Preparation and Characterization of DNA Films Induced by UV Irradiation

Masanori Yamada,[a, b] Kozue Kato,[a] Motoyoshi Nomizu,[a] Nobuo Sakairi,[a] Kousaku Ohkawa,[b] Hiroyuki Yamamoto,[b] and Norio Nishi*[a]

Abstract: Large amounts of DNA-enriched materials, such as salmon milts and shellfish gonads, are discarded as industrial waste. We have been able to convert the discarded DNA to a useful material by preparing novel DNA films by UV irradiation. When DNA films were irradiated with UV light, the molecular weight of DNA was greatly increased. The reaction was inhibited by addition of the radical scavenger galvinoxyl suggesting that the DNA polymerization with UV irradiation proceeded by a radical reaction. Although this UV-irradiated DNA film was water-insoluble and resistant to hydrolysis by nuclease, the structure of the DNA film in water was similar to non-irradiated DNA and maintained B-form structure. In addition, the UV-irradiated DNA film could effectively accumulate and condense harmful DNA-intercalating compounds, such as ethidium bromide and acridine orange, from diluted aqueous solutions. The binding constant and exclusion number of ethidium bromide for UV-irradiated DNA were determined to be $6.8 \pm 0.3 \times 10^4 \text{M}^{-1}$ and 1.6 ± 0.2, respectively; these values are consisted with reported results for non-irradiated DNA. The UV-irradiated DNA films have potential uses as a biomaterial filter for the removal of harmful DNA intercalating compounds.

Keywords: DNA structures · environmental chemistry · functional materials · intercalations · water-insolubilized DNA

Introduction

DNA, the most important genetic material of living organisms, has a unique double-stranded structure[1] consisting of nucleic acid base pairs. Since double-stranded DNA has highly specific functions,[2, 3] such as the accumulation of intercalating or groove binding compounds, it has a potential ability to be used as functional materials. DNA is readily purified from salmon milts or shellfish gonads but large amounts of the DNA-enriched materials have been discarded as waste in the industry. Conversion of this discarded DNA to be a useful material would be beneficial to utilize the unique property of DNA.

DNA is highly water-soluble and biochemically unstable. These properties have been making it difficult to utilize as a functional material. Overcoming these undesirable properties is important for the utilization of DNA as a functional material. DNA columns,[4, 5] DNA/nanoparticle hybrid materials,[6–8] DNA films,[9–12] and DNA gels[13, 14] have utilized DNA which was stabilized by immobilization on a solid support, such as cellulose powder or gold nanoparticle, or by making a stable complex with other polymers, such as cationic amphiphilic lipids or acrylamide. Recently, we also prepared DNA–polymer complexes by conjugating it with alginic acid, chitosan, or collagen.[15–18] Our DNA–polymer complexes were stable in water and functioned as filters to remove ethidium bromide[15] or even showed antibacterial activity.[18] The original properties of DNA seem to be reduced or eliminated by mixing with other materials. DNA films without any polymer supports may be advantages to determine property of DNA matrix.

In the present study, using UV irradiation, we have prepared a double-stranded DNA film with a three-dimensional network. We demonstrate that the UV-irradiated DNA film is water-insoluble and nuclease-resistant. We also describe the utilization of the UV-irradiated DNA film as functional materials for removing harmful DNA intercalating pollutants from aqueous solutions.

[a] Prof. Dr. N. Nishi, Dr. M. Yamada, K. Kato, Dr. M. Nomizu, Prof. Dr. N. Sakairi
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)
Fax: (+81) 11 7062256
E-mail: nishin@ees.hokudai.ac.jp

[b] Dr. M. Yamada, Dr. K. Ohkawa, Prof. Dr. H. Yamamoto
Faculty of Textile Science and Technology
Shinshu University
Ueda 386-8567 (Japan)

Results

Preparation of UV-irradiated DNA film: Aqueous double-stranded DNA solution was applied onto glass plates and dried overnight at room temperature. When the dried DNA

  0947-6539/02/0806-1407 $ 17.50+.50/0

film was irradiated with UV light for more than 1 h, a water-insoluble DNA film was produced. UV irradiation for less than 45 minutes resulted in water-soluble films. Furthermore, when UV irradiation was carried out using an aqueous double-stranded DNA solution or a dried single-stranded DNA, water-insoluble DNA film was not produced (data not shown). These results indicated that dried double-stranded DNA which was UV-irradiated for more than 1 h was critical for the preparation of a water-insoluble DNA film. The water-insoluble UV-irradiated DNA film on the glass plate was successfully stripped by the immersion to water. The reproducibility of the insoluble DNA film was very high. When the stripped UV-irradiated DNA films were stained with ethidium bromide and acridine orange, the clear films were dyed red and yellow, respectively (Figure 1).

