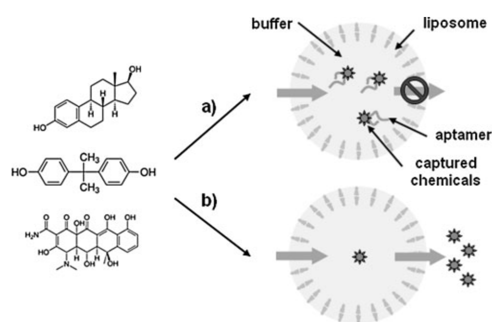
Aptamers-in-Liposomes for Selective and Multiplexed Capture of Small Organic Compounds[a]

Yeon Seok Kim, Javed H. Niazi, Yun Ju Chae, U-Ri Ko, Man Bock Gu*

Small, organic, toxic compounds are not well eliminated by water-treatment systems and eventually become concentrated in the human body. In this study, liposomes are employed to house aptamers with their own binding buffer. When small, organic, toxic compounds in water pass through a liposome barrier, only the target molecules are captured by the DNA aptamers inside the liposomes. The capture efficiency is not high when DNA aptamers are used in tap water. When DNA aptamers in liposomes are used, the capture efficiency increases more than 80%. The simultaneous and selective elimination of target toxicants is successfully performed for tap-water samples containing toxicant mixtures.



Introduction

Aptamers are single-stranded nucleic acids that can be selected in vitro from a large random DNA or RNA library to bind a number of molecules with high affinity and specificity.[1,2] They are emerging as new recognition molecules that can rival antibodies in various fields.[3,4] In some cases, aptamers show a better performance compared with antibodies, mainly due to their high selectivity, stability, and the convenience in their generation and modification.[5,6] Especially, small-molecule compounds such as organic toxicants or biological toxins could be potential targets for the selection and application of aptamers since aptamers are not limited in their screening targets, in contrast to antibodies. In recent years, therefore, numerous studies for the applications of aptamers have been reported for new-drug development, medicinal diagnostics, drug-delivery systems, in vivo imaging and biosensors.[7–12]

With regard to the binding characteristics of aptamers to target molecules, several reports have discussed the effects of ionic strength and pH for the binding capacity of the aptamers. The salt concentration in the reaction solution, for example, is known to highly affect the folding of aptamers, and subsequently the interaction between aptamers and their target molecules might be affected by a conformational change of the aptamer.[13–15] Use of aptamers for applications in the binding and the detection of particular small organic compounds in an aqueous environment should have several restrictions in terms of the activity of the aptamer, (i.e., the reaction conditions, such as the salt concentration, can drastically affect the binding ability of an aptamer). In light of this view, aptamers need to be placed in a suitable environment to

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protect them from such unwanted circumstances; one possible environment that could accomplish this requirement is a liposome as a holding moiety. Liposomes have been widely used as biomimetic materials for drug delivery in studies of cell biology and in biosensor applications.[16–20] Toxic chemicals such as endocrine-disrupting chemicals (EDCs), residual pharmaceutics, and antibiotics pose serious concern to the environment and public health. These small, toxic compounds cannot be completely eliminated by normal physico-chemical treatment systems and could be finally concentrated in the human body through contaminated drinking water.[21–23]

In this study, therefore, we have developed a novel biocomposite material (aptamers-in-liposomes) composed of aptamers and liposomes, with its application for the selective capture and elimination of small organic compounds in water.

