Functional DNA–Silica Composite Prepared by Sol–Gel Method

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ABSTRACT: The high water-solubility and the biochemical instability of DNA have been serious problems in applying DNA as a practical material. Improvement of these weak points would encourage the development of a DNA-based separating device for various DNA-interactive harmful chemicals such as some mutagens and endocrine disruptors. Recently, we designed various DNA-conjugating materials to improve such weak points. In this study, we describe a new composite which combined DNA with silica components *via* sol–gel method. The DNA–silica composite showed the advantages of mechanical and chemical stability in both aqueous and organic solvents, and the incorporated DNA molecules were stably retained and maintained the specific functions. The DNA–silica composite could adsorb the DNA-interactive chemicals in their diluted aqueous solution. The selective adsorbing effect to the DNA-interactive chemicals was confirmed by the competitive adsorption test and GC–MS analysis. The used composite could be recycled by washing with the appropriate solvents. Thus, the DNA–silica composite has a desirable property and potential utility as a tool for separating DNA-interactive chemicals, and for environmental clean-up.
[DOI 10.1295/polymj.37.94]
     KEY WORDS   DNA / Sol–Gel / Functional Material / Organic–Inorganic / Affinity-based Separation / Intercalation / Endocrine Disruptor /

DNA is one of the infinite natural resource which exists in the natural world. Large amounts of DNA can be easily obtained from valueless marine products, such as salmon milt and shellfish gonads. It must be valuable to find practical uses for DNA. Recently, DNA has been studied as an advanced functional material because of its unique functions. DNA has a double-helical frame and stacked structure of nucleic acid base pairs.[1,2] Such unique structures produce some functions of DNA, including intercalation, groove-binding, electron transfer, etc.[3–6] These are highly specific functions which are difficult to mimic by synthetic polymers.

In past years, we have focused on the utilization of DNA as an environmental clean-up material. Recently, endocrine disruptors in water and soil have become a worldwide problem.[7,8] Such chemicals are considered to show hormone-like effects and disturb the normal organism system even at the extremely low concentrations. Suitable techniques to remove them from the environment are required. It has been reported that certain kinds of endocrine disruptors, mutagens and carcinogens interact with DNA *via* intercalation.[7] Intercalation is the principal model of the association between DNA and certain kinds of small molecules which have planar aromatic ring systems. We thought that DNA would be applicable as an affinity adsorbent of environmental pollutants. Previously, we reported the concentration and removal of some endocrine disruptors from aqueous solution using DNA-conjugating materials.[8–12]

The high water-solubility and biochemical instability of DNA have been serious problems in applying DNA as a practical material. Previously, various trials[13–17] to overcome these weak points have been carried out. Some approaches have already utilized DNA-materials in chemosensory devices,[13] affinity chromatography,[14] etc. The chemical modification technique was mainly used. In these cases, the amount of immobilized DNA was limited, and complicated processes were often required. The adsorption capacity was considered to be important, when applying DNA as an affinity adsorbent, so a sufficiently large amount of DNA has to be immobilized. Some non-covalent linking methods, such as the construction of a polyelectrolyte complex, were performed, which enable us to immobilize large amounts of DNA.[18,19] However, the loss of the specific functions of DNA and elution of the immobilized DNA were often observed.[18,19] The desirable material would offer some

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advantages, such as good mechanical strength, chemical stability, and enough capacity for the DNA-interactive chemicals. In this paper, we describe the "DNA–silica composite". This material was prepared *via* a sol–gel process,[20] which well satisfied such requirements.

Sol–gel process is a cornerstone technique to prepare inorganic materials or organic-inorganic composite materials at relatively low temperatures. It enabled us to combine inorganic components with fragile biomolecules, such as enzymes,[21] antibodies,[22] general proteins[23] or even living cells,[24] and then a number of biotechnological applications were performed.[20,25] For example, the micro-reactor[26] is one of the promising products which conjugate the sol–gel-derived matrix with an enzyme. A number of the chromatography systems, which incorporated various ligands into the porous silica network, have also been developed.[22] Some researchers focused on the sensing and removal of contaminants from the environment. Because of the toughness, stability and good permeability, the sol–gel-derived materials were considered to be suitable for the large-scale processing of contaminated water. From such a viewpoint, some affinity-based separators[28–30] were developed *via* the sol–gel process, however, there has been no example applying DNA to the best of our knowledge.