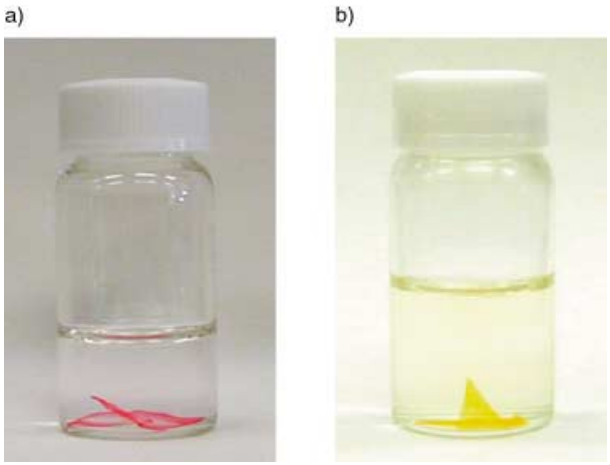

Figure 1. UV-irradiated DNA film in an aqueous solution with ethidium bromide (a) and acridine orange (b). UV-irradiated DNA films were incubated in these aqueous solutions for 24 h at room temperature. Clear DNA films were dyed red and yellow by ethidium bromide and acridine orange, respectively.

We examined the stability of the UV-irradiated DNA film in water (Figure 2). The UV-irradiated DNA films were incubated in water and the absorbance of the solution was measured at 260 nm at various times. The amount of eluted

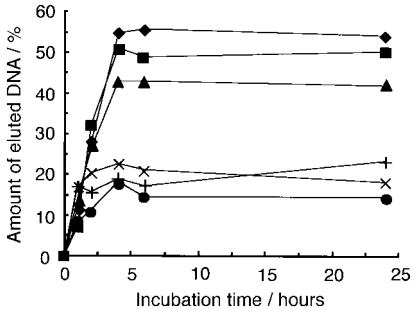

Figure 2. Stability in an aqueous solution of DNA films with UV irradiation for various times. The UV-irradiated DNA films were incubated in aqueous solution at room temperature and absorbance at 260 nm of the solution was measured at various times: ◆, 1 h; ■, 2 h; ▲, 4 h; ×, 6 h; +, 8 h; ●, 12 h.

DNA from the UV-irradiated DNA film increased with the incubation time and reached a constant value at 4 h. The amount of eluted DNA from the UV-irradiated DNA film decreased, when the sample was irradiated UV light. In addition, this DNA film did not dissolve in an aqueous solution even after incubation in water for up to one year. UV-irradiated DNA films were stored in ultra-pure water for more than one day to remove the small amount of water-soluble DNA and then used in the further experiments.

Effect of nuclease on the UV-irradiated DNA film: We tested the biochemical stability of UV-irradiated DNA films using *Micrococcal nuclease*. DNA films which were irradiated by UV light for various times were incubated with nuclease in aqueous solution. The amount of hydrolyzed DNA was determined by the absorbance at 260 nm (Figure 3). The

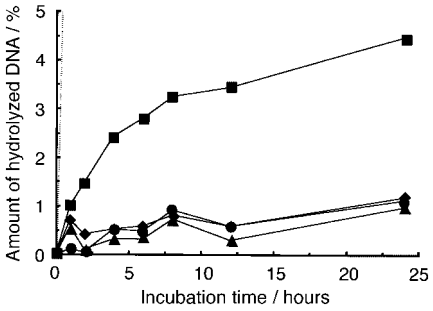

Figure 3. Effect of nuclease (*Micrococcal nuclease*) on UV-irradiated DNA films. ■ and ● are UV-irradiated DNA films with 2 and 6 h UV irradiation and incubation with nuclease, respectively. ◆ and ▲ are UV-irradiated DNA films with 2 h and 6 h UV irradiation and incubation without nuclease, respectively. Dashed line is the hydrolysis curve of a non-irradiated DNA in aqueous solution with nuclease.

UV-irradiated DNA films showed resistance to hydrolysis by the nuclease. The amount of hydrolyzed DNA from a 2 h UV-irradiated DNA film was much lower than that from non-irradiated DNA (shown in Figure 3 dashed line). When a 2 h UV-irradiated DNA film was incubated with the nuclease for 24 h, approximately 4.5 % of DNA film was hydrolyzed. The amount of hydrolyzed DNA from a 6 h UV-irradiated DNA film was approximately 1 % after 24 h and similar to that without the nuclease; that is DNA which was irradiated UV light for more than 6 h was not hydrolyzed by nuclease. These results indicate that the UV-irradiated DNA film is resistant to nuclease and the biochemical stability of the DNA could be controlled by the length of time of UV irradiation.

