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# Cationic surfactant mediated hybridization and hydrophobization of DNA molecules at the liquid/liquid interface and their phase transfer

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**Hybridization of complementary oligonucleotides mediated by a cationic surfactant at the water/hexane interface leads to hydrophobic, double-helical DNA which may be readily phase transferred to the organic phase and cast into thin films on solid substrates.**

The development of synthetic, virus-like DNA vectors is a topical problem of considerable interest. Following the report of Felgner *et al.*[1] demonstrating that DNA pre-complexed with a cationic surfactant leads to its enhanced uptake by eucaryotic cells, DNA–cationic liposome complexes are being investigated in great detail as non-viral DNA vectors.[2]

Many studies have dealt with DNA–surfactant complexes in an aqueous environment.[3] In aqueous solutions containing equimolar amounts of DNA and the cationic surfactant, the complex formed was water-insoluble but soluble in low-polarity organic solvents.[3a,d] Reimer *et al.*[4] have shown that DNA molecules may be hydrophobized by complexation with cationic surfactants in a Bligh and Dyer monophase[5] and thereafter transferred into the organic phase by partitioning the monophase. We describe herein a simple method for the hybridization of complementary oligonucleotides employing electrostatic complexation with a cationic lipid, octadecylamine (ODA), at the water/hexane interface followed by phase transfer of duplex DNA into the non-polar organic environment. Furthermore, films of the DNA–ODA complexes may be easily deposited on solid surfaces by solvent evaporation. To the best of our knowledge, this is the first report on the surfactant-mediated hybridization of complementary oligonucleotides at the interface between two liquids.

Oligonucleotides of the sequences GGAAAAAACTTCGTGC (ssDNA1), GCAC-GAAGTTTTTTCC (ssDNA2) and AGAAGAAGAAAAGAA (ssDNA3) were synthesized as described elsewhere.[6] ssDNA1 and ssDNA2 are complementary oligonucleotides while ssDNA3 is non-complementary to both ssDNA1 and ssDNA2. In typical experiments, 10 mL of a $10^{-4}$ M solution of ODA (Sigma USA) in hexane was added to: (a) 10 mL of $10^{-6}$ M aqueous solution of ssDNA1 and ssDNA2 taken in an equimolar ratio (experiment **A**); (b) 10 mL of $10^{-6}$ M preformed duplex DNA constituted from complementary pairs ssDNA1 and ssDNA2 in water[7] (experiment **B**) and (c) 10 mL of $10^{-6}$ M aqueous solution of non-complimentary pairs ssDNA1 and ssDNA3 in an equimolar ratio (experiment **C**). The pH of the DNA solutions in all cases was 6.8. The hybridization of the complementary oligonucleotides ssDNA1 and ssDNA2 and the intactness of the duplex structure after phase transfer of the preformed duplex DNA was followed using the fluorescent intercalator, ethidium bromide, added to the aqueous solutions at a concentration of $10^{-5}$ M along with the DNA molecules. Vigorous mixing of the biphasic mixture was carried out at room temperature using a motor-driven overhead stirrer operating at 3000 rpm immersed in the liquid phase for 20 min. During the mixing process, a uniform and milky microemulsion-like phase was observed to form which, within one minute of cessation of stirring, resulted in a rapid separation of two clear layers.

Fig. 1A shows UV-vis spectra recorded from the hexane phase before and after the mixing protocols described in experiments **A** and **B**.[8] There is no indication of the presence of DNA in the organic layer before mixing (Fig. 1A, curve 3) but a strong resonance at 270 nm is induced in the hexane phase by the mixing process in experiments **A** and **B** (curves 2 and 1 respectively, Fig. 1A) and indicates phase transfer of the DNA molecules into hexane.[9] The mixing procedure resulted in the formation of a microemulsion-like phase which is clearly indicative of formation of extremely small droplets of hexane stabilized in the aqueous phase by the ODA molecules. This is likely given that hexane has a finite solubility in water (0.0138 g per 100 mL of water).[10] These droplets would increase the interfacial area between the water and hexane phases facilitating the interaction of DNA with the ODA molecules at the interface. At pH 6.8, the ODA molecules are positively charged ($pK_B$ of ODA = 10.8) and the negatively charged DNA are thus electrostatically bound to the cationic ODA molecules. The DNA molecules are rendered hydrophobic upon complexation with ODA resulting in their phase transfer into hexane. The relative molar ratio of DNA–ODA is important in achieving a minimum critical hydrophobicity to accomplish the phase transfer. Equimolar ratios of DNA–ODA did not result in a detectable phase transfer of the DNA molecules into hexane. In the experiments described herein, a nearly 100-fold molar excess of ODA molecules (over DNA) was taken in the hexane phase.

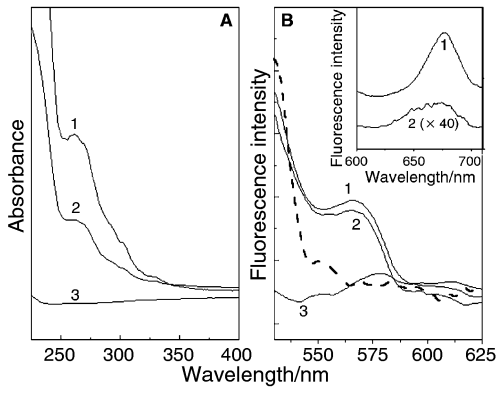


**Fig. 1** (A) UV-vis spectra of the hexane phase before (curve 3) and after shaking biphasic mixtures of $10^{-4}$ M ODA in hexane and aqueous solutions of **A** (curve 2) and **B** (curve 1). (B) Fluorescence spectra of the hexane phase after transfer of DNA from aqueous solutions of experiment **A** (curve 1) and experiment **B** (curve 2). The fluorescence spectrum from the hexane phase in experiment **C** is also shown (curve 3). The dashed curve corresponds to the fluorescence spectrum from a mixture of ssDNA1–ODA and ssDNA2–ODA separately phase-transferred into hexane. The inset shows fluorescence spectra recorded from DNA–ODA complex films cast from the hexane phase on quartz substrates from experiments **A** (curve 1) and **B** (curve 2).

