

# Involvement of DNA Conformational Change Induced by Rearrangement of Copper-coordination Geometry in Oxidative DNA Damages Caused by Copper and Dopamine

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Oxidative DNA damages involve reduction-oxidation reactions between certain metals and compounds, and result in genetic abnormalities leading to diseases including cancer and neurodegenerative disorders. Here, we report that DNA damages mediated by combination of a neurotransmitter dopamine and copper accompany a unique DNA conformational change. Copper(II) [Cu(II)] was shown as bound to consecutive guanines (G-G) of DNA, by demonstrating the G-G selective inhibition of restriction enzymes. When dopamine was added to calf thymus DNA in the presence of Cu(II), DNA guanines were oxidized. UV and  $^1\text{H}$  NMR analyses suggested that dopamine was oxidized to the quinone form in the presence of Cu(II). The circular dichroism experiments showed DNA spectral changes under the conditions of oxidative DNA damage, indicating a conformational change of the DNA strand by dopamine and copper. The DNA conformational change was suppressed by adding a Cu(I)-specific chelator bathocuproine. Therefore, the conformational change can be explained by a rearrangement of the copper-coordination geometry in DNA through Cu(II) reduction to Cu(I). Thus, changes in DNA conformation could be involved in metal-mediated genotoxicity as well as oxidative damage but via different mechanism.

**Key words** — oxidative damage, DNA conformation, metal toxicity, genotoxicity, coordination geometry

## INTRODUCTION

Oxidative DNA damage is a potential cause of metals/compounds-mediated genotoxicity. Reduction-oxidation reactions between certain metals and compounds produce reactive oxygen species that attack biomolecules including DNA. Numerous compounds have been reported to exert DNA damages. In these compounds, the catechol structure is an important key for the redox reactions with metals especially copper. Endogenous factors as well as xenobiotics include compounds possessing the catechol structure. A neurotransmitter dopamine, one of these endogenous catechols, is one factor responsible for the pathology of Parkinson's disease in which metals/compounds-mediated oxidative stress is also considered to be involved.<sup>1,2)</sup> Oikawa *et al.* showed a possible link between copper-mediated oxidative DNA damage and dopaminergic neuronal cell death.<sup>3)</sup> Several groups including us proposed mechanisms for the oxidative DNA damage induced by copper and catechol compounds.<sup>4,5)</sup> However, the detailed mechanisms remain to be elucidated. Followings are particularly concerned: damaging position on DNA (preferential vulnerability of consecutive guanines), the damaging forms of the reactive species, and physical distance between DNA and damaging factors.

In the present study, we monitored the DNA conformational changes using a circular dichroism (CD) spectrophotometry during the reaction between copper and dopamine to simulate oxidative DNA damage. We also examined spectral changes of dopamine by copper addition in UV and  $^1\text{H}$  NMR measurements, the copper binding to plasmid DNA using restriction enzymes, and oxidative damage in calf thymus DNA by monitoring the

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formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG). We propose potential effects of DNA conformational changes during the oxidative damage in metals/compounds-mediated diseases.

## MATERIALS AND METHODS

**Materials** — Methanol (99.7%), ethanol (99.5%), diethylenetriamine-*N,N,N',N'',N''*-pentaacetic acid (DTPA), dimethyl sulfoxide (DMSO), sodium dihydrogen phosphate, disodium hydrogen phosphate,  $\beta$ -nicotinamide adenine dinucleotide (NADH), copper(II) [Cu(II)] chloride dihydrate ( $\text{CuCl}_2$ , 99.9%), DMSO- $d_6$  (99.9%), bovine liver catalase and superoxide dismutase (SOD) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Bathocuproinedisulfonic acid disodium salt (bathocuproine) was purchased from Dojin Chemicals (Kumamoto, Japan). Catechol was from Tokyo Chemical Industry (Tokyo, Japan). Calf thymus DNA, 8-oxodG and 2'-deoxyguanosine (dG) were from Sigma (St. Louis, MO, U.S.A.). Nuclease P1 was from Yamasa Shoyu (Chiba, Japan). Calf intestine alkaline phosphatase, BamHI and EcoRI were from Roche Diagnostics (Mannheim, Germany). Ascorbic acid was from Katayama Chemical (Osaka, Japan). Plasmid DNA pCold (Takara Bio, Shiga, Japan) was prepared by the alkaline-sodium dodecyl sulfate (SDS) method from *Escherichia coli* (*E. coli*) DE21 (lab stock) transformed with the plasmid.

