

DNA Cleavage by Good's Buffers in the Presence of Au(III)

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DNA (pBluescript plasmid, 2.96 kbp) cleavage by Good's buffers, which are extensively used in laboratories of chemistry, biochemistry, or biology, was studied by gel electrophoresis, CD spectra, ESR spectra, and CV in the presence of Au(III). Incubation of plasmid DNA with Good's buffers and Au(III) resulted in DNA cleavage. DNA cleavage occurred due to the formation of nitrogen-centered cationic free radicals from the Good's buffers in the presence of Au(III). The formation of the nitrogen-centered free radicals was confirmed by ESR spectroscopy. Gel electrophoresis results indicated that Form I and Form II were converted into Form III and a DNA fragment. The molecular weight of the DNA fragment was estimated ca. 1.47 kbp by DNA marker gel electrophoresis. ESR spectra were not observed from the Good's buffers in the presence of other metal ions, such as Mn(II), Fe(III), Co(II), Ni(II), Zn(II), Pd(II), Cd(II), Hg(II), and Pb(II), resulting in no DNA cleavage. Gel electrophoresis results indicated a partial DNA cleavage during the incubation of DNA in the presence of Good's buffers and Au(III). This is because the DNA cleavage reaction stopped at 15 min, since the radical lifetime is ca. 15 min. The intensities of the CD spectra at 270 nm decreased with time by 15 min upon the addition of Au(III), and a constant CD spectrum was observed after 15 min. Further addition of Au(III) showed an alternation of the CD spectra from positive ellipticity to negative ellipticity at 270 nm, suggesting significant DNA cleavage. The mechanism of DNA cleavage is discussed based on the gel electrophoresis, ESR, CD, and CV results.

The selection of proper buffers for studies on the chemistry and biochemistry of transition metals is an important matter. Every buffer provides a potential ligand for cations. The use of a phosphate buffer is severely restricted because of the insolubility of many trace metal phosphates, or of its significant interaction with metal ions. The application of amine buffers, such as tris(hydroxymethyl)aminomethane (Tris) seemed to obviate these problems. However, it has been recognized that Tris, forms complexes with transition metals.[1] The search for more "innocent" buffers for biological research led to a series of buffers, named Good's buffers. In 1966, Good et al. reported[2] on a new series of buffers for use in biological research. The buffers had several important properties, including pK_a values between 6 and 8, maximum water solubility with minimum solubility in other solvents, minimum salt effects, low ability to cross biological membranes, and supposedly low affinities for transition-metal ions. However, Grady et al.[3] found that some of the Good's buffers, e.g., HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid), PIPES (piperazine-1,4-bis[2-ethanesulfonic acid]), or EPPS (3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid), give nitrogen-centered cationic free radicals in the presence of Fe(II), Fe(III)-polymer, and oxygen. It has also been shown that Cu(II) can oxidize HEPES in the presence of a ligand that stabilize the Cu(I).[4] Very recently, we also found that HEPES gives gold nanoparticles in the presence of Au(III) through the formation of nitrogen-centered free radicals,[5] which damages DNA.[6] In addition, Kawanishi et al.[7,8] found that semicarbazide caused DNA damage in the presence of Cu(II) or aromatic amine, 4-aminobiphenyl (4-ABP), and that its *N*-hydroxy(4-ABP(NHOH)) metabolites also caused DNA damage in the presence of Cu(II) and NADH. Moreover, in a previous paper,[9] we demonstrated that Au(III) shows higher toxicity to *Trypanosoma brucei brucei*, which causes African Trypanosomiasis, compared to other metal ions, e.g. Pd(II), in the HMI-9 medium (HMI-9 medium contains 0.05 mol dm^{-3} (=M) HEPES to maintain the physiological pH).

In this paper, we report that other Good's buffers (e.g., EPPS, MES (2-morpholinoethanesulfonic acid), MOPS (3-morpholinopropanesulfonic acid), MOPSO (2-hydroxy-3-morpholinopropanesulfonic acid), HEPPSO (2-hydroxy-3-[4-(2-hydroxyethyl)-1-piperazinyl]propanesulfonic acid), or PIPES as well as HEPES also have the ability to cleave DNA in the presence of Au(III). ESR results indicate the generation of nitrogen-centered cationic free radicals from Good's buffers with Au(III), which cleave DNA. A non-detectable inhibitory effect of reactive oxygen species (ROS) scavengers on DNA cleavage further suggests that the nitrogen-centered radicals are the only responsible radicals for the cleavage of DNA. However, no DNA cleavage was observed from these Good's buffers in the presence of other metal ions such as Mn(III), Fe(III), Co(II), Ni(II), Zn(II), Pd(II), Cd(II), Hg(II), and Pb(II). We will show here that radical formation and DNA cleavage are general phenomena for Good's buffers in the presence of Au(III).

