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# DNA–inorganic hybrid material as selective absorbent for harmful compounds

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ABSTRACT

Double-stranded DNA is one of functional polymers, but the large amounts of DNA sources, such as salmon milt and shellfish gonads, have been discarded as industrial wastes. Therefore, conversion of this discarded DNA to be a useful material would be beneficial to utilize the unique property of DNA. These materials including DNA have been prepared by mixing with the organic polymers, such as alginic acid, collagen, and chitosan. However, since these materials have consisted from entirely organic components, these do not have the mechanical strength for a material. So, we prepared the organic–inorganic hybrid materials by mixing DNA with silane coupling reagents bis(trimethoxysilylpropyl)amine or bis[(3-trimethoxysilyl)propyl]ethylenediamine. These hybrid materials with the flexibility were water-insoluble and resistant to hydrolysis by nuclease. In addition, the mechanical strength of this hybrid material was approximately twice as high as that of DNA without mixing with silane coupling reagents. Furthermore, the double-stranded DNA in the hybrid materials has been maintained in a B-form structure in aqueous solution. Thus, we demonstrated the utilization of DNA as a functional material. As a result, this material could selectively accumulate harmful DNA-intercalating compounds with the planar structure, such as dibenzo-*p*-dioxin, dibenzofuran, and ethidium bromide. Organic–inorganic hybrid material including double-stranded DNA has potential to serve as a useful biomaterial for medical, engineering, and environmental applications.



## 1. Introduction

DNA, one of the most important materials for the genetic process of living organisms, has a double-stranded structure with complemented nucleic acid base pairs [1]. Because double-stranded DNA has highly specific functions, such as the accumulation of DNA-intercalating compounds [2], DNA binding protein [1], endocrine disruptor [3,4], and heavy metal ions [5], it has the potential ability to be used as a functional polymer material [6]. DNA is readily purified from salmon milt or shellfish gonads but large amounts of the DNA-enriched materials have been discarded as industrial wastes around the world. Therefore, the conversion of this discarded DNA to a useful material would be beneficial to utilize the unique properties of DNA.

DNA is a highly water-soluble material. This property has made making it difficult to utilize as a functional material. Therefore, the water-insolubilization of DNA is important for the utilization of DNA as a functional material [3–8]. These water-insoluble DNA materials have been prepared by a cross-linking reaction with UV irradiation, as a composite with a cationic surfactant, or mixing with an organic polymer, such as alginic acid, collagen, chitosan, and artificial polymers [3,4,7–11]. However, these materials have consisted of entirely organic components, therefore they do not have the mechanical strength for a useful material. On the other hand, organic–inorganic hybrid materials with both the properties of the flexibility of an organic material and the mechanical strength of an inorganic material have been attractive as the novel functional materials [12–16], such as for electrical and optical devices, coating materials, and sensors. Additionally, the biological application of organic–inorganic hybrid material has also been reported [17–19]. Therefore, an organic–inorganic hybrid material including double-stranded DNA is interesting and important from the aspect of material, environmental, and biological sciences. As a result, a DNA–silica composite was prepared for the supporting material of a column and used for separating DNA–intercalative chemical and for environmental clean-up [20]. Additionally, the sol–gel reaction using plasmid DNA as a template has been also reported [21,22]. However, in this case, DNA was encapsulated in silica as the inorganic material, and the property, such as flexibility, for organic polymer materials of DNA was not used. Furthermore, DNA-containing organic–inorganic hybrid materials with flexibility and stability have not been reported as far as we know.

In the present study, we prepared a flexible DNA–inorganic hybrid material by mixing DNA with silane coupling reagents

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bis(trimethoxysilylpropyl)amine (SiNSi) or bis[(3-trimethoxysilyl)propyl]ethylenediamine (SiNNSi). These hybrid materials were water-insoluble and resistant to hydrolysis by a nuclease. In addition, the mechanical strength of this hybrid material was approximately twice as high as that of DNA without mixing with silane coupling reagents. Furthermore, the double-stranded DNA in the hybrid materials has been maintained in a B-form structure in aqueous solution. Thus, we demonstrated a utilization of DNA as an environmental material. As a result, this material could selectively accumulate harmful compounds with a planar structure, such as dibenzo-*p*-dioxin, dibenzofuran, biphenyl, and ethidium bromide.

## 2. Experimental

### 2.1. Materials

Double-stranded DNA (sodium salt from salmon milt, molecular weight $>5 \times 10^6$) was obtained from Yuki Fine Chemical Co., Ltd., Tokyo, Japan, and used without further purification. Silane coupling reagents bis(trimethoxysilylpropyl)amine (SiNSi) or bis[(3-trimethoxysilyl)propyl]ethylenediamine (SiNNSi) were purchased from Gelest, Inc., Morrisville, PA. *Micrococcal nuclease* was purchased from Worthington Biochemical Corp., Lakewood, NJ. Ethidium bromide, acridine orange, dibenzo-*p*-dioxin, dibenzofuran, biphenyl, benzophenone, bisphenol A, diethylstilbestrol, tetraethoxysilane (TEOS), and 3-aminopropyltriethoxysilane (APTES) were obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan, Tokyo Kasei Industries Ltd., Tokyo, Japan, or Nacalai Tesque, Inc., Kyoto, Japan. Ultra-pure water (Millipore Corporation, Billerica, MA) was used in all the experiments described.