Structure of UV-irradiated DNA films: The UV-irradiated DNA film was constructed onto a quartz plate and covered with another quartz plate. The DNA sandwiched quartz plate was put into the normal quartz cell and immersed in buffer solution, and then CD spectra were measured. Figure 4a shows the CD spectra of non-irradiated DNA and 15 – 45 min UV-irradiated DNAs. Clearly, non-irradiated and the 15 – 45 min UV-irradiated DNAs formed a B-form structure[19, 20] with the maximum peak at 280 nm and the minimum peak at 240 nm. Figure 4b shows the CD spectrum of 6 h UV-irradiated water-insoluble DNA film. This spectrum indicated

0947-6539/02/0806-1408 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 6

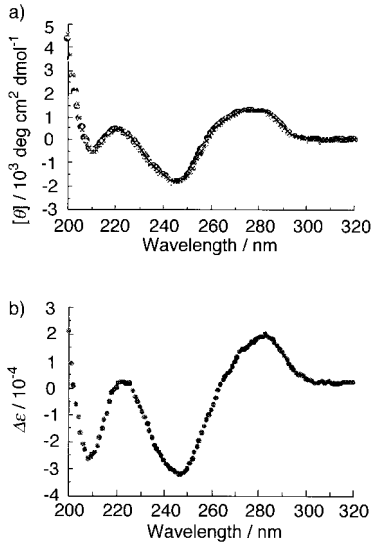

Figure 4. CD spectra of native DNA and water-insoluble DNA films in aqueous solution. a) Water-soluble DNA. ●, non-irradiated salmon milt DNA; ■, dried DNA; ▲, 15 min UV-irradiated water-soluble DNA; ◆, 30 min UV-irradiated water-soluble DNA; ×, 45 min UV-irradiated water-soluble DNA. b) 6 h UV-irradiated water-insoluble DNA film. The UV-irradiated DNA film was assembled onto a quartz plate and covered with another quartz plate. The DNA sandwiched quartz plate was put into the normal quartz cell and immersed in buffer solution, and then CD spectra were measured.

a maximum peak at 280 nm and a minimum peak at 240 nm as well, which means that this UV-irradiated DNA film had the B-form structure in aqueous solution. In contrast, the CD spectra of the UV-irradiated DNA film in dry conditions did not suggest B-form structure (data not shown). Similar results were obtained a normal DNA film without the UV irradiation. These results indicated that it is necessary for water molecules to maintain the B-form structure of the both UV-irradiated and non-irradiated DNA.

Accumulation of ethidium bromide by UV-irradiated DNA film: Double-stranded DNA specifically binds to intercalating compounds, such as ethidium bromide[21–26] and acridine orange.[27, 28] Next, we examined whether the UV-irradiated DNA film could bind these compounds. When the UV-irradiated DNA film was incubated with a dilute aqueous ethidium bromide solution for 24 h, the color of DNA film changed from clear to red (Figure 1a). A similar phenomenon was observed at diluted aqueous acridine orange solution as well (Figure 1b). UV absorption at 480 nm of the aqueous solutions which was incubated in the absence and presence of UV-irradiated DNA film was measured to quantitate the amount of ethidium bromide bound to the DNA film (Figure 5). When the UV-irradiated DNA film was added to aqueous ethidium bromide solution, the absorption peak at 480 nm disappeared. A similar result was obtained when acridine orange was used as a DNA intercalating compound (data not shown). These results indicate that the UV-irradiated DNA film has a strong binding affinity for the DNA intercalating compounds similar to that observed with intact double-stranded DNA.

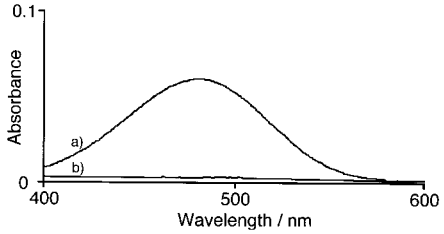

Figure 5. Absorption spectra of an aqueous ethidium bromide solution in the absence (a) and presence (b) of UV-irradiated DNA films. The UV-irradiated DNA films are put into an aqueous ethidium bromide solution and incubated at room temperature for 24 h. The UV-irradiated DNA films are removed from the aqueous solution and then UV spectra were measured from 400 to 600 nm.

Next, we determined the binding constant of ethidium bromide for UV-irradiated DNA films (Figure 6). UV-irradiated DNA immobilized on glass bead were incubated in Tris-HCl buffer containing various concentrations of ethidium bromide; the amount of ethidium bromide bound onto UV-irradiated DNA was then determined by the absorbance at 480 nm. The amount of accumulated ethidium bromide increased when the ethidium bromide concentration was increased, and reached a constant value (approximately 14 μM) (Figure 6). The binding constant K of ethidium bromide for DNA can be expressed as:

