Available online at www.sciencedirect.com
SCIENCE DIRECT
Polymer 46 (2005) 10102–10112
polymer
www.elsevier.com/locate/polymer

Preparation of novel bio-matrix by the complexation of DNA and metal ions

Masanori Yamada[1], Miho Yokota, Michiko Kaya, Shuya Satoh, Benjamas Jonganurakkun, Motoyoshi Nomizu[2], Norio Nishi*

Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Sapporo 060-0810, Japan

Received 26 January 2005; received in revised form 3 June 2005; accepted 25 July 2005
Available online 15 August 2005

Abstract

Large amounts of DNA-enriched biomaterials, such as salmon milts and shellfish gonads, are discarded as industrial waste around the world. We could convert the discarded DNA into a novel bio-matrix by the hybridization of DNA and metal ions, such as Al^{3+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Cu^{+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}. These water-insoluble DNA-metal ion matrices could be created in various desirable forms, such as a gel, capsule, film, or fiber. DNA-Al^{3+} matrices were found to maintain a B-form DNA structure, which was the native double-stranded DNA structure in water. The DNA-Al^{3+} fiber showed flexibility with the molecular orientation in the direction of drawing. When a DNA-Cu^{2+} matrix was incubated in an aqueous hydroquinone or ascorbic acid solution, benzoquinone or dehydroascorbic acid was produced, respectively, by the oxidative effect of Cu^{2+} in the DNA-metal ion matrix. These results suggest that metal ions in the DNA-metal matrix maintained the oxidative function. The water-insoluble DNA-metal ion matrices may have a potential utility as a functional bio-material, such as an antibacterial, oxidative, bio-sensor, and ion conductive materials.

Keywords: Bio-matrix; DNA-metal ion matrices; Water-insoluble DNA fiber

1. Introduction

DNA, the most important genetic material of living organisms [1], can easily be purified from either salmon milts or shellfish gonads, which are generally discarded as industrial waste around the world. DNA is a natural product and is safe for humans and for the environment. In addition, double-stranded DNA has highly specific functions [1], such as the accumulation of compounds into its helical structure [2], the complementary binding of nucleic acid bases [3], and the stacked aromatic bases as the electron transfer medium [4]. Utilization of the double-stranded DNA as a functional material has various advantages, e.g. adsorption materials of harmful compounds [5–11], biomedical materials [12–14], electrical [15,16], or optical materials [17]. Additionally, DNA has also been considered as a nanotechnological material [18,19]. However, DNA is highly water-soluble and biochemically unstable. These properties make it difficult to utilize DNA as a functional material.

The water-insolubilization procedures of DNA have used the covalent immobilization on a solid support, such as cellulose powder [20] or gold [21] nanoparticles, or complexation with other polymers, such as alginic acid [9], collagen [22], cationic amphiphilic lipids [15] or acrylamide [23]. However, these insolubilizing methods were sometimes hard to maintain the full original DNA function because the disappeared of charge or the transformation of the DNA structure by the electrostatic composite formation with other large molecules. So, we reported the preparation of the three-dimensional supramolecular DNA matrix with the intermolecular cross-linking induced by UV irradiation [5–8]. The UV-irradiated

* Corresponding author. Address: Laboratory of Bio-Material Chemistry, Division of Bioscience, Graduate School of Environmental Earth Science, Hokkaido University, Kita-10, Nishi-5, Kita-ku Sapporo 060-0810, Japan. Tel.: +81 11 7062256; fax: +81 11 7062256.
E-mail address: nishin@ees.hokudai.ac.jp (N. Nishi).
[1] Present address: Energy Technology Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba, Ibaraki 305-8568, Japan.
[2] Present address: School of Pharmacy, Tokyo University of Pharmacy and Life Science, Tokyo 192-0392, Japan.

0032-3861/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2005.07.086

double-stranded DNA had water-insoluble and nuclease-resistant properties [5]. Furthermore, the UV-irradiated DNA maintained a B-form DNA structure, which was the native double-stranded DNA structure in water. However, this method had to irradiate UV light ($\lambda = 254$ nm), which was not safe for the human body or the environment, to induce the cross-linking reaction.

In the living body, metal ion binding to DNA is important for the maintenance of the DNA structure and for DNA replication, repair, and transcription [24]. Some metal ions, such as Fe^{2+}, Cu^{2+}, Zn^{2+}, Hg^{2+}, and Cd^{2+}, strongly bind to not only the phosphate group but also the nucleic acid base in the center of the double stranded structure by the electrostatic interaction and the coordination bonding [24–26]. Therefore, metal ion binding to DNA may be used for a novel cross-linking reagent. Additionally, metal ion has also oxidative or reducible properties. Furthermore, these functions can control under the pH, thermal, and electric potential conditions. So, the metal ion-containing DNA matrix can be used as novel bio-/electric-matrices.

In this study, we prepared novel water-insoluble bio-matrices by the hybridization of aqueous DNA and metal ion solutions, such as Al^{3+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Cu^{+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}. These DNA-metal ion matrices were found to be an extremely stable in water. The DNA-Al^{3+} matrices possess the native double-stranded DNA structure. Additionally, the DNA-metal ion fibers, prepared by drawing the liquid/liquid interface, had a molecular orientation in the drawing direction. Furthermore, the matrices have maintained the oxidative function based on the property of metal ion.

2. Experimental section

2.1. Materials

Double-stranded DNA (sodium salt from salmon milt, purity >98%, molecular weight $1–5 \times 10^6$) was obtained from Yuki Fine Chemical Co. Ltd, Tokyo, Japan, and used without further purification. The water-soluble metal ion salts, such as copper(II) chloride, copper(I) chloride, aluminum chloride, zinc(II) chloride, cadmium(II) chloride, manganese(II) chloride, nickel(II) chloride, vanadium(III) chloride, yttrium(III) chloride, lanthanum(III) chloride, iron(II) chloride, iron (III) chloride, chromium(III) chloride, indium(II) chloride, silver(I) nitrate, ruthenium(II) chloride, titanium(IIII) chloride, cobalt(II) chloride, gold(III) chloride, platinum(IV) chloride, sodium chloride, lithium chloride, potassium chloride, calcium chloride, barium chloride, and magnesium chloride were purchased from Wako Pure Chemical Industries, Ltd, Osaka, Japan. The MES buffer solution and GelStar [27] were purchased from Dojindo Co., Kumamoto, Japan, and FMC Bioproducts, Rockland, ME, respectively. GelStar stain can be used for fluorescent detection of both double-stranded and single-stranded DNAs, oligonucleotide, and RNA [27]. Ultra-pure water (Nanopure Infinity Basic, Barnstead/Thermolyne, Dubuque, IA) was used in all the experiments.

