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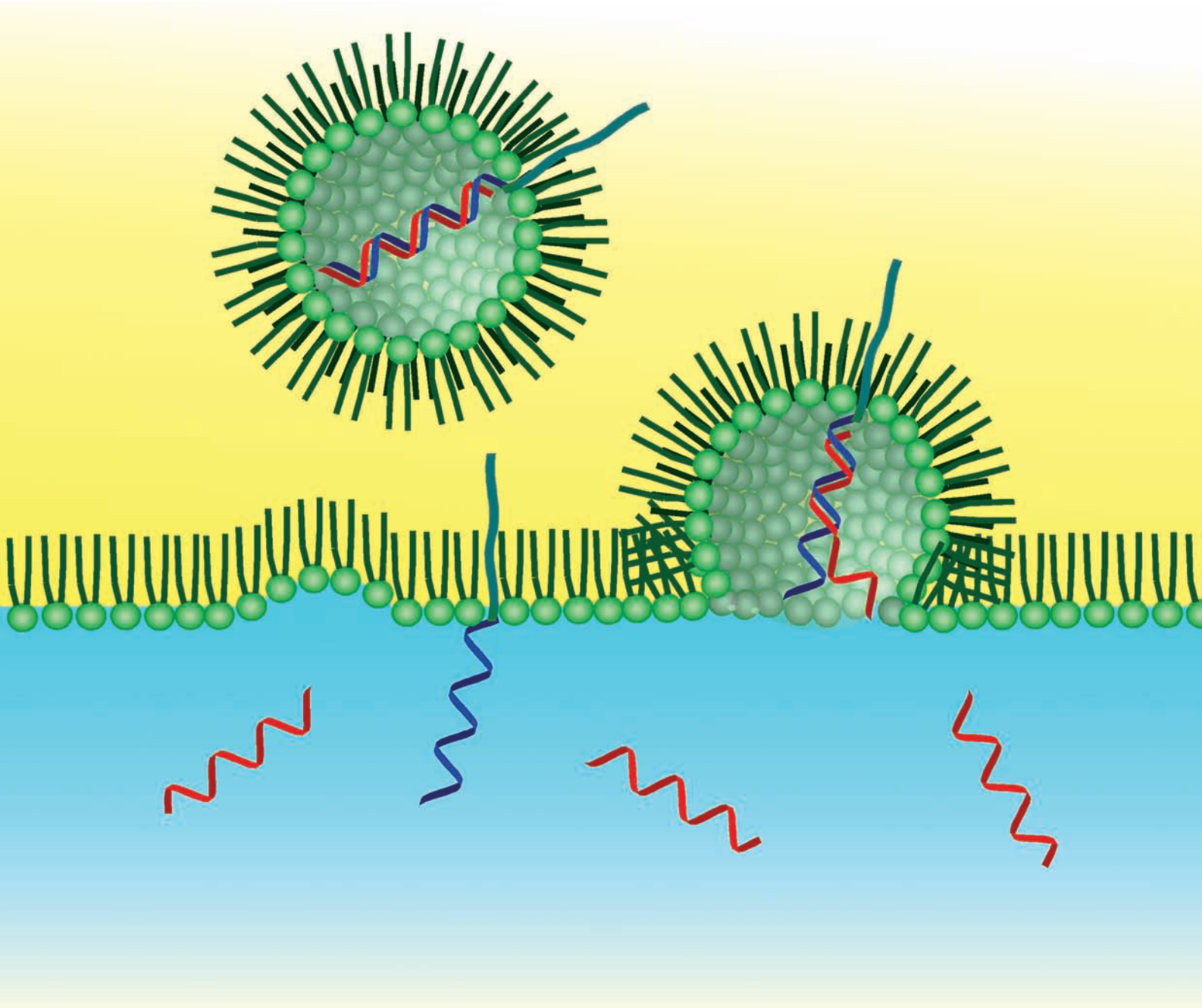


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# Sequence-selective extraction of single-stranded DNA using DNA-functionalized reverse micelles†