### 2.2. Preparation and characterization of DNA–inorganic hybrid materials

DNA–inorganic hybrid materials were prepared as follows: DNA aqueous solution (10 mg/ml) and silane coupling reagents, SiNSi or SiNNSi, were immediately mixed by the vortex mixer. This mixed solution was applied onto Teflon® plates and dried at room temperature overnight. These dried-hybrid films were stripped from the plate and stored in water. The amount of DNA in the hybrid material was determined by the following procedure [3,4]: the hybrid material was hydrolyzed with 1 M HCl solution at 100 °C for 1 h and quantitated by absorption at 260 nm using a UV–vis spectrophotometer U-2010 (Hitachi Co., Ltd., Tokyo, Japan).

The stability in an aqueous solution of the hybrid material was confirmed by the following method [3,4]: the hybrid materials (1 mg) were incubated in ultra-pure water (20 ml) for various time intervals. The absorbance at 260 nm of the solution was measured, and the eluted DNA from the hybrid material was determined. The effect of a nuclease on the hybrid material was confirmed by the following method [3]: the hybrid materials were added to 20 ml of 20 mM Tris–HCl buffer containing 5 mM NaCl and 2.5 mM $CaCl_2$ (pH 7.4) in the presence of the nuclease (*Micrococcal nuclease*, 2 units/ml) at 37 °C. The amount of hydrolyzed DNA by the nuclease was measured by the absorption at 260 nm at various time intervals. The swelling ratio of the DNA–inorganic hybrid material was determined by the following test: the hybrid materials were immersed in water–ethanol mixed solvents for 1 min, and the weight of these swelling materials was measured. The swelling ratio was estimated from Eq. (1).

$$\text{Swelling ratio} = \frac{W_S - W_0}{W_0} \quad (1)$$

where $W_0$ and $W_S$ are the initial and swelling weights of the hybrid material, respectively.

### 2.3. Structural analysis of DNA–inorganic hybrid materials

The infrared (IR) absorption spectra of the DNA–inorganic hybrid materials were measured by the attenuated total reflection (ATR) method using a Fourier transform infrared spectrometer FT-IR 8200 (Shimadzu Corp., Kyoto, Japan). The IR spectrum was measured with a resolution of 4 $cm^{-1}$. The structure of the DNA–inorganic hybrid materials in water was measured using a circular dichroism (CD) spectrophotometer. The DNA–inorganic hybrid material was constructed on a quartz plate ($w9.9 \times h40 \times t1\ mm^3$) and covered by another quartz plate [3]. The DNA-sandwiched-quartz plate was put into a normal quartz cell ($w10 \times h40 \times t10\ mm^3$) and immersed in buffer solution (20 mM Tris–HCl, pH 7.4, containing 100 mM NaCl) for more than 8 h. At several times, the buffer solution in the cell was exchanged with new buffer solution to remove the water-soluble DNA from the hybrid material. CD spectra were recorded on a Jasco Model J-820 CD spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan) at 20 °C. The optical path length is ca. 0.1 mm.

### 2.4. Thermal stability of DNA–inorganic hybrid material

The thermal stability of DNA–inorganic hybrid materials was analyzed by thermogravimetric–differential thermal analysis (TG–DTA) (DTG-60, Shimadzu Corp.). The TG–DTA measurement was carried out at a heating rate of 10 °C $min^{-1}$ under a dry nitrogen flow.

### 2.5. Tensile strength of DNA–inorganic hybrid materials

The double-stranded DNA membrane and DNA–23 wt% SiNSi hybrid membrane were cut into $30 \times 4.5\ mm^2$. The thickness of the membrane was 30 μm. The tensile strain and stress were measured using a tensile machine (Autograph AGS-J, Shimadzu Corp.). The measurement condition of the tensile strength was controlled by the laboratory air conditioner and the temperature and humidity conditions were 25 °C and 50 ± 5%, respectively. The initial gauge length of the membrane was 5 mm and the drawing speed was 10 mm $min^{-1}$. The value of tensile strain and stress was expressed as an average of ten measurements.

### 2.6. Accumulation of harmful compounds

Dibenzo-*p*-dioxin, dibenzofuran, biphenyl, bisphenol A, diethylstilbestrol, and benzophenone were used as model harmful compounds, such as endocrine disruptors. The aqueous harmful compound solutions were prepared by the reported method [4]. The accumulation of harmful compounds was confirmed by the following procedure [4]: The DNA–inorganic hybrid material (1 mg) was incubated in the respective aqueous harmful compound solution (10 ml) for 24 h at room temperature. The hybrid materials were then separated from the aqueous solutions. The amount of accumulated compounds was determined by the absorption spectra of the aqueous solutions in the absence or presence of the hybrid material. The aqueous ethidium bromide (10 μM in Tris–HCl buffer, pH 7.4) and acridine orange (5 μM in Tris–HCl buffer, pH 7.4) solutions were used as the intercalating reagent into double-stranded DNA [1,2].