2.2. Preparation of DNA-metal ion matrix

Aqueous DNA (100 μl, 0.01–10 mg ml^{-1}) and various metal ion (100 μl, 0.001–1 M or 1.7 μM–1.7 mM) solutions were mixed on a glass plate (Matsunami Glass Ind., Ltd, Osaka, Japan). The gelation of the aqueous DNA and metal ion solutions was detected by the construction of a water-insoluble gel. These DNA-metal ion matrices were rinsed with pure water (5 ml×5 times) to remove the DNA and metal ion which were not gelled, and then stored in water. The amount of DNA in the DNA-metal ion matrix was determined by the following procedure: The DNA-metal ion matrix was dissolved by incubation with aqueous EDTA solution (10 mM) for 24 h. GelStar solution was added to the dissolved DNA-metal ion matrix solution and the fluorescence emission at 525 nm was determined using a F-4500 fluorescence spectrophotometer (Hitachi Co. Ltd, Tokyo, Japan) with excitation at 493 nm. The concentrations of DNA were quantified using the fluorescence intensity of the dissolved sample. The amount of metal ion in the dissolved DNA-metal ion matrix solution was analyzed using a SPCA-626D atomic absorption spectrophotometer (Shimazu Co. Ltd, Kyoto, Japan).

In contrast, we used the DNA-Mg^{2+} mixed material for the water-soluble DNA-metal ion complex. The preparation of this material is as following: Aqueous DNA and magnesium chloride solutions were mixed in beaker and stirred by the magnetic stirrer. This mixed solution was clear without the precipitation. This solution was cast on the teflon plate and dried overnight at room temperature. This dried DNA-Mg^{2+} mixed material was used for the sample of thermal analysis (Section 2.5).

2.3. Preparation of DNA-metal ion fiber [28]

An aqueous metal ion solution (0.01 M) was put on the DNA aqueous solution (10 mg ml^{-1}) without mixing and the DNA-metal ion matrix at the liquid/liquid interface was drawn from the liquid interface by tweezers at the pulling rate of approximately 10 mm s^{-1}. These DNA-metal ion fibers were rinsed with pure water (50 ml×5 times) and dried under an air or nitrogen flow at room temperature. The DNA-metal ion fiber was observed using an optical or polarization microscope (BX51, Olympus Optical Co. Ltd, Tokyo, Japan) with two polarizing filters.

2.4. Scanning electron microscope (SEM) images

The DNA-metal ion fibers were dried in a vacuum chamber for 3 h. The fiber breaking was performed in liquid nitrogen. The dried fibers were coated with gold at 10 mA,

1 kV for 5 min using an ion sputter (JFC-1000, JEOL, Ltd, Tokyo, Japan). The surface and cross-section profiles were observed using a scanning electron microscope (SEM) (S-2400, Hitachi, Co. Ltd, Tokyo, Japan) with an accelerating voltage of 15 kV.

2.5. Characterization of DNA-metal ion matrix

The stability of the DNA-Cu^{2+} matrix in water was measured by the following procedure: The DNA-Cu^{2+} matrix was incubated in 50 mM MES buffer solution, pH 7.0, at room temperature during several hours. The time course of absorbance at 260 nm was measured using a UV–vis spectrophotometer U-2000A (Hitachi Co. Ltd, Tokyo, Japan) and the eluted DNA from the DNA-Cu^{2+} matrix was estimated from the calibration curve. The effect of EDTA on the DNA-Cu^{2+} matrix was demonstrated by the following method. The DNA-Cu^{2+} matrix was incubated with an aqueous EDTA solution (10 mM or 50 mM, 50 mM MES buffer solution (pH 7.0)) at room temperature during several hours. The eluted DNA from the DNA-Cu^{2+} matrix was determined by the fluorescence emission with GelStar. The thermal stability of the DNA-metal ion matrices was analyzed by thermogravimetric–differential thermal analysis (TG–DTA) (TG–DTA 2000S, Mac Sciences Co. Ltd, Yokohama, Japan). The TG–DTA measurement was carried out at the heating rate of 10 °C min^{-1} under a dry-nitrogen flow.

2.6. Structure of DNA-metal ion matrix

The structure of the DNA-metal ion matrix was investigated using a circular dichroism (CD) spectrophotometer. The matrices were prepared by the mixing of DNA and the metal ion solutions on a quartz plate (w11×h40×t1 mm^3) and then covered by another quartz plate [29]. The optical path length was 0.1 mm. The CD measurements were performed under the constant DNA concentration condition and the absorption at 260 nm was approximately two at the quarts cell with the optical path length of 0.1 mm. The CD spectra were recorded using a Jasco Model J-720 CD spectropolarimeter (Japan Spectroscopic Co. Tokyo, Japan) at 20 °C with five integrations. The infrared absorption spectra of the DNA-metal ion matrices were measured by KBr methods using a Fourier transform infrared spectrometer RT-210 (Horiba Co. Ltd, Kyoto, Japan). The IR spectrum was measured with the resolution of 4 cm^{-1}. The mixing ratio (r value) of DNA-metal ion complex was indicated by

$$ar = \frac{\text{[molar concentration of metal ion]}}{\text{[molar concentration of DNA (nucleotide)]}}$$

2.7. Oxidation of hydroquinone and ascorbic acid

p-Hydroquinone and ascorbic acid were used as the reducing reagent. *p*-Hydroquinone (5 μM) was incubated with the DNA-Cu^{2+} matrix in 50 mM MES buffer solution (pH 5.0) at room temperature. The amount of *p*-hydroquinone oxidized by the DNA-Cu^{2+} matrix was quantified by the absorbance at 246 nm. The amount of oxidized ascorbic acid was determined by absorbance at 265 nm. The *p*-Hydroquinone and ascorbic acid solutions in the absence of the DNA-metal ion matrix were used for the control of the natural oxidation.

3. Results and discussion

3.1. Preparation of DNA-metal ion matrix

The DNA-metal ion matrix was prepared by mixing of aqueous DNA and the metal ion solutions. The construction of the matrix was detected by the formation of a water-insoluble gel. Table 1 shows the screening for the construction of the matrices by the hybridization of DNA and the metal ion solutions at various concentrations. When the highly concentrated solutions of DNA (10 mg ml^{-1}) and metal ions (1 M), such as Al^{3+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+}, Y^{3+}, La^{3+}, In^{2+}, and Cd^{2+}, were mixed, the water-insoluble DNA-metal ion matrices were immediately produced by the metal ion-assisted cross-linking (denoted by open circles in Table 1). Fig. 1(a) and (b) shows a photograph of the DNA-Al^{3+} and DNA-Cu^{2+} matrices on a glass plate, respectively. These DNA-metal ion matrices were an elastic material and were not broken by pinching

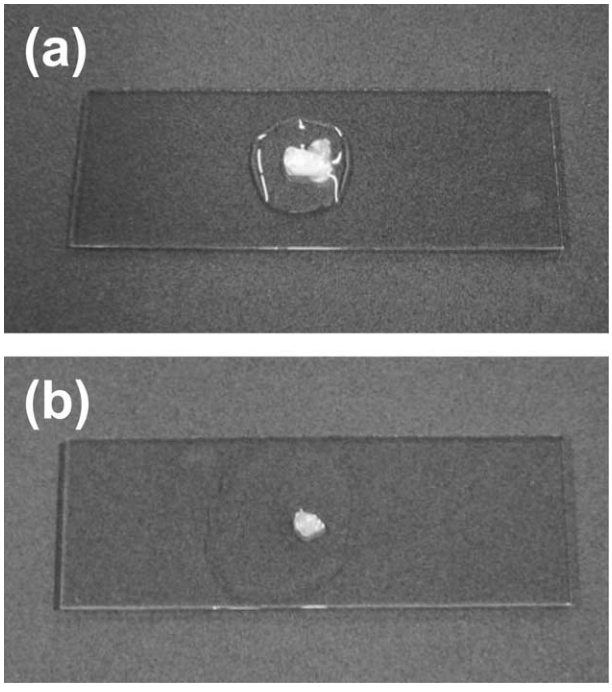

Fig. 1. Photographs of water-insoluble DNA-Al^{3+} matrix (a) and DNA-Cu^{2+} matrix (b) on glass plate. These matrices were prepared by the hybridization of aqueous DNA and aluminum chloride or copper(II) chloride solutions.

