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Properties and Applications of Novel DNA-Based Surfactants

Vesselin N. Paunov*, Chun Xu, Pietro Taylor, Mustafa Ersoz and Paul D.I. Fletcher
Department of Chemistry, University of Hull, Hull, HU6 7RX, United Kingdom.

ABSTRACT

We have designed a novel class of functional surfactants based on DNA and have explored their properties in relation to several possible applications. The DNA-surfactants consist of short chain DNA oligonucleotides covalently bound to a large hydrophobic group, which makes the DNA molecules amphiphilic. We demonstrate that these materials behave like common detergents and are surface-active at various fluid surfaces, e.g. air-water, oil-water interfaces, and lipid bilayers. We also show that once adsorbed the DNA-surfactants remain on the liquid surface upon hybridisation with a complementary DNA chain. We use complementary DNA-surfactants to functionalise fluid surfaces and to program the interactions between them based on Watson-Crick pairing. By selecting the appropriate DNA base sequences, the interaction between the fluid surfaces functionalised with DNA-surfactants can be programmed with a very high specificity. We have also developed a novel procedure for micro-patterning of solid surfaces with DNA by a microcontact printing with aqueous inks of DNA-surfactants which can be utilised for rapid fabrication of DNA assays and genetic biochips.

INTRODUCTION

We designed and used a new class of DNA-based surfactants,[1] produced by covalent conjugation of a hydrophobic group to one of the ends of a short-chain DNA-oligonucleotide (Fig. 1A). This makes the modified DNA molecules amphiphilic and allows DNA chains to be adsorbed and orientated at fluid surfaces, including the air-water and oil-water interfaces, lipid bilayers (Fig. 1A) and also at the interface between water and a hydrophobic solid.[1] Modified oligonucleotides containing hydrophobic ends-group have been synthesized previously,[2-9] but have been looked only as "anti-sense" oligonucleotides, where the hydrophobic group may facilitate their penetration through cellular membranes[2,3,7] and may serve to stabilise either duplex or triplex strand formation.[4,5,9] Here we focus our attention on the possibility to use DNA-surfactants to functionalise fluid surfaces with DNA chains (Fig. 1B). We explore the surfactant properties of these materials at the air-water and oil-water interfaces and address the question related to the stability of their attachment at the liquid interface upon hybridisation with complementary DNA chains. We also use DNA-surfactants adsorbed at liquid surfaces to program the interactions between them based on complementary DNA-base sequences and Watson-Crick pairing (Fig. 1B). For successful hybridisation, the adsorbed DNA-surfactants should have an anti-parallel orientation which is realised only if they are adsorbed at opposite fluid interfaces. Similar strategy has been already used by Mirkin et al.[10-13] for DNA-functionalized solid particles. Recently, we have fabricated DNA-recognising liposomes by adsorption of a DNA-surfactant within the liposome bilayer[14] and have use then as inks to

*Corresponding author. Email: V.N.Paunov@hull.ac.uk , Phone +44 1482 465660, Fax: +44 1482 466410.

fabricate DNA-micropatterns on solid substrates by microcontact printing. We also demonstrate the specific Watson-Crick interaction between complementary DNA-surfactants by deposition of DNA-recognising liposomes on DNA-micropatterned solid surfaces obtained by a microcontact printing technique.[14] We also use aqueous DNA-surfactant solutions[1] as inks for micropatterning of suitable solid substrates with DNA by using similar microcontact printing technique.

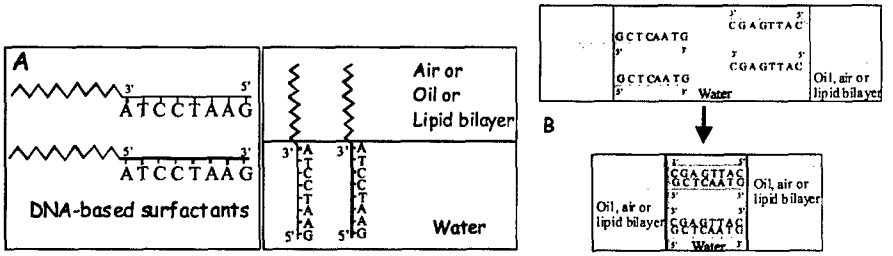

Figure 1: Schematic representation of the idea for the preparation of DNA-based surfactants and their adsorption properties. (A) DNA-surfactants are conjugates of a short-chain oligonucleotide, covalently bond to a hydrophobic group at its 3'- or 5'-end. The DNA-based surfactant molecules can adsorb at various liquid surfaces and the DNA chain orientates with respect to the liquid interface. Watson-Crick interactions between DNA-surfactant adsorbed at liquid surfaces or lipid bilayers can lead to specific programmable interactions between such surfaces.