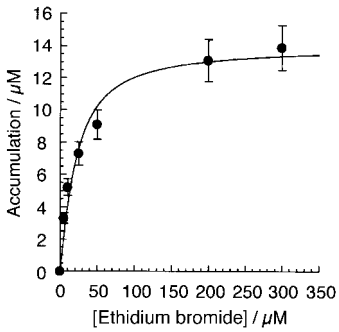

Figure 6. Binding of ethidium bromide to UV-irradiated DNA immobilized on glass bead. DNA-immobilized on glass bead were incubated in buffer solution containing various concentrations of ethidium bromide, and then the amount of ethidium bromide bound onto the UV-irradiated DNA was determined by the absorbance at 480 nm. Solid lines are the results of the non-linear least-squares fit of Equation (3).

$$\mathrm{DNA} + \mathrm{EB} \rightleftarrows \mathrm{DNA} \cdot \mathrm{EB} \quad (1)$$

$$K = \frac{[\mathrm{DNA}\cdot\mathrm{EB}]}{[\mathrm{DNA}][\mathrm{EB}]} \quad (2)$$

where K = binding constant of ethidium bromide for DNA, [DNA] = a concentration of free DNA without ethidium bromide, [EB] = a concentration of non-intercalating ethidium bromide, and [DNA·EB] = a concentration of complex between DNA and ethidium bromide. Equation (2) was transformed to Equation (3):

$$[\mathrm{A}] = \frac{\frac{1}{K} + \frac{[\mathrm{DNA}]}{n} + [\mathrm{EB}] - \sqrt{\left(\frac{1}{K} + \frac{[\mathrm{DNA}]}{n} + [\mathrm{EB}]\right)^2 + 4\cdot\frac{[\mathrm{DNA}]}{n}\cdot[\mathrm{EB}]}}{2} \quad (3)$$

where [A] = a concentration of accumulated ethidium bromide by the UV-irradiated DNA, [DNA] = a concentration of base pair in UV-irradiated DNA, [EB] = a concentration of ethidium bromide, and n = exclusion number. When this Equation (3) was applied to the data obtained in Figure 6 by the non-linear least-squares method (shown in Figure 6 solid line), the binding constant and exclusion number were calculated to be $6.8 \pm 0.3 \times 10^4\,\text{M}^{-1}$ and 1.6 ± 0.2, respectively. The binding constant and exclusion number have been previously determined between intact double-stranded DNA and ethidium bromide in aqueous solution by UV/Vis spectroscopy,[22] fluorescence spectroscopy,[23, 26] NMR,[24] and AFM[25] measurements as $6.0-12.0 \times 10^4\,\text{M}^{-1}$ and 2.0–2.8, respectively. These reported binding constants and exclusion numbers were consistent with the data we obtained with UV-irradiated DNA.

Electrophoresis of UV-irradiated DNA film: We analyzed the effect of UV irradiation on molecular size using agarose gel electrophoresis. We used 2680 bp DNA (from *E. coli*) as a standard for this experiment and prepared the DNA film by drying and UV irradiation as well. This DNA film was solubilized by boiling at 100 °C for 20 minutes and analyzed by the agarose gel electrophoresis (Figure 7). Lane 1 indicated the non-irradiated 2680 bp DNA. When the UV-irradiated DNA was analyzed, the 2680 bp band decreased and the most of sample stayed on top of the gel (Figure 7, lane 2). These results suggested that the molecular weight of the UV-irradiated DNA film increased and that the DNA film with UV irradiation was formed by intermolecular cross-linking reactions. Next, we examined the cross-linking reaction in UV-irradiated DNA film using a radical scavenger galvinoxyl.[29] Figure 7 in lane 3 indicated the UV-irradiated DNA sample with galvinoxyl containing ethanol solution. The molecular weight of UV-irradiated DNA with galvinoxyl was similar to that of non-irradiated 2680 bp DNA (Figure 7, lane 3 and lane 1); this indicates that the radical scavenger galvinoxyl inhibited the intermolecular cross-linking reactions of DNA induced by UV irradiation.

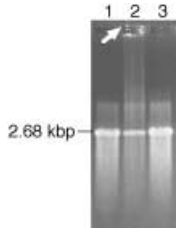

Figure 7. Agarose gel electrophoresis of UV-irradiated DNA films. Lane 1, non-irradiated 2680 bp DNA (from *E. coli*). Lane 2, 2680 bp DNA with UV irradiation for 45 min. Lane 3, 2680 bp DNA with UV irradiation for 45 min in the present of the radical scavenger galvinoxyl containing ethanol solution. An arrow indicates the increase of molecular weight. DNAs were analyzed by 1% agarose gel electrophoresis and detected on the gels by staining with ethidium bromide under UV illumination.