Table 1
Construction of matrix by the hybridization of DNA and metal ion at various concentrations

Metal ion	Concentration of metal ion (M)	Concentration of DNA (mg ml^{-1})			
		10	1	0.1	0.01
Cu^{2+}	1	○	△	×	×
	0.1	○	×	×	×
	0.01	○	×	×	×
	0.001	×	×	×	×
Cu^{+}	1.7 (mM)	○	×	×	×
	0.17 (mM)	×	×	×	×
	0.017 (mM)	×	×	×	×
	0.0017 (mM)	×	×	×	×
Al^{3+}	1	○	×	×	×
	0.1	○	×	×	×
	0.01	○	×	×	×
	0.001	△	×	×	×
Zn^{2+}	1	○	△	△	×
	0.1	○	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Cd^{2+}	1	○	×	×	×
	0.1	○	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Mn^{2+}	1	△	×	×	×
	0.1	△	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Ni^{2+}	1	△	×	×	×
	0.1	△	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
V^{3+}	1	△	×	×	×
	0.1	△	×	×	×
	0.01	△	×	×	×
	0.001	×	×	×	×
Y^{3+}	1	○	△	×	×
	0.1	○	△	×	×
	0.01	○	△	×	×
	0.001	△	△	×	×
La^{3+}	1	○	△	×	×
	0.1	○	△	×	×
	0.01	○	△	×	×
	0.001	△	△	×	×
Fe^{2+}	1	○	×	×	×
	0.1	△	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Fe^{3+}	1	○	×	×	×
	0.1	△	×	×	×
	0.01	△	×	×	×
	0.001	×	×	×	×
Cr^{3+}	1	○	△	×	×
	0.1	○	△	×	×
	0.01	△	△	×	×
	0.001	×	×	×	×
In^{2+}	1	○	×	×	×
	0.1	○	×	×	×
	0.01	○	×	×	×
	0.001	×	×	×	×
$AgNO_3$	1	△	×	×	×
	0.1	△	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Ru^{2+}	1	△	×	×	×

(*continued on next page*)

Table 1 (*continued*)

Metal ion	Concentration of metal ion (M)	Concentration of DNA (mg ml^{-1})			
		10	1	0.1	0.01
	0.1	△	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Ti^{4+}	1	+	+	×	×
	0.1	+	×	×	×
	0.01	+	×	×	×
	0.001	×	×	×	×
Co^{2+}	1	△	×	×	×
	0.1	△	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
$HAuCl_3$	1	+	+	×	×
	0.1	×	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
H_2PtCl_6	1	+	+	×	×
	0.1	+	+	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Na^+	1	×	×	×	×
	0.1	×	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Li^+	1	×	×	×	×
	0.1	×	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
K^+	1	×	×	×	×
	0.1	×	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Ca^{2+}	1	×	×	×	×
	0.1	×	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Ba^{2+}	1	×	×	×	×
	0.1	×	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×
Mg^{2+}	1	×	×	×	×
	0.1	×	×	×	×
	0.01	×	×	×	×
	0.001	×	×	×	×

○, Water-insoluble DNA-metal ion matrix; △, liquid with high viscosity; ×, no construction; +, precipitation.

with tweezers. The elasticity of the matrices decreased by the decrease of concentration of the DNA (1–0.1 mg ml^{-1}) and metal ion (0.1–0.01 M) solutions and produced a liquid with a high viscosity (denoted by open triangles in Table 1). These results suggest that the concentration of DNA solution is more important for the construction of insoluble DNA-metal ion matrices than the ratio of the DNA and metal ion. When DNA and alkali metal or alkali earth metal ions, such as Mg^{2+}, Ca^{2+}, Ba^{2+}, Na^+, K^+, and Li^+, solutions were mixed, the water-insoluble matrices were never produced (denoted by cross marks in Table 1). These results suggest that alkali and alkali earth metal ions do not so strongly bind to DNA as it constructs the water-insoluble matrix. These interactions of DNA and various metal ions have been consistent with our previous reports [8]. In contrast, the mixing of Ti^{4+}, $HAuCl_3$, and H_2PtCl_6 produced a precipitation (denoted by plus marks in Table 1). This reason is as following: the aqueous solution of $TiCl_4$, $HAuCl_3$, and H_2PtCl_6 indicate the acidity, as a result, the molecular weight of DNA decreases by the hydrolysis and constructs the water-insoluble precipitation but not the water-insoluble DNA-metal ion gel.

An insoluble spherical droplet could be formed when the aqueous DNA solutions were dropped into metal ion solutions. Fig. 2(a) and (b) shows the spherical droplets of the DNA-Al^{3+} and DNA-Cu^{2+} matrices in distilled water,

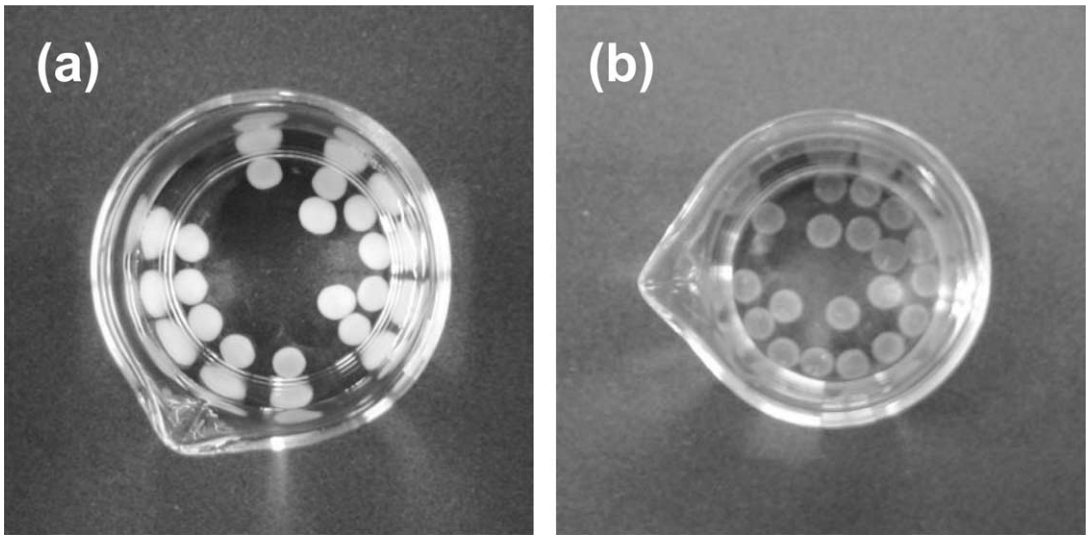

Fig. 2. Photographs of DNA-metal ion capsules in distilled water. These capsules were prepared by the dropwise addition of DNA solution into aqueous metal ion solution. (a) DNA-Al^{3+} capsules; (b) DNA-Cu^{2+} capsules.

respectively. These spherical capsules were stable in water and were not broken by pinching with finger or magnetic stirring. The surface of the capsules was dyed red when these spherical capsules were incubated in an aqueous ethidium bromide solution, a well-known DNA binding reagent [2]. These results suggest that the outside surface of the droplet is coated with the DNA-metal ion matrices and the inside of the capsule is filled with the DNA solution. On the other hand, the converse spherical capsule, of which the inside material is filled with the metal ion solution, can be prepared by the addition of the metal ion solution into the DNA solution.