Experimental

Materials. Good's buffers of MES, MOPS, MOPSO, EPPS, PIPES, HEPES, CAPS (*N*-cyclohexyl-3-aminopropanesulfonic acid), HEPPSO, and $Na_2H_2edta \cdot 2H_2O$ (disodium dihydrogen eth-

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ylenediaminetetraacetate dihydrate) were purchased from Dojindo laboratories Ltd., Japan; Sodium tetrachloroaurate(III) dihydrate, sodium chloride, sodium hydroxide, ethanol, dimethyl sulfoxide (DMSO), superoxide dismutase (SOD), catalase, tris(hydroxymethyl)aminomethane, mercury(II) nitrate, iron(III) chloride, cobalt(II) chloride, nickel(II) chloride, zinc(II) chloride, and palladium(II) chloride were purchased from Wako Chemicals Co., (Osaka, Japan); D(-)Mannitol was purchased from TCI-GR (Tokyo Kasei Kogyo Co., Ltd., Japan); sodium formate was purchased from TCI-GR (Tokyo Kasei Kogyo Co., Ltd.); manganese(II) chloride, cadmium(II) nitrate, and lead(II) nitrate were purchased from Katayama Chemical Co., (Japan). DNA ladder was purchased from Biolabs (USA). pBluescript II plasmid DNA was prepared from a plasmid bearing the *Escherichia coli* strain using a standard procedure,[10] and then dissolved in deionized water. A stock solution of DNA was prepared by dissolving pBluescript plasmid DNA in deionized water, and the concentration in base pairs was determined by its known molar extinction coefficient at 260 nm (1.32×10^4 mol^{-1} dm^3 cm^{-1}).[11,12] Doubly deionized water was used throughout the experiments.

Methods and Apparatus. Detection of DNA Cleavage by Good's Buffers in the Presence of Au(III): Samples for gel electrophoresis were prepared by mixing Good's buffers, NaCl (0.10 M), plasmid DNA (2.00×10^{-4} M in base pairs) and Au(III) in a series of microtubes (1.5 cm^3; Eppendorf). The samples were then incubated at 37 °C for 60 min in a constant-temperature bath (Yamato, Japan). After incubation, the samples were stained with 1 μdm^3 of a loading buffer (containing 30% glycerol, 0.1 M EDTA, 0.25% xylene cyanol, and 0.25% bromophenol blue), and then run in 1% neutral agarose slab horizontal gel containing TAE buffer of pH 8.30 (tris(hydroxymethyl)aminomethane, 2.40 g; $Na_2H_2edta \cdot 2H_2O$, 0.37 g; glacial acetic acid, 0.57 cm^3 (99.7%) in 500 cm^3 doubly deionized water) for 32 min using a Mupid-2 Cosmo Bio Company apparatus (Japan). The gel was stained with ethidium bromide (0.50 $\mu g/mL$) for 35 min and photographed under a transilluminator using a Polaroid MP-4 land camera with a Polapan black and white coatless film. Similar gel electrophoresis experiments were conducted with these Good's buffers e.g., EPPS: (i) with DNA ladder, (ii) with increasing concentration of Au(III), (iii) as a function of the incubation time (5, 10, 15, 30, and 60 min), (iv) in the presence of other metal ions (e.g., Mn(II), Fe(III), Co(II), Ni(II), Zn(II), Pd(II), Cd(II), Hg(II), and Pb(II)), and (v) with scavengers of reactive oxygen species (ROS) (e.g., ethanol, DMSO, superoxide dismutase, and catalase). Sodium chloride (0.10 M) was used to adjust the ionic strength for all of the metal ions, except for Hg(II), where 0.10 M $NaNO_3$ was used. The concentrations of the buffering agents and Au(III) were maintained at 2.00×10^{-2} and 2.00×10^{-4} M, respectively. All experiments were conducted at physiological pH (7.40) and under room light.

Detection of Free Radicals Derived from Good's Buffers in the Presence of Au(III): ESR spectra were recorded to detect radicals derived from Good's buffers in the presence of Au(III). The spectra were measured at room temperature using a JES-TE 300 (JEOL, Tokyo, Japan) spectrometer with a microwave power of 2.00 mW and a modulation amplitude of 0.63 mT. Moreover, time-dependence ESR spectra of the free radicals generated from Good's buffers were recorded in the presence of Au(III). Similar ESR experiments were conducted with the Good's buffers in the presence of other metal ions, as mentioned above. No ESR spectra were observed for these buffer agents in the presence of these metal ions. The concentrations of the buffering agents and the metal ions were maintained at 4.00×10^{-1} and 2.00×10^{-4} M, respectively.