MATERIALS

In this study we present results with several examples of DNA-surfactants produced by conjugation of a hydrophobic groups to one of the ends (3' or 5') of 10-12 based single strand oligonucleotides The hydrophobic groups used include: (i) Cholestyeryl-group (e.g. Cholesteryl-5'-TTTTTTCCCCCC-3') and (ii) from 2 to 4 subsequent dodecyl groups (dodecyl spacers), $(Dodecyl)_2$-5'-GGGGGGAAAAAA-3', $(Dodecyl)_3$-5'CAGTGACTGGCC-3' and $(Dodecyl)_4$-5'-GGGTTGGGAATT-3'. Using different number of dodecyl spacers allows control over the surface activity of the produced DNA-surfactants.

RESULTS AND DISCUSSION

a) Surfactant properties. We have used Krüss drop shape analysis tensiometry to demonstrate that the cholesteryl-DNA is surface active and reduces the interfacial tension of the dodecane-water interface (Fig. 2A). We also studied the surface pressure vs area curves of various DNA-surfactants spread at the air-water interface in a Langmuir through where they demonstrate a typical hysteresis in the surface pressure upon compression and expansion (Fig. 2B). Fig. 2C shows the decane-water interfacial tension isotherm for the palindromic DNA-surfactant Cholesteryl-5'-AAAAAATTTTTT-3'. The critical micellization concentration (CMC) of the same Cholesteryl-DNA was determined by tensiometric measurements to be about 6 μM without the presence of a background electrolyte. We discovered that for palindromic DNA-surfactants there is an interesting interplay between DNA-DNA hybridization and micellization as the DNA melting point overlaps with the CMC (Fig. 2C). Further research is in progress to determine

wherther the sharp drop in the interfacial tension at 6 μM DNA-surfactant is only due to micelle formation or hybridization of palindromic DNA-surfactants in bulk of the solution which produces dimers of higher surface activity than the monomers.