## 3. Results and discussion

### 3.1. Preparation and characterization of DNA–inorganic hybrid materials

The chemical structures of SiNSi and SiNNSi are shown in Fig. 1. SiNNSi has two imino groups in its molecule and indicates a higher

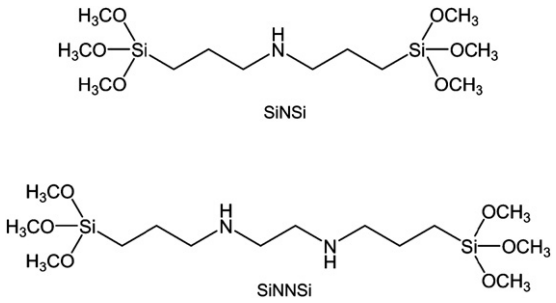


**Fig. 1.** Chemical structures of bis(trimethoxysilylpropyl)amine (SiNSi) or bis[(3-trimethoxysilyl)propyl]ethylenediamine (SiNNSi).

hydrophilicity than SiNSi (see chemical structure in Fig. 1). Generally, the trimethoxysilyl group, $-Si(OCH_3)_3$, hydrolyzes stepwise in water to give the corresponding silanols, which ultimately condense to siloxanes [23]. The hydrolysis of the trimethoxysilyl group is relatively fast, while the condensation reaction of the silanol group is much slower [23]. The mixed solution of double-stranded DNA and silane coupling reagents SiNSi or SiNNSi was applied onto a Teflon® plate and dried at room temperature overnight. The flexibility of the hybrid material decreased with an increase in the mixing ratio of the silane coupling reagent, and at a mixing ratio of >50 wt%, the hybrid material was rigid and like a glass material. At <50 wt%, these DNA–inorganic hybrid materials were flexible and transparent. Fig. 2 shows the photograph of the DNA–23 wt% SiNSi hybrid material in an aqueous ethidium bromide solution. The ethidium bromide molecules were accumulated by the hybrid material; as a result, the hybrid film containing double-stranded DNA was dyed red. In contrast, during incubation in aqueous acridine orange solution, the hybrid material was dyed yellow (data not shown). These phenomena suggest that the DNA in the hybrid material has the ability to accumulate the intercalating reagents, such as ethidium bromide and acridine orange.

Fig. 3(a) and (b), respectively, shows the stability of DNA–SiNSi and DNA–SiNNSi hybrid materials with a different mixing ratio in water. The amount of eluted DNA from the hybrid material increased with the incubation time and reached a constant value at 6 h. This amount of eluted DNA decreased with the increase of the mixing ratio of the silane coupling reagents. Additionally, the stability in water of the DNA–SiNSi hybrid materials was higher than that of the DNA–SiNNSi hybrid material. Especially, the DNA–23 wt% inorganic hybrid material showed not only the water-insolubility but also the high flexibility. Therefore, the DNA–inorganic hybrid material at the 23 wt% has corresponded with our purpose for the water-insolubility of DNA and the flexibility of material. On the other hand, these hybrid materials did not dissolve in an aqueous solution even after incubation in water for up to 1 month. The hybrid materials were stored in ultra-pure water for more than one day to remove the small amount of water-soluble DNA and then used for the further experiments.

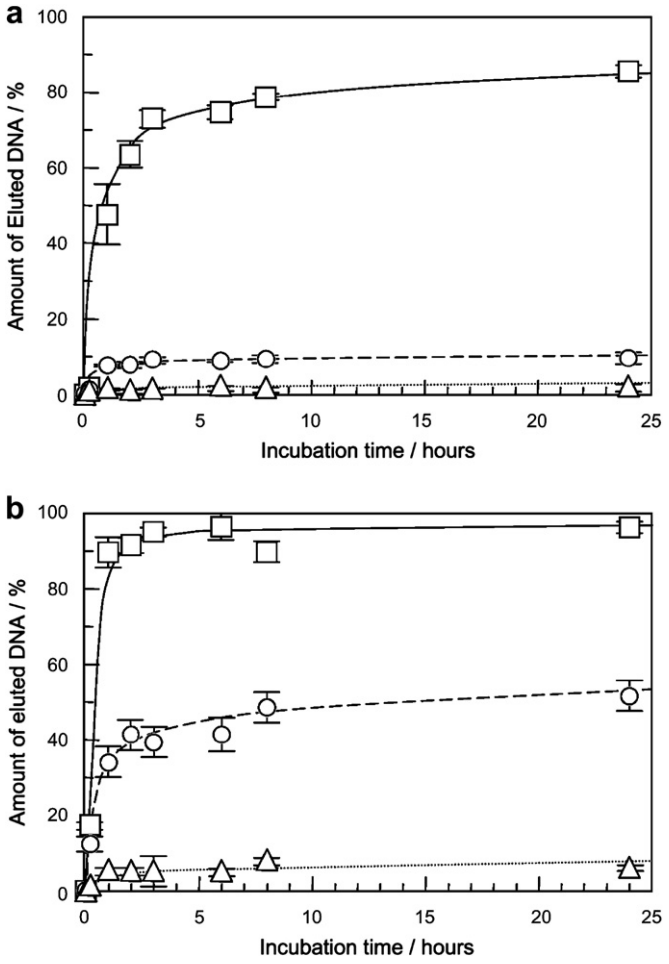


**Fig. 3.** Stability of DNA–inorganic hybrid materials in water. (a) DNA–SiNSi hybrid materials. The mixing ratios of sSiNSi are (□) 9 wt%; (○) 23 wt%; (△) 33 wt%. (b) DNA–SiNNSi hybrid materials. The mixing ratios of SiNNSi are (□) 9 wt%; (○) 23 wt%; (△) 33 wt%. Each value represents the mean of three separate determinations ± standard deviations (SD). Triplicate experiments gave similar results.

**Fig. 2.** Photograph of DNA–23 wt% SiNSi hybrid material in aqueous ethidium bromide solution. The hybrid material was dyed red by the accumulation of ethidium bromide.