**Detection of Copper Binding to DNA** — Plasmid DNA pCold has BamHI- and EcoRI-recognition sequences in the multiple cloning site. Plasmid DNA (300  $\mu\text{M}$ /base) was mixed with 0, 20 or 40  $\mu\text{M}$   $\text{CuCl}_2$  and incubated overnight at 37°C. Following incubation, the mixture was ultrafiltered to remove free copper ion using centrifugal filter units Microcon MWCO 30000 (Millipore, Bedford, MA, U.S.A.). Filtered DNA was dissolved in distilled water and incubated for 1 hr at 37°C with or without 1 unit of restriction enzyme. Reaction solutions were analyzed by electrophoresis with 1% agarose gel including ethidium bromide in Tris-acetate-ethylenediamine tetraacetic acid (TAE) buffer (40 mM Tris-acetate, 20 mM sodium acetate, 1 mM EDTA, pH 8.0).

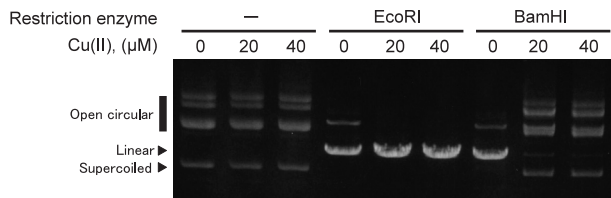
**Measurement of 8-oxodG Formation** — Reaction mixtures of 10  $\mu\text{M}$  dopamine, calf thymus DNA (100  $\mu\text{M}$ /base), 20  $\mu\text{M}$   $\text{CuCl}_2$  and 100  $\mu\text{M}$  NADH in 400  $\mu\text{l}$  of 4 mM sodium phosphate buffer

(pH 7.8) containing 5  $\mu\text{M}$  DTPA were incubated for 1 hr at 37°C. Following the ethanol precipitation, DNA was digested to the nucleosides with nuclease P1 (3.4 units) and calf intestine alkaline phosphatase (1.3 units) and analyzed with an HPLC (model LC-10 series, Shimadzu, Kyoto, Japan) equipped with an electrochemical detector (ECD, model Coulochem II, ESA, Chelmsford, MA, U.S.A.). HPLC conditions were as follows: column, ODS-80Ts (Tsk-gel, 150  $\times$  4.6 mm *i.d.*, Tosoh, Tokyo, Japan); column temperature, 25°C; flow rate, 1 ml/min; and detection wavelength, 254 nm (for dG). The amount of 8-oxodG was determined by ECD analysis. ECD conditions were: guard cell, 400 mV; channel 1, E 150 mV/R 100  $\mu\text{A}$ /filter 2/output 1 V; channel 2, E 300 mV/R 200 nA/filter 10/output 1 V. To examine the reactive species involved in 8-oxodG formation, reactive oxygen species scavengers (10% ethanol, 5% DMSO, 50 units SOD and 50 units catalase) and a Cu(I)-specific chelator (50  $\mu\text{M}$  bathocuproine) were added to the reaction mixtures prior to  $\text{CuCl}_2$  addition.

**UV Spectrophotometry** — UV spectra of dopamine were measured with a UV-visible (UV-Vis) spectrophotometer (model V-560, JASCO, Tokyo, Japan) at room temperature in measurement cell of path length 10 mm. Reaction mixtures consisting of 60  $\mu\text{M}$   $\text{CuCl}_2$ , 30  $\mu\text{M}$  dopamine, or both of them in 2 ml of 4 mM sodium phosphate buffer (pH 7.8) containing 5  $\mu\text{M}$  DTPA were subjected to measurement immediately after preparation.

**$^1\text{H}$  NMR** —  $^1\text{H}$  NMR spectra were obtained on a model ECP500 spectrometer (JEOL, Tokyo, Japan) for 200  $\mu\text{M}$  dopamine with or without 10 mM  $\text{CuCl}_2$  in DMSO- $d_6$ . Chemical shifts are expressed in parts per million (ppm) based on the reference signal of DMSO at  $\delta$  2.49. The data were analyzed by ALICE2 software (JEOL).