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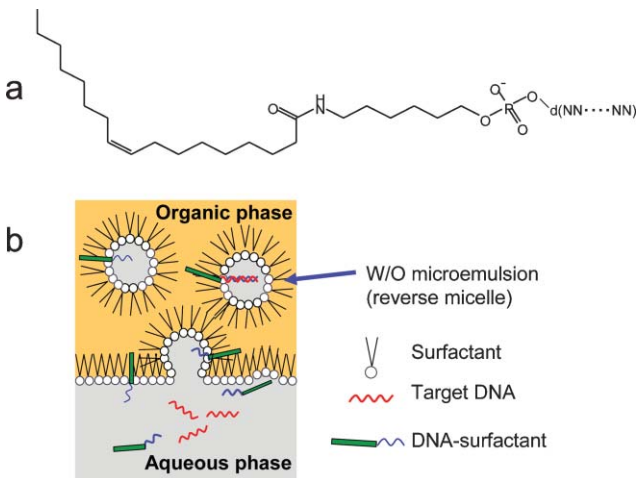
**We report here sequence-specific liquid/liquid extraction of single-stranded DNA using reverse micelles (water-in-oil microemulsions), in which hybridization between a DNA-surfactant and a target DNA having a complementary sequence allows selective transport of the target DNA to an organic phase from a mixture of DNA oligonucleotides.**

Molecular recognition plays a significant role not only in chemistry but also in technology.[1] Numerous efforts have been made to find new synthetic compounds and biomolecules that can recognize a target molecule in a specific manner. Then the new discovery can be applied to a novel and efficient analytical or separation technique. Liquid/liquid extraction is one of the useful separation techniques involving a molecular recognition event. Despite a number of studies on liquid/liquid extraction, it is still a challenge to extract a target molecule selectively to another phase based on the precise recognition at a liquid/liquid interface. Even if a good extractant with precise recognition is developed for a certain target molecule, further effort is required for the discovery of a different extractant suitable for another target. In particular, if the target is a biomacromolecule, the presence of multifunctional groups prevents selectivity and versatility in liquid/liquid extraction.

We here propose the sequence-specific liquid/liquid extraction of DNA oligonucleotides using reverse micelles (water-in-oil microemulsions) with a DNA–surfactant (Scheme 1). A reverse micelle, which is a nanoscale water pool surrounded by surfactant molecules in an organic solvent, is one of the highly useful tools for extraction of water-soluble compounds from an aqueous solution into an organic solvent.[2,3] The advantages of reverse-micellar extraction are i) the capability to scale-up the process, ii) the inhibition of microbial contamination in an organic solvent, thus avoiding biological decomposition of oligonucleotides, and iii) to reduce contamination with water-soluble biomacromolecules (when there is no attractive interaction between biomolecules and a surfactant[4]), due to the isolated and restricted nano-space of a reverse micelle. In the present study we introduce the DNA–surfactant, which is composed of short- and single-stranded DNA with a hydrophobic moiety, as a molecular recognition agent in reverse-micellar liquid/liquid extraction. Using this, we succeeded in the sequence-selective extraction of single-stranded DNA oligonucleotides from an aqueous phase to an organic solvent driven by DNA hybridization.

An oleoyl group was introduced at the N-terminus of a single-stranded 5′-aminated DNA oligonucleotide (20-mer), and the resulting product was designated a DNA-surfactant, which was used as an extractant in a phospholipid-based reverse-micellar system.[5] The sequences of the DNA-surfactants and target DNA are listed in Table 1. Fig. 1a shows the effect of the molar ratio of DNA–surfactant to target DNA on the extraction of a FITC-labeled target DNA (Target 1a). In each case, we observed a decrease of the FITC fluorescence in the aqueous phase and the coinciding increase of fluorescence in the organic phase. As the molar ratio increased, the percent extraction of the target DNA increased. In the absence of the DNA–surfactant, there was no significant fluorescence in the organic phase, even though the reverse-micellar phase contained 25 g $l^{-1}$ water. It means that the target DNA was not extracted to the organic phase in the absence of DNA–surfactant. Indeed several groups also reported that proteins were not transported to the organic phase in the absence of interaction between proteins and surfactant.[4] Our results obtained here indicate that the DNA–surfactant allowed the extraction of the FITC-labeled target DNA from the aqueous phase to the organic phase. It should be noted that there was no detectable difference in fluorescence between single-stranded and double-stranded Target **1a** and between Target **1a** in an aqueous solution and that in a reverse-micellar phase.




**Scheme 1** Schematic illustrations of DNA–surfactant (a) and sequence-selective extraction of DNA oligonucleotides using DNA-functionalized reverse micelles (b).