Next, we tested the biochemical stability of hybrid materials using *Micrococcal nuclease*, which is one of the DNA-hydrolyzing enzymes [1]. In Fig. 4, (△), (○), and (□), respectively, show the biochemical stability of pure DNA without mixing of a silane coupling reagent, DNA–23 wt% SiNSi, and DNA–23 wt% SiNNSi hybrid materials. The amount of hydrolyzed DNA was determined by the absorbance at 260 nm. The control DNA without mixing of the silane coupling reagents, water-soluble DNA, was completely hydrolyzed in a few minutes. However, the DNA–inorganic hybrid material showed resistance to hydrolysis by the nuclease and was

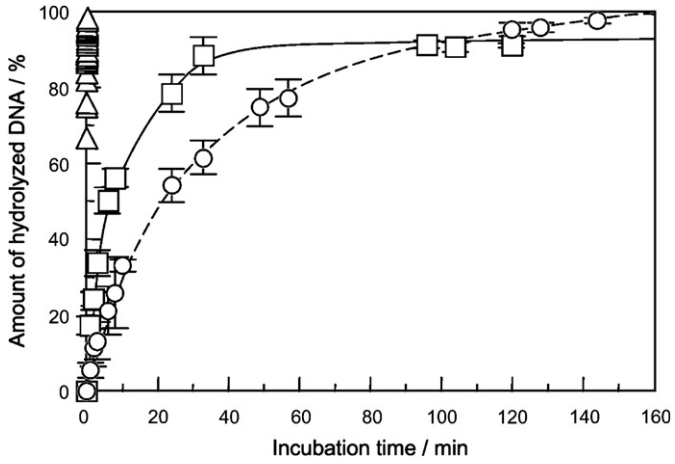


**Fig. 4.** Biochemical stability of DNA–inorganic hybrid material. The hybrid materials were incubated with nuclease (*Micrococcal nuclease*) in aqueous solution. (△), (○), and (□) showed the pure DNA without the mixing of silane coupling reagent, DNA–23 wt% SiNSi, and DNA–23 wt% SiNNSi materials, respectively. Each value in represents the mean of three separate determinations ± SD. Triplicate experiments gave similar results.

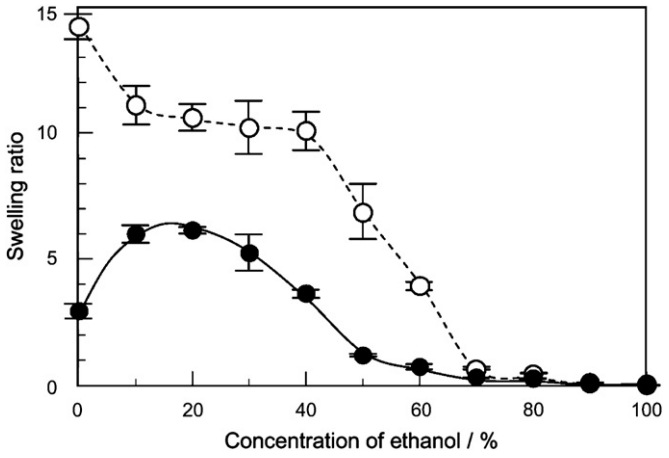


**Fig. 5.** Swelling ratio of DNA–inorganic hybrid materials in water–ethanol mixed solvents. (●) and (○) showed the DNA–23 wt% SiNSi and DNA–23 wt% SiNNSi hybrid materials. Swelling ratio was estimated by Eq. (1). Each value represents the mean of five separate determinations ± SD. Triplicate experiments gave similar results.

completely hydrolyzed at approximately 120 min. Additionally, the DNA–SiNSi hybrid material indicated a higher biochemical stability than the DNA–SiNNSi hybrid material. On the other hand, the biochemical stability of the hybrid material increased with an increase in the mixing ratio of the silane coupling reagent (data not shown). These results indicate that the DNA–inorganic hybrid material is resistant to the nuclease and that the biochemical stability of the DNA can be controlled by the mixing ratio of the silane coupling reagents.

In Fig. 5, (●) and (○), respectively, show the swelling ratio of the DNA–23 wt% SiNSi and DNA–23 wt% SiNNSi hybrid materials in water–ethanol mixed solvent. The swelling ratio of the hybrid material indicated the highest value at <20% ethanol condition and then decreased with an increase in the ethanol content. This result suggests that the hybrid material can encapsulate the much water in its matrix; especially, the SiNNSi hybrid material has a higher affinity for water molecules than the SiNSi hybrid material. In fact, regarding the water-stability of the hybrid materials, the DNA–SiNNSi hybrid material showed a lower stability than the DNA–SiNSi (see Fig. 3(a) and (b)). Furthermore, the difference in biochemical stability of the SiNNSi and SiNSi hybrid materials can be explained as follows: the SiNNSi molecule has more imino groups than the SiNSi molecule. Therefore, the SiNNSi hybrid material in aqueous solution contains more water in its matrix than the SiNSi material, and as a result, it can take in more nuclease into the matrix. In consequence of these phenomena, the SiNNSi material released the hydrolyzed DNA from the matrix to the aqueous solution.

Since tetraethoxysilane (TEOS) or 3-aminopropyltriethoxysilane (APTES) forms a glass-like material by a sol–gel reaction, these silane coupling reagents have been used as the inorganic components of organic–inorganic hybrid materials [17–23]. So, we also prepared a DNA–TEOS or DNA–APTES composite material by mixing DNA and TEOS or DNA and APTES, respectively. However, these materials were rigid and like a glass material without flexibility. The similar result, such as non-flexibility, has been reported in DNA–silica composite material [20]. Generally, the silanol group is condensed to siloxanes by the dehydration [23]. Therefore, in the case of TEOS- or APTES-condensed materials, many parts of silane coupling molecules are connected by the siloxane bonding and constructed the non-flexible three-dimensional network. However, in our research, since SiNSi or SiNNSi molecules have an alkyl chain, these chains can link between the siloxane bondings, and as a result; the inorganic parts in hybrid material have higher degree of freedom than TEOS- or APTES-condensed materials. Therefore, the dipodal silane coupling reagents with the alkyl chain are effective to construct the flexible DNA–inorganic hybrid material. In fact, these molecular designs, such as dipodal silane coupling reagents with the alkyl chain, have been reported for the preparation of proton conducting membrane with the flexibility [24–26].