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Fig. 1B shows the fluorescence spectra[11] from the hexane phase after transfer of DNA from aqueous solutions in experiments **A** (curve 1) and **B** (curve 2). A strong fluorescence signal at *ca.* 570 nm is seen in both cases. Ethidium bromide intercalates between the base pairs of DNA double helical structures and this process is detected by enhanced fluorescence.[12] The fluorescence results thus clearly indicate that the preformed double helical DNA molecules are phase transferred into hexane with retention of their double helical structure (curve 2, Fig. 1B). More importantly, the results show that not only have the single-stranded oligonucleotides ssDNA1 and ssDNA2 been transferred to the organic phase by complexation with ODA molecules, *they have hybridized into a duplex* (curve 1, Fig. 1B). No fluorescence was detected in the organic phase after the phase transfer of mixtures of ssDNA1 and ssDNA3 (non-complementary oligonucleotides, experiment **C**) even though the presence of the single-stranded DNA molecules was indicated by UV-vis measurements. To distinguish whether the transfer of DNA occurs in single-strand form followed by duplexation in hexane or directly as a duplex, hexane solutions of ssDNA1 and ssDNA2 separately phase transferred by complexation with ODA were mixed and the fluorescence measured (dashed curve, Fig. 1B). It is observed that there is no fluorescence in this experiment either. Thus, the hybridization inferred by the fluorescence measurements shown in Fig. 1B (curve 2) is clearly due to recognition of the complementary base sequences and occurs only at the hexane/water interface. In the absence of any added salts, the cationic ODA molecules act like counterions and screen the repulsive electrostatic interactions between the individual DNA strands to facilitate the hybridization process. The formation of double helical structures of ssDNA1 and ssDNA2 does not occur in the bulk of the aqueous phase in the absence of salt and these results clearly imply an interfacial process mediated by the cationic lipid molecules.

An exciting aspect of this is the possibility of depositing films of ODA-stabilized DNA by simple solvent evaporation. The fluorescence spectra obtained from films of DNA–ODA complexes cast from hexane solution onto quartz from experiments **A** and **B** are shown in the inset of Fig. 1B as curves 1 and 2 respectively. Strong emission signals from ethidium bromide are observed in both the films with an emission at *ca.* 675 nm. A large red shift in the emission wavelength is seen in the DNA–ODA films relative to the solution wavelength (main part of Fig. 1B). This is likely to be a consequence of the large increase in polarity of the intercalator environment in the film form and has support from the literature.[12] The low intensity fluorescence spectrum from the preformed DNA–ODA (curve 2, inset of Fig. 1B) is perhaps a consequence of non-uniformity of the film during evaporation of hexane and not due to a deterioration of the degree of hybridization of the DNA double helices.

Fig. 2 shows UV-melting curves from DNA–ODA complex films cast onto quartz from experiments **A** (curve 2) and **B** (curve 1). The preformed duplex DNA complexed with ODA shows a single melting transition at 55 °C (curve 1, Fig. 2) which is higher than the aqueous solution melting transition temperature of 41 °C[3b] indicating significant thermal stabilization of the duplex structure by the ODA molecules. The UV–melting transition curve for the DNA hybridized at the hexane/water interface-ODA film shows two $T_M$ values at 39 and 61 °C (curve 2, Fig. 2). Such behaviour has been recently observed by Pattarkine and Ganesh in aqueous DNA–lipid complexes[3b] and was attributed to phase separation of lipid-free DNA double helical structures (lower $T_M$) and DNA double helices capped with a layer of surfactant molecules (higher $T_M$). DNA cannot exist in the organic phase without some degree of hydrophobization provided by electrostatically complexed ODA molecules. The above result may be attributed to two phase-transferred components wherein the degree of complexation of the DNA duplex with ODA molecules is different.

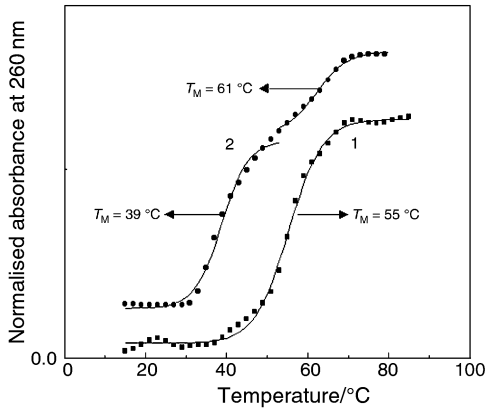


**Fig. 2** UV-temperature plots of films of DNA–ODA complexes on quartz substrates in experiment **A** (curve 2) and experiment **B** (curve 1).

In conclusion, electrostatic complexation of DNA molecules with cationic lipid molecules at the organic/water interface and phase transfer of DNA into the organic phase has been demonstrated. An important finding is the surfactant-facilitated hybridization (and consequent hydrophobization and phase transfer) of complementary single-stranded DNA molecules at the liquid/liquid interface under conditions where the hybridization to form double helical structures does not occur in the bulk of the aqueous phase. The DNA molecules may be conveniently cast in the form of thin films onto any solid support by solvent evaporation. This approach is expected to facilitate the generation of lipid–DNA complexes for possible application in gene-transfer systems *etc.*

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