formation is thus completely reversible [12].

We have noticed that during visible light irradiation of the complexes dissolved from the precipitate, the irradiation dose needed to trigger the precipitation decreased with increasing Z. This behavior was similar to that observed for colloidally stable systems ($Z > 2.4$) under *trans-cis* isomerization. To investigate this effect in detail, solutions of C_4-Azo-OC_6TMAB were irradiated with UV light prior to mixing with DNA, and the resulting mixtures were kept under exposure before irradiation with visible light. An example of how the UV-visible spectra of the surfactant change during *cis-trans* isomerization is shown in Fig. 7(a). One observes the typical behavior of the surfactant until the onset of precipitation. After that point, the system starts to scatter light and the absorption of the DNA and the surfactant drops. Although the behavior was very similar to

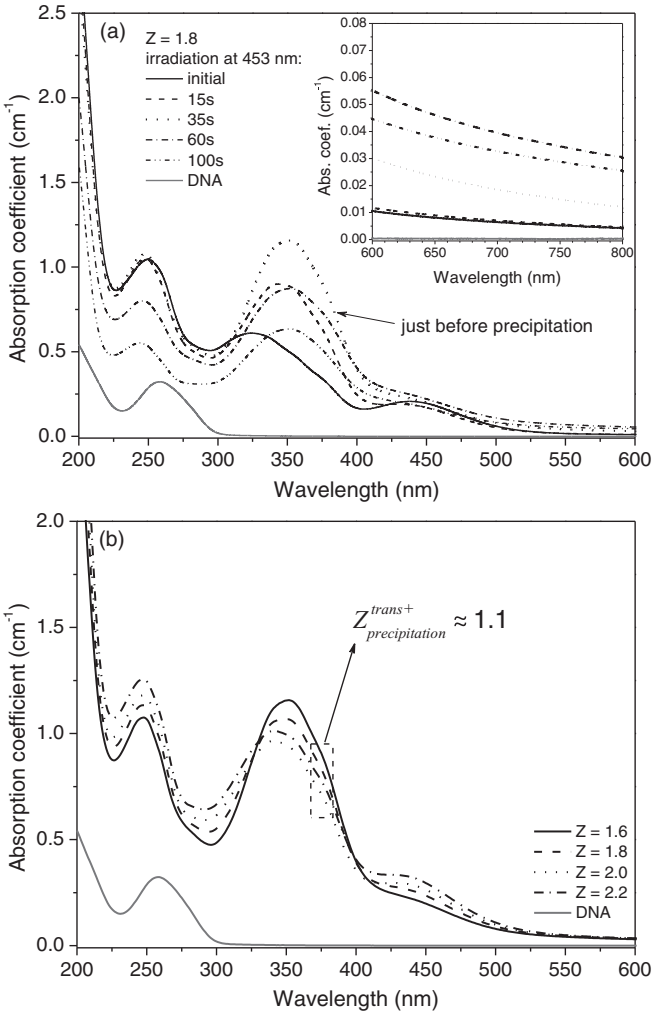

FIG. 7. (a) Changes in UV-visible spectra of the C_4-Azo-OC_6TMAB–DNA complex ($Z = 1.8$) in response to the *cis-trans* isomerization of azobenzene (visible irradiation). Inset: The corresponding changes of light scattering in the long-wavelength region. (b) Spectra of the complexes for $1.2 < Z < 2.4$ irradiated with visible light, measured just before the onset of precipitation.

that for the colloidally stable systems [cf. Figs. 7(a) and 6(a)], its interpretation is completely different. The main differences are that the initial systems contained extended DNA coils and the amount of *trans* isomer increased with irradiation.

We assigned the onset of precipitation to the increase in scattering. This was not as easy as for systems with $Z > 2.4$. The reasoning is that before precipitation, compaction of DNA should take place, leading to scattering in solution. Moreover, the *cis* isomer also changes the DNA conformation and probably takes part in the compaction because its concentration is larger than CAC_{cis}. One could also follow the onset of precipitation by tracking the extinction coefficient, that is, the decrease of peak height at 245 nm (absorption of benzene rings within the azobenzene units of surfactant + DNA absorption), which is insensitive to isomerization. However, the decrease of this peak was compensated for by an increase of scattering during the first stages of precipitation. All these factors hindered the exact determination of the point of precipitation onset. Nevertheless, we could quantitatively analyze the spectra just before the onset of precipitation, as shown in Fig. 7(b). The average value of the absorption coefficient at 376 nm was used to calculate the concentration of *trans* isomers just before precipitation, $5.5 \pm 0.5 \times 10^{-5}$ M, corresponding to $Z^{trans+}_{precipitation} \approx 1.1 \pm 0.1$. This value is very close to the ratio of $Z = 1.2$, signifying the onset of precipitation in the experiments without irradiation (see above). This result demonstrates that the compaction of DNA and further aggregation of the globules are triggered predominantly by *trans* isomers of the surfactant.