Results and Discussion

The Effect of Salt Concentration on an Aptamer

It has already been reported that various factors, especially ionic strength, can affect the capacity of aptamers.[13–15] To evaluate the effect of salt concentration on the binding capacity of an aptamer to a target molecule, a DNA aptamer that binds with high affinity to the endocrine-disrupting chemical 17β-estradiol (K_d: 0.13×10^{-6} M) was examined.[24] This DNA aptamer was immobilized on the surface of magnetic beads via avidin-biotin interaction. Around 2.3×10^5 aptamers were coated on the surface of a single magnetic bead. Then, the magnetic beads (10^9 beads) coated with 17β-estradiol-binding aptamer (EBA) were suspended in specific buffer solution (100×10^{-3} M NaCl, 20×10^{-3} M Tris-HCl, 5×10^{-3} M KCl, 2×10^{-3} M $MgCl_2$, 1×10^{-3} M $CaCl_2$, pH 7.6) containing 1×10^{-6} M 17β-estradiol as a standard condition for the binding reaction and incubated with mild shaking for 30 min at room temperature. The concentration of unbound 17β-estradiol in the buffer solution was analyzed by high-performance liquid chromatography (HPLC) after magnetic separation (Figure 1A). The results showed that $90.4 \pm 14.6\%$ of the 17β-estradiol in the buffer solution was captured by the aptamer-coated magnetic beads (Figure 1B), whereas the magnetic beads alone, without aptamers, did not reduce the amount of 17β-estradiol in the solution. The magnetic beads coated with the 17β-estradiol-binding aptamers were then added to tap water containing 1×10^{-6} M 17β-estradiol, in which the tap water showed a very-low ionic strength ($Ca^{2++} < 0.3 \times 10^{-3}$ M, $Mg^{2+} < 0.1 \times 10^{-3}$ M), and incubated under the same conditions. As shown in Figure 1C, only $12.7 \pm 5.3\%$ of the 17β-estradiol was captured with the same number of EBA-coated magnetic beads. This suggests that the 17β-estradiol-binding aptamer was not functionally active under this very-low salt concentration in tap water, and consequently resulted in loss of the binding ability of the aptamer.[13–15]

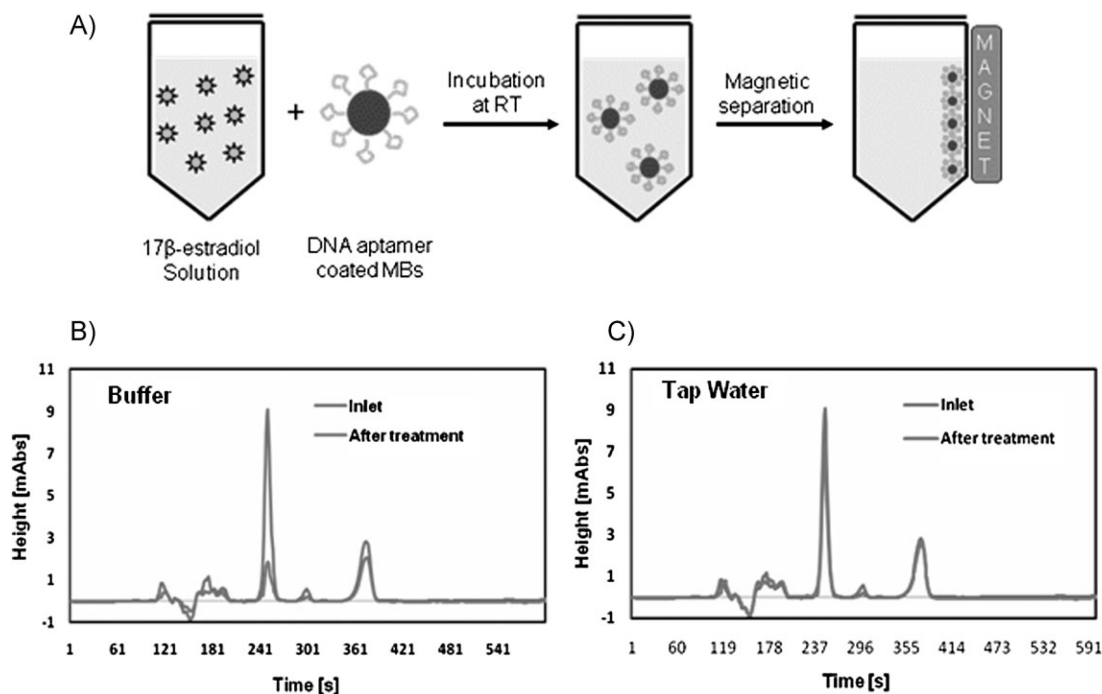

Figure 1. A) Scheme of toxicant capture using aptamer-coated magnetic beads. B) HPLC profiles of 17β-estradiol in binding buffer before and after the treatment. C) HPLC profiles of 17β-estradiol in tap water before and after the treatment.