### 3.2. Structure of DNA–inorganic hybrid materials

The molecular structure of the DNA–inorganic hybrid material was confirmed by infrared (IR) spectrometry with an attenuated total reflection (ATR) prism. Fig. 6 shows the IR spectra of pure DNA, DNA–SiNSi hybrid materials with various mixing ratios of the SiNSi molecule, and pure SiNSi material. The absorbance band at 1000–1200 $cm^{-1}$ is attributed to the stretching vibration of Si–O–Si

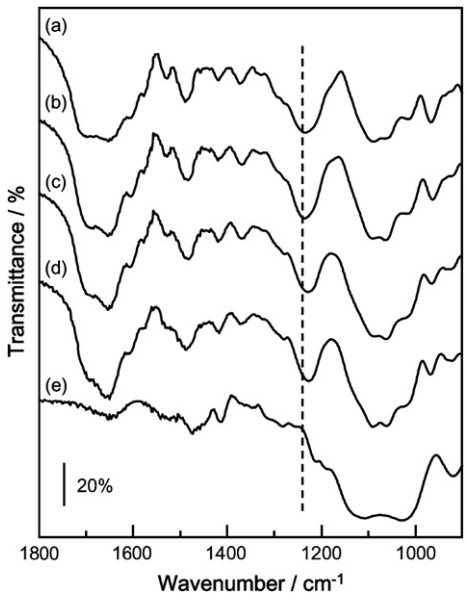


**Fig. 6.** IR spectra of DNA–SiNSi hybrid materials with different mixing ratios of SiNSi. (a) pure DNA; (b) DNA–9 wt% SiNSi hybrid; (c) DNA–33 wt% SiNSi hybrid; (d) DNA–50 wt% SiNSi hybrid; (e) pure polymerized SiNSi materials.

[23,27,28] and SiNSi molecules polymerized by the hydrolysis of the methoxy group in the DNA–SiNSi hybrid material. Therefore, the SiNSi molecules have formed a three-dimensional network structure in the hybrid material. These polymerizations of silane coupling reagents have been well known [23]. Additionally, the absorption band at 1234 $cm^{-1}$, related to the antisymmetric vibration of the phosphate group [5,29–32], was shifted to a lower wavenumber with an increase in the mixing ratio of SiNSi (see dashed line in Fig. 6). These results suggest that the phosphate group of DNA binds to the imino group of the silane coupling reagent by the electrostatic interaction. Similar phenomena have been reported as the electrostatic interaction of a heavy metal ion and DNA [5,33]. Therefore, the composite structure of DNA–silane coupling reagents hybrid material can be explained as follows: The SiNSi or SiNNSi molecules form the polymerized silane coupling reagents in hybrid material. The portion of double-stranded DNA is entrapped into the three-dimensional network of polymerized silane coupling material. Additionally, the phosphate group of DNA binds to the positively charged imino group of the silane coupling reagent by the electrostatic interaction. However, at the >50 wt% condition, many parts of DNA have been entrapped in polymerized silane coupling material and the hybrid material constructed the non-flexible material. As a result, double-stranded DNA and 23 wt% silane coupling reagent construct the water-insoluble and flexible hybrid materials. In addition, the absorption band at 1300–1600 $cm^{-1}$, related to the stretching vibration of the nucleic acid base [5,11,30], did not change. This result suggests that the conformation of the double-strand in the DNA–inorganic hybrid material has been maintained. On the other hand, the sol–gel reaction using DNA as a template has been reported [21,22]. However, in our research, we have not observed the DNA–inorganic hybrid material at the nano-scale by the transmission electron microscopy (TEM) or atomic force microscopy (AFM) and not found the influence of DNA and silane coupling reagents as a template.

We determined the double-stranded DNA structure of the DNA–inorganic hybrid material in aqueous solution by a circular dichroism (CD) spectrophotometer [3]. Fig. 7(a) and (b), respectively, shows the CD spectrum of pure double-stranded DNA and the DNA–23 wt% SiNSi hybrid film in aqueous solution. The CD spectrum of the pure DNA shows the B-form structure, which is the native double-stranded DNA structure in water [1,3,34,35], with positive peaks at 225 nm and 275 nm and negative peak at 245 nm (see Fig. 7(a)). The similar CD spectrum obtained for the DNA–SiNSi hybrid material, i.e., the double-stranded DNA in the hybrid material possessed the B-form structure in aqueous solution. In contrast, the CD spectra of the hybrid material under dry conditions did not suggest the B-form structure. These results suggest that the B-form structure of the DNA–SiNSi hybrid material has been maintained in aqueous solution.