The DNA–silica composite was prepared as follows: Tetraethoxysilane (TEOS) was hydrolyzed under acidic conditions to form a precursor solution. DNA dissolved in buffer solution was then added to this precursor solution. Polycondensation was induced by the mixing them, and as a result, DNA was immobilized by the formation of a silica network. To avoid denaturation or precipitation of the DNA, the incorporation of DNA into the silica network was performed under mild conditions. Parameters, such as pH, concentration of DNA and salts, were optimized. Some silane coupling reagents was added to improve the DNA-holding capacity. The obtained DNA–silica composite was evaluated from the standpoints of the selective adsorption effect, the mechanical or chemical stability, the DNA-holding capacity and the resistance to nuclease.

EXPERIMENTAL

Materials and Chemicals

Double-stranded DNA (sodium salt from salmon milt, M_w; *ca.* 5×10^6) was purchased from Yuki Fine Chemical Co., Ltd. (Tokyo, Japan) and used without further purification. Tetraethoxysilane (TEOS) and γ-aminopropyltriethoxysilane (APTES) were purchased from Shin-Etsu Chemistry Co., Ltd. (Tokyo, Japan). Ethidium bromide, dibenzofuran, benzophe-

non, biphenyl, isopropyl alcohol, dichloromethane and all other chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Isopropyl alcohol and dichloromethane were analytical grade. Water was purified by a MilliQ-water system (Millipore, Bedford, MA, USA).

Preparation of DNA–Silica Composites

The DNA–silica composites were prepared by the following procedure: The precursor solution was prepared by the acid-catalyzed hydrolysis of TEOS. TEOS, distilled water and hydrochloric acid were mixed at a $1 : 5 : 1 \times 10^{-4}$ molar ratio. The mixture was regularly stirred at 25 °C until a transparent solution was obtained. This precursor solution was used throughout this experiment. The double-stranded DNA was dissolved in acetate buffer solution (0.2 M, pH 4) and the absorbance of the solution was measured using a UV–vis spectrophotometer (V-550, JASCO Corporation, Tokyo, Japan). The DNA concentration was determined using the extinction coefficient which is expressed in terms of base molarity ($\varepsilon_{260} = 6600\,M^{-1}\,cm^{-1}$).[31] The concentrations of the DNA solutions were adjusted to 1.0, 3.0, and 5.0 mg/mL. APTES, which is known as a concern silane coupling agent, was diluted with ethanol and adjusted to the concentrations ranging between 0.24 and 6.6 wt %. Six grams of the DNA aqueous solution and 3.0 g of the APTES ethanol solution were gently mixed. The final solution was then obtained by mixing 6.0 g of the precursor solution and 9.0 g of the DNA–APTES mixed solution. In order to allow the formation of the silica network, the mixture was sonicated for 1 min and incubated in a closed vial at 50 °C for 48 h. The obtained gel was repeatedly washed with distilled water to remove the salts, ethanol and unreacted components. This gel was lyophilized in a freeze-dryer (FDU-830, EYELA Co., Ltd., Tokyo, Japan) and was ground into a powder. In this study, three kinds of the DNA–silica composites were prepared using DNA aqueous solutions of 1.0, 3.0, and 5.0 mg/mL. The composites were defined as groups "A", "B" and "C". Additionally, they were classified from No. 1 to 5 by each APTES ratio which were defined using the equation: APTES ratio = [APTES]/ ([APTES] + [TEOS]). Their compositions are summarized in Table I.