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Selective Capture of Small Molecules using Aptamer-Liposome Composites

To overcome the limitations of the poor performance due to the lowered aptamer function in tap water, aptamer-in-liposome composites were designed, and it was expected that the aptamers' binding function would be maintained in such a well-protected environment, which was supplied by the liposomes harboring optimized buffer solution, regardless of aqueous samples. Figure 2A shows a schematic diagram for the specific capture method for small organic compounds using the aptamer-in-liposome composites. Three different DNA aptamers, 17β-estradiol-binding aptamer (EBA), bisphenol-A-binding aptamer (BBA), and oxytetracycline-binding aptamer (OBA), which bind to their specific target molecules, were examined.[25,26]

At first, liposomes composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) were prepared; then EBA, BBA and OBA were encapsulated into different liposomes based on the ethanol and calcium-ion induced method (Figure S1, Supporting Information). In the preparation of the aptamer-in-liposome composites, the specific buffer (100×10^{-3} M NaCl, 20×10^{-3} M Tris-HCl, 5×10^{-3} M KCl, 2×10^{-3} M $MgCl_2$, 1×10^{-3} M $CaCl_2$, pH 7.6) as an aptamer-binding buffer was filled in the core regions of the liposomes. The efficiency of the encapsulation of the DNA aptamers in the liposomes was calculated as ≈7–8% by measuring the concentration of non-encapsulated aptamers after liposome precipitation; consequently, ≈1.1–1.2 nmol of the DNA aptamers were placed inside the liposomes. Then, the aptamer-in-liposome composites were incubated in tap water containing 17β-estradiol, bisphenol A and oxytetracycline, respectively, with a mild shaking condition at room temperature. It was anticipated that these small organic molecules enter the liposomes by diffusion and are captured by the aptamers-in-liposomes, which provides a favourable binding condition for the aptamers. Consequently, small organic molecules in water can be specifically eliminated by aptamers within the core region of the liposomes. Liposomes with no encapsulated aptamers could simply allow the small organic molecules to be easily diffused out of the liposomes. During mixing of the samples of the aptamer/liposome composites with tap water, water also entered into the liposomes. Subsequently, we also tested the effects of ionic strength in terms of the duration of the mixing time with water, and found that the ionic strength inside the liposomes might be decreased with increasing mixing time. If the mixing time is short, the change of ionic strength is not significant, and the target molecules cannot diffuse into the liposomes sufficiently. In contrast, if the mixing time is too long, the target molecules can be diffused in enough, but the ionic strength seems to be changed greatly. Therefore, we found the optimum mixing time to be 30 min for the elimination of contaminants. When the mixing time was over 40 min, the liposomes were significantly ruptured. It was confirmed that the aptamer/liposome complex was stable and worked well within 30 min.

It is clear from Figure 2B that 87.6 ± 7.5% of 1×10^{-6} M 17β-estradiol in tap water was captured by the EBA-in-liposome composite within 30 min. However, only 28.2 ± 4.6% of the 17β-estradiol was non-specifically captured from the tap water when liposomes without the 17β-estradiol-binding aptamers were used. This non-specific removal of a small portion of 17β-estradiol, despite of the absence of the specific aptamer in the liposomes, is attributed to the retention of a small portion of 17β-estradiol in the liposomes. This result implies that the core region in the liposomes ideally facilitates sustaining the