When an aqueous aluminum chloride solution was carefully applied on an aqueous DNA solution without mixing, a film of the DNA-Al^{3+} matrix was formed at the liquid/liquid interface. This film was continuously produced at the interface by drawing the film and the DNA-Al^{3+} fiber was prepared (Fig. 3(a)). These DNA-Al^{3+} fibers were flexible and did not snap, even if rounded (Fig. 3(b)). Fig. 3(c) and (d) are showed the non-polarized and polarized light microscopic images of DNA-Al^{3+} fiber, respectively. The image of the DNA-Al^{3+} fiber can be observed under the crossed nicols condition. These images under the crossed nicols condition were obtained in all parts of the DNA-Al^{3+} fiber. Therefore, DNA-metal ion fiber has an ordered structure with a molecular orientation at the drawing direction. Additionally, this fiber under the crossed nicols condition showed the core structure (see the arrow in Fig. 3(d)). This core structure in fiber has been also reported at other materials [28] and the inside and outside of DNA-metal ion fiber has been constructed by different components. We think that the core in fiber has been constituted by the metal ion of high concentration. On the other hand, a similar phenomenon, such as the construction of fiber at the liquid/liquid interface, was confirmed at other metal ion, which constructs the water-insoluble DNA-metal ion matrix.

Fig. 4 shows the scanning electron microscopic (SEM) images of the DNA-metal ion fibers. The arrows in Fig. 4(a) and (c) indicate the drawing direction of the fibers at the liquid/liquid interface. The structure of the DNA-Al^{3+} fiber had a fibril structure in the drawing direction (Fig. 4(a)) and its surface morphology was smooth (Fig. 4(b)). The cross-section profile of the fiber showed a layer structure (Fig. 4(b) insert), which was not the annual ring type but the pile type. The DNA-Cu^{2+} fiber could be prepared by a similar method. However, the DNA-Cu^{2+} fiber tended to break and was not flexible like a DNA-Al^{3+} fiber. The structure of the DNA-Cu^{2+} fiber also showed the fibril structure (Fig. 4(c)), and its surface morphology was rough (Fig. 4(d)). The cross-section profile was smooth (Fig. 4(d) insert). The difference of morphology between DNA-Al^{3+} and -Cu^{2+} fibers are considered as following: The aluminum ion of trivalent induces the divergence of fiber and Al^{3+} fiber was bundled at the parallel by the drawing process. As a result, the cross section of DNA-Al^{3+} fiber showed the layer structure.

3.2. Characterizations of DNA-metal ion matrix

We examined the stability of the DNA-Cu^{2+} matrix in water (Fig. 5). The DNA-Cu^{2+} matrix was incubated in water and the absorbance at 260 nm of the solution was measured at various time intervals. The eluted DNA from the matrix was not detected at the incubation time of 500 h (closed circles in Fig. 5). The eluted Cu^{2+} was also not detected by the atomic absorption spectroscopic (AAS) analysis (data not shown). These results suggest that the DNA-Cu^{2+} matrix is extremely stable in water. Next, we tested the chemical stability of the DNA-Cu^{2+} matrix in an aqueous ethylenediaminetetraacetic acid (EDTA) solution, one of the strong chelating reagents. When the DNA-Cu^{2+}

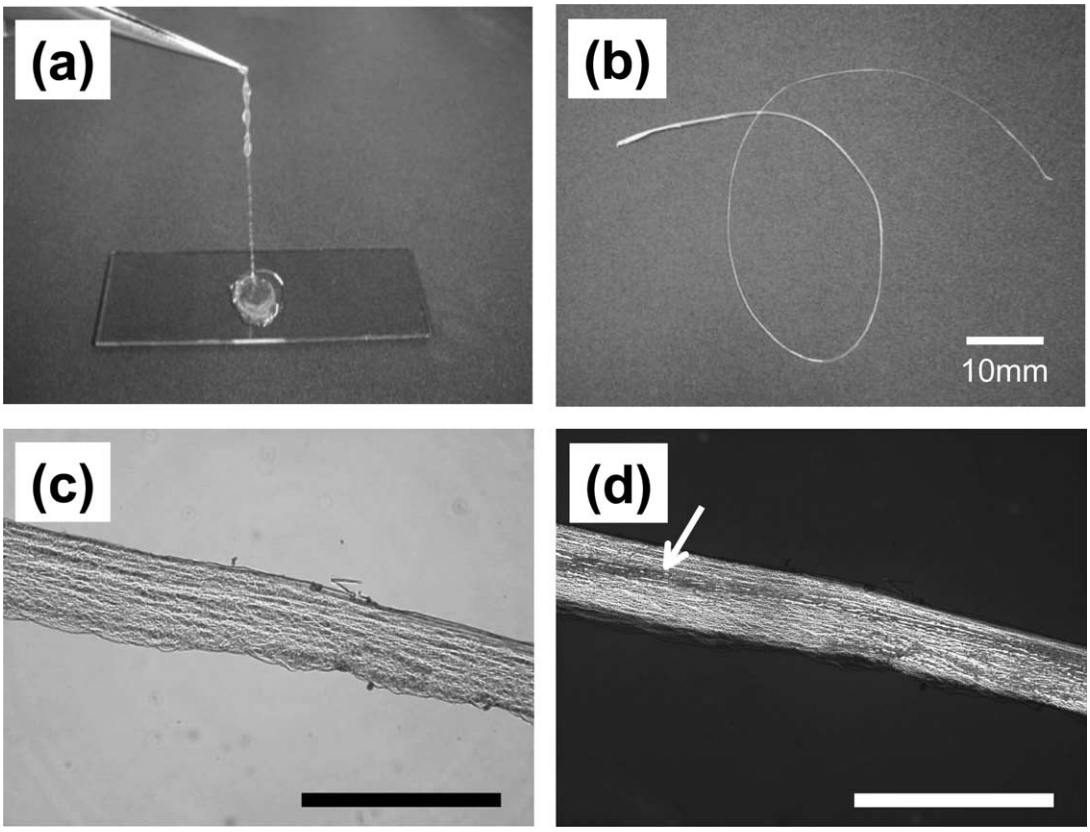

Fig. 3. Photographs of DNA-Al^{3+} fiber and its optical microscopic images. (a) the continuous reaction at the interface of aqueous DNA and Al^{3+} solutions; (b) the flexible DNA-Al^{3+} fiber after drying; (c) and (d) the non-polarized and polarized light microscopic images, respectively. A scale bar is 500 μm. The arrow indicates the core structure.

matrix was incubated with 10 mM EDTA, the matrix gradually dissolved and completely disappeared in 6 h (closed squares in Fig. 5). The dissolving rate of the matrices depended on the concentration of the aqueous EDTA solution and became faster with the increasing of EDTA concentration (closed triangles in Fig. 5). These results indicate that the DNA-metal ion matrices are constructed by the metal ion-assisted cross-linking through the metal-ion-binding to DNA. The DNA-Al^{3+} matrices also showed the identical characteristics (data not shown).