**CD Spectrophotometry** — CD spectra of calf thymus DNA were measured with a CD spectrophotometer (model J-725, JASCO). Reaction mixtures contained calf thymus DNA (100  $\mu\text{M}$ /base) and either 20  $\mu\text{M}$   $\text{CuCl}_2$ , 10  $\mu\text{M}$  dopamine or both of them; for Cu(I) production, 20  $\mu\text{M}$   $\text{CuCl}_2$  was treated with 20  $\mu\text{M}$  ascorbic acid in 2 ml of 4 mM sodium phosphate buffer (pH 7.8) containing 5  $\mu\text{M}$  DTPA. The mixture was immediately subjected to CD spectrophotometry under the following measurement conditions: light path length, 1 cm; range, 200–350 nm; band width, 2.0 nm; resolution, 1 nm; speed, 100 nm/min.



**Fig. 1.** Sequence-specific Binding of Cu(II) on Plasmid DNA. Plasmid DNA incubated with Cu(II) was digested by restriction enzymes as described in Materials and Methods. Forms of plasmid are indicated near the bands.

## RESULTS

### Sequence-specific Binding of Cu(II) to DNA

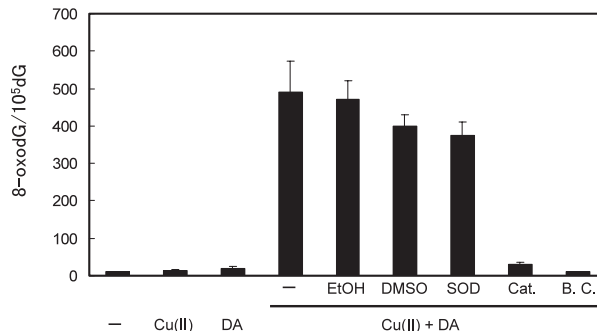
Interaction between Cu(II) and DNA was investigated using restriction enzymes (Fig. 1). BamHI digestion at the recognition site (5'-G/GATCC-3') was inhibited by pretreatment of plasmid DNA with Cu(II), whereas EcoRI digestion (5'-G/AATTC-3') was not inhibited. Direct inhibitions of the enzyme activities by Cu(II) were unlikely because unbound Cu(II) with DNA was removed by centrifugal filtration before the enzymatic reaction with DNA. These results suggest that Cu(II) preferentially binds to consecutive guanines (G-G) in DNA.

### Formation of 8-oxodG in Calf Thymus DNA in the Presence of Copper and Dopamine

Dopamine induced 8-oxodG formation in calf thymus DNA in the presence of Cu(II) (Fig. 2). Neither dopamine nor Cu(II) alone induced 8-oxodG. Catalase and bathocuproine, a Cu(I)-specific chelator, inhibited the 8-oxodG formation, whereas ethanol and DMSO, typical hydroxyl radical scavengers, as well as SOD showed almost no effects. These results indicate that Cu(I) and H<sub>2</sub>O<sub>2</sub> are responsible for the 8-oxodG formation in the dopamine/copper system.

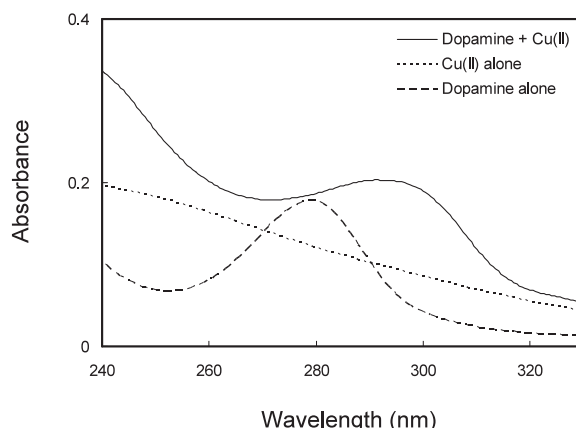
### UV Spectral Changes of Dopamine in the Presence of Copper

The reaction between dopamine and Cu(II) was monitored based on the UV spectral changes (Fig. 3). Absorption peak of dopamine was observed at 278 nm. In the presence of Cu(II), the peak was shifted to around 295 nm with slight increase of the absorbance. This change was not an additive effect of Cu(II). This suggests the formation of a certain product from dopamine through the reaction with Cu(II).



**Fig. 2.** Formation of 8-oxodG in Calf Thymus DNA in the Presence of Copper and Dopamine and Effect of Reactive Oxygen Species Scavengers and Cu(I)-specific Chelator

Formation of 8-oxodG was measured as described in Materials and Methods. DA, dopamine; EtOH, 10% (v/v) ethanol; DMSO, 5% (v/v); SOD, 50 units; Cat., 50 units catalase; B.C., 50 μM bathocuproine. Each Column and bar indicates mean  $\pm$  SD of at least three independent reactions.