Evaluation of DNA-holding Capacity

Evaluation of the DNA-holding capacity was performed for all the composites. The DNA–silica composites were treated with hydrochloric acid solution (1.0 M) under reflux for 1 h. The total amounts of incorporated DNA were spectrophotometrically determined from the absorbance of the supernatant at

Table I. Compositions of DNA–silica composite

Sample	DNA solution[a]	[APTES]/[Nucleotide][b]	[APTES]/[Si] (%)	DNA (mg/g-beads)	Percentage of eluted DNA (%)[c]
A-1	1 mg/mL	0.0	0.0	2.8	5.60 ± 0.22
A-2	1 mg/mL	2.4	0.2	5.0	3.99 ± 0.20
A-3	1 mg/mL	4.8	0.5	5.1	3.66 ± 0.18
A-4	1 mg/mL	7.2	0.7	5.5	3.54 ± 0.18
A-5	1 mg/mL	9.5	1.0	6.0	2.25 ± 0.27
B-1	3 mg/mL	0.0	0.0	7.1	5.08 ± 0.25
B-2	3 mg/mL	2.4	0.7	16.2	2.19 ± 0.22
B-3	3 mg/mL	4.8	1.4	16.5	0.82 ± 0.16
B-4	3 mg/mL	7.2	2.2	17.0	0.27 ± 0.07
B-5	3 mg/mL	9.5	2.9	17.0	0.14 ± 0.08
C-1	5 mg/mL	0.0	0.0	12.3	5.69 ± 0.34
C-2	5 mg/mL	2.4	1.2	19.4	2.12 ± 0.17
C-3	5 mg/mL	4.8	2.4	19.6	0.47 ± 0.16
C-4	5 mg/mL	7.2	3.6	19.2	0.22 ± 0.13
C-5	5 mg/mL	9.5	4.8	18.4	0.19 ± 0.15

[a]The concentration of initial DNA solution. [b]The molar ratio of amino groups of APTES to phosphate groups of DNA.
[c]Percentages of eluted DNA were expressed by an average of four measurements (means $\pm$ SD).
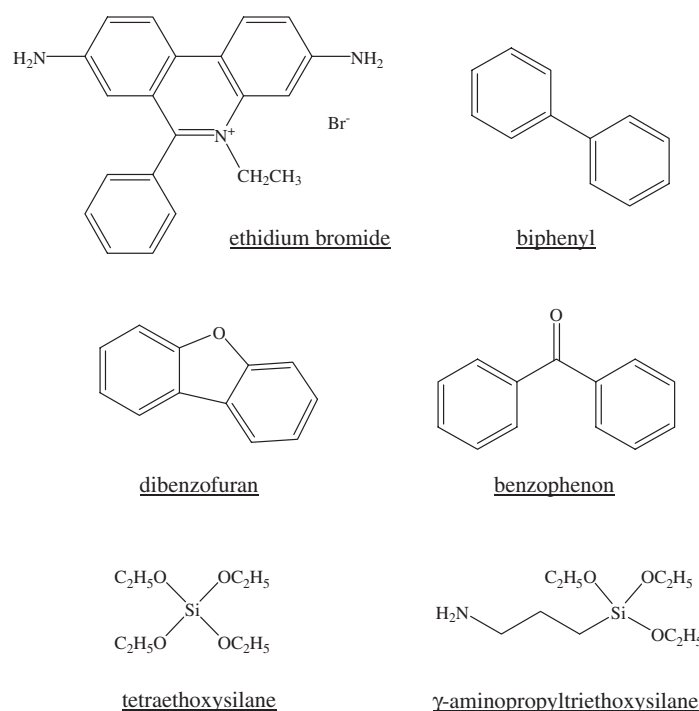
Figure 1. Chemical structures of reagents.

260 nm. Separately, the composites were incubated in the neutral buffer solution (10 mM Tris–HCl, 100 mM NaCl, and pH 7.5) at room temperature for 48 h. The amounts of eluted DNA were determined in the same way. The percentages of the DNA elution were calculated from these values, and were used as the indicator of DNA-holding capacity of each composite.

Adsorption Test Using Ethidium Bromide
    The property of the DNA–silica composites as adsorbents for DNA-interactive chemicals was examined using a model reagent, ethidium bromide (EtBr), which is one of the most popular "DNA-intercalative" reagents. The chemical structure of EtBr is shown in Figure 1. EtBr was dissolved in the neutral buffer solution (10 mM Tris–HCl, 100 mM NaCl, and pH 7.5) to prepare a stock solution. The concentration of EtBr was correctly adjusted to 100 μM using the molar extinction coefficient ($\varepsilon_{480} = 5800 \, M^{-1} \, cm^{-1}$). The stock solution of EtBr was stored in a dark vial at 4 °C and used for the following experiments after adequate dilution. A 100 mg sample of the DNA–silica