The observation of cross-linking DNA by atomic force microscopy: Next, we examined the intermolecular cross-linking reaction of pBR322 plasmid DNA (from *E. coli*) using tapping mode atomic force microscopy[30] (TMAFM). Figure 8a and b show AFM images (scan size; $2 \times 2\ \mu\text{m}^2$) of the non-irradiated plasmid DNA and the UV-irradiated plasmid DNA, respectively. The non-irradiated plasmid DNA formed supercoiled DNA on the mica surface (shown in Figure 8a). Linear and open-ringed DNAs were not observed in the AFM images. Supercoiled DNA on either the mica surface or and amino-group modified mica surface have been reported previously.[31–33] The AFM image of the 45 min UV-irradiated plasmid DNA indicated intermolecular cross-linking structures on the mica surface (Figure 8b). This intermolecular cross-linking DNA was composed of three open-ringed DNAs; that is this cross-linked DNA is trimeric complex of plasmid DNA. Figure 8c shows the AFM image of other cross-linked DNAs (scan size; $5 \times 5\ \mu\text{m}^2$) with both linear and open-ringed DNAs. These network structures were spread more than 2 μm. Taken together, the data from the agarose gels and AFM measurements indicate that intermolecular cross-linked DNA forms a three-dimensional network.

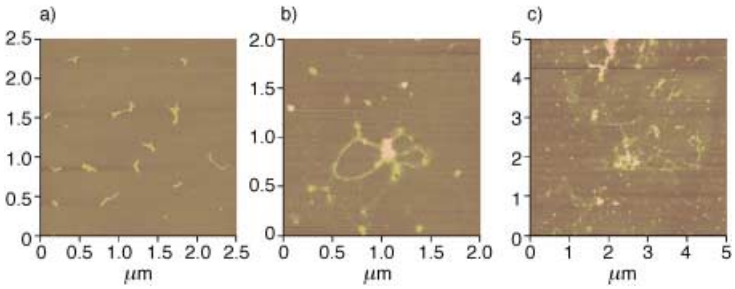

Figure 8. Tapping mode AFM image of UV-irradiated DNA films on cleaved mica in air. a) Non-irradiated pBR322 plasmid DNA. b) and c) 45 min UV-irradiated pBR322 plasmid DNA. The height range of the gray-scale on these images is less than 2 nm.

Discussion

We have prepared a novel DNA film using UV irradiation. The UV-irradiated DNA film was stable in aqueous solution and did not dissolve even after a long incubation (approximately one year). In addition, UV-irradiated DNA films showed resistance to hydrolysis by nuclease. These properties were different from those of non-irradiated DNA. The UV-irradiated DNA showed an increase of molecular weight which suggested the formation of DNA intermolecular cross-links with a three-dimensional network. However, the structure of the UV-irradiated DNA film in aqueous solution showed the B-form structure, which was native double-stranded DNA structure in water. UV irradiation did not induce the transformation of the DNA structure, such as the formation of a hydrophobic structure. AFM images of UV-irradiated plasmid DNA demonstrated multi-open-ring and network structures. We conclude that UV irradiation of the DNA did not cause decomposition of either the nucleic acid bases or phosphate groups.

The UV-irradiated DNA film could bind DNA-intercalating compounds, such as ethidium bromide and acridine orange. The binding constant and exclusion number of ethidium bromide for UV-irradiated DNA film were consistent with reported results for non-irradiated DNA.[21–26] Thus, an essential function of double-stranded DNA, such as

  0947-6539/02/0806-1410 $ 17.50+.50/0 *Chem. Eur. J.* **2002**, *8*, No. 6

intercalation, was not lost with UV irradiation. Nuclease degradation of UV-irradiated DNA film was blocked by steric hindrance of the three-dimensional network structure with the close packing of DNA. These biochemical properties were regulated by the length of time of UV irradiation.

Damage of DNA by UV light, such as the decomposition of nucleic acid bases, the formation of pyrimidine dimmers,[34, 35] and nicks to phosphodiester-bonding,[31] has been reported previously. Since the UV-irradiated DNA film remained the original properties of DNA, the effect of small changes in the DNA by UV irradiation is not a significant problem. UV-irradiated DNA film could effectively accumulate and condense harmful DNA-intercalating compounds from diluted aqueous solutions. The binding constant and exclusion number between UV-irradiated DNA and ethidium bromide was consistent with reported results and the double-stranded DNA acted as a functional material. Since many DNA-intercalating compounds are carcinogens,[3] the accumulation and condensation of these harmful compounds are important for maintenance of both a safe water supply and the environment. The UV-irradiated DNA film may be useful to remove the harmful compounds.

UV-induced DNA intermolecular cross-linking was caused by a radical reaction. The dimer of thymine bases[34, 35] or these derivatives[36–39] have been reported previously. When aqueous poly(dA)–poly(dT) or poly(dG)–poly(dC) solution was applied onto a glass plate and treated with UV irradiation, water-insoluble DNA films were produced and their molecular weight increased to 1×10^6. Infrared spectra by the KBr method of either the UV-irradiated poly(dA)–poly(dT) or poly(dG)–poly(dC) showed the formation of pyrimidine dimers (data not shown). Since the IR spectra of the UV-irradiated DNA also indicated the disappearance or decrease at the absorption bands of phosphate group and deoxyribose, we conclude that one of the formation mechanisms of water-insoluble DNA film involved the intermolecular dimerization of pyrimidine. Thus, UV-irradiated DNA formed a supramolecular cross-linking structure with a three-dimensional network resulting in an increase of molecular weight.