**Fig. 3.** UV Spectra of Dopamine and Its Reaction Product with Cu(II)

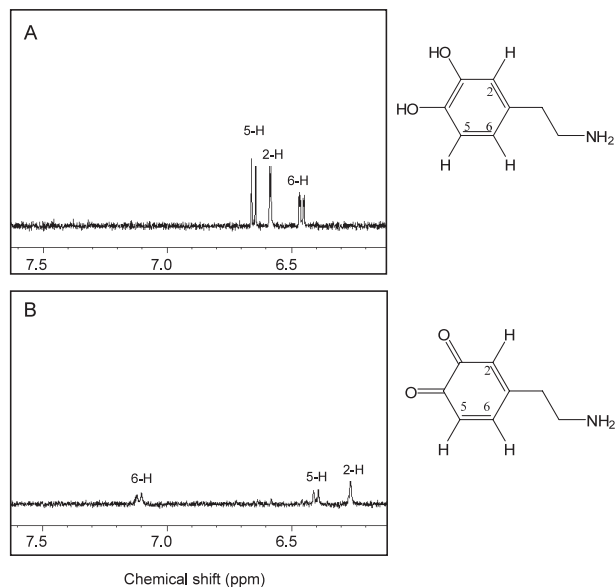
The measurements were performed as described in Materials and Methods. Solid line, 30 μM dopamine + 60 μM Cu(II); dashed line, 60 μM dopamine; dotted line, 60 μM Cu(II).

### <sup>1</sup>H NMR Spectral Changes of Dopamine in the Presence of Copper

To further investigate the reaction between dopamine and Cu(II), <sup>1</sup>H NMR analysis was performed (Fig. 4). Proton signals of dopamine (2-H,  $\delta$  6.60, d; 5-H,  $\delta$  6.66, d; 6-H,  $\delta$  6.47, dd) disappeared in the presence of sufficient Cu(II), and instead, signals corresponding to 3,4-dioxodopamine (2-H,  $\delta$  6.27, s; 5-H,  $\delta$  6.41, d; 6-H,  $\delta$  7.12, d) appeared. Only 3,4-dioxodopamine signals were observed without other products.

### CD Spectral Changes of DNA in the Presence of Dopamine and Copper

A conformational change in the DNA strand



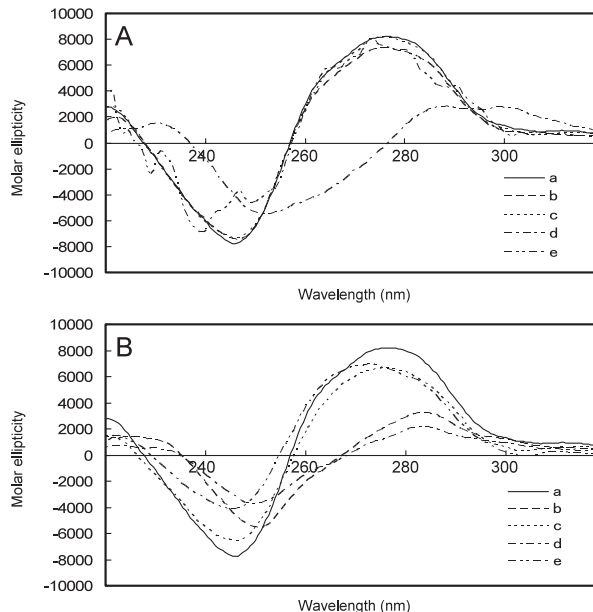
**Fig. 4.**  $^1\text{H}$  NMR Spectra of Dopamine and Its Reaction Product with Cu(II)

A, 200  $\mu\text{M}$  dopamine solution. B, 200  $\mu\text{M}$  dopamine and 10 mM Cu(II). The reactions and measurements were performed as described in Materials and Methods.

in the presence of dopamine and copper was detected by CD spectrophotometry. The CD spectrum of calf thymus DNA was drastically changed by co-addition of dopamine and Cu(II), although dopamine and Cu(II) alone showed no spectral change (Fig. 5A, a–d). Bathocuproine suppressed the spectral change (Fig. 5A, e), while neither ethanol, DMSO, SOD nor catalase had any effect (data not shown). Moreover, Cu(I) reduced from Cu(II) by ascorbic acid induced the spectral change irrespective of  $\text{H}_2\text{O}_2$  presence (Fig. 5B). These results suggest that Cu(I) plays a crucial role in this DNA conformational change induced by the dopamine/copper system.

