

UV-Irradiated DNA Matrix Selectively Accumulates Heavy Metal Ions

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We recently demonstrated the preparation of a water-insoluble DNA matrix by UV irradiation. The UV-irradiated DNA matrix selectively accumulated DNA-intercalating compounds and some endocrine disruptors. We evaluated the accumulation of metal ions in the UV-irradiated DNA matrix using a UV-irradiated DNA-film and DNA-immobilized glass beads. When DNA-immobilized glass beads were incubated with an aqueous solution of Hg^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, or Fe^{3+}, the heavy metal ions were accumulated in the DNA-beads. The maximum amounts of the accumulated Hg^{2+}, Cd^{2+}, and Cu^{2+} in the DNA-beads were approximately 0.21, 0.13, and 0.22 mmol per gram of immobilized-DNA, respectively. The amounts of the accumulated Pb^{2+}, Zn^{2+}, and Fe^{3+} were lower than that of Hg^{2+}, Cd^{2+}, and Cu^{2+}. Further, Fourier transform infrared (IR) studies using a UV-irradiated DNA-film with heavy metals suggested that the heavy metal ions interacted with not only the nucleic acid bases but also the phosphate groups. In contrast, the DNA-immobilized glass beads could not accumulate Ca^{2+} and Mg^{2+}. These results suggested that the UV-irradiated DNA could selectively accumulate metal ions. The UV-irradiated DNA matrix has potential utility as a functional material to remove harmful heavy metal ions from contaminated water.

DNA has a unique double-stranded structure and promotes specific functions, such as intercalation or a groove binding interaction of drug reagents.[1,2] These functions due to the double-stranded DNA structure are difficult to mimic using synthetic polymers. In the living body, the binding of metal ions to DNA is important for maintenance of the DNA structure and for the process of DNA replication, repair, and translation.[3] However, heavy-metal ions, such as Hg^{2+}, Cd^{2+}, and Cu^{2+}, were found to induce mismatching of the replication and translation.[4–7]

Although DNA can be extracted and purified from salmon milts and shellfish gonads, large amounts of the DNA sources have been discarded as industrial waste. It is important to find a use for DNA as a functional material for industrial recycling and environmental science. However, DNA is highly water-soluble and biochemically unstable. Overcoming these undesirable properties is important for utilizing DNA as a functional material. DNA-columns,[8,9] DNA-cloth,[10] DNA-films,[11–14] and DNA-gels[15–17] have utilized DNA stabilized by immobilization on a solid support, such as cellulose powder or gold nanoparticles, or by making a stable complex with other polymers, such as cationic amphiphilic lipids, acrylamide, alginic acid, or collagen. Recently, we described the preparation of a water-insoluble DNA matrix with an intermolecular cross-linked structure induced by UV irradiation.[18] The UV-irradiated DNA was nuclease-resistant and possessed a double-stranded DNA structure with the B-form.[18] Further, the UV-irradiated DNA selectively accumulated endocrine disruptors with a planar structure, such as dioxin- and PCB-derivatives and benzo[*a*]pyrene, and harmful DNA intercalating compounds, such as ethidium bromide and Acridine Orange.[10,18,19] The UV-irradiated DNA was suggested to be a useful biomaterial to separate harmful chemical compounds in water. In addition, an Ag^{+}-containing DNA-immobilized nonwoven cellulose fabric showed the antibacterial activity.[10] UV-irradiated DNA has a potential to be applied to medical, engineering, and environmental biomaterials.

In this study, we immobilized double-stranded DNA onto glass beads using UV irradiation. We demonstrated the selective accumulation of harmful heavy-metal ions by the DNA-immobilized glass beads. The selective interaction of the metal ions with the UV-irradiated DNA matrix was also examined using infrared absorption spectroscopy.

Experimental

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. Porous MIS-20 glass beads (particle size; 1–2 mm, pore size; < 120 μm) were purchased from Tokyorikakikai Co., Ltd., Tokyo, Japan. 3-Aminopropyltriethoxysilane was obtained from Nacalai Tesque, Inc, Kyoto, Japan. The metal-ion salts were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. Ultra-pure water (Nanopure Infinity Basic, Barnstead | Thermolyne, Dubuque, IA) was used in all of the experiments.