Determination of DNA Conformation in the Presence of Good's Buffers with Au(III): CD measurements were carried out to determine the DNA conformation in the presence of Good's buffers and Au(III). The CD spectra of DNA were measured: (i) in the presence of Good's buffers with a fixed concentration of Au(III) as a function of time (singly scanned) and (ii) with each addition of Au(III) in the presence of Good's buffers (five times scanned and then averaged). A Jasco J-720 spectropolarimeter (Japan) was used to scan the CD spectra of DNA at 37 °C. The concentrations of the buffer agents (e.g., EPPS) and DNA were maintained at 2.00×10^{-2} M and 1.50×10^{-4} M in base pairs, respectively, where the sodium chloride concentration was 0.10 M.

Electrochemistry of Good's Buffers: Cyclic voltammetry measurements were performed with an electrochemical analyzer, ALS 802A. Glassy carbon (GC) was used as working electrode in conjunction with a Pt counter electrode and a silver/silver chloride reference electrode. An aqueous solution of 0.10 M of Good's buffers was used for cyclic voltammetric measurements. Sodium sulfate of 0.20 M was maintained in these solutions as a supporting electrolyte. Each sample solution was flushed by nitrogen gas for 5 min to remove dissolved oxygen before each CV scan. All of the CV experiments were conducted at pH 7.40, except for CAPS, where the pH values were maintained at 7.40 and 10.40.

All of the experiments were conducted in the presence of 0.10 M sodium chloride. The main chemical species of Au(III) were $[AuCl(OH)_3]^-$ and $[Au(OH)_4]^-$ with a few percentage of $[AuCl_2(OH)_2]^-$ at pH 7.40.[13,14] In addition, the reagents were added in the order of NaCl, Good's buffers, DNA, and Au(III). Au(III) was the last, since radical formation occurred immediately after the addition of Au(III).

Results

Detection of DNA Cleavage by Good's Buffers in the Presence of Au(III). Good's buffers caused DNA (pBluescript plasmid, 2.96 kbp) cleavage in the presence of Au(III) (2.00×10^{-4} M), as shown in Fig. 1, lanes 2 to 6, where lane 1 is DNA control. A 1.47 kbp of DNA fragment was formed due to DNA cleavage, which was confirmed by DNA marker gel electrophoresis, as shown in Fig. 2 (lane M, DNA marker; lane 1, EPPS + Au(III) and lane 2, DNA control). Figure 3 shows the effect of the concentrations of Au(III) on DNA

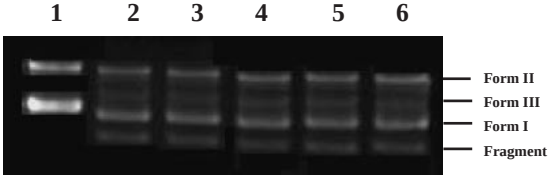

Fig. 1. Gel electrophoresis of plasmid DNA (2.00×10^{-4} M in base pairs) with different Good's buffers (pH 7.40) in the presence of 2.00×10^{-4} M of Au(III). Lane 1, DNA alone; Lane 2, EPPS + Au(III); Lane 3, MES + Au(III); Lane 4, MOPS + Au(III); Lane 5, MOPSO + Au(III); Lane 6, HEPPSO + Au(III). The concentrations of the buffering agents and sodium chloride were maintained at 2.00×10^{-2} M and 0.10 M, respectively. Incubation temperature and time were maintained at 37 °C and 60 min, respectively.

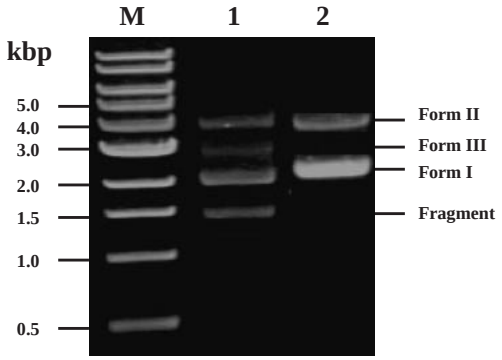

Fig. 2. Determination of molecular weight of DNA fragments with DNA marker. Lane M, DNA marker; Lane 1, EPPS + Au(III); Lane 2, DNA control. The concentrations of EPPS and sodium chloride were maintained at 2.00×10^{-2} M (pH 7.40) and 0.10 M, respectively. Incubation temperature and time were maintained at 37 °C and 60 min, respectively.

