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Photocontrol of Spatial Orientation and DNA Cleavage Activity of Copper(II)-Bound Dipeptides Linked by an Azobenzene Derivative

Halan Prakash,[†] Akemi Shodai,[‡] Hiroyuki Yasui,[§] Hiromu Sakurai,[§] and Shun Hirota*[,†]

Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan, JST, Kawaguchi, Saitama 332-0012, Japan, and Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, 5 Nacauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan

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Copper(II) ion-bound CysGly dipeptides linked by an azobenzene derivative were photoisomerized between the trans and cis forms. The two copper(II) ion centers were positioned close to each other in the cis form, whereas they were far away from each other in the trans form. The copper complex in the cis form exhibited DNA cleavage activity, whereas the activity in the trans form was negligible. The DNA cleavage activity of the cis form is attributed to the cooperation of the closely located copper(II) centers. The present results show the photocontrol of the cooperation of metal ions for DNA cleavage.

DNA cleavage by metal complexes has been actively studied to design artificial metallonucleases.[1,2] For this purpose, many dinuclear metal complexes have been shown to be effective, in which the metal ion centers could exhibit cooperation for DNA cleavage.[1,2] Peptide and amino acid metal complexes have also been shown to be effective for DNA cleavage.[3] On the other hand, photoresponsive molecules are of enormous interest in view of control of the structure and function of biomolecules.[4,5] The helical structure and spatial orientation of DNA binding peptides have been photocontrolled by modification of the peptides with an azobenzene cross-linker, which was photoisomerized between the trans and cis forms.[5] We envisaged that the cooperation of the metal ion centers in DNA cleavage could be photocontrolled by linking two metal complexes with an azobenzene derivative, in which the distance between the metal ion centers could be modulated due to the change in their spatial orientation by photoisomerization of the azobenzene linker. Therefore, we linked CysGly (cystylglycine) dipeptides by an azobenzene derivative, *trans*-4,4′-bisbromomethylazobenzene, and obtained a photoisomerizable peptide (**1**) as a ligand and its dicopper complex (**1**-2Cu^{2+}; Figure 1). The copper complex **1**-2Cu^{2+} was reactive for DNA cleavage in the cis form (**1**-cis-2Cu^{2+}), in which the copper(II) ion centers are oriented close to each other. However, the reactivity of **1**-2Cu^{2+} was negligible in the trans form (**1**-trans-2Cu^{2+}), where the copper(II) ions are far away from each other. These results demonstrate the photocontrol

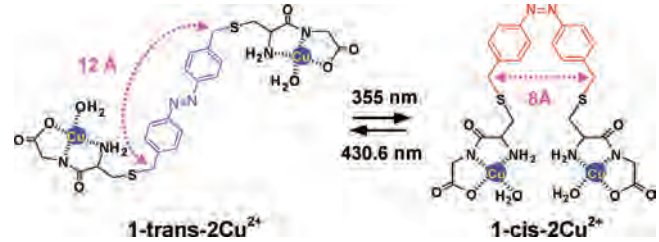

Figure 1. Photoconversion between the trans and cis forms of **1**-2Cu^{2+}, in which the azobenzene chromophore links Cu(II)-bound CysGly peptides.

* To whom correspondence should be addressed. E-mail: hirota@ms.naist.jp.
† Nara Institute of Science and Technology.
‡ JST.
§ Kyoto Pharmaceutical University.

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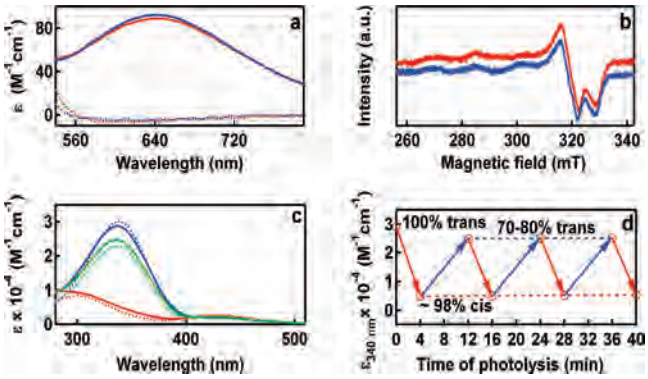

Figure 2. Absorption spectra of **1** and **1**-2Cu^{2+} and ESR spectra of **1**-2Cu^{2+}. (a) Absorption spectra of **1**-trans-2Cu^{2+} (blue, solid line), **1**-cis-2Cu^{2+} (red, solid line), **1**-trans (blue, dotted line), and **1**-cis (red, dotted line). (b) ESR spectra of **1**-trans-2Cu^{2+} (blue) and **1**-cis-2Cu^{2+} (red) recorded at 77 K. The spectra were offset for clarity. (c) Changes in the absorption spectra upon photoisomerization of **1**-trans-2Cu^{2+} (blue, solid line) to **1**-cis-2Cu^{2+} (red, solid line) and **1**-cis-2Cu^{2+} to the photostationary state (70−80% **1**-trans-2Cu^{2+}, green, solid line). Corresponding changes of **1** upon photoisomerization (dotted lines) are depicted. (d) Absorbance change at 340 nm on reversible photoisomerization of **1**-2Cu^{2+}. The cis and trans forms were obtained by photolysis at 355 nm (7 mJ, 4 min) and 430.6 nm (4 mJ, 8 min), respectively.

of the spatial orientation of the copper(II)-bound dipeptides linked by an azobenzene derivative for DNA cleavage.