Preparation of DNA-Immobilized Glass Beads. Amino groups were incorporated onto the surface of porous glass beads by a silane coupling method, as follows:[20–23] porous glass beads (100 mg) were heated in a 2 mL mixture of 30% hydrogen peroxide water and concentrated sulfuric acid (30:70, v/v) at 70 °C for 30 min. The porous glass beads were rinsed with distilled water (5 mL × 10 times) and dried at room temperature for 2 h. The

beads were immersed in 1 mL of 1% 3-aminopropyltriethoxysilane/hexane solution for 2 h and rinsed with hexane (2 mL × 5 times), then dried at room temperature for 2 h. A modification of the 3-aminopropyltriethoxysilane onto the support was confirmed by XPS 7000 X-ray photoelectron spectroscopy (Rigaku Co., Tokyo, Japan).

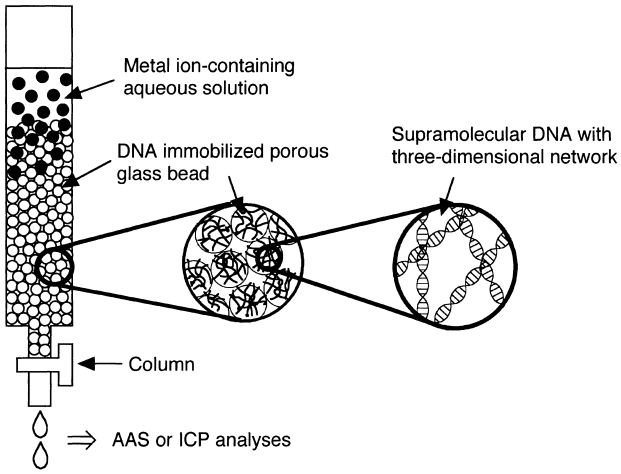
An aqueous DNA solution (300 µl, 10 mg/mL) was applied onto the amino group modified glass beads (100 mg) and dried overnight at room temperature, then irradiated with UV light (R-52G, Ultraviolet Inc., Upland, CA) at 254 nm for 6 h. The intensity of the UV irradiation was 5600 µW/cm^2 at the sample position. The UV-treated glass beads were rinsed with distilled water (10 mL × 7 times) to remove any non-immobilized DNA and the DNA-immobilized glass beads were stored in water at room temperature. The amount of immobilized DNA was determined by the following procedure:[19] after DNA-immobilized glass beads (100 mg) were hydrolyzed with a 1 M hydrochloric acid solution (5 mL) at 100 °C for 1 h, the amount of DNA in the aqueous solution was determined from the absorbance at 260 nm using the U-2000A UV-Vis Spectrophotometer (Hitachi Co., Ltd., Tokyo, Japan). The immobilized amount was approximately 6 mg per gram of glass beads.

Accumulation of Metal Ions by DNA-Immobilized Glass Beads. Mercury(II) chloride, cadmium chloride, cadmium sulfate, cadmium nitrate, lead(II) chloride, zinc chloride, zinc sulfate, zinc nitrate, copper(II) chloride, copper(II) sulfate, copper(II) nitrate, iron(III) chloride, magnesium chloride, magnesium nitrate, and calcium chloride were dissolved in ultrapure water. DNA-immobilized porous glass beads (100 mg) were put into respective metal ion solutions (10 mL) and incubated at room temperature for 24 h. The DNA-immobilized glass beads were removed from the aqueous solution and then the amount of metal ions in the aqueous solution was analyzed using a SPCA-626D atomic absorption spectrophotometer (Shimazu Co., Ltd., Kyoto, Japan) and an ICAP-575(II) inductivity coupled argon plasma atomic emission spectrophotometer (Nippon Jarrell-Ash Co., Ltd., Kyoto, Japan).

Preparation of DNA-Immobilized Porous Glass Bead Column (Scheme 1). DNA-immobilized porous glass beads (approximately 5 g) were dried overnight at room temperature and packed into a column (ϕ 10 mm, total length 300 mm, Kiriyama Glass Co., Ltd., Tokyo, Japan). The length of the mobile phase was approximately 200 mm. This DNA-immobilized column was rinsed with distilled water (10 mL × 10 times) to remove the non-immobilized DNA or the contamination, and the DNA-immobilized glass beads in the column were stored in water.