Table II. GC–MS conditions

Gas chromatograph		Mass spectrometer	
Column	DB-5MS	Interface temperature	310 °C
	length: 30 m, ϕ: 0.25 mm, film: 0.25 μm	Electron multiplier voltage	1.50 kV
Carrier gas	He	Scan mode	SIM mode
Total flow	49.5 mL/min	m/z range	100–200
Septum purge	1.2 mL/min		
Column pressure	100 kPa		
Injector temperature	250 °C		
Detector temperature	310 °C		
Oven temperature program	120 °C (3 min) to 240 °C at 40 °C/min		

composites ("B-1" and "B-5") was immersed into 5 mL of EtBr aqueous solution ([EtBr] = 10 μM) for 10 min. The amounts of adsorbed EtBr were spectrophotometrically determined from the absorbance at 480 nm, and the color of the composites was observed. In this experiment, silica ("B-1" without DNA) and AP-Silica ("B-5" without DNA) were also used to estimate the physical adsorption of EtBr.

Reusability of DNA–Silica Composites

Reusability of the composites was evaluated using the following procedure: First, 100 mg of the DNA–silica composite ("B-5") was incubated in 5.0 mL of EtBr solution ([EtBr] = 50 μM, water:acetonitrile = 50:50 (v/v)) for 10 min. The molar amounts of the adsorbed EtBr were calculated from the decrease in the absorbance. Subsequently, the composites were collected by centrifugation and repeatedly washed with acetonitrile to extract the adsorbed EtBr. These recycled composites were air-dried and the adsorption tests were performed using them again. These procedures were repeated 5 times, and the decrease in the efficiency for adsorbing EtBr was evaluated.

Affinity-based Adsorption Test Using Dibenzofuran and Benzophenon

The applicability of the DNA–silica composites for the selective adsorption of DNA-interactive chemicals was examined using two model chemicals, dibenzofuran (DF) and benzophenon (BPN). DF has been known to be a DNA-interactive reagent,[7,11] while BPN is not DNA-interactive. DF and BPN were dissolved in an isopropanol/water mixed solution ([DF] = [BPN] = 10 μM, isopropanol:water = 5:95 (v/v)). The competitive adsorption test was performed by the following procedure: 100 mg of the DNA–silica composite ("B-1" and "B-5") was incubated in 10 mL of the DF/BPN mixture solution at room temperature for 30 min. Two milliliters of the supernatant and 200 μL of dichloromethane was mixed and mechanically shaken for 1 min. An adequate amount of bi-

phenyl (BP) was dissolved in dichloromethane, which was added to the extract as an internal standard. The GC–MS analysis was then performed using a GC–MS system (QP5050A, Shimadzu Corp., Tokyo, Japan). Two microliters of the sample solution containing DF, BPN and BP was injected using the splitless injection mode. The parameters of the gas chromatograph were as follows: carrier gas was He, total flow 49.5 mL/min, septum purge 1.2 mL/min, column pressure 100 kPa, injector temperature 250 °C, detector temperature 310 °C and oven temperature programmed at 120 °C (3 min) to 240 °C at 40 °C/min, then remaining constant for 1 min. The mass spectra were obtained using electron impact ionization. These conditions are summarized in Table II. The concentrations of the chemicals were calculated by the internal standard method, and the [DF]/[BPN] ratios were compared with the ratio before the adsorption processing. In this experiment, silica ("B-1" without DNA) and AP-silica ("B-5" without DNA) were also used to estimate the non-specific adsorption of these chemicals.