In conclusion, we have prepared water-insoluble and nuclease resistant DNA films by UV irradiation. The UV-irradiated DNA films had properties of double-stranded DNA in both the solid and liquid state. The DNA film in water maintained a B-form structure and was cross-linked. In addition, these chemical and biochemical properties of the DNA films were found to be controlled by the length of time of UV irradiation. Furthermore, UV-irradiated DNA films effectively accumulated and condensed harmful DNA-intercalating compounds, and the binding constant of ethidium bromide was strong enough to remove it from dilute aqueous solution. Therefore, UV-irradiated DNA films may have a potential utility as a biomaterial, such as a filter for removing harmful DNA intercalating compounds from aqueous solution.

Experimental Section

Materials: Double-stranded DNA (Na salt from salmon milt, molecular weight; 5×10^6) and single-stranded DNA (Na salt from salmon milt, molecular weight; approximately 2×10^5) were obtained from Yuki Fine Chemical Co. Ltd., Tokyo, Japan and Nissan Chemical Industries, Ltd., Tokyo, Japan, respectively. These DNAs were used without further purification and were dissolved in ultra-pure water. Polydeoxyadenylic acid–polydeoxythymidylic acid (poly(dA)–poly(dT)) and polydeoxyguanylic acid–polydeoxycytidylic acid (poly(dG)–poly(dC)) was purchased from Sigma Chemical Co., St. Louis, MO or Amersham Pharmacia Biotech, Inc. Uppsala, Sweden. *Micrococcal nuclease* and pBR322 plasmid DNA was purchased from Sigma Chemical Co. and Fermentas. Inc., Vilnius, Lithuania. Ethidium bromide, acridine orange, and galvinoxyl was purchased from Wako Pure Chemical Industries Ltd., Osaka, Japan. HEPES buffer solution and mica for sample substrate of atomic force microscopy was purchased from Dojindo Co., Kumamoto, Japan, and Okenshoji Co., Ltd., Tokyo, Japan. Ultra-pure water (Nanopure Infinity Basic, Barnstead/Thermolyne, Dubuque, IA) was used in all the experiments described.

Preparation of DNA film with UV irradiation: Aqueous double-stranded DNA solution (100 μL, 10 mg mL^{-1} DNA in H_2O) was applied onto glass plates, dried at room temperature overnight, and then irradiated with UV light[40] (R-52G, Ultraviolet Inc., Upland, CA) at 254 nm for various times. The intensity of UV irradiation was 5600 μW cm^2 at the sample position. The UV-treated DNA applied on glass plate was immersed in water and the DNA film was striped from the glass plate and stored in water. The preparation of UV-irradiated DNA film was reproducible. The amount of DNA in the UV-irradiated DNA film was determined by the following procedure: UV-irradiated DNA film was hydrolyzed with 1M HCl solution at 100 °C for 1 h and quantitated by absorption at 260 nm using a UV/Vis spectrophotometer U-2000A (Hitachi Co. Ltd., Tokyo, Japan). A preparation of UV-irradiated single-stranded DNA was prepared similarly.

Characterization of UV-irradiated DNA film: The stability in an aqueous solution of the UV-irradiated DNA film was confirmed by the following method: The UV-irradiated DNA films were incubated in ultra-pure water (20 mL) for various times. The absorbance at 260 nm of the solution was measured and the eluted DNA from the UV-irradiated DNA film was determined.

The effect of nuclease on UV-irradiated DNA film was confirmed by the following method: The 2 and 6 h UV-irradiated DNA films were incubated with nuclease (*Micrococcal nuclease*, 2 units per mL) in 10 mM Tris-HCl buffer (10 mL, pH 7.4) in the presence of 5 mM NaCl and 2.5 mM $CaCl_2$ at 37 °C. The amount of hydrolyzed DNA by the nuclease was measured by the absorption at 260 nm at various times. Double-stranded salmon milts DNA (50 μg mL^{-1}) was used for the control.

The structure of UV-irradiated double-stranded DNA film in water was measured using a circular dichroism (CD) spectrophotometer. The 6 h UV-irradiated DNA film was constructed onto a quartz plate (w9.9 × h40 × t1 mm^3) and covered by another quartz plate. The DNA-sandwiched-quartz plate was put into the normal quartz cell (w10 × h40 × 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 UV-irradiated DNA film. CD spectra were recorded on a Jasco Model J-720 CD spectropolarimeter (Japan Spectroscopic Co., Tokyo, Japan) at 20 °C with the integrating of five times. Double-stranded salmon milts DNA (30 μg mL^{-1}, 20 mM Tris-HCl, pH 7.4, containing 100 mM NaCl) was used for the control of DNA.