3.3. Thermal characterization of DNA-metal ion matrix

The thermal stability of the DNA-metal ion matrices was analyzed by thermogravimetric–differential thermal analysis (TG–DTA). Fig. 6(a) and (b) shows the TG and DTA curves of pure DNA and DNA-metal ion matrices with a heating rate of 10 °C min^{-1} up to 300 °C under a dry nitrogen flow, respectively. Pure DNA without the metal ion had a TG weight loss of approximately 10% at 150 °C (line (1) in Fig. 6(a)). This weight loss is due to the evaporation of water from the DNA material. The DTA analysis of the DNA indicated an exothermic peak at 205.9 °C by the pyrolysis. Similar results, such as thermal decomposition at approximately 200 °C, have been reported from differential scanning calorimetry (DSC) measurements [30]. The TG weight loss of the DNA-Mg^{2+} mixed material which did not form the water-insoluble matrix, was larger than that of pure DNA, and the DTA analysis showed an exothermic peak at approximately 200 °C (line (2) in Fig. 6(a) and (b)). The large TG weight loss of DNA-Mg^{2+} material is due to the evaporation of hydration water, which increases by the addition of Mg^{2+}. In contrast, the exothermic peak at approximately 200 °C disappeared with the addition of Cu^{2+} or Al^{3+} (lines (3) and (4) in Fig. 6(b)). Furthermore, the TG weight loss of the DNA-Cu^{2+} or DNA-Al^{3+} matrices below 150 °C was identical with the pure DNA (lines (3) and (4) in Fig. 6(a)). We think that this disappearance of exothermic peak at approximately 200 °C is due to the thermal-stabilization of DNA-metal ion matrices by the cross-linking of DNA molecules through the metal ion, such as Cu^{2+} or Al^{3+}. In fact, these thermal stabilizations by the strong interaction between polymer and metal ion have been reported at metal-chelating polymers [31]. These results indicate that the thermal property of DNA in water-insoluble DNA-metal ion matrices has been maintained.

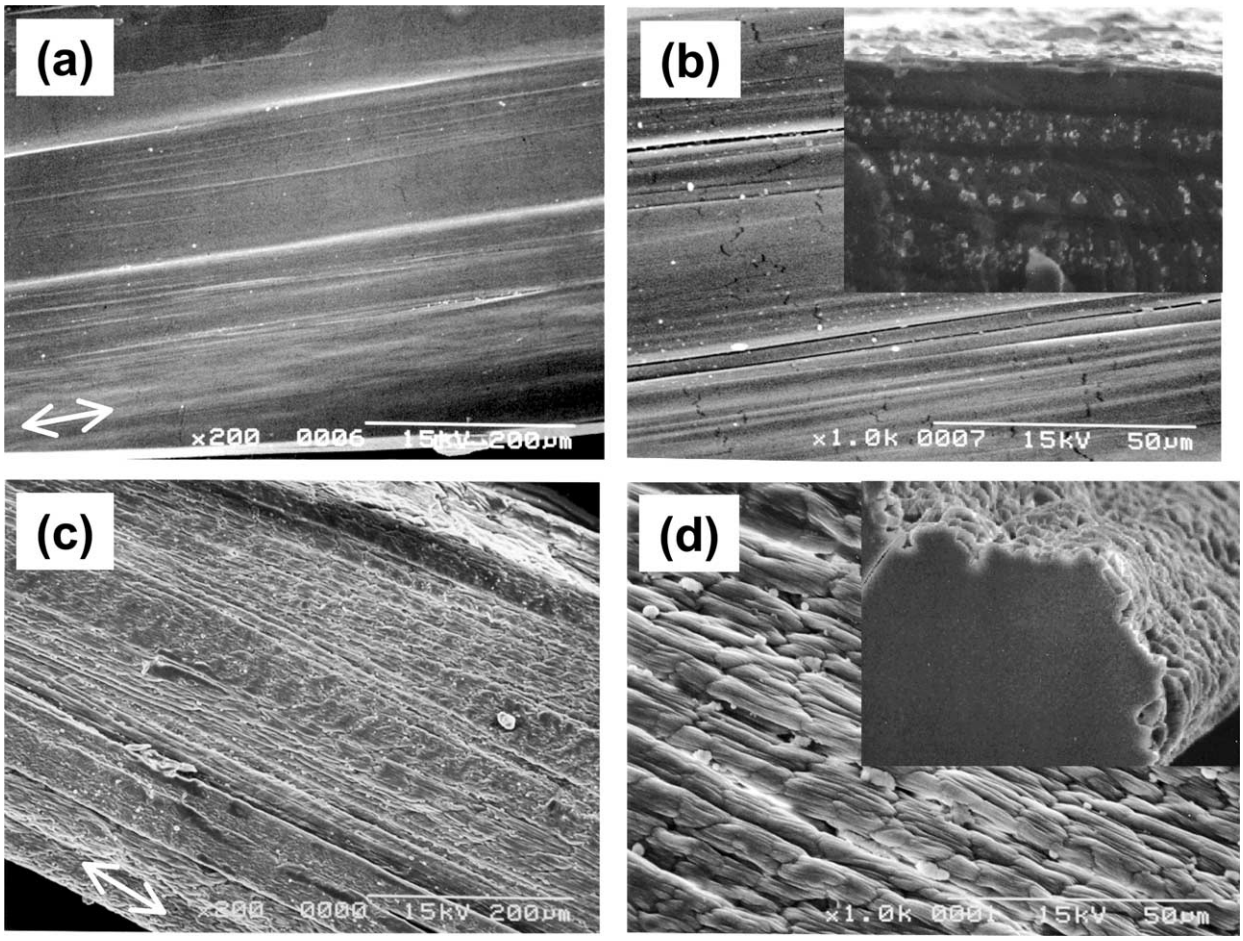

Fig. 4. Scanning electron microscopic (SEM) images of DNA-metal ion fiber. (a) DNA-Al^{3+} fiber (×200); (b) DNA-Al^{3+} fiber (×1000); (c) DNA-Cu^{2+} fiber (×200); (d) DNA-Cu^{2+} fiber (×1000). Inserts indicate the cross-section profile of DNA-metal ion fiber (×1000). Arrows indicate the direction of drawing.

