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Electron Tomography Shows Molecular Anchoring Within a Layer-by-Layer Film

ARTICLE *in* JOURNAL OF THE AMERICAN CHEMICAL SOCIETY · SEPTEMBER 2008

Impact Factor: 12.11 · DOI: 10.1021/ja804930d · Source: PubMed

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Insights in the Organization of DNA–Surfactant Monolayers Using Cryo-Electron Tomography

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The organization and complexation of DNA has been a subject of intensive research, not only to gain insight into biological processes¹ but also to understand its behavior in biomedical applications.^{2,3} In particular, the behavior of DNA in the presence of cationic surfactants is of interest to understand phenomena involved in gene transfection,² DNA sensing,³ and more recently the layer-by-layer synthesis of DNA-based biomaterial coatings.⁴ In addition to the DNA–surfactant complexes studied in the bulk phase,⁵ Langmuir monolayers are frequently used to obtain information about DNA–surfactant interactions.⁶ The model for such a system generally involves nucleic acid molecules that are orderly bound via electrostatic interactions to a closely packed monolayer of cationic surfactants. Several techniques, such as Brewster angle microscopy (BAM),⁷ in situ grazing incidence X-ray diffraction, and infrared reflection absorption spectroscopy (IR-RAS), have been applied to obtain information about the organization of these monolayer systems; however, from these studies, no generalizing conclusions could be drawn.⁸ Whereas Möwald et al. concluded that DNA becomes aligned due to the compression of the complex, Okahata showed that the presence of an intercalating dye was required for the ordering of DNA under a surfactant monolayer.⁹ Significantly, Yamaoka et al. demonstrated that the complex present at the air–water interface changes its structure upon deposition to a solid substrate.

Here we present the first direct 3D in situ imaging of DNA molecules bound to a monolayer of a bisurea-stabilized surfactant (**1**) using cryo-electron tomography. It is demonstrated that, for the present system, individual DNA strands do not organize in an orderly fashion at the monolayer surface, but bind only partially with a part of the chain extending down into the subphase.

Surface pressure versus surface area (Π – A) isotherms were recorded for the bisureido-based surfactant **1**¹⁰ spread on a PBS (pH 7.4) subphase (Figure 1). The isotherm was dominated by a liquid condensed state as was evidenced by a steep increase in surface pressure upon compressing the layer below 25 Å²/molecule. By extrapolation of the slope of the curve to zero pressure, a limiting molecular area of 22 Å²/molecule was deduced (Figure 1). These results are indicative for the preorganization of the surfactant dictated by the formation of strong intermolecular hydrogen bonds already at low degrees of compression (see Supporting Information).

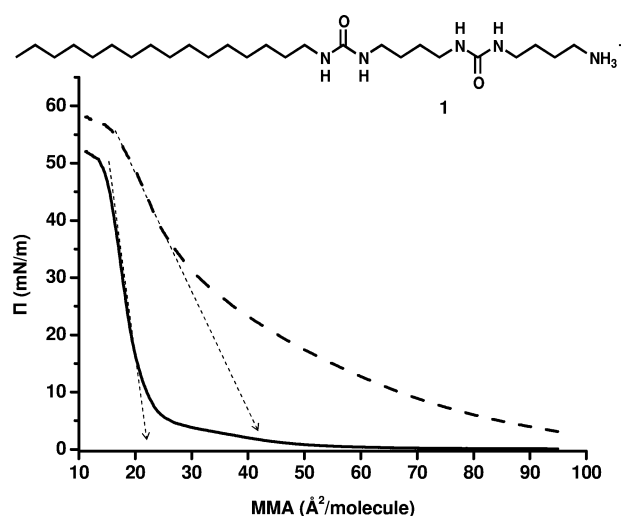


Figure 1. Top: The molecular structure of surfactant **1**. Bottom: surface pressure versus surface area isotherms (Π – A) of bisurea surfactant spread and compressed on a PBS buffer (—) and on 3 mg/mL DNA/PBS subphase (---). The arrows indicate the extrapolated mean molecular area (MMA) at zero pressure.

The Π – A isotherm of a cationic surfactant monolayer is known to change significantly when it is compressed on a DNA-containing subphase.¹¹ When **1** was spread and compressed on a buffered DNA-containing subphase ([DNA] = 3 mg/mL), an increase in the surface pressure was indeed observed, indicating a liquid expanded phase from ~100 to 30 Å²/molecule (Figure 1). Although the DNA alone also displayed some surface activity, summation of the curves recorded with pure **1** and pure DNA, respectively, demonstrated that this fact cannot account for the observed changes in the isotherms (see Supporting Information). This suggests that the presence of DNA in the subphase leads to the formation of complexes that no longer allow for a close packing of the surfactant molecules despite the strong H-bonding capacity of the bisurea units. In contrast, when DNA was injected under a preformed monolayer of **1** kept at a constant pressure of 35 mN/m, no expansion of the monolayer was observed. This suggests that, once the monolayer is formed, the strong hydrogen bonds of the bisurea units prevent penetration of the DNA in between the surfactant molecules. Cryo-TEM images of a vitrified sample of a 3 mg/mL DNA solution ([DNA] in the subphase) without an applied monolayer clearly showed individual DNA chains (Figure 2A).