Electrophoresis of UV-irradiated DNA films: This DNA (2680 bp DNA from *E. coli*) was applied onto the glass plate, dried at room temperature for overnight, and then irradiated with UV light at 254 nm for various times. This UV-irradiated DNA film was solubilized by incubation at 100 °C for 20 min and analyzed by 1% agarose gel electrophoresis. DNA was detected on the gels by staining with ethidium bromide under UV illumination.

Inhibition effect by radical scavenger: The 2680 bp DNA (1 μL, 1 mg mL^{-1}) was applied to mini vials and dried at room temperature overnight. Next, 1 μM galvinoxyl ethanol solution (100 μL) was added to mini vials and the vials were covered with a polyvinylidene chloride film. UV light was irradiated onto the ethanol solution in the presence and absence of galvinoxyl for 45 min. UV-irradiated DNA was rinsed with ethanol (5 × 500 μL) to remove galvinoxyl and dried at room temperature. The dried-DNA was solubilized in buffer (20 mM Tris-HCl, pH 7.4, containing 100 mM

NaCl) and was analyzed by 1% agarose gel electrophoresis. DNA was detected on the gels by staining with ethidium bromide under UV illumination.

Accumulation of DNA intercalating compounds by UV-irradiated DNA films: The accumulation of ethidium bromide and acridine orange in water were measured by the following methods: The UV-irradiated DNA film (1 film) was incubated in 10 μM aqueous ethidium bromide solution (7 mL) for 24 h at room temperature, and then removed from the solution. The level of ethidium bromide in solution was determined by the comparison of absorption at 480 nm in the absence and presence of the UV-irradiated DNA film.

Binding constant of UV-irradiated DNA and ethidium bromide: Since the amount of DNA in the UV-irradiated DNA film was difficult to maintain constant, DNA immobilized on porous glass bead[41] were used to determine the binding constant value. DNA immobilized on glass bead (100 mg, the immobilized-amount of DNA onto porous glass bead was 0.20 mg) were incubated with 10 mL of various concentrations of aqueous ethidium bromide solutions (20 mM Tris-HCl, pH 7.4, containing 100 mM NaCl) at room temperature for 24 h. The amount of ethidium bromide bound to the DNA was determined by comparison of absorption at 480 nm in the absence and presence of DNA-immobilized on glass bead.

Atomic force microscopy images: pBR322 plasmid DNA (1 μL, 0.5 mg mL^{-1}) was applied to mini vials, dried at room temperature overnight, and then irradiated with UV light at 254 nm for 45 min. This DNA treated by UV was solubilized in 10 mM HEPES buffer (50 μL, pH 7.5), containing 10 mM $MgCl_2$. This DNA solution (10 μL) was applied onto freshly cleaved mica (10×10 mm^2) and incubated in a small box with 100% humidity for 20 min.[20] The DNA immobilized on mica plates were rinsed with ultra-pure water (5×500 μL) and dried at room temperature. These samples were measured in air by the tapping mode AFM[31] using a Nanoscope IIIa (Digital Instruments, Santa Barbara, CA).

Acknowledgements

We thank Prof. Akihiko Yamagishi (Graduate School of Science, Hokkaido University, Sapporo, Japan) for helpful discussion on the AFM measurements and Dr. Hynda K. Kleinman (National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, Maryland, USA) for critical reading of the manuscript. This work was supported by the Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (No. 11694114, No. 11450359, No. 10555327, and No. 13132201) and also by Hokkaido Foundation for the Promotion of Scientific and Industrial Technology (Hokscitec).