Based on our results, we suggest a model of complex formation for different values of Z and a fixed concentration of DNA (DNA $= 5 \times 10^{-5}$ M); see Fig. 8. For $Z < 1.2$, the surfactant in both the *trans* and *cis* states binds to DNA. The DNA compaction process is promoted by the *trans* isomer starting from some critical charge ratio $Z^{trans+}_{precipitation} \approx 1.1 \pm 0.1$. In the compacted state ($1.2 < Z < 2.4$), the surfactant seems to be forming a hydrophobic shell around the DNA globule. Such systems are colloidally unstable and start aggregating and finally precipitate from the solution. With an increase of the surfactant ratio exceeding a critical value of $Z > 2.4$,

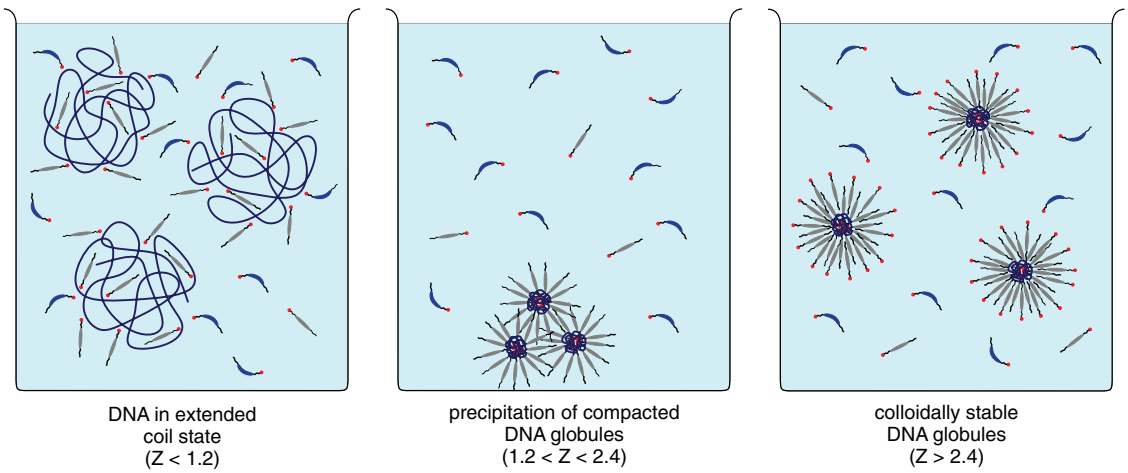

FIG. 8. (Color online) Model of DNA conformations for different mixing charge ratios Z with the surfactant C_4-Azo-OC_6TMAB containing azobenzene. The concentration of DNA is 5×10^{-5} M (of nucleotides).

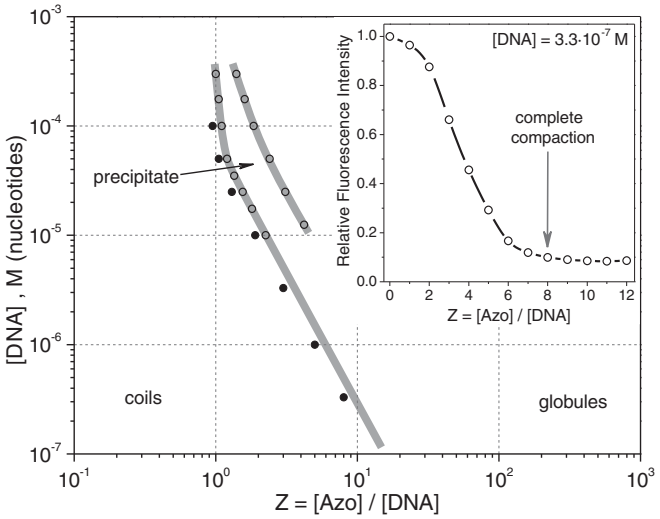

FIG. 9. Phase diagram of the C_4-Azo-OC_6TMAB–DNA complex. Open circles: visual observations and UV-visible spectroscopic measurements (example in Fig. 3); closed circles: fluorescence measurements. The inset shows an example of the relative change of the integrated fluorescence intensity of ethidium bromide on the addition of the surfactant (EtBr: DNA = 1:10, λ_{ex} = 546 nm).