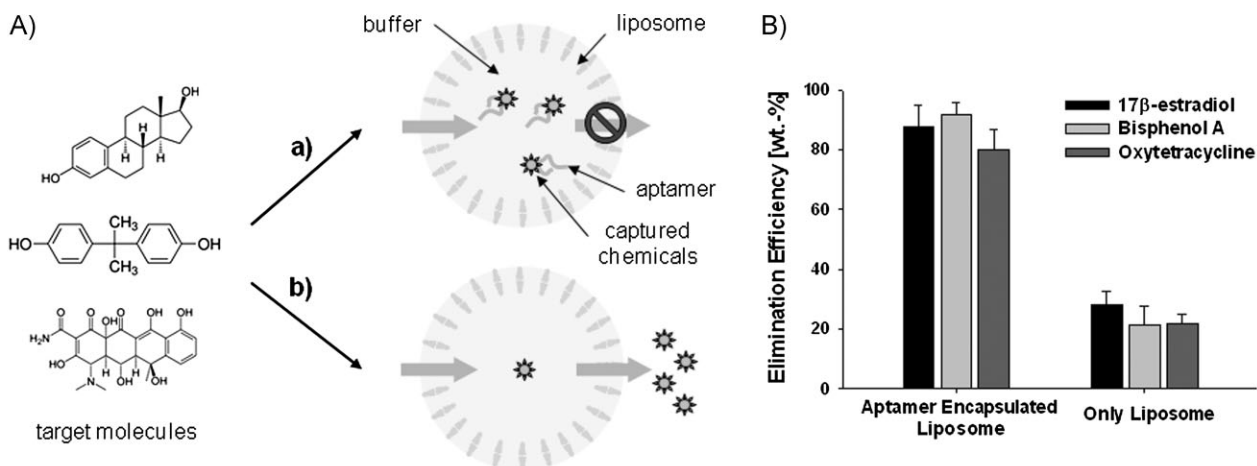

Figure 2. A) Schematic diagram showing aptamer-in-liposome composite (a) and liposomes without aptamer encapsulation (b). B) Chemical capturing efficiency for three small organic compounds (17β-estradiol, bisphenol A, and oxytetracycline) in tap water using target-specific DNA aptamers encapsulated in liposomes and liposomes with no aptamer encapsulated.

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binding buffer and consequently helps the binding ability of the aptamer to the target within the liposome, thus leading to retaining the bound complex of the aptamers and targets. The same protocol was repeatedly applied for the other small organic molecule targets, such as bisphenol A (endocrine-disrupting chemical) and oxytetracycline (antibiotic), using BBA-in-liposome and OBA-in-liposome composites, respectively. As a result, $91.9 \pm 4.0\%$ and $79.8 \pm 6.8\%$ of the bisphenol A and oxytetracycline in tap water was captured by the aptamer-in-liposome composites, respectively, while the liposomes without BBA or OBA eliminated about $21.4 \pm 6.1\%$ and $21.7 \pm 3.3\%$ of bisphenol A and oxytetracycline, respectively. In this result, the elimination efficiency of oxytetracycline was around 10% lower than that of 17β-estradiol or bisphenol-A removal. This could probably be caused by the differences in the diffusing ability of each molecule into the liposomes. The 17β-estradiol and bisphenol A are highly nonpolar, unlike oxytetracycline, and, therefore, they could be easily diffused into the liposomes and thus captured efficiently by the aptamers in the liposomes.

Selective and Multiplexed Elimination of Small Molecules using Mixtures of Aptamer-Liposome Composites

The most-attractive advantage of this method for the elimination of small organic compounds is the selective elimination or recovery of a mixture of small organic compounds. To verify the performance of the selective capture, a mixture of small organic chemicals containing 17β-estradiol and bisphenol A was tested using two different types of aptamer-liposome composites (EBA-in-liposome and BBA-in-liposome composites). At first, aptamers for 17β-estradiol and bisphenol A were encapsulated in liposomes. These two different types of aptamer-in-liposome composites were then incubated individually, and also together in the tap-water sample containing 1×10^{-6} M each of a 17β-estradiol and bisphenol-A mixture (Figure 3A). Incubation of the EBA-in-liposome composite with the 17β-estradiol and bisphenol-A mixture showed specific capture of $74.3 \pm 2.2\%$ of the 17β-estradiol but only $7.1 \pm 4.0\%$ of the bisphenol A was captured non-specifically (Figure 3B). Similarly, when the BBA-in-liposome composite was incubated with a mixture of 17β-estradiol and bisphenol A (1×10^{-6} M each), a maximum amount of bisphenol A ($75.5 \pm 6.1\%$) was specifically captured, compared to only $8.3 \pm 1.8\%$ of 17β-estradiol captured non-specifically. Finally, when the EBA-in-liposome and BBA-in-liposome composites were incubated together with a mixture of 17β-estradiol and bisphenol A in tap water, a significant amount of both the 17β-estradiol and bisphenol A were eliminated simultaneously ($75.3 \pm 2.9\%$ and $78.4 \pm 3.4\%$, respectively). Additionally, liposomes without any aptamers showed a small reduction of only $17.4 \pm 6.3\%$ and $11.6 \pm 5.3\%$ 17β-estradiol and bisphenol A, respectively. Based on the above results, it is clear that the selective and multiplexed elimination of each small organic molecule was promoted effectively by the aptamers-in-liposomes.