The CysGly dipeptides were linked to an azobenzene chromophore by chemical modification of the side-chain thiol group of the CysGly dipeptide with *trans*-4,4′-bisbromomethylazobenzene. The obtained ligand (**1**) was characterized by matrix-assisted laser desorption ionization−time of flight mass spectrometry, ^{1}H NMR, and absorption spectra and found to be 100% in the thermodynamically stable trans form (**1**-trans; Figures S1−S4, Supporting Information).[6] The addition of 2 equiv of Cu(II) ions to **1**-trans produced **1**-trans-2Cu^{2+}, which could be photoisomerized to **1**-cis-2Cu^{2+} (Figure 1). The copper complexes **1**-trans-2Cu^{2+} and **1**-cis-2Cu^{2+} showed similar absorption bands with maximum wavelengths around 638 nm (Figure 2a) and electron spin resonance (ESR) spectra with parameters of $g_{||} = \sim 2.255$ and $A_{||} = \sim 174 \times 10^{-4}$ cm^{-1} (Figure 2b). These spectroscopic results are closely related to the characters of the Cu(II) complexes of GlyCys disulphide and GlyGly dipeptides, which reveal that the terminal amino group, the carboxyl group, the deprotonated amide nitrogen, and labile water are coordinated to the Cu(II) ion in **1**-2Cu^{2+} (Figure 1).[7,8]

1-trans and **1**-trans-2Cu^{2+} exhibited similar $\pi-\pi^*$ (280−390 nm) and $n-\pi^*$ (390−500 nm) absorption bands due to the azobenzene chromophore (Figure 2c). The photolysis of **1**-trans and **1**-trans-2Cu^{2+} at 355 nm led to the formation of the cis form of ligand **1** (**1**-cis) and **1**-cis-2Cu^{2+}, respectively, with a decrease in the intensity of the $\pi-\pi^*$ band and an increase in that of the $n-\pi^*$ band (Figure 2c and Figure S4 of the Supporting Information). Subsequent photolysis of **1**-cis and **1**-cis-2Cu^{2+} at 430.6 nm resulted in a photostationary state with the corresponding trans forms as the predominant species (70−80%; Figure 2c and Figure S4 of the Supporting Information). The photoisomerization of **1** and **1**-2Cu^{2+} could be repeatedly performed (Figure 2d and Figure S4 of the Supporting Information), and the photoisomerization properties of **1** and **1**-2Cu^{2+} were comparable to those of the earlier reported azobenzene-based peptides.[5,6a] The azobenzene chromophore linking the CysGly dipeptides undergoes effective photoisomerization, and therefore the spatial orientation of the Cu(II)-bound dipeptides can be externally controlled by light (Figure 1).

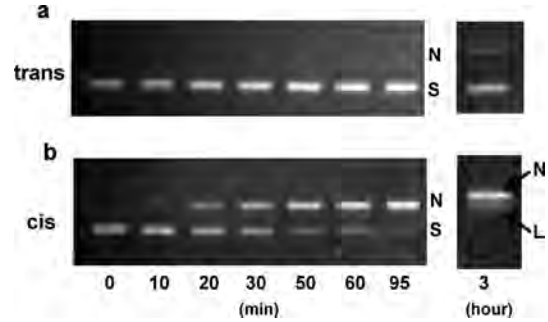

Figure 3. Agarose gel of DNA observed at various time intervals during cleavage by **1**-2Cu^{2+}: (a) **1**-trans-2Cu^{2+} and (b) **1**-cis-2Cu^{2+}. Concentrations of ligand **1** and Cu(II) were 35 and 70 μM, respectively. The amount of DNA was 1 μg, and the reaction was carried out at 37 °C. S, N, and L, represent supercoiled, nicked, and linear DNA, respectively.

1-trans-2Cu^{2+} and **1**-cis-2Cu^{2+} showed clear difference in their ability to cleave the supercoiled DNA to the nicked and linear forms, which was monitored by agarose gel electrophoresis.[2] Incubation of supercoiled DNA (1 μg) with **1**-trans-2Cu^{2+} (70 μM Cu(II) ion concentration) up to 3 h did not produce nicked DNA in significant amounts (Figure 3a). This result reveals that there is no appreciable DNA cleavage activity by **1**-trans-2Cu^{2+} (Figure 3a). Under the same experimental conditions, incubation of supercoiled DNA with **1**-cis-2Cu^{2+} for 3 h produced both the nicked and linear DNA forms (Figure 3b), whereas the Cu(II) ions and ligand **1** showed no appreciable DNA cleavage activity (Figure S5, Supporting Information). The rate constant for the decay of the amount of supercoiled DNA by **1**-cis-2Cu^{2+} (4.7 μM) was $\sim 3.2 \times 10^{-5}$ s^{-1}, which was close to the rate constants reported for artificial metallonucleases[1a] (Figure S6, Supporting Information). The results show that **1**-cis-2Cu^{2+} has higher DNA cleavage activity than **1**-trans-2Cu^{2+}.