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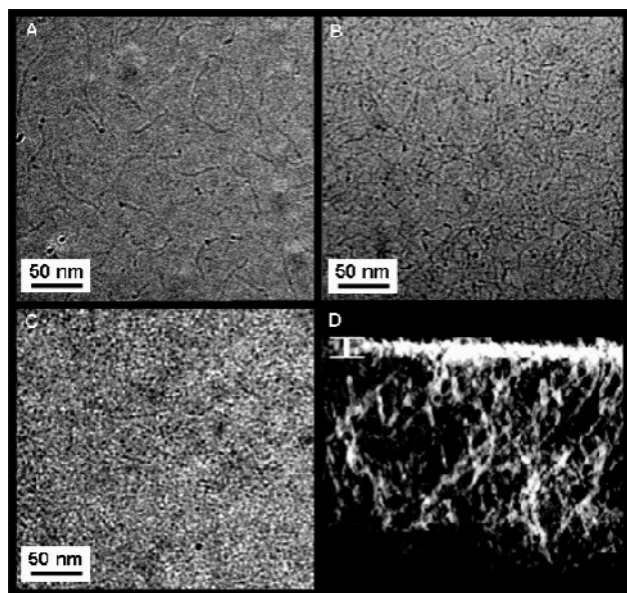


Figure 2. Cryo-TEM images of (A) a 3 mg/mL DNA solution, (B) a DNA-surfactant monolayer with surfactant molecules spread on a DNA subphase, (C) same as B with DNA injected underneath the preformed monolayer. (D) Projection in the xz plane of the 3-D reconstructed volume of the surfactant-DNA complex shown in C; white bar represents 10 nm.

To directly image the DNA-surfactant monolayers¹² using cryo-TEM, a self-organized monolayer of **1** was formed on top of a DNA-containing subphase in a glass dish, with a density corresponding to a surface pressure of ~ 35 mN/m. A TEM grid coated with a holey carbon layer was placed underneath the monolayer surface prior to spreading. The dish was situated inside a humidity- and temperature-controlled glovebox and placed underneath a fully automated vitrification robot to ensure 100% humidity throughout the complete experiment. Following spreading and equilibration, the subphase was drained to lower the monolayer onto the grid. After subsequent transfer of the grid to the vitrification robot, the sample was blotted and vitrified by plunging into melting ethane. Cryo-electron microscopy was used to evaluate the samples under low electron dose conditions. Since most of the solution is removed from the grid during the blotting process, the co-localization of DNA and surfactant at the air-water interface can be assessed from these images. The absence of an obvious concentration of DNA molecules supports the formation of a mixed DNA-surfactant phase, rather than a compact monolayer with a dense ordered packing of DNA chains underneath; individual DNA chains could still be distinguished (Figure 2B). However, when a monolayer of surfactant **1** was prepared prior to the injection of DNA under this layer, indeed a higher concentration of the nucleic acid molecules was observed in the thin vitrified films. Cryo-TEM micrographs now showed a remarkably dense coverage of DNA chains, indicating that indeed a DNA-surfactant monolayer is formed using this method (Figure 2C).

Although these projection images clearly show a high concentration of DNA in the upper layer of the solution, these do not show whether the macromolecules are actually bound to the monolayer

at the air-water interface. Using low-dose cryo-electron tomography, a tilt series of 86 images from -70 to $+70^\circ$ was recorded for the injected DNA-surfactant monolayer and subsequently reconstructed to a 3-D volume which clearly shows a ~ 10 nm thin concentrated layer at the air-water interface representing the DNA-surfactant monolayer. Surprisingly, it also showed with unprecedented detail that individual DNA strands do not bind completely to the monolayer surface in an orderly fashion, but are partially suspended from the surface extending down into the subphase (Figure 2D).

In summary, we have shown that in the present system the formation of a self-assembling surfactant monolayer in the presence of DNA leads to a disordered mixed phase complex rather than to the assembly of DNA under an organized monolayer. It is reasonable to assume that also in most other systems the formation of a continuous monolayer is inhibited in a similar manner by the complexation with DNA as most surfactants have a lower self-organizing capacity than **1**.

Acknowledgment. This work was supported by The Technology Foundation STW (NKG 5758). We thank the Spinoza Junior Researchers Institute for providing **1**, and E. W. Meijer for stimulating discussions.

Supporting Information Available: Experimental details and movie of the reconstructed volume. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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JA0736515