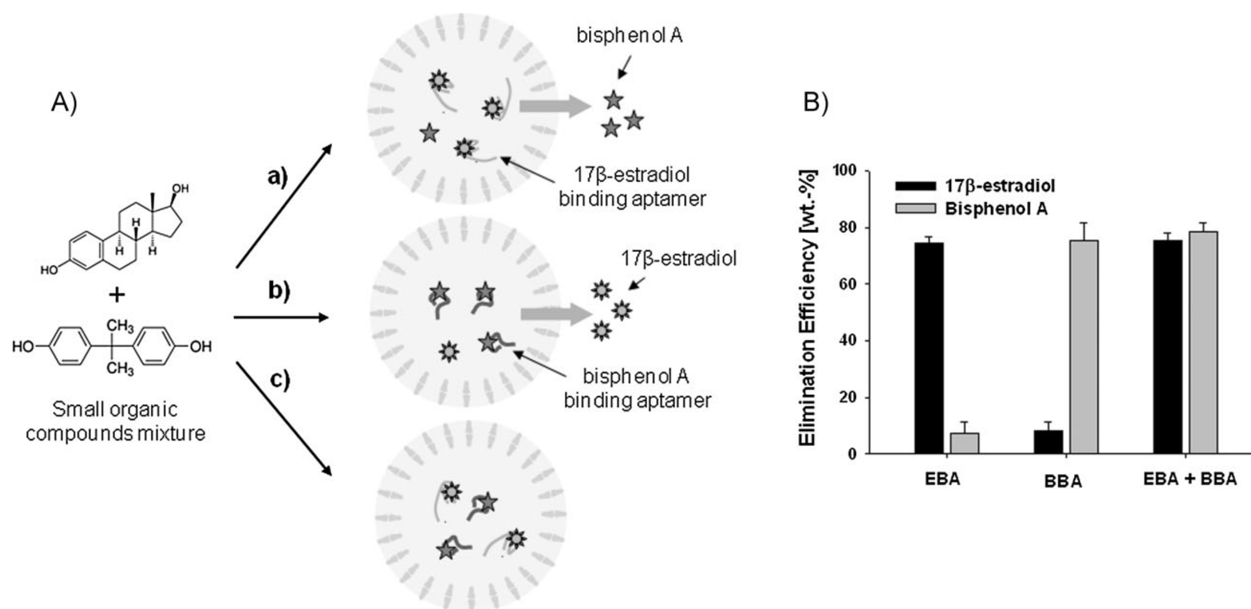

Figure 3. A) Schematic diagram of selective elimination of small organic molecules from the mixture using different aptamer-in-liposome composites: liposomes encapsulated with aptamers specific for 17β-estradiol (a); liposomes encapsulated with aptamers specific for bisphenol A (b) and liposomes encapsulated with both 17β-estradiol and bisphenol A (c). B) Elimination efficiency of the mixture of 17β-estradiol and bisphenol A in tap water using the liposomes containing the different aptamers.

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Comparison between the elimination of the individual target and the mixture by using the aptamer-in-liposome composites indicated that the elimination of the 17β-estradiol and bisphenol A as a mixture was slightly lower than that of the 17β-estradiol or bisphenol A alone. This could be possibly due to the fact that the diffusion of 17β-estradiol and bisphenol A into the liposomes may be competitive, and consequently, the extent of elimination for each compound declined when the solution contained a mixture of targets.

Conclusion

We have demonstrated the novel design of aptamer-in-liposome composites, which can allow the restoration of the aptamer's activity from external environmental perturbations, such as ionic strength, pH and various other contaminants. Additionally, the liposomes were found to serve as a selective barrier that facilitates restrictive passage of other contaminants that affect the binding ability of the aptamer to its target molecule. The capture efficiency was found to be more than 80%, compared to the case without liposomes. These aptamer-in-liposome composites can be used for real applications, especially in the detection and monitoring of environmental contaminants, pharmaceutical preparations, and synthetic chemicals. Furthermore, this aptamer-in-liposome-composite system can be applied to the selective capture and recovery of high-value molecules in industry or the scavenging of hazardous compounds in the pharmaceutical and environmental fields.

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