RESULTS AND DISCUSSION

Preparation of DNA–Silica Composites

Organic/inorganic composite based on DNA and silica was prepared *via* the sol–gel method. The sol–gel reaction involves the following processes. First process is the hydrolysis of alkoxysilane. Another one is the condensation process to form siloxane bonding and network. The preparation of the composite containing DNA has certain requirements. In order to maintain the native conformation and the function of DNA, it requires carrying out mainly in aqueous environment with mild pH, ionic strength and temperature. From the viewpoint of practical uses, the composite has to be stable enough to prevent a leakage of incorporated DNA. It is also required that the external solutes are accessible to the incorporated DNA.
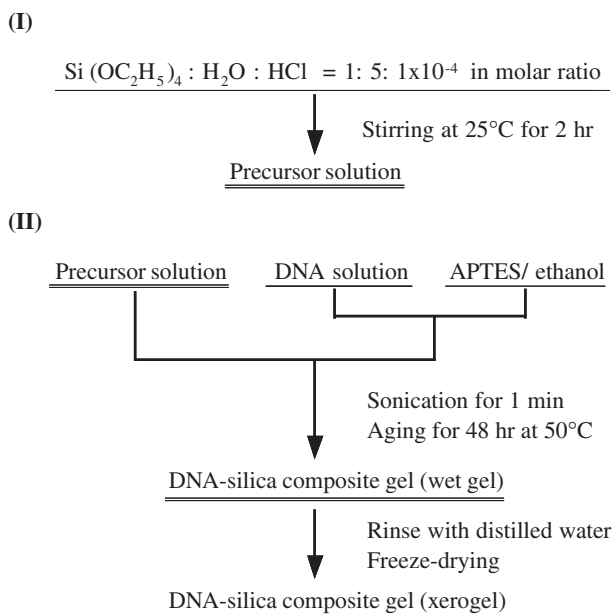
(I)

Si $(OC_2H_5)_4$: H_2O : HCl = 1: 5: 1x10^{-4} in molar ratio

↓ Stirring at 25°C for 2 hr

Precursor solution

(II)

Precursor solution   DNA solution   APTES/ ethanol

↓ Sonication for 1 min
  Aging for 48 hr at 50°C

DNA-silica composite gel (wet gel)

↓ Rinse with distilled water
  Freeze-drying

DNA-silica composite gel (xerogel)

Figure 2. Schematic diagram of the DNA–silica composite preparation: (I) Preparation of precursor solution by acid-catalyzed hydrolysis of tetraethoxysilane (TEOS). (II) Incorporation of DNA into silica matrix was performed. Obtained gel was ground into a powder-form.

Figure 3. SEM image of the DNA–silica composite ("B-5"). The sample was coated by Au layer in high-vacuum conditions. The pictures were obtained using a Hitachi S-4800 field emission scanning electron microscope: (a) ×50,000, (b) ×100,000.

In this study, TEOS, APTES and DNA were used as the components to form the DNA–silica composites. Figure 2 shows the schematic diagram of our procedure. The precursor solution was prepared by hydrolysis of TEOS as described above. DNA was dissolved in acetate buffer solution (200 mM, pH 4) to protect the DNA conformation from the critical change in pH. APTES was selected as a linking agent to improve DNA-holding capacity of the DNA–silica composite. APTES is a bifunctional silane compound, which has been reported to construct water-soluble siloxane oligomers in aqueous solution.[32,33] This cationic oligomer is possible to gather DNA molecules electrostatically, and binds with other silanol groups.[32,33] Thus, the oligomers would connect DNA molecules to the siloxane matrix. APTES was highly diluted with ethanol before addition, because APTES in the aqueous environment is basic and it often causes precipitation of DNA and sudden gelation of the precursor solution.[34] The APTES ethanol solution was gradually added to the DNA aqueous solution. Final solution was obtained by mixing of the precursor solution and the DNA–APTES mixture solution. Polycondensation and gelation of the hydrolyzed TEOS solution were induced by the change in pH and addition of salts. Then DNA molecules were incorporated by the produced silica network. In our experiment, transparent or translucent monolithic gels were produced in a few hours. After aging in closed vials, the gels were w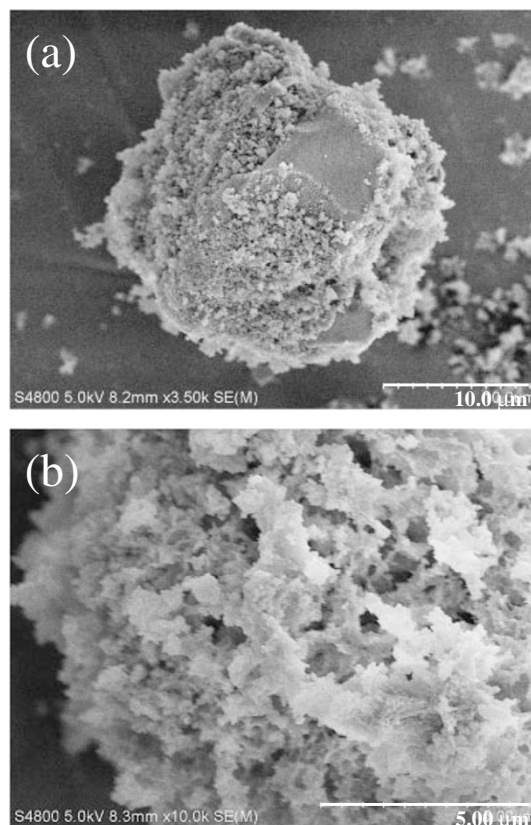ashed and lyophilized. The obtained gels were ground into powders. Figure 3 shows a scanning electron micrograph of the DNA–silica composite with the highly porous structure. Addition of DNA did not show measurable effect on the time to gelation, nor did it induce significant change of the structure. As shown in Table I, 15 types of DNA–silica composites were prepared with different compositions. The combination of DNA and APTES was confirmed from the FT-IR measurement (data not shown).