[1] W. Saenger, *Principles of Nucleic Acid Structure*, Springer, Berlin, **1987**.
[2] M. J.Waring, *Annu. Rev. Biochem.* **1981**, *50*, 159 – 192.
[3] H. M. Berman, P. R. Young, *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 87 – 114.
[4] R. M. Litman, *J. Biol. Chem.* **1968**, *243*, 6222 – 6233.
[5] B. Alberts, G. Herrick, *Methods Enzymol.* **1971**, *21*, 198 – 217.
[6] C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature* **1996**, *382*, 607 – 609.
[7] R. C. Mucic, J. J. Storhoff, C. A. Mirkin, R. L. Letsinger, *J. Am. Chem. Soc.* **1998**, *120*, 12674 – 12675.
[8] P. Alivisatos, K. P. Johnsson, X. Peng, T. E. Wilson, C. J. Loweth, M. P. Bruchez, P. G. Schults, *Nature* **1996**, *382*, 609 – 611.
[9] K. Tanaka, Y. Okahata, *J. Am. Chem. Soc.* **1996**, *118*, 10679 – 10683.
[10] Y. Okahata, T. Kobayashi, K. Tanaka, M. Shimomura, *J. Am. Chem. Soc.* **1998**, *120*, 6165 – 6166.
[11] S. Machida, M. Morisada, K. Horie, Y. Okahata, *Polym. J.* **1999**, *31*, 1179 – 1184.
[12] Y. Kawabe, L. Wang, S. Horinouchi, N. Ogata, *Adv. Mater.* **2000**, *12*, 1281 – 1283.
[13] D. Umeno, T. Kano, M. Maeda, *Anal. Chim. Acta* **1998**, *365*, 101 – 108.
[14] D. Umeno, M. Kawasaki, M. Maeda, *Bioconjugate Chem.* **1998**, *9*, 719 – 724.
[15] K. Iwata, T. Sawadaishi, S. Nishimura, S. Tokura, N. Nishi, *Int. J. Biol. Macromol.* **1996**, *18*, 149 – 150.
[16] H. Kitamura, E. Matsuura, A. Nagata, N. Sakairi, S. Tokura, N. Nishi, *Int. J. Biol. Macromol.* **1997**, *20*, 75 – 77.
[17] H. Kitamura, C. Iwamoto, N. Sakairi, S. Tokura, N. Nishi, *Int. J. Biol. Macromol.* **1997**, *20*, 241 – 244.
[18] H. Kitamura, Y. Kondo, N. Sakairi, N. Nishi, *Nucleic Acids Symp. Ser.* **1997**, *37*, 273 – 274.
[19] F. S. Allen, D. M. Gray, G. P. Roberts, Jr., I. Tinoco, *Biopolymers* **1972**, *11*, 853 – 879.
[20] N. Nishi, R. Ogawa, H. Hayasaka, K. Hasegawa, *Pept. Chem.* **1989**, 325 – 330.
[21] W. Fuller, M. J. Waring, *Ber. Bunsenges. Phys. Chem.* **1964**, *68*, 805 – 808.
[22] M. J. Waring, *J. Mol. Biol.* **1965**, *13*, 269 – 282.
[23] J. B. LePecq, C. Paoletti, *J. Mol. Biol.* **1967**, *27*, 87 – 106.
[24] S. Gopalakrishnan, S. Byrd, M. P. Stone, T. M. Harris, *Biochemistry* **1989**, *28*, 726 – 374.
[25] J. E. Coury, L. McFail-Isom, L. D. Williams, L. A. Bottomley, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 12283 – 12286.
[26] X. Qu, J. B. Chaires, *J. Am. Chem. Soc.* **1999**, *121*, 2649 – 2650.
[27] R. W. Armstrong, T. Kurucsev, U. P. Strauss, *J. Am. Chem. Soc.* **1970**, *92*, 3174 – 3181.
[28] W. Müller, D. M. Crothers, *Eur. J. Biochem.* **1975**, *54*, 267 – 277.
[29] P. D. Bartlett, T. Funahashi, *J. Am. Chem. Soc.* **1962**, *84*, 2596 – 2601.
[30] Q. Zhong, D. Innis, K. Kjoller, V. B. Elings, *Surf. Sci. Lett.* **1993**, *290*, L688 – L692.
[31] B. Samorì, C. Nigro, A. Gordano, I. Muzzalupo, C. Quagliariello, *Angew. Chem.* **1996**, *108*, 569 – 570; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 529 – 530.
[32] M. Tanigawa, T. Okada, *Anal. Chim. Acta* **1998**, *365*, 19 – 25.
[33] K. Rippe, N. Mücke, J. Langowski, *Nucleic Acids Res.* **1997**, *25*, 1736 – 1744.
[34] R. Beukers, J. Ijlstra, W. Berends, *Recl. Trav. Chim.* **1958**, *77*, 729 – 732.
[35] R. Beukers, W. Berends, *Biochim. Biophys. Acta* **1960**, *41*, 550 – 551.
[36] T. Sugita, N. Tohnai, Y. Wang, T. Wada, Y. Inaki, *Bull. Chem. Soc. Jpn.* **1996**, *69*, 1777 – 1786.
[37] N. Tohnai, Y. Inaki, M. Miyata, N. Yasui, E. Mochizuki, Y. Kai, *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1143 – 1151.
[38] T. Young, R. Nieman, S. D. Rose, *Photochem. Photobiol.* **1990**, *52*, 661 – 668.
[39] A. Joseph, G. Prakash, D. E. Falvey, *J. Am. Chem. Soc.* **2000**, *122*, 11219 – 11225.
[40] M. Yamada, K. Kato, K. Shindo, M. Nomizu, M. Haruki, N. Sakairi, K. Ohkawa, H. Yamamoto, N. Nishi, *Biomaterials* **2001**, *22*, 3121 – 3126.
[41] M. Yamada, K. Kato, M. Nomizu, K. Ohkawa, H. Yamamoto, N. Norio, *Environ. Sci. Technol.*, in press.

Received: July 26, 2001 [F3442]

  0947-6539/02/0806-1412 $ 17.50+.50/0