Accumulation of Metal Ions by DNA-Immobilized Porous Glass Bead Column. An aqueous solution containing a mixture of $CdCl_2$, $ZnCl_2$, $CuCl_2$, and $MgCl_2$ was applied to a DNA-immobilized glass-bead column. The amount of applied solution was approximately 50 mL. The concentration of the mixed-metal ion solution was 2 or 5 ppm. The flow rate of the DNA-immobilized glass-bead column was approximately 0.5 mL/min. The accumulation of these metal ions was quantified by atomic absorption spectroscopy for the solutions before and after application to the column.

Fourier Transform Infrared Spectroscopy of UV-Irradiated DNA-Film with Metal Ion. The water insoluble DNA-film with UV irradiation was prepared by a method described in a previous paper.[18] UV-irradiated DNA-film (0.5 mg) was immersed in 1 mL of an aqueous $CuCl_2$ or $MgCl_2$ solution at room temperature for 24 h. The UV-irradiated DNA-film with the metal ions was rinsed with ultrapure water (10 mL × 3 times) and dried overnight at room temperature on a Teflon® plate. The infrared absorption spectra for these DNA-films with metal ions were measured by KBr methods using a RT-210 Fourier transform infrared spectrometer (Horiba Co., Ltd., Kyoto, Japan). The IR spectrum was measured with a resolution of 4 cm^{-1}.



Scheme 1. Schematic illustration of DNA-immobilized porous glass bead column and the three-dimensional supramolecular network of UV-irradiated DNA matrix. The amount of removed metal ions was quantified by AAS and ICP analyses for their aqueous solution before and after being applied to the DNA-immobilized column.

Results and Discussion

Accumulation of Metal Ion by DNA-Immobilized Glass Beads. DNA-immobilized porous glass beads were prepared as previously described,[19] and then incubated with aqueous solutions of Hg^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}, Zn^{2+}, Fe^{3+}, or Mg^{2+}. After 24 h, the DNA-immobilized glass beads were removed and the amount of metal ions in the solutions was determined using an atomic absorption spectrophotometer (AAS) or an inductivity coupled plasma (ICP) atomic emission spectrophotometer.

When DNA-immobilized porous glass beads were incubated with various concentrations of $CdCl_2$, Cd^{2+} was accumulated in the DNA-beads (Fig. 1(a)). When the concentration of Cd^{2+} was increased, the amount of accumulated Cd^{2+} increased and reached a constant value at 3 µmol (Fig. 1(a), closed circle). The maximum amount of accumulated Cd^{2+} was 0.14 mmol per gram of the immobilized-DNA. A similar result was obtained when DNA-immobilized beads were incubated with $CdSO_4$ (Fig. 1(a), closed triangle). The porous glass beads alone did not accumulate any Cd^{2+}. In addition, the maximum amount of accumulated Cd^{2+} by amino-group modified glass beads was less than 10% of that of Cd^{2+} by DNA-immobilized glass beads. However, the surface of the DNA-immobilized beads was completely covered with a DNA matrix. These results indicated that Cd^{2+} was accumulated into the UV-irradiated DNA matrix.

We also examined the accumulation of metal ions using $Cu(NO_3)_2$ and $Zn(NO_3)_2$. Cu^{2+} and Zn^{2+} were also accumulated in DNA-immobilized porous glass beads (Fig. 1(b)). The