Evaluation of DNA-holding Capacity

The stability of the DNA–silica composites was evaluated by the following procedure: First, hydrolysis of the incorporated DNA was performed. The composites were processed in hydrochloric acid solution (1.0 M) under reflux for 1 h. The amounts of incorporated DNA in the composites were spectrophotometrically determined from the absorbance of the supernatant. Separately, the composites were incubated in a neutral Tris buffer solution at 25 °C for 48 h. The amounts of eluted DNA were determined in the same way. The molar percentage of the eluted DNA with respect to the amount of incorporated DNA was then calculated ($N = 4$). In Figure 4, the percentage of eluted DNA was plotted versus the APTES ratio. In
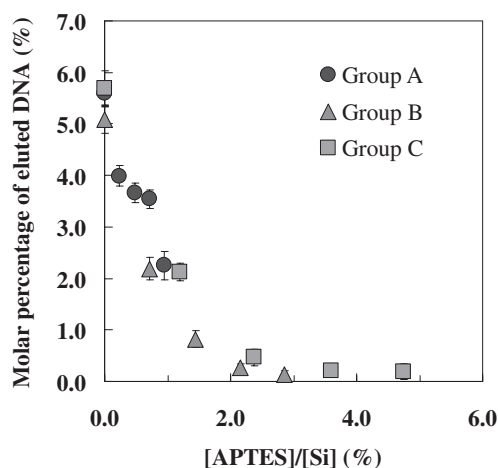
Figure 4. Stability of the DNA–silica composite in aqueous solution. Group A, B and C were defined as the class of samples which were prepared by the DNA solution (1, 3 and 5 mg/mL, respectively). Each composite was incubated in a neutral buffer solution (10 mM Tris–HCl, 100 mM NaCl, and pH 7.5) and the amount of eluted DNA was determined. Vertical axis indicates the percentage of eluted DNA with respect to incorporated DNA. APTES ratio was defined as the following: [APTES]/([APTES] + [TEOS]).

Figure 5. The amounts of adsorbed EtBr by the DNA–silica composite ("B-5"). The composite was incubated in EtBr aqueous solution or EtBr acetonitrile-water mixed solution for 10 min. The absorbance of the supernatant was measured. Physical adsorptive capacity was estimated using control as an adsorbent ("B-5" without DNA).

every case, the increase in APTES ratio contributed to preventing the leakage of DNA from the composites. Good results were obtained when the APTES ratio exceeded about 2%, in which the molar ratio of nucleotide to APTES was 4.8 or more. For example, the sample "B-5" contains 17 mg of DNA per gram of gel, in which the APTES ratio was 2.85% and the molar ratio of nucleotide to APTES was 9.5. Then the molar percents of eluted DNA were controlled to 0.2% or less. It was confirmed that more than 90% of the initial amounts of DNA was incorporated into the final product. Inside the silica matrix, DNA molecules were considered to be dispersedly fixed, and the aggregation of DNA molecules had been prevented. Therefore, incorporation of DNA into the silica matrix contributed to stabilizing DNA against some denaturing forces. For example, precipitation, aggregation or irreversible collapse of the DNA did not occur even at high alcohol concentrations. In fact, the DNA–silica composite efficiently maintained the initial function even after rinsing by various organic solvents or concentrated salt solutions. The DNA–silica composite showed a good resistance to nuclease (data not shown).