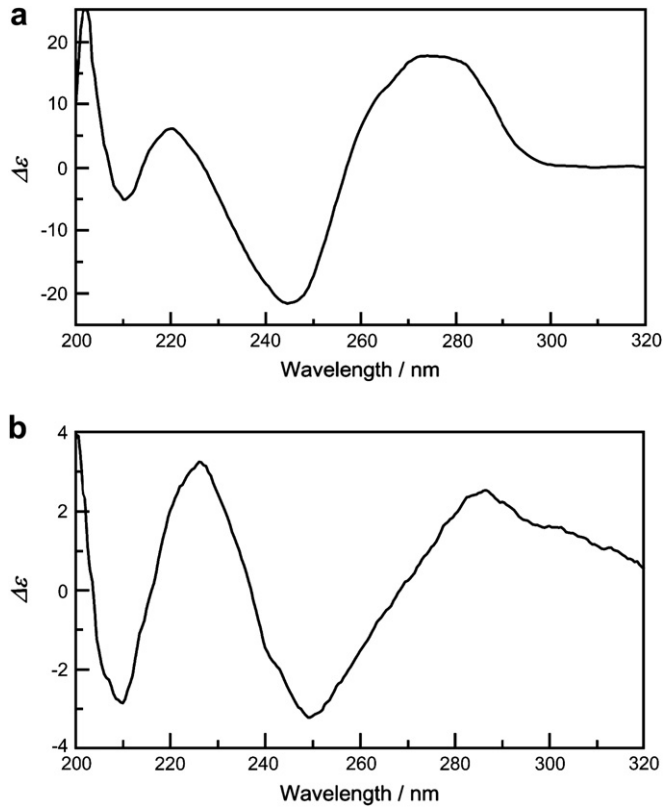


**Fig. 7.** CD spectra of (a) double-stranded DNA and (b) DNA–23 wt% SiNSi hybrid material in aqueous solution. The DNA–23 wt% SiNSi hybrid material was constructed on the quartz plate and covered with another quartz plate.

### 3.3. Thermal property of DNA–inorganic hybrid materials

The thermal stability of the DNA–inorganic hybrid material was analyzed by thermogravimetric–differential thermal analysis (TG–DTA). Fig. 8(a) and (b), respectively, shows the TG and DTA curves of (1) pure DNA (2) DNA–23 wt% SiNSi hybrid material, and (3) DNA–23 wt% SiNNSi hybrid material at a heating rate of 10 °C $min^{-1}$ up to 300 °C under a dry nitrogen flow. Pure DNA had a TG weight loss of approximately 15% at 150 °C (line (1) in Fig. 8(a)). This weight loss is due to the evaporation of water from the DNA material [5]. The DTA analysis of the pure DNA showed an exothermic peak at 232.6 °C due to the pyrolysis [5]. Similar results, such as thermal decomposition at approximately 200 °C, have been reported from differential scanning calorimetry (DSC) measurements [36–38]. The TG weight loss of the DNA–inorganic hybrid material was lower than that of pure DNA, and DTA analysis showed an exothermic peak at

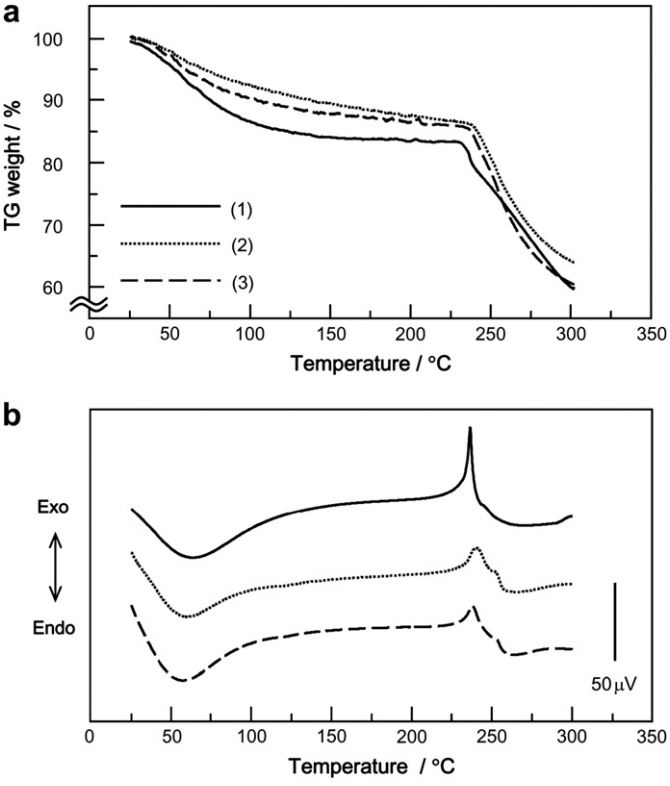


**Fig. 8.** TG (a) and DTA (b) curves of DNA–inorganic hybrid materials with the heating rate of 10 °C $min^{-1}$ under dry nitrogen. (1) pure DNA; (2) DNA–23 wt% SiNSi hybrid; (3) DNA–23 wt% SiNNSi hybrid materials.

approximately 230 °C (lines (2) and (3) in Fig. 8(a) and (b)). These TG weight losses and exothermic peaks are due to the evaporation of water from the hybrid material and pyrolysis of the DNA, respectively. These results indicate that the thermal properties of DNA in the DNA–inorganic hybrid material have been maintained. In contrast, pure polymerized SiNSi and SiNNSi materials did not show the exothermic peak based on pyrolysis at <300 °C.