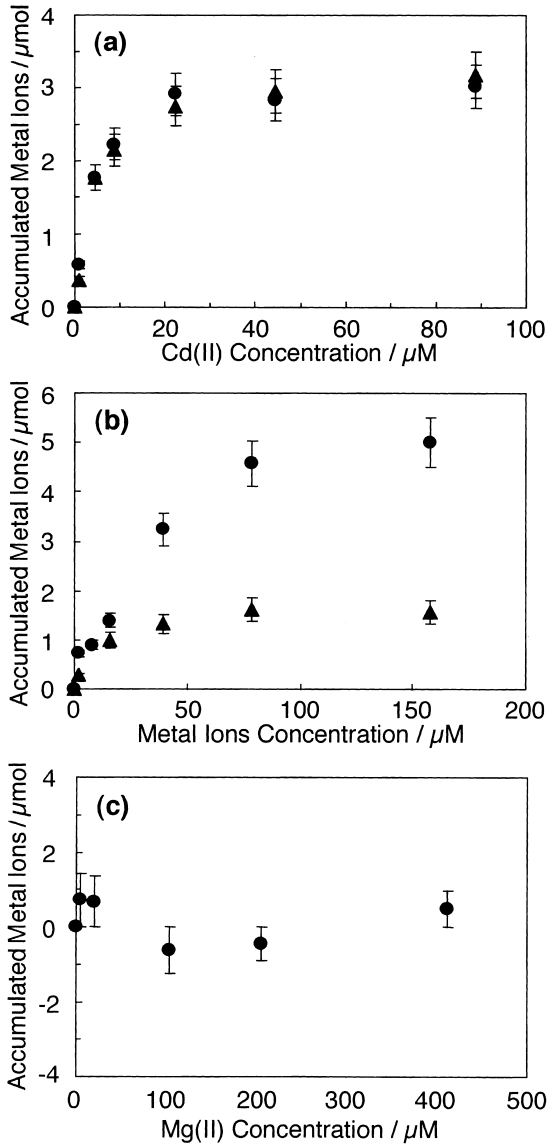


Fig. 1. The accumulation of metal ions by the DNA-immobilized glass beads. DNA-immobilized porous glass beads were placed in an aqueous solution of metal ions and incubated for 24 h. The accumulations of these metal ions were confirmed by AAS. (a) ●, $CdCl_2$; ▲, $CdSO_4$. (b) ●, $Cu(NO_3)_2$; ▲, $Zn(NO_3)_2$. (c) $MgCl_2$. Error bars represent ±10% error.

maximum amounts of accumulated Cu^{2+} and Zn^{2+} were approximately 0.20 and 0.11 mmol per gram of the UV-irradiated-DNA, respectively. The accumulated amount of Zn^{2+} was lower than that of Cd^{2+}. These chloride and sulfate salts were also similarly accumulated in DNA-immobilized porous glass beads. When $MgCl_2$ was incubated with DNA-immobilized porous glass beads, Mg^{2+} was not accumulated in the beads (Fig. 1(c)). When other magnesium salts, such as $Mg(NO_3)_2$, were also incubated with the DNA-immobilized porous glass beads, Mg^{2+} was not accumulated (data not shown). These results indicated that the UV-irradiated DNA accumulated metal ions with metal-specific affinity.

Figure 2 shows the maximum amount of accumulated Hg^{2+}, Pb^{2+}, and Fe^{3+}. All of the heavy metal ions were highly accumulated in DNA-immobilized porous glass beads. The efficiency for the accumulation of the various metal ions by UV-irradiated DNA glass beads was indicated as follows: $Mg^{2+} \ll Zn^{2+} < Pb^{2+} < Cd^{2+} < Fe^{3+} < Hg^{2+} \approx Cu^{2+}$. Previously, the relative affinity of various metal ions for phosphate groups and nucleic acid bases on DNA have been suggested as follows: $Mg^{2+} < Co^{2+} < Ni^{2+} < Mn^{2+} < Zn^{2+} < Cd^{2+} < Cu^{2+} < Ag^{+} < Hg^{2+}$.[24,25] These affinity orders for metal ions are similar, suggesting that the phosphate group and nucleic acid bases have potential to interact with the metal ions.[24,25]

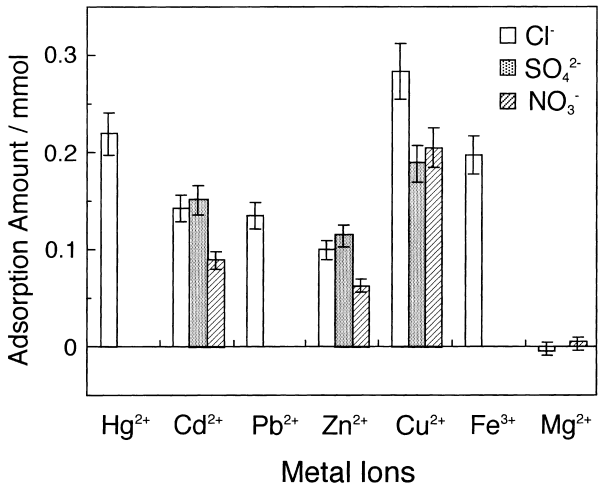


Fig. 2. The amount of various accumulated metal ions by UV-irradiated DNA (1 g). The counter ion of metal ions was chloride, sulfate, and nitrate ions. The accumulation of Cd^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Fe^{3+}, and Mg^{2+} was confirmed by AAS. The amount of Hg^{2+} was quantified by ICP. $HgSO_4$, $Hg(NO_3)_2$, $PbSO_4$, $Pb(NO_3)_2$, $Fe_2(SO_4)_3$, and $Fe(NO_3)_3$ were not examined since they were insoluble in water. Error bars represent ±10% error.