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† Electronic supplementary information (ESI) available: experimental and the sequences of DNAs (Table S1). See DOI: 10.1039/b708082d

 

**Table 1** Nucleobase sequences of DNA surfactants and target DNA oligonucleotides

| Name | Sequence | Type |
|---|---|---|
| DNA–surfactant **1** | Oleoyl-5′-CCAATACCACATCATCCATA-3′ | Linear 20-mer |
| DNA–surfactant **2** | Oleoyl-5′-CCATAACATACGGTAATCTT′-3′ | Linear 20-mer |
| DNA–surfactant **3** | Oleoyl-5′-TCTCATGTTGAATCTGTGTA-3′ | Linear 20-mer |
| DNA–surfactant **4** | Oleoyl-5′-GTGAG**CATCATCCATATAGCT**CTCAC-3′ | Hairpin 26-mer (loop 16-mer: bold) |
| Target **1a** | FITC-5′-TATATGGATGATGTGGTATTGG-3′ | Linear 22-mer |
| Target **1b** | FITC-5′-GATGATGTGG-3′ | Linear 10-mer |
| Target **1c** | FITC-5′-CAGTTATATGGATGATGTGGTATTGGGGGC-3′ | Linear 30-mer |
| Target **1d** | FITC-5′ CCCACTGTTTGGCTTTCAGTTATATGGATGAT-GTGGTATTGGGGGCCAAGTC-3′ | Linear 52-mer |
| Target **2** | TAMRA-5′-TTAAGATTACCGTATGTTATGG-3′ | Linear |
| Target **3** | Cy5-5′-TTTACACAGATTCAACATGAGA-3′ | Linear |
| Target **4a** | FITC-5′-AGCTATATGGATGATG-3′ | Perfectly-matched to DNA surfactant **4** |
| Target **4b** | FITC-5′-AGCTATAT*T*GATGATG-3′ | Single-base mismatch to DNA surfactant **4** |
| Target **4c** | FITC-5′-AGCTAT*TAC*GATGATG-3′ | Three-base mismatch to DNA surfactant **4** |

[a] *Italic nucleobases represent mismatches to DNA surfactant **4**.

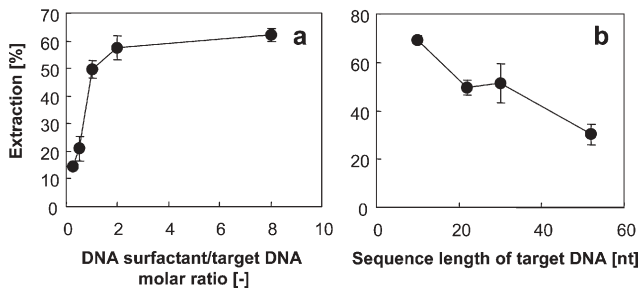


**Fig. 1** a) Effect of the concentration of DNA surfactant 1 on the extraction of Target **1a**. b) Effect of sequence length of target DNA on the extraction efficiency using DNA surfactant **1**. The aqueous phase contained Targets **1a**, **1b**, **1c** or **1d** at 25 nM each and the organic phase contained DLPC (10 mM) and 1-hexanol (3 vol%).

There are several reports on the solubilization of DNA in reverse micelles.[3,6] Some of them reported that DNA was forced to solubilize into a reverse-micellar phase by the microinjection method. Our previous reports described that a cationic surfactant transferred DNA to a reverse-micellar phase non-selectively based on the electrostatic interaction.[3] Taking account of these reports, the extraction results obtained here are reasonable. Above a molar ratio of two, the percent extraction reached a plateau and was constant at approximately 60%. The reason why the percent extraction reached a plateau might be attributable to the partitioning of the target DNA/DNA–surfactant complex between the organic phase and the aqueous phase. Since the hydrophobic moiety was just one oleoyl group in the target DNA/DNA–surfactant complex, increasing the hydrophobicity might result in higher extraction of the target DNA.

The extracted target DNA in the organic phase was found to be back-extracted to an aqueous phase simply by the addition of 2-butanol at high temperature, under which conditions reverse micelles are decomposed and the DNA duplex is dissociated. After the forward extraction of Target **1a** at the molar ratio of two described above, 1 ml of the organic phase containing Target **1a** was added to 1 ml of 10 mM Tris–HCl buffer (pH 8), followed by the addition of 0.5 ml 2-butanol. The mixture was stirred vigorously at 80 °C for 3 h and the fluorescence in the resultant aqueous phase was measured. We observed that 86 ± 7% of the extracted Target **1a** was back-extracted from the organic phase to the aqueous phase. Therefore, approximately 50% of the initial Target **1a** was recovered through the forward and backward extractions.

The extraction of a non-labeled target DNA (22-mer) to an organic phase was also examined, because of the possibility that the fluorophore conjugated to the target DNA acted as a hydrophobic moiety to facilitate the DNA extraction. The non-labeled target DNA was also successfully extracted to the organic phase in the presence of an equivalent mole of DNA–surfactant, and the fluorescent measurement using double-stranded DNA-specific dye, SYBR Green I[7] revealed 45 ± 10% extraction of a non-labeled target DNA, while 50 ± 3% of the FITC labeled target was extracted under the same conditions. The good agreement in percent extraction between labeled and non-labeled targets means that the fluorophore did not assist the extraction of the labeled target DNA.