Adsorption Test Using Ethidium Bromide

In this test, some types of the DNA–silica composites were incubated in aqueous EtBr solution. They turned red while the orange color of the EtBr aqueous solution faded, suggesting that EtBr molecules permeated the gel matrix and interacted with the incorporat-
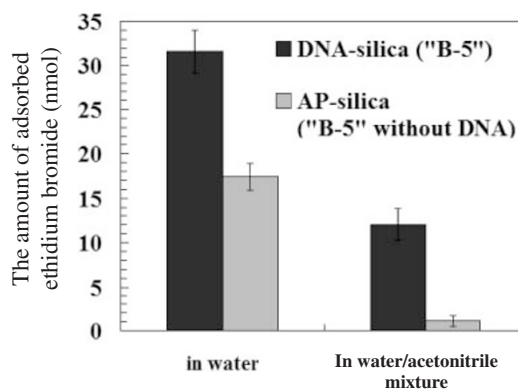
ed DNA. Such phenomena were remarkable in the composites containing both DNA and APTES. In contrast, the composites not containing DNA turned slightly orange. It was considered to be a result of the physical adsorption of EtBr to the composites. The amounts of adsorbed EtBr were calculated from the decrease in absorbance of the EtBr solution at 480 nm. The composites, which contain both of DNA and APTES, were more effective for adsorbing EtBr than the other samples (without DNA). As shown in Figure 5, in the cases of AP-silica ("B-5" without DNA) and "B-5", the amounts of adsorbed EtBr reached approximately 18 and 32 nmol, respectively. The difference was considered to be due to the existence of DNA. Non-specific adsorption of EtBr to AP-silica was induced by the physical adsorption effect, and the slight electrostatic interaction with silanol groups. The amount of incorporated DNA affected the adsorption capacity of EtBr. In the mixture solution containing acetonitrile (water:acetonitrile = 50:50 (v/v)), the physical adsorption of EtBr was prevented and the difference became significant, as shown in Figure 5. These results suggested that the interaction between DNA and the external solute occurred, and the incorporated DNA molecules maintained the specific function of the native DNA. The adsorption efficiency of the present DNA–silica composite was less than previous materials which immobilized DNA molecules on the surface.[35] It is desirable to improve the amount of effectively working DNA in the composite.

Reusability of DNA–Silica Composites

It is possible to separate DNA and the adsorbed chemicals by adequate separating procedures. Extraction using acetonitrile was found to be effective for
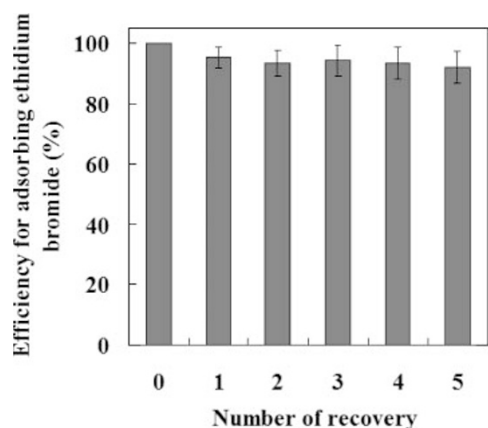
Figure 6. Reusability of the DNA–silica composite. Used composite was recycled by washing with acetonitrile, and the decrease in the efficiency for adsorbing EtBr was estimated.
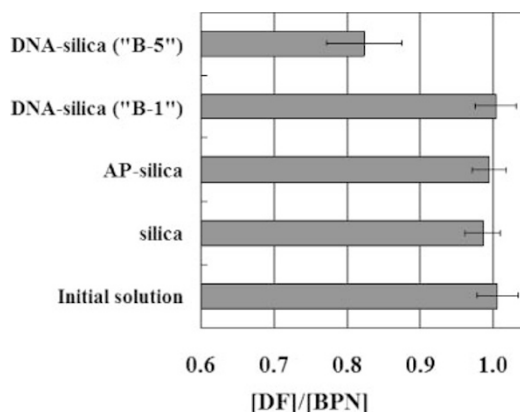