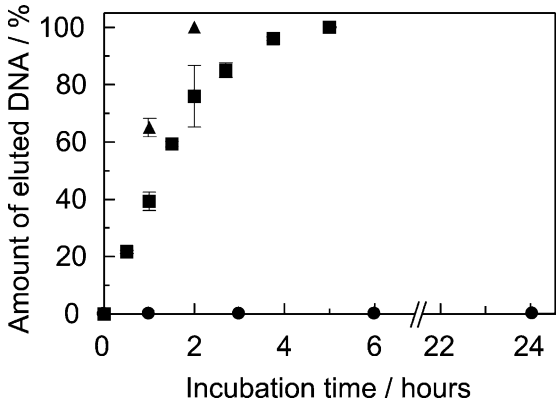

Fig. 5. Stability of DNA-Cu^{2+} matrix in aqueous solution. DNA-Cu^{2+} matrices were incubated in 50 mM MES buffer solution (pH 7.0). ● was incubated in absence of EDTA solution. ■ and ▲ were incubated in presence of 10 and 50 mM EDTA solution, respectively. The amount of eluted DNA from matrix was measured by the absorbance at 260 nm or the fluorescence intensity of GelStar (λ_{ex}=493 nm, λ_{em}=525 nm).

3.4. Structure of DNA-metal ion matrices

Fig. 7(a)–(c) showed the CD spectra of the DNA-Mg^{2+} mixed material, DNA-Al^{3+} matrix, and DNA-Cu^{2+} matrix, respectively. The CD spectrum of the pure DNA material shows the B-form structure, which is the native double-stranded DNA structure in water, with the positive peak at 275 nm and the negative peak at 245 nm (open circles in Fig. 7). The DNA-Mg^{2+} mixed material, which did not construct the water-insoluble matrix, maintained the B-form structure (Fig. 7(a)). The spectra of the water-soluble (r= 0.001) and water-insoluble (r=0.07) DNA-Al^{3+} matrices indicated a positive peak at 275 nm and a negative peak at 245 nm as well, i.e. the DNA in the water-insoluble DNA-Al^{3+} matrix possessed the B-form structure (Fig. 7(b)). In contrast, the CD spectra of the DNA-Cu^{2+} matrices were different from that of the DNA-Mg^{2+} and DNA-Al^{3+} matrices. The characteristic peaks at 275 and 245 nm of the B-form DNA structure diminished with the construction of the water-insoluble matrices and disappeared. Until now, the denaturation of DNA by the addition of Cu^{2+} was been reported [32–34] and our result of CD spectra has supported

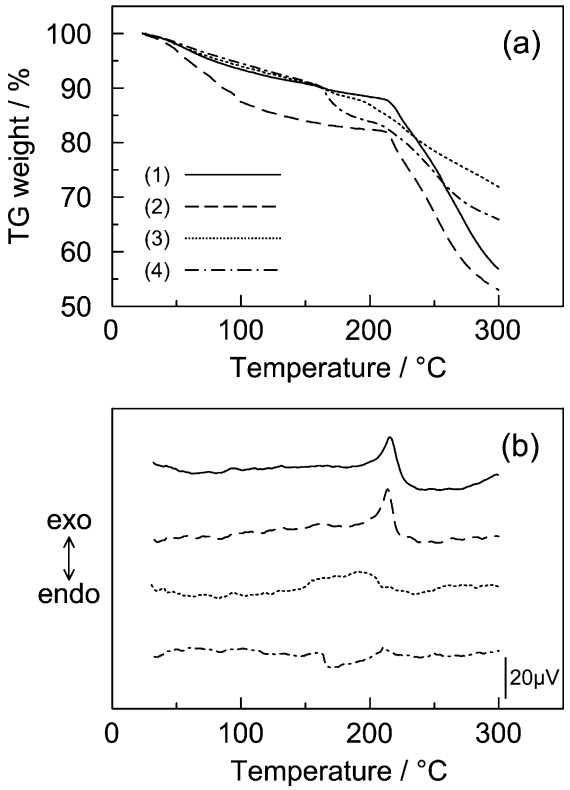

Fig. 6. TG (a) and DTA (b) curves of DNA-metal ion matrices with the heating rate of 10 °C min^{-1} under dry-nitrogen flow. (1) Pure DNA-film; (2) DNA-Mg^{2+} mixed material; (3) DNA-Cu^{2+} matrix; (4) DNA-Al^{3+} matrix.

these reports. These results indicate that the conformation of DNA in the matrix can be controlled by choosing the type of metal ions. Especially, the addition of Cu^{2+} induced a drastic conformational change in the B-form DNA structure.

Fig. 8(A)–(C) shows the infrared (IR) spectra (KBr methods) of the DNA-Mg^{2+} mixed material, DNA-Al^{3+} matrix, and DNA-Cu^{2+} matrix, respectively. The IR spectra of the DNA-Mg^{2+} mixed material, not constructing the water-insoluble matrix, did not change in the presence of the various concentrations of Mg^{2+}, and maintained the native DNA structure (Fig. 8(A)). The IR spectra of the DNA-Al^{3+} matrix changed with the construction of the water-insoluble matrices. The absorption band at 1234 cm^{-1}, the antisymmetric vibration of the phosphate group [8,34–36], shifted to a lower wave number with the increase in the Al^{3+} concentration (Fig. 8(B)). We have reported these phenomena for the interaction of heavy metal ions with the UV-irradiated DNA film [8]. A similar phenomenon was also obtained for the DNA-Cu^{2+} matrix (Fig. 8(C)). Additionally, the absorption band at 1500–1600 cm^{-1}, related to the stretching vibration of C=N of guanine and cytosine bases [8,34–36], changed when the water-insoluble DNA-Cu^{2+} matrix was constructed. The decrease of intensity at 1500–1600 cm^{-1} by the addition of Cu^{2+} is due to the binding of metal ion into this coordination site. Especially, in our researches, this phenomenon appeared clearly as DNA-

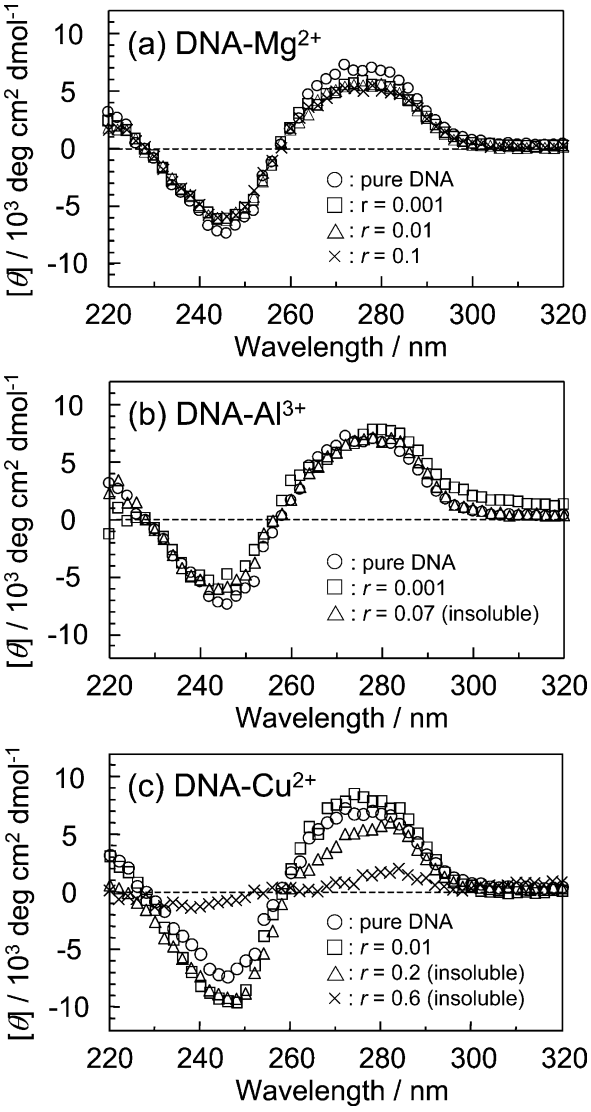

Fig. 7. CD spectra of DNA-metal ion matrices. The DNA-metal ion matrix was constructed on the quarts plate and covered with another quartz plate. The optical path length is 0.1 mm. (a) DNA-Mg^{2+} mixed material; (b) DNA-Cu^{2+} matrix; (c) DNA-Al^{3+} matrix. The r values indicated at Section 2.