The present extraction system utilized the water pool of a reverse micelle for dissolving a target DNA in an organic phase. The diameter of the reverse micelle was 16.7 nm, as determined by the dynamic light scattering method. The balance between the reverse-micelle size and the target DNA length would play a key role in the extraction of DNA. The effect of the sequence length of a target DNA was evaluated using oligonucleotides ranging from 10 to 52 nucleotides in length (Targets **1a**–**1d**). It was found that the shorter the sequence of a target DNA, the higher the percent extraction was (Fig. 1b). Even in the case of 52-mer target DNA, 30% of the target DNA was extracted. The tendency of shorter oligonucleotides to be extracted more easily to the organic phase is expected, being probably due simply to the size of the oligonucleotides.

We then investigated the sequence-selective extraction of a target DNA from a mixture of different oligonucleotides. DNA–surfactant **1**, **2** or **3** was added to an aqueous phase containing Targets **1a**, **2** and **3**, followed by addition of an organic phase containing DLPC and 1-hexanol. After gently stirring for 3 h, the concentration of each of the target oligonucleotides was determined by measuring the fluorescence derived from fluorophores conjugated to each target oligonucleotide (Fig. 2a). Using DNA–surfactant **1**, over 50% of Target 1a, which was complementary to DNA–surfactant **1**, was extracted to the organic phase, while Targets 2 and 3 were scarcely extracted (less than 3%). Likewise, the use of DNA–surfactants **2** or **3** instead of DNA–surfactant **1**

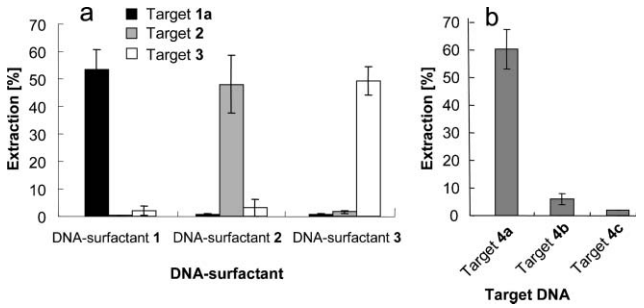


**Fig. 2** a) Sequence-selective extraction of a target oligonucleotide from a mixture of oligonucleotides. The aqueous phase contained DNA–surfactant, Targets **1a**, **2** and **3** at 50 nM each and the organic phase contained DLPC (10 mM) and 1-hexanol (3 vol%). Black bars, grey bars and white bars represent the extraction ratios of Targets **1a**, **2** and **3**, respectively. b) Effect of nucleotide mismatches on the extraction of 16-mer DNA oligonucleotides using hairpin DNA–surfactant at 40 °C (DNA–surfactant **4**). Targets **4a** (perfectly matched to DNA–surfactant **4**), **4b** (single-base mismatch) and **4c** (three-base mismatch).

facilitated selective extraction of the target oligonucleotides (Targets 2 and 3) complementary to each DNA–surfactant. These results revealed that the present system affords sequence-selective extraction of DNA oligonucleotides from a mixture of oligonucleotides, to an organic phase.

Finally, we employed hairpin DNA conjugated to an oleoyl group as a DNA–surfactant. Hairpin DNA precisely recognizes its perfect complement, and not an oligonucleotide having a single-base mismatch.[8] The DNA–surfactant having hairpin DNA (loop 16-mer, DNA–surfactant **4**) was synthesized and applied to the liquid/liquid extraction of oligonucleotides (16-mer). The extraction of each target oligonucleotide was carried out independently (Fig. 2b). The DNA–surfactant **4** recognized the perfect complement (Target **4a**) and over 60% of Target **4a** was extracted to an organic phase, while the percent extractions of Target **4b** (a single-base mismatch) and Target **4c** (three-base mismatches) were only 6% and 2%, respectively.

We here employed reverse micelles coupled with molecular-recognition chemistry and achieved the first sequence-selective extraction of DNA to an organic phase. The present study reveals that molecular recognition can be utilized in liquid/liquid extraction for separation of oligonucleotides. In the last decade, new functions of short-stranded DNA and RNA have been discovered.[1b,9] The recent great progress in the functional DNA and RNA oligonucleotides suggests a necessity for the sequence-specific purification of nucleic acids.[1d,10] But there are still considerable uncertainties on the factors affecting the extraction efficiency. Controls of the micellar size and of the hydrophobicity of the DNA–surfactant are thought to improve the extraction efficiency and to make the present system more suitable to a wide application. We are currently trying to modify the DNA-facilitated reverse-micellar system to various functional DNA and RNA oligonucleotides.

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