Figure 7. Comparison of molar ratio of dibenzofuran (DF) to benzophenon (BPN) in each solution was performed after the competitive adsorption. Selectivity was estimated using the DNA–silica composites and controls. The amounts of each chemical were calculated from the peak area on GC–MS chart.

separating DNA and EtBr. The reusability of the used DNA–silica composites was examined using "B-5". The composite was incubated in EtBr solution ([EtBr] $= 20\,\mu M$, water:acetonitrile $= 50{:}50$ (v/v)) as described above. Here, the water:acetonitrile mixture solution was used to prevent the physical adsorption of EtBr by the gel. The composites were repeatedly washed with acetonitrile and air-dried. Subsequently, the adsorption tests were performed again using the recycled composites. These cycles were repeated 5 times. The efficiency for adsorbing EtBr was plotted versus the number of recycling process. As shown in Figure 6, the efficiency for adsorbing EtBr did not show a critical decrease even after five recycling processes.

Affinity-based Separation Using Dibenzofuran and Benzophenon

The applicability of the DNA–silica composites for the selective adsorption of DNA-interactive chemicals was evaluated from the competitive adsorbing test using DF and BPN. DF is one of the dioxin derivatives which are considered to be some of the most dangerous compounds.[7] According to our previous study,[11] DF was selectively adsorbed by DNA in the intercalation manner, while BPN was not. The difference in the interaction to DNA is based on their molecular structures. DF is a molecule which has a rigid planar structure, while BPN does not. BPN has two aromatic rings which can freely rotate. Consequently, BPN can not take the rigid planar structure. Thus, the manners of their interaction with DNA become different. In this experiment, four types of gels were evaluated as the selective affinity adsorbent to DF.

The DNA–silica composite ("B-1" and "B-5") was incubated in the DF/BPN mixture solution. Silica ("B-1" without DNA) and AP-silica ("B-5" without

DNA) were then used to estimate the non-specific adsorption. As a result, there was a significant change in the DF/BPN ratio when using "B-5". The DF/BPN ratio in the initial solution was approximately 1, then the ratio after processing changed to approximately 0.8 (Figure 7). In this experiment, about 45 nmol of DF and 34 nmol of BPN were adsorbed to "B-5". In contrast, "B-1" (which did not contain APTES but DNA), silica ("B-1" without DNA) and AP-silica ("B-5" without DNA)) did not induce any significant change in the DF/BPN ratios, and the amounts of adsorbed substances were ranging between 30 and 50 nmol. These results suggested that not only the amount of DNA but another factor would be required in order to efficiently maintain the function of DNA. For decades, many researchers studied the immobilization of proteins, such as various enzymes. They suggested that it is important to understand the various conditions, such as the conformation of the incorporated protein, the dynamics of the protein, and the accessibility of analytes to the protein.[20,25] The knowledge of such parameters would make it possible to optimize the material property so as to maintain the initial function of the proteins. It must also be important for DNA. As shown in the case of "B-1", DNA which has been confined in a rigid silica network did not offer a specific DNA function. It is desirable to reserve enough space around the DNA molecules, thus allowing its natural behavior. Therefore, it must be reasonable that DNA be connected *via* a suitable linker, such as APTES.

CONCLUSIONS

We developed the DNA–silica composite as a novel affinity adsorbent *via* the sol–gel method. Due to the

formation of the silica-based composite, DNA acquired a mechanical toughness and resistance to some denaturation effects. Undesirable precipitation, aggregation and the irreversible collapse of DNA did not occur even under the conditions of high alcohol and salt concentrations. The DNA–silica composite also showed a good resistance to nuclease because of the selective permeation effect due to the molecule size. The DNA–silica composite selectively adsorbed the DNA-interactive chemicals in their aqueous solution. The used composite could be recycled by washing with appropriate solvents. The DNA–silica composite has the potential utility as an effective tool for separating DNA-interactive chemicals, and for environmental clean-up.

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