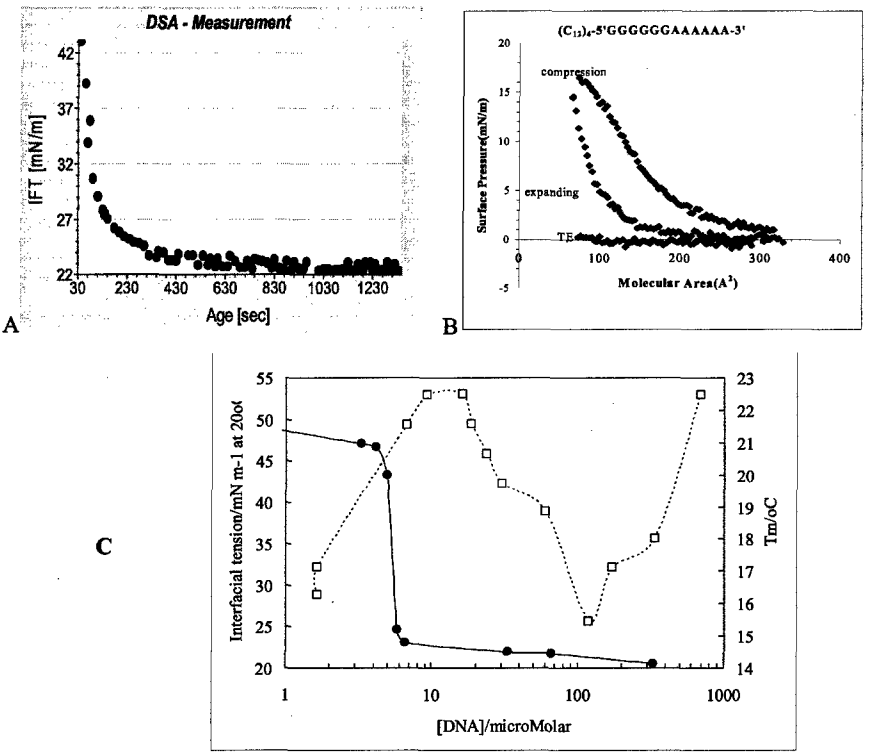

Figure 2: (A) Dodecane-water interfacial tension vs time for 65.8 μM Cholesteryl-5'-AAAAATTTTT-3' at 20 ^{0}C. (B) Surface pressure vs area curve at the air-water interface for $(C_{12})_4$-5'-GGGGGGAAAAAA-3' at 20 ^{0}C (solid symbols). In this case 25 μL of 148 μM DNA-surfactant in 50:50 water-IPA were spread in the Langmuir through and compressed immediately. Compressing and expanding rate were 50 cm^2/min. (C) Interfacial tension isotherm of Cholesteryl-5'-AAAAAATTTTTT-3' at the decane-water interface at 20 ^{0}C. The RHS axis gives the melting point of the palindromic DNA-surfactant in bulk of the solution (open square symbols). The lines are guides to the eye.

b) hybridization at a liquid interface. To test whether the DNA-surfactant molecules remain adsorbed at the liquid interface when hybridization with a complementary DNA chain takes place we conducted the following experiment. We exposed a hexadecane drop to a solution of Cholesteryl-DNA and then to a solution of fluorescently tagged complementary DNA. The drops were imaged with a confocal fluorescence microscopy to check the presence of a fluorescent

signal from the surface of the drop. The image in Fig. 3A shows the case of complementary DNA sequences of the DNA-surfactant adsorbed on the hexadecane drop surface and the TAMRA-DNA. The image in Fig. 3A is taken by confocal fluorescence microscopy. We observe a fluorescence signal at the drop interface which indicates a specific adsorption of TAMRA-DNA hybridized with the complementary cholesteryl-DNA. We also conducted the control experiment where the drops treated with same cholesteryl DNA were exposed to a solution of non-complementary TAMRA-DNA. The confocal image in Fig. 3B shows that there is no fluorescence from the drop surface at the same conditions, which is a result of the lack of complementarity between the two DNA chains. This result indicates that the adsorbed DNA-surfactant remains at the oil-water interface even after hybridization with complementary DNA at the same liquid interface.

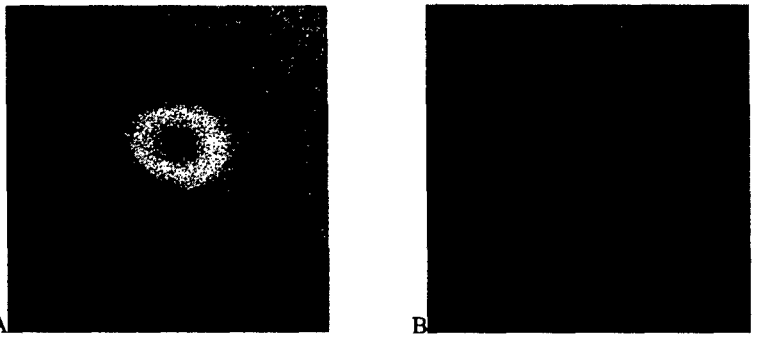

Figure 3: (A) A confocal fluorescence microscope image of hexadecane drops deposited on a glass slide under water and treated (i) with 66 μM solution of Cholesteryl-5'-GGGGGGAAAAAA-3' followed by (ii) 85 μM solution of complementary TAMRA-5'-TTTTTTCCCCCC-3'. The image shows the fluorescence signal from TAMRA-DNA localized at the drop surface. Here the confocal plane is set close to the oil-glass boundary. (B) The control experiment: a confocal fluorescence microscope image of hexadecane drops deposited on a glass slide under water and treated (i) with a 66 μM solution of Cholesteryl-5'-GGGGGGAAAAAA-3' followed by (ii) 85 μM solution of a non-complementary oilgonucleotide TAMRA-5'-GGCCAGTCACTG-3'. No fluorescence signal from TAMRA-DNA is detected at the drop surface.