Accumulation of Metal Ions in DNA-Immobilized Glass Bead Column. We next examined the accumulation of metal ions in UV-irradiated DNA from a multi-component solution. DNA-immobilized porous glass beads were packed into a column (Scheme 1). A mixed-metal ionic solution of Cd^{2+}, Cu^{2+}, Zn^{2+}, and Mg^{2+} was applied to a DNA-immobilized porous glass bead column, and the accumulation of metal ions from the multi-components solution was analyzed by AAS. Cd^{2+} and Cu^{2+} were highly accumulated in the DNA-immobilized column, and the removal efficiency from the multi-component solution was more than 80% (Fig. 3). Although Zn^{2+} was also accumulated in the column, the removal efficiency was lower than that of Cd^{2+} and Cu^{2+}. In contrast, Mg^{2+} was not accumulated in the DNA-immobilized column. A similar result was obtained for an aqueous solution containing 5 ppm mixed-metal ions (data not shown). These results indicated the affinity of metal ions under multi-component conditions, as follows: $Mg^{2+} < Zn^{2+} \ll Cd^{2+} < Cu^{2+}$. This relative affinity order was consistent with previous reports.[24,25]

Fourier Transform Infrared (IR) Spectrometry Analysis for the Interaction of the UV-Irradiated DNA with Metal Ions. Next, we evaluated the interaction of UV-irradiated DNA with metal ions using infrared (IR) absorption spectros-

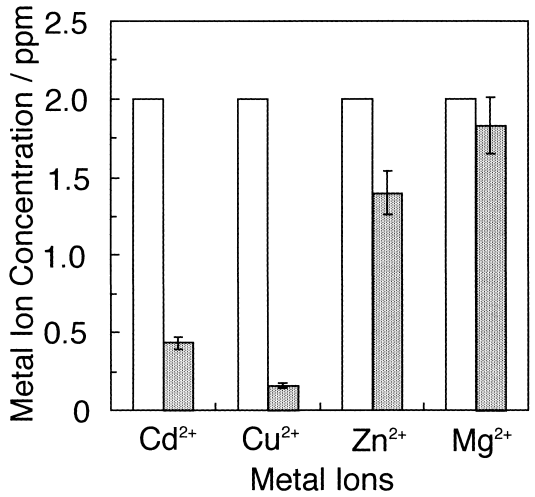


Fig. 3. Accumulation of metal ion from multi-component solution by DNA-immobilized porous glass bead column. The demonstrated solution was the mixed aqueous solution of Cd^{2+}, Cu^{2+}, Zn^{2+}, and Mg^{2+}. The concentration of each aqueous metal ion was 2 ppm. The amount of removed metal ions was quantified by the AAS analysis for their aqueous mixed-metal ion solutions, before (□) and after (■) being applied to the column.

copy. UV-irradiated DNA-film was incubated in an aqueous solution containing $CuCl_2$ or $MgCl_2$ for 24 h and rinsed with water and dried overnight. The DNA-films were analyzed by IR spectrometry using the KBr method. Figure 4 shows the IR spectrum of the UV-irradiated DNA-film with or without Cu^{2+}. The absorption band at 1500–1700 cm^{-1}, related to the nucleic acid bases,[26–28] changed when Cu^{2+} was added. This result indicated that Cu^{2+} interacted with the nucleic acid base in the UV-irradiated DNA-film. In addition, the absorption band at 1234 cm^{-1}, an antisymmetric vibration of the phosphate group,[26–31] was shifted to a lower wavenumber along with an increase in the Cu^{2+} concentration. The absorption band at 1090 cm^{-1}, a symmetric stretching vibration of the phosphate group,[26–30] decreased relatively with an increase in the Cu^{2+} concentration (Fig. 4(c)). The absorption band at 1060 cm^{-1}, a symmetric stretching vibration of ribose,[26–29] increased relatively. These results indicated that Cu^{2+} had a strong interaction with not only the nucleic acid bases but also the phosphate groups. The IR spectrum of UV-irradiated DNA-film with or without Mg^{2+} is shown in Fig. 5. The absorption bands for the nucleic acid base (1500–1700 cm^{-1}) and phosphate group (1234 cm^{-1}) were not affected in the presence of Mg^{2+} (Figs. 5(a) and (c)).