### 3.4. Tensile strength of DNA–inorganic hybrid materials

The tensile strength of a DNA membrane and DNA–inorganic hybrid materials was measured by the tensile machine. These measurements were demonstrated at 25 °C under 50 ± 5% humidity conditions. The initial length of the membrane was 5 mm and the separation rate was 10 mm min$^{-1}$. The tensile strength was expressed by an average of ten measurements. Fig. 9(a) and (b), respectively, shows the stress–strain curves of the double-stranded DNA membrane and the DNA–23 wt% SiNSi hybrid material. When the stress was loaded on the double-stranded DNA without mixing of inorganic compounds, this membrane was broken at 29 ± 3 MPa. The strain was ca. 15%. As for the DNA–23 wt% SiNSi hybrid material, the breaking stress was 67 ± 5 MPa, and this value was approximately twice as high as that of pure DNA. Additionally, the strain of the DNA–inorganic hybrid material was ca. 11%. These results suggested that the physical strength of the DNA material increases with the addition of the silane coupling reagents, such as SiNSi or SiNNSi. In contrast, the strain decreased slightly with the addition of the silane coupling reagent. This decrease in strain is due to the molecular structure, such as cross-linking by the electrostatic interaction, in the DNA–inorganic hybrid material.

### 3.5. Accumulation of harmful compounds

Previously, we reported the accumulation of harmful compounds with a planar structure, such as dibenzo-*p*-dioxin, dibenzofuran, and benzo[*a*]pyrene, by the UV-irradiated double-stranded DNA [4]. This phenomenon is due to intercalation of the planar molecules into the double-stranded DNA. Therefore, the DNA–inorganic hybrid material with the B-form structure of DNA might also show the accumulation of harmful compounds with the planar structure. We examined the interaction between the DNA–inorganic hybrid material and various harmful compounds, such as dibenzo-*p*-dioxin, dibenzofuran, biphenyl, bisphenol A, diethylstilbestrol, and benzophenone. The DNA–23 wt% SiNSi hybrid material was incubated for 24 h in aqueous solutions of the harmful compound, and then the amounts of compound were determined by measuring the absorption spectra of the solutions. Fig. 10(a) and (b), respectively, shows the absorption spectra of aqueous dibenzofuran and benzophenone solutions in the absence (solid line) and presence (dashed line) of hybrid material. In this case, dibenzofuran and benzophenone have a planar and a non-planar structure, respectively. When the hybrid material was added to the aqueous dibenzofuran solution, the absorbance of the solutions decreased. Namely, ca. 35% of dibenzofuran was accumulated by the DNA–inorganic hybrid material. The absorbance of aqueous benzophenone solution, which has non-planar structure, did not show the decrease and benzophenone was not accumulated. So, we demonstrated the accumulation of various harmful compounds with the planar and non-planar by the DNA–inorganic hybrid material. Fig. 11 shows the molecular structures and the accumulative ratio of various harmful compounds. The planar structure-containing harmful compounds, such as dibenzo-*p*-dioxin and biphenyl, were accumulated by the DNA–inorganic hybrid materials. Especially, ethidium bromide and acridine orange, which are famous intercalators into the double-stranded DNA [1,2,39,40], indicated a high accumulative ratio. In contrast, the hybrid material did not show the affinity to the non-planar structure-containing harmful compounds, such as bisphenol A and diethylstilbestrol.

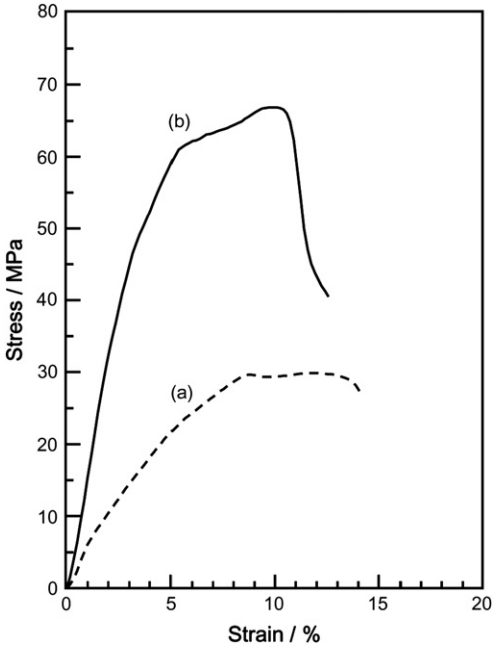


**Fig. 9.** Stress–strain curves of (a) double-stranded DNA and (b) DNA–23 wt% SiNSi hybrid material. These measurements were demonstrated at 25 °C under 50 ± 5% humidity conditions. The initial length of the membrane was 5 mm and the separation rate was 10 mm min$^{-1}$.

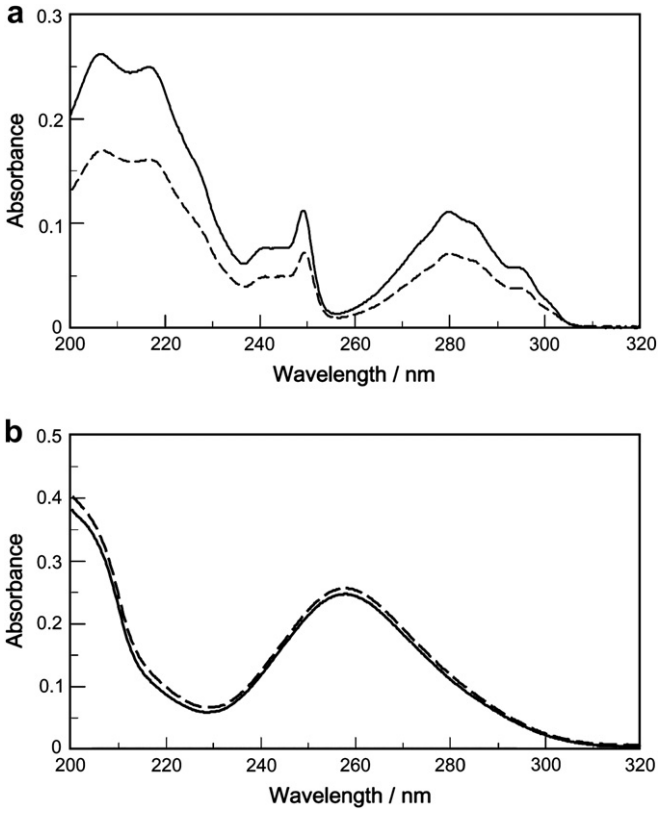


**Fig. 10.** Absorption spectra of aqueous (a) dibenzofuran and (b) benzophenone solutions in the absence (solid line) and presence (dashed line) of the DNA–23 wt% SiNSi hybrid material.