c) micropatterning of solid surfaces with DNA-surfactants. We have demonstrated that DNA-surfactants can also adsorb on solid surfaces of suitable hydrophobicity which allowed us to develop a new way of attaching DNA-chains to solid substrates. We have used microcontact printing with DNA-surfactant "inks" spread on hydrophilized PDMS stamps to produce patterns of DNA-surfactant on solid surfaces. Using an alternative technique for delivering the DNA-surfactant ink to the solid surface can also be used to create DNA-microarrays (see the LHS image of Fig. 4 for details). The RHS part of Fig. 4 shows typical images of our PDMA stamps produced by molding laser ablated glass masters or photoresist masters with 10μm x 10μm and 50μm x 50 μm squares, respectively. They were successfully hydrophilized by surface oxidizing with piranha solution treatment.[1,14] Our experiments showed that the microcontact printing

technique works successfully with DNA-surfactant inks only for solid substrates of intermediate hydrophobicity. If the solid surface is too hydrophilic, the ink just spreads and creates no pattern, and there is no adsorption and retention of DNA-surfactant on the surface. If the substrate is too hydrophobic (e.g. like polystyrene slide), the ink on the pattern retracts and forms microdrops. We found that optimal result is obtained with a moderately hydrophilic microscope cover slip of contact angle about 50^0, where the pattern remains, but produces the well known coffee ring effect.

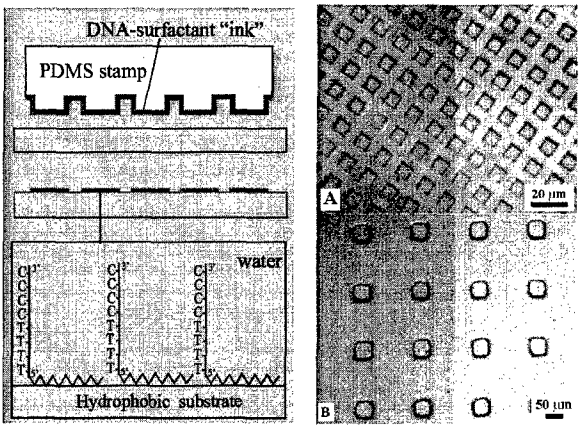

Figure 4 LHS image: schematics of our method for micropatterning with DNA-surfactants. (A) the hydrophilized PDMS stamp is inked with a solution of DNA surfactant (ink). (B) the DNA-surfactant ink is printed on the surface of the solid substrate. (C) The DNA-surfactant adsorbs to the solid surface like an ordinary detergent. RHS: (A) and (B) optical images of the used hydrophilized PDMS stamps for μCP.

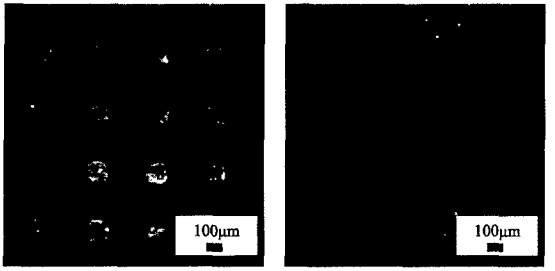

Figure 5. Fluorescence microscope images of $(Dodecyl)_3$-5'CAGTGACTGGCC-3' aqueous solution microcontact-printed on a glass cover slip, blocked with BSA and exposed to:
(LHS image) complementary TAMRA-5'- GGCCAGTCACTG-3'
(RHS image) non-complementary TAMRA-5'-GAATCCTACC-3'.

We have microcontact-printed with aqueous solutions of DNA-surfactants with hydrophilized PDMS stamps inked on such substrates and have successfully created DNA patterns which are visible in fluorescence mode after exposure to complementary TAMRA-DNA but show no fluorescence signal when exposed to a non-complementary TAMRA-DNA (see Fig.5) at the same conditions.

CONCLUSIONS

We have designed and prepared DNA-surfactants by conjugating a hydrophobic group to a short-chain oligonucleotide. We show that DNA-surfactants can adsorb at air-water and oil-water interfaces as well as at solid surfaces and can hybridize with complementary DNA when adsorbed at fluid surfaces. We have developed a new method for preparation of DNA microarrays by microcontact printing with DNA-surfactants on solid substrates.

ACKNOWLEDGEMENTS

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