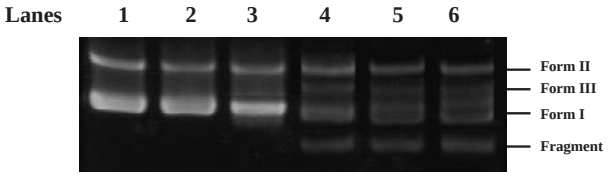

Fig. 3. The effect of the concentrations of Au(III) in the presence of EPPS (2.00×10^{-2} M, pH 7.40) on DNA cleavage. Lane 1, DNA alone; Lane 2, 2.00×10^{-6} M Au(III); Lane 3, 2.00×10^{-5} M Au(III); Lane 4, 2.00×10^{-4} M Au(III); Lane 5, 6.00×10^{-4} M Au(III); Lane 6, 1.00×10^{-3} M Au(III). The concentrations of DNA and sodium chloride were maintained at 2.00×10^{-4} M in base pairs and 0.10 M, respectively. Incubation temperature and time were maintained at 37 °C and 60 min, respectively.

cleavage. The degree of DNA cleavage depended on both the concentrations of the Good's buffers and Au(III). In contrast, no DNA cleavage was observed for the Good's buffers (e.g., EPPS) in the presence of other metal ions, such as Mn(II), Fe(III), Co(II), Ni(II), Zn(II), Pd(II), Cd(II), Hg(II), and Pb(II). The results show that Forms I (supercoiled) and II (circular) of plasmid DNA were converted into Form III (linear) and a DNA fragment of 1.47 kbp, as shown in lanes 2 to 6 (Fig. 1), in the presence of Good's buffers and Au(III). Figure 4 shows the gel electrophoresis of DNA (a) and its change in intensities (b) in the presence of EPPS and Au(III) (2.00×10^{-4} M) at different incubation timed. The results show the DNA cleavage occurred as soon as the addition of Au(III), and continued for 15 min due to the lifetime of the free radicals (vide infra). Moreover, gel electrophoresis experiments were conducted in the presence of the scavengers for reactive oxygen species, ROS (e.g., $^{\bullet}OH$, $O_2^{-\bullet}$, and H_2O_2). No detectable inhibitory effect was observed on the DNA cleavage (data not shown).

Detection of Free Radicals Caused from Good's Buffers in the Presence of Au(III). Figure 5 shows the ESR spectra of free radicals generated from the aqueous solutions of Good's buffers (e.g., EPPS) in the presence of Au(III)

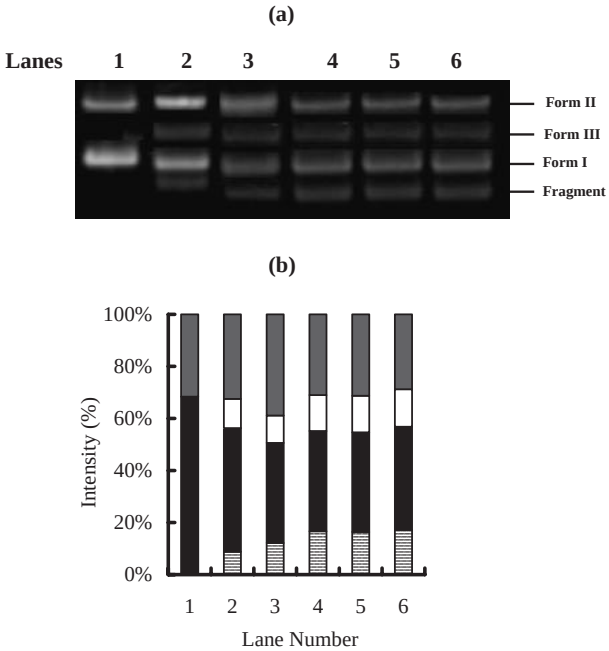

Fig. 4. (a) Gel electrophoresis of plasmid DNA (2.00×10^{-4} M in base pairs) with 2.00×10^{-2} M EPPS (pH 7.40) in the presence Au(III) at different incubation times. Lane 1, DNA alone; Lane 2, 5 min; Lane 3, 10 min; Lane 4, 15 min; Lane 5, 30 min; Lane 6, 60 min. The concentrations of Au(III) and sodium chloride were maintained at 2.00×10^{-4} and 0.10 M, respectively. Incubation temperature was maintained at 37 °C. (b) Intensities (%) of Form-I (■), Form-II (■), Form-III (□), and DNA fragment (▤) for gel electrophoresis at different incubation time in the presence of EPPS and Au(III) (2.00×10^{-4} M). Concentrations of EPPS and sodium chloride were maintained at 2.00×10^{-2} (pH 7.40) and 0.10 M, respectively. Lane 1, 0 min; Lane 2, 15 min; Lane 3, 30 min; Lane 4, 60 min; Lane 5, 90 min; Lane 6, 150 min. Incubation temperature was maintained at 37 °C.