Figure 6 shows the wavenumber of the absorption band for the phosphate group at various concentrations of Cu^{2+} and Mg^{2+}. When the Cu^{2+} concentration increased, the absorption band of the phosphate group (1234 cm^{-1}) shifted to a lower wavenumber and reached constant value at approximately 1215 cm^{-1}. In contrast, the wavenumber of the absorption band for the phosphate group was not changed in the presence of various concentrations of Mg^{2+}. These results indicated that Cu^{2+} interacted with the phosphate groups in the UV-irradiated DNA matrix, while Mg^{2+} did not. These results suggest

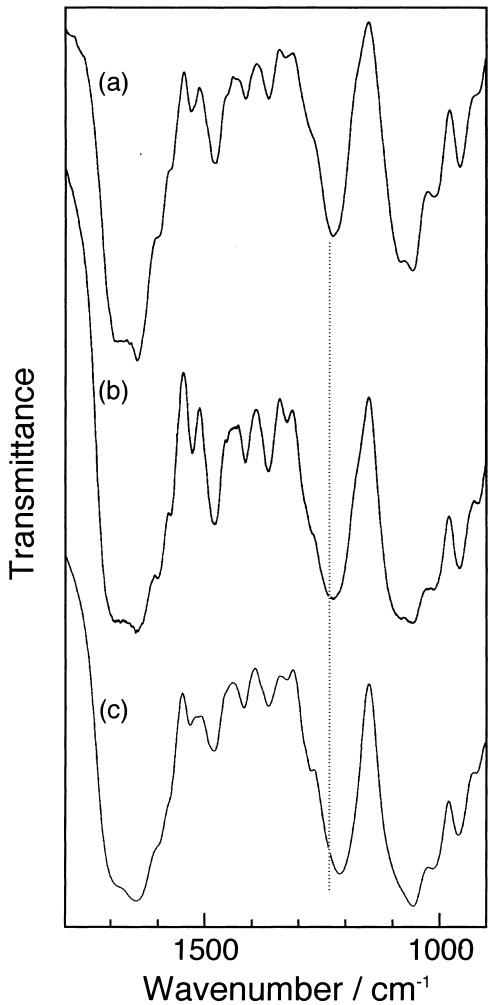


Fig. 4. IR spectrum (KBr method) of the UV-irradiated DNA-film with or without Cu^{2+}. The UV-irradiated DNA-film was incubated in aqueous $CuCl_2$ solution. This DNA-film was rinsed with water and dried. The concentrations of the aqueous Cu^{2+} solutions were (a), 0 μM; (b) 4.1 μM; (c) 410 μM.

that the phosphate groups are involved with the accumulation mechanism of metal ions into the DNA-matrix.

Cu^{2+} has been reported to strongly bind with inter-nucleic acid bases compared to phosphate groups.[24–26] Mg^{2+} has also been reported to bind with the phosphate groups more strongly than the nucleic acid bases.[24,25,32] However, the ratio of the amount for the maximum accumulated-Cu^{2+} and nucleic acid base pair was less than 1, and the accumulation mechanism of metal ions can not be explained only by binding to the nucleic acid base. In fact, the IR spectrum of the DNA-film with $CuCl_2$ significantly changed the vibration of the nucleic acid base having an absorption band at 1500–1700 cm^{-1} as well as that of the phosphate group with the absorption band at 1234 cm^{-1} (Figs. 4 and 6). Furthermore, our measurements with Mg^{2+} did not observe a large change in the absorption band for the nucleic acid base at 1500–1700 cm^{-1} and the phosphate group at 1234 cm^{-1} (Figs. 5 and 6). Therefore, we propose that the heavy-metal ion selectively interacted with the UV-irradiated DNA matrix through the phosphate groups. The UV-