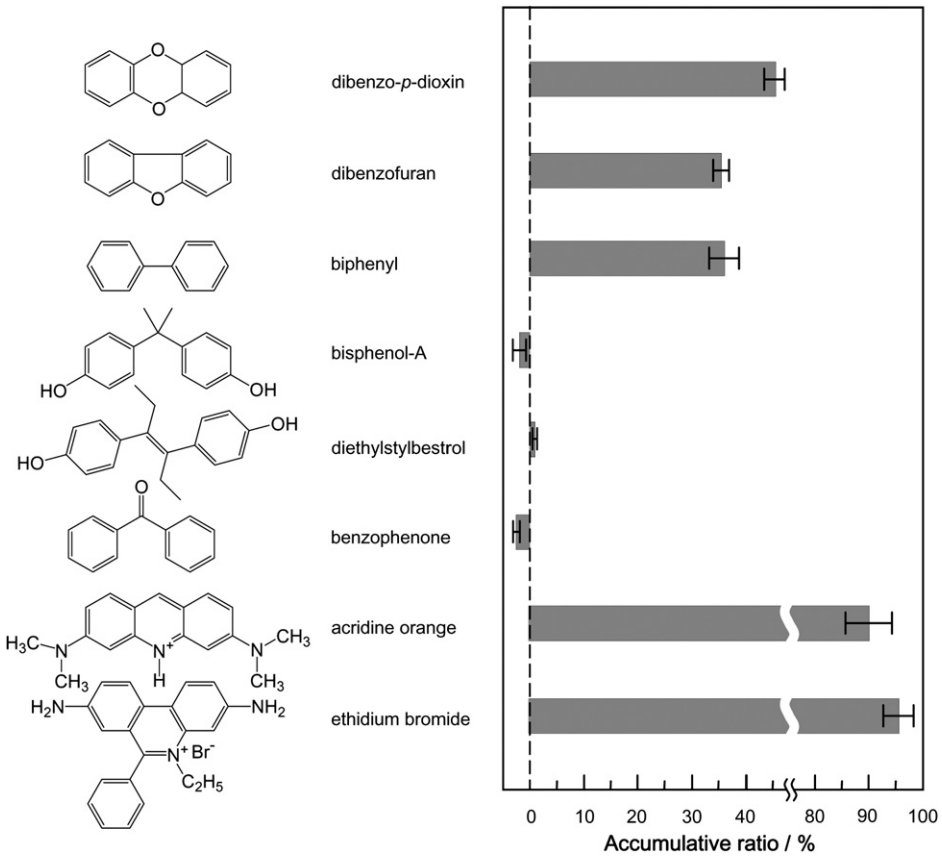


**Fig. 11.** Molecular structures and accumulative ratio of harmful compounds. The accumulative ratio was determined by the absorption spectra in the absence and presence of the DNA–23 wt% SiNSi hybrid material. Each value represents the mean of three separate determinations ± SD. Triplicate experiments gave similar results.

Furthermore, polymerized SiNSi and SiNNSi materials without mixing of DNA did not show the accumulation of harmful compounds. These results suggest that the double-stranded DNA–inorganic hybrid material accumulates the chemical compounds by molecular selectivity.

Dioxin- and PCB-derivatives and benzo[*a*]pyrene, which are designated as endocrine disruptors, with a planar structure have been shown to be DNA-intercalating compounds [4,40–43]. Bisphenol A, diethylstilbestrol, and benzophenone without the planar structure do not intercalate into double-stranded DNA [4,44]. Therefore, the DNA–inorganic hybrid material was not effective for the accumulation of the non-planar structure-containing compounds, such as bisphenol A, diethylstilbestrol, and benzophenone. These double-stranded DNA–inorganic hybrid materials have a potential for the selective removal of dioxin- and PCB-derivatives and benzo[*a*]pyrene, with the planar structure, from human drinking sources or industrial drainage.

## 4. Conclusions

We have prepared a water-insoluble DNA–inorganic hybrid material by mixing DNA with a silane coupling reagent, SiNSi or SiNNSi. This hybrid material was stable in an aqueous nuclease (*Micrococcal nuclease*) solution. In addition, this hybrid material indicated high mechanical strength. Furthermore, the DNA hybrid materials have been maintained in the B-form structure in aqueous solution. Furthermore, this material could selectively accumulate harmful DNA-intercalating compounds, such as ethidium bromide, dibenzo-*p*-dioxin, and dibenzofuran. Though the accumulated amount of the harmful compound by the DNA–inorganic hybrid film is 30–45% and is not so high, these accumulated amounts might be improved by a DNA–immobilized column with the DNA–inorganic hybrid material. These DNA–inorganic hybrid materials have the potential not only for the absorption of harmful compounds but also for engineering, biosensor, biomedical materials, and so on.

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