(Fig. 5a and 5b) and Pd(II) (Fig. 5c). Figures 5a and b indicate the ESR spectra of the free radicals generated from EPPS without and with DNA in the presence of Au(III), respectively, while Fig. 5c indicates no ESR signal for EPPS in the presence of Pd(II). Similar ESR spectra were observed for the other Good's buffers (e.g., MES, MOPS, MOPSO, PIPES, or HEPPSO) in the presence of Au(III) (data not shown). Figure 6 shows the time dependence of the ESR spectra of the free radicals generated from EPPS with Au(III) in the presence of DNA (1.50×10^{-4} M in base pairs). The radicals disappeared completely within 15 min (Fig. 6c). No ESR spectra were observed for these Good's buffers in the presence of other metal ions, as mentioned above. CAPS ($pK_a = 10.40$) did not show any ESR signal in the presence of Au(III) at pH 7.40. Free-radical generation was also observed from the Good's buffers at different chloride concentrations (0.10, 0.20, and 0.25 M, NaCl) at pH 7.40, where the predominant species of Au(III) were $[AuCl_2(OH)_2]^-$, $[AuCl(OH)_3]^-$, and $[Au(OH)_4]^-$.[13,14] The results indicate that the generation of free radicals is independent of the Au(III) species.

DNA Conformation Change in the Presence of Good's

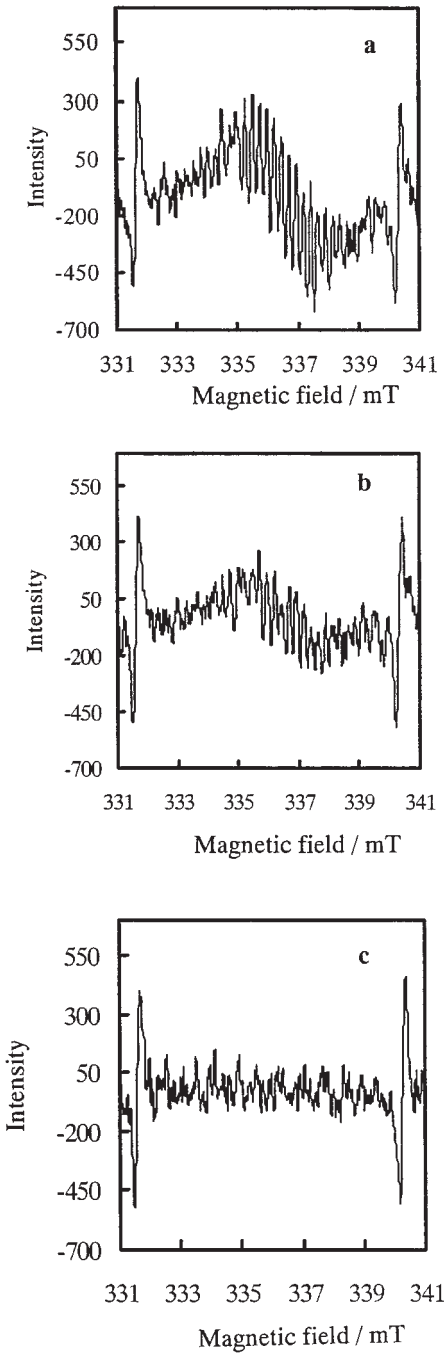

Fig. 5. ESR spectra of free radicals generation from Good's buffers (e.g., EPPS) in the absence (a) and the presence (b) of DNA with Au(III) at room temperature. Figure 5c denotes ESR spectra for Pd(II). The concentrations of buffer agents and Au(III) were maintained at 4.00×10^{-1} and 2.00×10^{-4} M, respectively. Instruments settings: field set, 335 mT; sweep, 10 mT; scan rate, 4 min; modulation amplitude, 0.63 mT; gain 790; and power, 2.00 mW.