metal ion matrices were measured under dry condition. These results suggest that Al^{3+} and Cu^{2+} strongly bind to the phosphate group by an electrostatic interaction. Moreover, Cu^{2+} strongly binds to not only the phosphate group but also to the nucleic acid base pairs, such as cytosine and guanine.

Table 2 shows the property of the DNA-metal ion fiber, thermal stability, DNA structure, and metal ion binding site of the DNA-metal ion matrix. Cu^{2+} ions have been reported to strongly bind with nucleic acid bases in the helical structure and also with the phosphate groups in an aqueous solution [24–26]. In fact, the DNA-Cu^{2+} matrix also indicated a strong binding to the nucleic acid base pairs, such as the cytosine-guanine base pair, and phosphate group. As a result, the double-stranded structure of DNA

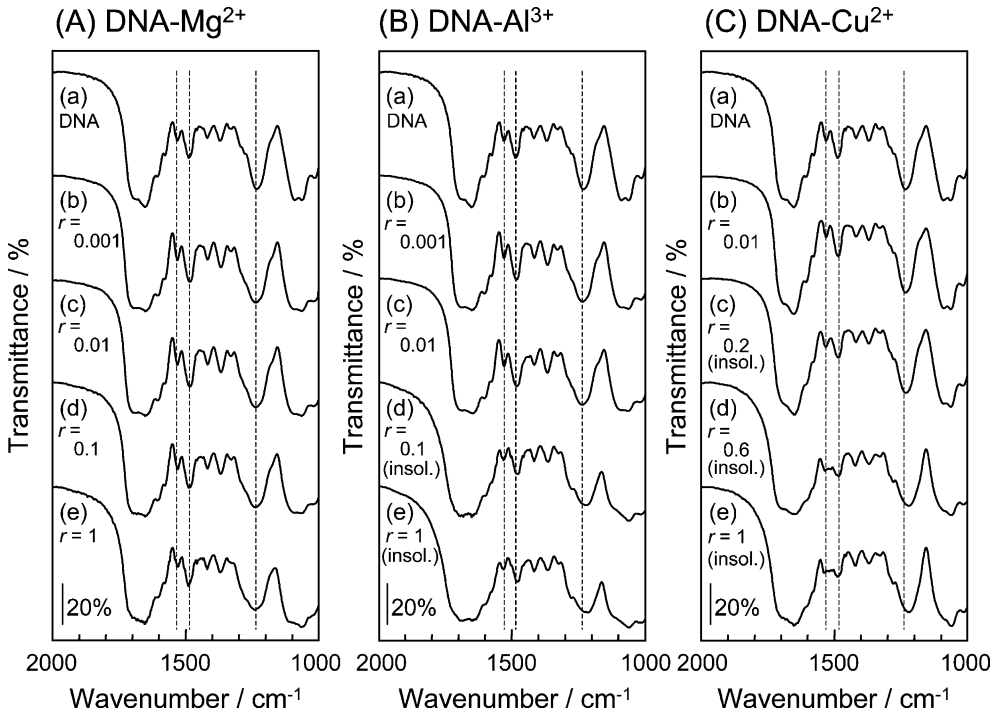

Fig. 8. IR spectra of DNA-metal ion matrices. The spectra for the dried DNA-metal ion matrices were measured by KBr method. (A) DNA-Mg^{2+} mixed material; (B) DNA-Cu^{2+} matrix; (C) DNA-Al^{3+} matrix. The *r* values indicated at Section 2.

became unstabilized and the CD spectra did not show the B-form structure (Fig. 7(c)). The thermal stability of the DNA-Cu^{2+} matrix increased by the metal ion-assisted cross-linking through the coordination of Cu^{2+} and the DNA-Cu^{2+} matrix produced the fiber without flexibility. In contrast, Al^{3+} binds to only the phosphate group of the double-stranded DNA (Fig. 7(b)), and the DNA-Al^{3+} matrix possessed the B-formed DNA structure (Fig. 8(B)). In this case, the metal-ion assisted cross-linking of DNA occurs at the surface of the double-stranded DNA molecules through the phosphate group and the nucleic acid base pairs in the helical structure did not suffer the effect of the Al^{3+} binding. As a result, the DNA-Al^{3+} matrix produce a soft fiber with flexibility. The property and conformation of the DNA-Mg^{2+} mixed material, not constructing the water-insoluble matrix, was identical with that of the double stranded DNA. These results suggest that the binding of metal ions to the phosphate group is necessary for the construction of the DNA-metal ion fiber. However, the strong interaction of metal ion to the nucleic acid base pairs in the helical structure induces the destruction of the B-form DNA structure and the decrease of flexibility.

Table 2
Property and structure of DNA-metal ion matrices

DNA matrix	Flexibility of DNa-metal fiber	Thermal stability	DNA structure	Metal binding site
DNA-Mg	–	×	B-form	–
DNA-Ai	Flexible	○	B-form	Phosphate group
DNA-Cu	Not flexible	○	Not B-form	Phosphate group base pair
DNA	–	×	B-form	–

3.5. Oxidative property of DNA-Cu^{2+} matrix

Maintenance of the functions of the metal ions in the matrices is an important factor for the utilization of the DNA-metal ion matrices. We demonstrated the oxidation by

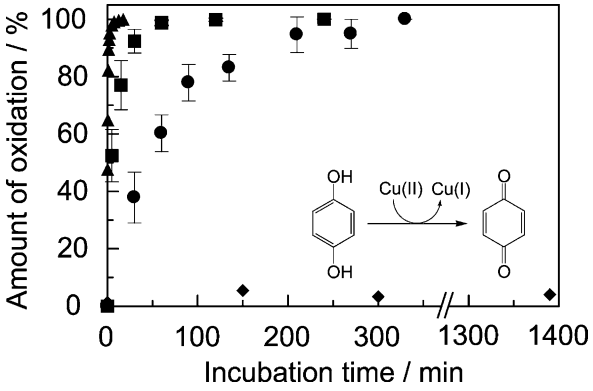

Fig. 9. Oxidation of *p*-hydroquinone by the DNA-Cu^{2+} matrix. *p*-Hydroquinone was incubated with DNA-Cu^{2+} matrix at room temperature and the absorbance at 246 nm was measured at various time intervals. The concentration of DNA in matrix is 3.5 mg (11 mmol). The insert indicates the oxidative reaction of *p*-hydroquinone by the Cu^{2+}. ♦ and ▲ are *p*-hydroquinone solution in the absence and presence of $CuCl_2$ (1.4 mM), respectively. ■ and ● are incubation with the $[Cu^{2+}]$/[DNA (nucleotide)] = 1.4 and $[Cu^{2+}]$/[DNA (nucleotide)] = 0.7, respectively.