## DISCUSSION

In the present study, Cu(II) preferentially bound to G-G in the DNA strand was reduced by dopamine to Cu(I), which induced the oxidative DNA damage (8-oxodG formation) together with concomitantly produced  $\text{H}_2\text{O}_2$ . A conformational change of DNA was also observed during the oxidative DNA damage by a copper-dopamine redox system. In  $^1\text{H}$  NMR experiments, only 3,4-dioxodopamine (quinone) signals were observed without other products such as semiquinone. This is consistent with



**Fig. 5.** CD Spectral Changes of DNA in the Presence of Dopamine and Copper

Panel A, CD spectra of DNA in the presence of dopamine/Cu(II) system. Spectrum a, 100  $\mu\text{M}$  calf thymus DNA; b, DNA + 20  $\mu\text{M}$  Cu(II); c, DNA + 10  $\mu\text{M}$  dopamine; d, DNA + 20  $\mu\text{M}$  Cu(II) + 10  $\mu\text{M}$  dopamine; e, DNA + 20  $\mu\text{M}$  Cu(II) + 10  $\mu\text{M}$  dopamine + 50  $\mu\text{M}$  bathocuproine. Panel B, CD spectra of DNA in the presence of  $\text{H}_2\text{O}_2$  or Cu(I) reduced from Cu(II) by ascorbic acid. Spectrum a, 100  $\mu\text{M}$  calf thymus DNA; b, DNA + 20  $\mu\text{M}$  Cu(II) + 20  $\mu\text{M}$  ascorbic acid; c, DNA + 20  $\mu\text{M}$   $\text{H}_2\text{O}_2$ ; d, DNA + 20  $\mu\text{M}$  Cu(II) + 20  $\mu\text{M}$  ascorbic acid + 20  $\mu\text{M}$   $\text{H}_2\text{O}_2$ ; e, DNA + 20  $\mu\text{M}$  ascorbic acid.

the results of UV spectrophotometry that only one absorption peak appeared at the longer wavelength than that of dopamine. Therefore, during the conformational change, dopamine was oxidized to a quinone form when Cu(II) was reduced to Cu(I). Based on these results, we postulate the mechanism for copper/dopamine-mediated DNA damage as follows: first, DNA-bound Cu(II) is reduced to Cu(I) together with dopamine oxidation; second, Cu(I) causes a DNA conformational change by a coordination geometry rearrangement; third, a reactive oxygen species such as  $\text{H}_2\text{O}_2$  produced concomitantly forms a reactive complex with Cu(I) which resides on G-G sequences with an advantageous positioning for oxidative DNA attacking.

Sequence-specific binding of Cu(II) supports the reported preference of G-G for 8-oxodG formation in the copper-redox system.<sup>6)</sup> The square planar Cu(II) coordination with guanines is rearranged to the tetrahedral Cu(I) geometry on Cu(II) reduction by dopamine. The stronger Cu(I) binding with guanines than Cu(II) strengthens the interaction of copper with DNA.<sup>7)</sup> This rearrangement in coordination

geometry may cause the conformational change in the DNA strand. The decrease in positive Cotton effect at around 270 nm could be related to an increase in the winding angle of the DNA helix,<sup>8)</sup> and the total pattern of the dopamine/Cu(II)-treated DNA spectrum was not consistent with any of the established forms of DNA (A, B, C and Z).<sup>9)</sup> Cleavage of the DNA strand by oxidative damage has also been suggested to cause this conformational change.<sup>10)</sup> However, our data strongly suggest that the conformational change is independent of oxidative damage, because catalase inhibited 8-oxodG formation without any effect on the CD spectrum, and actually H<sub>2</sub>O<sub>2</sub> was shown to be dispensable for the CD change.

Changes in the DNA conformation can potentially affect various DNA reactions, including replication, transcription and epigenetic modification.<sup>11)</sup> Copper exists in the cell nucleus at a relatively high concentration and closely associates with chromosomes and bases,<sup>12,13)</sup> although its free ion form is absent in the body.<sup>14)</sup> Thus, changes in DNA conformation could be involved in metal-mediated genotoxicity as well as oxidative damage but via different mechanism.

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