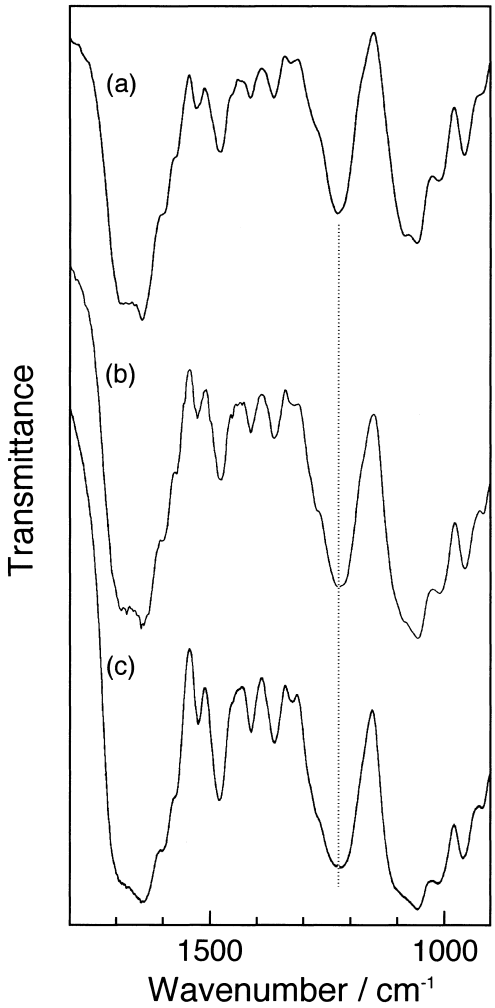


Fig. 5. IR spectrum (KBr method) of the UV-irradiated DNA-film with or without Mg^{2+}. The UV-irradiated DNA-film was incubated in aqueous $MgCl_2$ solution. This DNA-film was rinsed with water and dried. The concentrations of the aqueous Mg^{2+} solutions were (a), 0 μM; (b) 1.6 μM; (c) 160 μM.

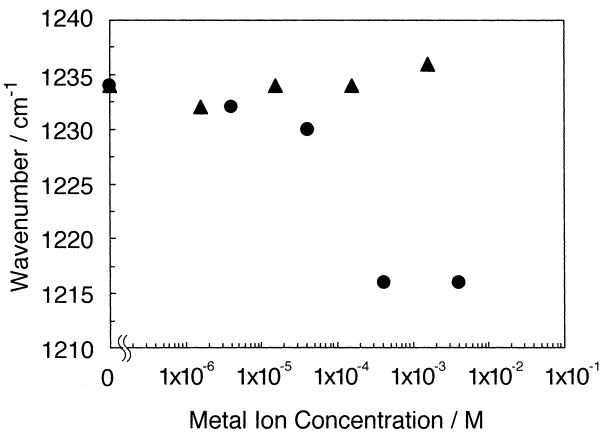


Fig. 6. The shift in transmittance peak for the phosphate group at 1234 cm^{-1} with or without Cu^{2+} and Mg^{2+}. ●, aqueous $CuCl_2$ solution; ▲, aqueous $MgCl_2$ solution.

irradiated DNA has constructed a three-dimensional supramolecular matrix by an intermolecular cross-linking reaction.[18] The UV-irradiated DNA matrix has maintained a B-form structure,[18] and the heavy-metal ion binds to the nucleic acid base. In addition, the DNA matrix may have a chance to form a coordination pocket surrounded by some phosphate groups. Therefore, the heavy-metal ions are accumulated in the UV-irradiated DNA through not only binding to the inter-nucleic acid base, but also trapping into the coordination pocket of the phosphate groups.

In conclusion, we have converted DNA into a functional material and described its applications. DNA-immobilized beads were found to accumulate heavy metal ions, such as Hg^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, and Fe^{3+}. However, it could not accumulate Ca^{2+} and Mg^{2+}. These results suggested that DNA-immobilized glass beads could selectivity accumulate heavy metal ions. In addition, we proposed that the selectivity for metal ions is due to the coordination pocket, which is surrounded by some phosphate groups in a three-dimensional supramolecular DNA-matrix. The DNA-immobilized supports may have potential utility as a biomaterial, such as a material to remove harmful heavy metal ions from contaminated water.

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