the DNA-Cu^{2+} matrix (Fig. 9). The DNA-Cu^{2+} matrix was incubated in an aqueous hydroquinone solution, and the absorbance at 254 nm of the solution was measured at various time intervals. Generally, hydroquinone is oxidized to benzoquinone (see insert in Fig. 9) by the oxidative reaction in the presence of Cu^{2+} [37]. When hydroquinone was incubated with the DNA-Cu^{2+} ($[Cu^{2+}]/[DNA] = 0.7$) matrix, the reagent was gradually oxidized and the oxidized amount reached 100% in 300 min (closed circles in Fig. 9). At the higher concentration of Cu^{2+} ($[Cu^{2+}]/[DNA] = 1.4$), hydroquinone was completely oxidized in 120 min (closed squares in Fig. 9). These oxidative effects of the DNA-metal ion matrices were slow in comparison with that of the copper chloride solution without DNA-Cu^{2+} matrices (closed triangles in Fig. 9). In contrast, the oxidized amount of hydroquinone without Cu^{2+} was approximately 5% at the incubation time of 300 min (closed diamonds in Fig. 9). These results indicate that Cu^{2+} in the DNA-Cu^{2+} matrix possesses the function of an oxidizing reagent. A similar effect was obtained for the ascorbic acid solution, a well known reducing reagent material (data not shown).

4. Conclusions

We prepared novel water-insoluble DNA-metal ion matrices by the mixing of DNA and metal ion solutions, such as Al^{3+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Cu^{+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}. These DNA-metal ion matrices were stable in water and retained the function of the metal ion, such as their oxidative property. The DNA-Al^{3+} matrix maintained a B-form DNA structure, its native double-stranded DNA structure in water. The matrices could be created in various forms, such as a gel, capsule, film, or fiber. The DNA-metal ion fibers had a highly ordered molecular orientation. However, some metal ions have toxicity or an element of the allergic reaction [38]. Therefore, the utilization of these matrices for the implantable material to human body must be careful. The DNA-metal ion matrices may have a potential utility as a novel bio-/electric-material with the oxidizing and reducing effect, an environmental material for removing harmful DNA intercalating compounds, and an electrical device with ionic or electrical conductivities.

Acknowledgements

This work was supported by the Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 13132201, No. 14350491, and No. 15303).

References

[1] Saenger W. Principles of nucleic acid structure. Berlin: Springer; 1987.
[2] Waring MJ. Ann Rev Biochem 1981;50:159–92.
[3] Brunsveld L, Folmer BJB, Meijer EW, Sijbesma RP. Chem Rev 2001; 101:4071–98.
[4] Boon EM, Barton JK. Curr Opin Struct Biol 2002;12:320–9.
[5] Yamada M, Kato K, Nomizu M, Sakairi N, Ohkawa K, Yamamoto H, et al. Chem Eur J 2002;8:1407–12.
[6] Yamada M, Kato K, Shindo K, Nomizu M, Haruki M, Sakairi N, et al. Biomaterials 2001;22:3121–6.
[7] Yamada M, Kato K, Nomizu M, Ohkawa K, Yamamoto H, Nishi N. Environ Sci Technol 2002;36:949–54.
[8] Yamada M, Kato K, Nomizu M, Haruk Mi, Ohkawa K, Yamamoto H, et al. Bull Chem Soc Jpn 2002;75:1627–32.
[9] Iwata K, Sawadaishi T, Nishimura S, Tokura S, Nishi N. Int J Biol Macromol 1996;18:149–50.
[10] Satoh S, Yamada M, Nomizu M, Nishi N. Polym J 2003;35:872–8.
[11] Liu XD, Murayama Y, Yamada M, Nomizu M, Matsunaga M, Nishi N. Int J Biol Macromol 2003;32:121–7.
[12] Jonganurakkun B, Liu XD, Nodasaka Y, Nomizu M, Nishi N. J Biomater Sci Polym Ed 2003;14:1269–81.
[13] Zhao C, Liu XD, Nomizu M, Nishi N. Biomaterials 2003;24:3747–55.
[14] Fukushima T, Inoue Y, Hayakawa T, Taniguchi K, Miyazaki K, Okahata Y. J Dent Res 2002;80:1772–6.
[15] Okahata Y, Kobayashi T, Tanaka K, Shimomura M. J Am Chem Soc 1998;120:6165–6.
[16] Nishimura N, Ohno H. J Chem Mater 2002;12:2299–304.
[17] Kawabe Y, Wang L, Nakamura T, Ogata N. Appl Phys Lett 2002;21: 1372–4.
[18] Seeman NC. Trends Biotech 1999;17:437–43.
[19] Richter J. Physica E 2003;16:157–73.
[20] Litman RM. J Biol Chem 1968;243:6222–33.
[21] Mirkin CA, Letsinger RL, Mucic RC, Storhoff JJ. Nature 1996;382: 607–9.
[22] Kaya M, Toyama Y, Kubota K, Nodasaka Y, Ochiai M, Nomizu M, et al. Int J Biol Macromol 2005;35:39–46.
[23] Umeno D, Kawasaki M, Maeda M. Bioconjugate Chem 1998;9: 719–24.
[24] Lippard SJ, Berg JM. Principles of bioinorganic chemistry. Mill Valley: University of Science Books; 1994.
[25] Flessel CP. Adv Exp Med Biol 1977;91:117–28.
[26] Hartwig A. Toxicol Lett 1998;102/103:235–9.
[27] White HW, Vartak NB, Burland TG, Curtis FP, Kusukawa N. Bio Techniques 1999;26:984–8.
[28] Yamamoto H, Horita C, Senoo Y, Nishida A, Ohkawa K. J Appl Polym Sci 2001;79:437–46.
[29] Yamada M, Nomizu M, Satoh S, Ohkawa K, Yamamoto H, Nishi N. ChemBiochem 2003;4:232–4.
[30] Lee SL, Debenedetti PG, Errington JR, Pethica BA, Moore DJ. J Phys Chem B 2004;108:3098–106.
[31] Kavakli C, Tuncel SA, Salih B. Thermochim Acta 2003;398:249–58.
[32] Liebe DC, Stuehr JE. Biopolymers 1972;11:145–66.
[33] Liebe DC, Stuehr JE. Biopolymers 1972;11:167–84.
[34] Hackl EV, Kornilova SV, Blagoi YP. Int J Biol Macromol 2005;35: 175–91.
[35] Tajmir-Riahi HA, Naoui M, Ahimad R. Biopolymers 1993;33: 1819–27.
[36] Banyay M, Sarkar M, Gräslund A. Biophys Chem 2003;104:477–88.
[37] Sato M, Inaki Y, Kondo K, Takemoto KJ. Polym Sci, Polym Chem Ed 1977;15:2059–65.
[38] Shi H, Hudson LG, Liu KJ. Free Radical Biol Med 2005;37:582–93.