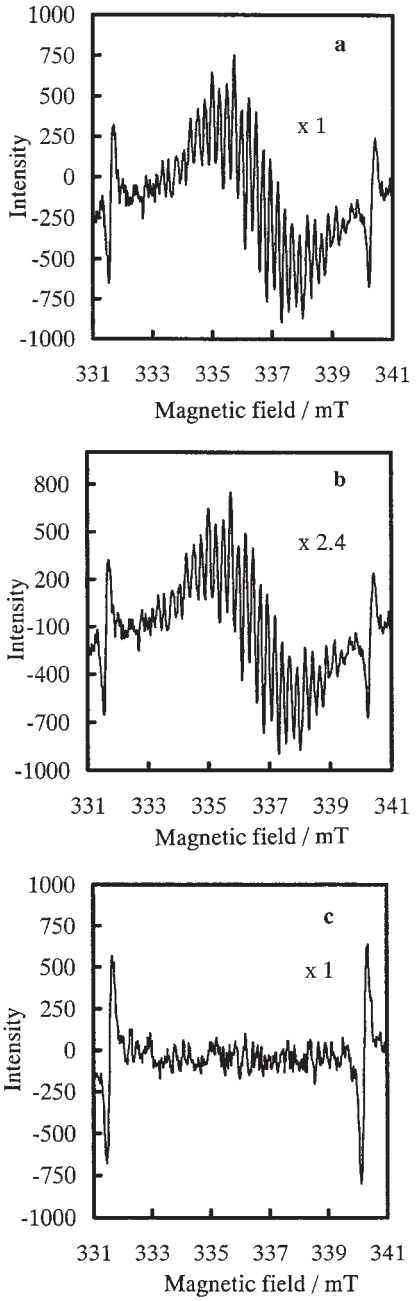

Fig. 6. ESR spectra of the free radicals generated from EPPS at (a) 5 min, (b) 10 min, and (c) 15 min after the addition of Au(III) in the presence of DNA. The concentrations of EPPS and Au(III) were maintained at 4.00×10^{-1} and 2.00×10^{-4} M, respectively. Instruments settings: field set, 335 mT; sweep, 10 mT; scan rate, 4 min; modulation amplitude, 0.63 mT; gain 790; and power, 2.00 mW.

Buffers with Au(III). We measured the CD spectra of DNA in the presence of different Good's buffers and Au(III), as shown in Fig. 7. Figures 7a and b indicate the CD spectra of DNA as a function of time, and with increasing concentrations of Au(III), respectively. The intensities of the CD spectra decreased at 270 nm with time, and a significant hypochromicity was observed at 15 min after the addition of Au(III) (Fig. 7a). However, an alternation of the CD spectra from positive ellipticity to negative ellipticity at 270 nm in the presence of EPPS with increasing concentrations of Au(III) was observed (Fig. 7b). Similar hypochromicity and a drastic change in the CD spectra of DNA were also observed in the presence of other Good's buffers (e.g., MES, MOPS, MOPSO, or PIPES).

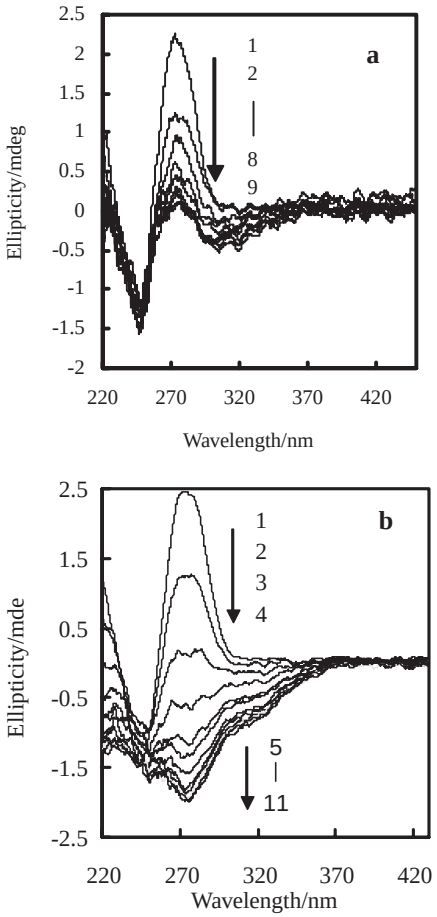

Fig. 7. Change in CD spectra of DNA in the presence of EPPS (2.00×10^{-2} M, pH 7.40) as a function of time with a fixed concentration of Au(III) (2.00×10^{-4} M) (a); (1) 0 min, (2) 3 min, (3) 5 min, (4) 8 min, (5) 11 min, (6) 15 min, (7) 20 min, (8) 30 min, and (9) 60 min. CD spectra of DNA were measured at 15 min after each addition of Au(III) (b). Final concentrations of Au(III) after the each addition: (1) 0.00, (2) 0.80, (3) 1.50, (4) 3.00, (5) 4.50, (6) 7.00, (7) 8.50, (8) 10.00, (9) 11.50, (10) 13.00, and (11) 15.00×10^{-5} M. The concentrations of DNA and sodium chloride were maintained at 1.50×10^{-4} M in base pairs and 0.10 M, respectively. Cell path length is 10 mm.