the residual amount of surfactant in the *trans* configuration, which does not take part in the DNA compaction, starts to form a second layer around the globules, compensating for the hydrophobic effect. With a further increase of Z, the size of the aggregates decreases.

C. Phase diagram

In the above investigations, the concentration of DNA was kept at 5×10^{-5} M (of nucleotides). This choice was motivated by obtaining good signals in the UV-visible spectra. In practical applications of nonviral vectors, DNA concentrations are usually difficult to control. Therefore, the knowledge of DNA conformation at different concentrations and mixing ratios with a compacting agent is of crucial importance.

Several series of samples with different DNA concentrations and values of Z were prepared to visually and spectroscopically detect at which concentrations the precipitate forms. The results are presented as a phase diagram in Fig. 9. The precision of identifying the points of precipitate formation is about 10%. Above a DNA concentration of about 10^{-5} M, there is a range of charge ratios where the precipitate forms. The corresponding values of Z for the precipitation onset and the formation of colloidally stable systems increase with a decrease of DNA concentration. For a DNA concentration below 10^{-5} M, no formation of a precipitate was observed. To determine points of the DNA compaction below the critical DNA concentration, the fluorescence of ethidium bromide (EtBr) was used. It is well known that the fluorescence of EtBr significantly increases in the presence of DNA because of its intercalation between DNA base pairs [22]. The addition of a cationic surfactant leads to a decrease of the fluorescence due to competition for electrostatic binding sites on DNA and/or proton transfer with the solvent [24]. An example of changes in the relative fluorescence intensity is shown in the inset of Fig. 9. The point of complete DNA compaction was assigned to the value of Z where the decrease of fluorescence reached its minimum (plateau). The obtained transition ratios Z versus the corresponding DNA concentration are shown in Fig. 9 as closed circles. Values of Z determined in this way are about 10% smaller because of the influence of ethidium bromide on the conformation of the DNA molecule.

There is one important feature to note. The line of the transition from the compacted to extended coil DNA state should not be taken as exact. It corresponds to the complete DNA compaction, whereas the first compacted globules could be detected already at lower values of Z [15,16]. The range of partially compacted states might depend on the DNA concentration. Therefore, the line represents the transition between partially and completely compacted DNA molecules.

IV. CONCLUSIONS

In this study, the interaction of cationic surfactant containing azobenzene with DNA molecules was investigated by analyzing the changes in the UV-visible spectra of the surfactant under photoisomerization. The results of this analysis, supplemented with dynamic light scattering, atomic force microscopy, and fluorescence spectroscopy measurements, revealed the dominant role of the *trans* isomer of the surfactant in the DNA compaction process and the colloidal stability of globules. The addition of the surfactant in the *trans* configuration to DNA above some charge ratio (e.g., Z = surfactant/DNA = 1.2 for DNA = 5×10^{-5} M) promotes DNA compaction. The critical value of Z for complete DNA compaction increases with the decrease of DNA concentration.

There is a critical DNA concentration ($\sim 10^{-5}$ M) above which compacted globules precipitate from the solution. To stabilize the compacted globules, one should add more surfactant to compensate for the hydrophobic shell created around the DNA by the surfactant. Above some critical ratio (e.g., $Z = 2.4$ for DNA = 5×10^{-5} M), the system becomes colloidally stable and the size of the aggregates decreases with increasing values of Z. The critical value of Z for colloidal stability increases with a decreasing DNA concentration and should consider only the *trans* isomer, which is responsible for the stability. The system with DNA $> 10^{-5}$ M cannot be transferred from the water-suspended globular state to the extended DNA coil state with optical stimuli without passing the precipitation. To avoid precipitation, one should work with DNA concentrations below the critical value ($\sim 10^{-5}$ M).

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