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It has been shown that metal ion centers placed ~8 Å apart from each other exhibit cooperativity for DNA cleavage.[2a] The higher DNA cleavage activity of **1**-cis-2Cu^{2+} is attributed to the cooperation of closely located Cu(II) ion centers, while the Cu(II) ion centers are positioned relatively far away from each other in **1**-trans-2Cu^{2+} and showed no appreciable DNA cleavage activity.

The interdistance between the benzylic carbon atoms of the central azobenzene chromophore in the cis form is approximately 8 Å according to the molecular model,[5b] and the Cu(II)-bound dipeptides could be oriented close to each other in **1**-cis-2Cu^{2+} to exhibit effective cooperation to cleave DNA (Figure 1 and Figure S7 of the Supporting Information). In the case of **1**-trans-2Cu^{2+}, the benzylic carbon atoms are ~12 Å away from each other (Figure S7, Supporting Information). Therefore, the Cu(II)-bound dipeptides are far away from each other and are not favorable for cooperation and DNA cleavage in **1**-trans-2Cu^{2+}.

The binding constants of **1**-trans-2Cu^{2+} and **1**-cis-2Cu^{2+} to *calf thymus* DNA were determined to be $\sim 1.7 \times 10^4$ and $\sim 0.7 \times 10^4$ M^{-1}, respectively (Figure S8, Supporting Information). Fluorescence quenching of ethidium bromide-bound *calf thymus* by **1**-cis-2Cu^{2+} indicated no significant intercalation into DNA (Figure S9, Supporting Information). Therefore, there was no significant difference in the DNA binding constant between **1**-trans-2Cu^{2+} and **1**-cis-2Cu^{2+}, although the DNA cleavage activity was different.[1,2a] The present results exemplify the importance of the cooperation of two metal ions for DNA cleavage. To the best of our knowledge, this is the first example of photocontrolling the cooperation of Cu(II) ion centers in a dicopper complex for DNA cleavage.

The DNA cleavage activity of **1**-cis-2Cu^{2+} was not effectively inhibited by the removal of oxygen or the presence of radical scavengers such as dimethyl sulfoxide, potassium iodide, or glycerol (Figure S10, Supporting Information). The pH-dependence of the nicked DNA produced by **1**-cis-2Cu^{2+} showed a bell-shaped feature with maximum activity around pH 9, which was close to the pK_a of the coordinated water molecule (Figure S11, Supporting Information).[2,7,8] This pH-dependence is typical for dinuclear complexes, which show hydrolytic DNA cleavage activity.[1a,2a] The nicked DNA produced by **1**-cis-2Cu^{2+} could be effectively transformed into competent cells, and supercoiled DNA could be obtained from the subsequently grown cells (Figure S12, Supporting Information). Similar results were reported for the nicked DNA produced hydrolytically by Cu(II) complexes.[9] The ligation of linearized plasmid DNA produced by **1**-cis-2Cu^{2+} was not observed in the presence of T4 ligase using gel electrophoresis. This result suggests random cleavage of DNA and blunt end formation, which may reduce ligation efficiency, as reported earlier.[1,9,10] It has also been reported that metal complexes including Cu(II) complexes may predominantly generate 3′ phosphate and 5′ hydroxyl end fragments that are not suitable for ligation.[1,10] However, in the presence of ascorbic acid, a reducing agent for the reduction of Cu(II) ions, **1**-trans-2Cu^{2+} and **1**-cis-2Cu^{2+} showed similar DNA cleavage activity (Figure S13, Supporting Information). These results show that the difference in DNA cleavage reactivity was observed only under hydrolytic conditions.

In summary, a new photoresponsive molecule with Cu(II)-bound dipeptides linked by an azobenzene chromophore is reported. The spatial orientation of the Cu(II) dipeptides were externally controlled by photoisomerization of the azobenzene linker. The copper complex in the cis form showed DNA cleavage activity, whereas there was no appreciable activity with the trans form. The higher reactivity of the cis form is attributed to the cooperation of closely located metal ions for DNA cleavage. The present results provide a new way to control the DNA cleavage activity of the metal complexes by photocontrol of the relative distance between the metal ion centers.

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Supporting Information Available: Syntheses of **1** and **1**-2Cu^{2+}, experimental conditions, and results of photoisomerization and DNA cleavage. This material is available free of charge via the Internet http://pubs.acs.org.

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