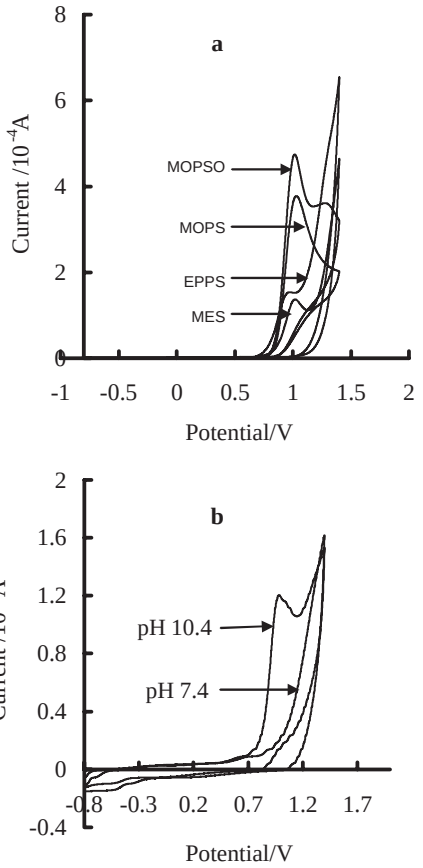

Fig. 8. Cyclic voltammograms of Good's buffers (a) EPPS, MES, MOPS, and HEPPSO at pH 7.40 and (b) CAPS at pH 7.40, 10.40. A glassy carbon (GC) was used as a working electrode in conjunction with a Pt counter electrode and a silver/silver chloride reference electrode. The concentration of Good's buffers was maintained at 0.10 mol dm^{-3}. Sodium sulfate of 0.20 mol dm^{-3} was used as a supporting electrolyte.

Electrochemistry of Good's Buffers. Figure 8 shows cyclic voltammograms of the Good's buffers. Voltammograms of the Good's buffers that have pK_a values at between 6 and 8 showed only one anodic peak, approximately at +1.00 V vs Ag/AgCl with different current intensities at pH 7.40 (Fig. 8a). Interestingly, CAPS of pK_a value 10.40 did not show any anodic peak at pH 7.40 (8b), but showed an anodic peak at +1.00 V vs Ag/AgCl at pH 10.40 (Fig. 8b).

Discussion

We previously reported that HEPES can induce oxidative DNA damage in the presence of Au(III) through the formation of nitrogen-centered cationic free radicals.[6] The aim of the present study was to investigate DNA cleavage in detail for other Good's buffers (e.g., EPPS, MES, MOPS, MOPSO, HEPPSO, or PIPES) in the presence of Au(III).

Gel Electrophoresis. Our results show that the above Good's buffers induce DNA cleavage in the presence of aqueous Au(III), as shown in Fig. 1, lanes 2 to 6. The DNA cleavage is due to the formation of free radicals during the incubation of DNA with the Good's buffers and Au(III), as shown in Fig. 5. However, no DNA cleavage was observed for these Good's buffers in the presence of other metal ions, as mentioned above. This is because that other metal ions cannot generate any free radicals from the Good's buffers. Interestingly, CAPS did not induce DNA cleavage in the presence of Au(III) at pH 7.40. Radical formation is prevented due to protonation at the secondary nitrogen atom of the buffer molecule at the pH. The percentages of unprotonated Good's buffers are 0.1 (CAPS, p$K_a = 10.40$), 21 (EPPS, p$K_a = 8.00$), 24.0 (HEPPSO, p$K_a = 7.90$), 62.0 (MOPS, p$K_a = 7.20$), 74.0 (MOPSO, p$K_a = 6.95$), and 95.0 (MES, p$K_a = 6.15$). EPPS was protonated by 79%, but DNA cleavage was observed. This was because the total concentration of EPPS as the unprotonated form was 20-times higher than that of Au(III).

ESR Spectra. Free radicals generated from different Good's buffers in the presence of Au(III) show a common

hyperfine ESR spectra with $g = 2.0056 \pm 0.0003$ and $a = 2.41 \pm 0.02$ G (Fig. 5). These determined parameters are in agreement with those reported for HEPES in the presence of Au(III),[6] or in the presence of Fe(II), Fe(III)-polymer, and oxygen.[3,15] This concordance in the "g" and "a" values for the free radicals formed from different Good's buffers suggest that the generated free radicals are nitrogen-centered cationic free radicals.[3,6,15] These free radicals are responsible for causing DNA cleavage, instead of any ROS (e.g., $^{\bullet}OH$, $O_2^{-\bullet}$, and H_2O_2), since no detectable inhibitory effect on DNA cleavage (gel electrophoresis) was observed using ROS scavengers (e.g., ethanol, sodium formate, mannitol, SOD, and catalase). An ESR experiment was also carried out to confirm the generation of free radicals from the Good's buffers-Au(III) system in the presence of DNA. The results show that identical nitrogen-centered free radicals were also generated in the presence of DNA (Fig. 5b), but the spectral intensity was reduced to approximately 50%, although the Au(III) concentration was maintained at 1.5-times higher compared to without a DNA sample. This ESR intensity reduction is due to the consumption of free radicals for the cleavage of DNA.[6] Moreover, it is also likely to be due to the interaction of Au(III) with DNA, resulting in a decrease of the concentration of free Au(III), leading to the reduction of free radicals. The lifetime of the free radicals generated from the Good's buffers was ca. 15 min (Fig. 6), which is not sufficient to cleave DNA completely, leading to partial DNA cleavage (Figs. 1–4).

CD Spectra. The cleavage of DNA with these Good's buffers in the presence of Au(III) was also explained by CD spectroscopic studies. The CD spectra of DNA in the presence of the Good's buffers (e.g., EPPS) and Au(III) showed a significant hypochromicity at 270 nm with time at 15 min (Fig. 7a). On the other hand, each addition of Au(III) to a DNA solution containing Good's buffers generated fresh radicals that cleaved DNA at a high total concentration of Au(III) (Fig. 7b), resulting in an alternation the CD spectra at 270 nm. As a result, the CD spectra were observed as irregular CD patterns, which suggest the production of unwound DNA fragments. It is also suggested that the irregular CD pattern may have been due to changes in the DNA structure by interaction with Au(III).

Cyclic Voltammogram. Good's buffers having pK_a values of between 6 and 8 (e.g., EPPS, MES, MOPS, MOPSO, HEPPSO, or PIPES) showed an anodic peak at $\sim$+1.00 V vs Ag/AgCl at pH 7.40, indicating that these buffer agents had mild reducing abilities (Fig. 8a). These buffer reagents generated free radicals during the incubation of DNA (pH 7.40) with Au(III), resulting in DNA cleavage. In contrast, CAPS showed neither any anodic peak nor an ESR signal in the presence of Au(III) at pH 7.40.

Interestingly, the incubated samples of DNA with Good's buffers plus Au(III) showed different colors (e.g., pinkish, blue or bluish, and dark blue) and absorption maxima (530 to 600 nm), depending on the types of Good's buffers that support the formation of gold nanoparticles.[16,17] The formation of gold nanoparticles during the incubation of DNA with HEPES was also considered in our previous study.[5,6]

DNA Cleavage Mechanism. The possible mechanism of the cleavage of DNA induced by Good's buffers with Au(III) is given in Eqs. 1–6.

$$\text{Au(III)} + \text{Good's Buffers} \rightarrow \text{Au(II)} + \text{Free radicals of Good's Buffers}, \quad (1)$$

$$2\text{Au(II)} \rightarrow \text{Au(III)} + \text{Au(I)}, \quad (2)$$

$$3\text{Au(I)} \rightarrow 2\text{Au(0)} + \text{Au(III)}, \quad (3)$$

$$n\text{Au(0)} \rightarrow (\text{Au(0)})_n, \text{(gold nanoparticle)}, \quad (4)$$

$$\text{Free radicals of Good's Buffers} + \text{DNA} \rightarrow \text{(Free radicals of Good's Buffers)DNA}, \quad (5)$$

$$\text{(Free radicals of Good's Buffers)DNA} \rightarrow \text{Cleaved DNA}. \quad (6)$$

Good's buffers generate nitrogen-centered cationic free radicals in the presence of Au(III), leading to the formation of gold nanoparticles. The radicals interact with DNA through intercalation and electrostatic interaction, resulting in DNA cleavage. The gel electrophoresis results indicate that Form I and Form II are converted into Form III and a DNA fragment of 1.47 kbp. Highly reactive species, such as $^{\bullet}OH$, cause DNA cleavage at every nucleotide, and cleave DNA into many fragments, whereas the nitrogen-centered radical is a less reactive species, which causes DNA cleavage specifically at guanines.[18] Good's buffers-derived radicals induce guanine-specific DNA cleavage, particularly at the 5′-G in GG sequence, because of the lowest oxidation potential among the guanine-containing dinucleotides.[19] This is supported by reports that sequence-specific DNA cleavage at G in 5′-GG is induced by hydrazine derivatives, which produce nitrogen-centered radicals.[20,21]

Conclusion

Good's buffers (e.g., EPPS, MES, MOPS, MOPSO, HEPPSO, or PIPES) induce DNA cleavage in the presence of Au(III) at pH 7.40. ESR results indicate that these buffer agents generate nitrogen-centered cationic free radicals in the presence of Au(III), resulting in DNA cleavage. Moreover, no detectable inhibitory effect on the DNA cleavage using ROS scavengers further confirms that the nitrogen-centered radicals are the only radicals for the cleavage of DNA. However, no DNA cleavage was observed with these buffer agents in the presence of other metal ions, such as Mn(II), Fe(III), Co(II), Ni(II), Zn(II), Pd(II), Cd(II), Hg(II), and Pb(II). This is due to the inabilities for the formation of any free radicals from these Good's buffers in the presence of these metal ions. Moreover, the Good's buffers having pK_a values near to 10 (e.g., CAPS) did not show any DNA cleavage effect in the presence of Au(III) at pH 7.40. At this pH (7.40), free-radical generation was prevented due to protonation (99.9%) at the nitrogen center of CAPS molecules. However, only Good's buffers or Au(III), itself, did not cleave DNA. The nitrogen-centered cationic radicals would participate to guanine-specific DNA cleavage, and lead to a DNA fragment of molecular weight 1.47 kbp. The site-specific cleavage of DNA in the presence of Good's buffers and Au(III) may lead to the development of a new type of restriction agent.

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