Metal ion-directed DNA recognition by chelating DNA ligands

Toshihiro Ihara, Takashi Ikegami, Shinji Sueda, Makoto Takagi and Akinori Jyo
Department of Applied Chemistry and Biochemistry, Faculty of Engineering, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555, Japan and Department of Chemical Systems and Engineering, Graduate School of Engineering, Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

ABSTRACT
Novel DNA ligand bearing oxine and pyridinium group has been synthesized. The DNA binding of this ligand was regulated by coexisting Cu²⁺ over the range of more than three orders in magnitude of binding constant. This should be due to the metal-mediated dimerization of the ligand and concomitant emergence of cationic charges. There were definite two DNA binding modes for the ligand dimer depending on the P/L ratio.

INTRODUCTION
In all living systems, every biological reaction has to be mutually connected and reconciled with each other for attaining their homeostasis. It is well known that many of these homeostasis-related regulatory reactions are achieved at a DNA transcription level, in which the interaction of gene regulatory factors with DNA is controlled directly or indirectly by several means, e.g., dimerization, phosphorylation, binding with the third species such as proteins, hormones, metal ions, and so on. Among these regulatory means, metalloregulation of DNA-ligand interaction is one of the most promising strategy for the construction of artificial DNA ligands possessing on/off switching ability for DNA-ligand binding, because it is possible as occasions demand to choose an appropriate combination of metal ions and chelating reagents from the enormous library of assets in metal complex chemistry.

The authors had designed the series of DNA ligands that are composed of two functionally different parts: one part carries a DNA binding function and the other has an ability to bind metal ions. The DNA binding characteristics of these synthetic ligands were regulated by metal ions coexisting in solution through metal complexation by the ligands, because the metal complexation by the ligands should lead to the change in the net electric charge as well as the change in the whole conformation of the ligands. We have added a novel compound to this series of metal ion-depended DNA ligands. The ligand is an azo derivative, 1, containing a aromatic quaternary ammonium and an oxine group for binding with DNA and a metal ion, respectively (Figure 1).

RESULTS AND DISCUSSION
The DNA ligand, 1, was synthesized through usual azo coupling between oxine and 3-aminopyrididine and subsequent quarternarization of the aromatic amine using methyl iodide. Metal binding properties of 1 were studied using UV/vis spectrophotometry. In the experiment of Cu²⁺ titration, the spectral changes of 1 clearly indicated that 1 form a dimer through 1 : 2 complexation with a Cu²⁺ ion in the absence and in the presence of DNA (from calf thymus) (data not shown). Although it is known that Cu²⁺ can bind to the phosphate groups and the bases of DNA, in the present system, bind strongly to the oxine moiety of 1 even in the presence of a large excess of DNA phosphates and bases. This is a good indication of an intimate ternary interaction among DNA strand, 1, and Cu²⁺ ion.

Figure 2 shows the DNA binding behavior of 1 in the absence and in the presence of a half moles of Cu²⁺. It is apparent that Cu²⁺ drastically affects the behavior. In the absence of Cu²⁺, the absorbance solely decreased with the amount of DNA increasing (saturated point is out of the range of Figure 2(a)). The binding constant of 1 with DNA was about 10³ M⁻¹. This small value is reasonable considering the pKₐ of the oxine moiety (pKₐ = 6.5); the major species of 1 was electrically neutral in the present conditions. The DNA binding behavior of 1 in the presence of Cu²⁺ was biphasic. As the DNA increased, the absorbance steeply declined and touched bottom at the equivalence point of the charge of 1 and DNA (phase I). Subsequently, the absorbance exhibited gentle increase (phase II); isosbestic points were observed in this phase. That is, there were definitely different two DNA
binding modes for metal-mediated dimer of 1 according to the P/L (DNA polymer / ligand) ratio.

The dimer of 1 bound DNA stoichiometrically in the phase I. Quantitative analysis of the spectral change observed in this phase was failed because the binding constant was too strong to be precisely estimated by spectrophotometry. The constant seems to be more than $10^6$ M$^{-1}$. The addition of one half moles of Cu$^{2+}$ enhanced the binding constant of 1 at least for thousand times in phase I (Figure 2(b)). The binding constant of 1 dimer surpasses those of typical low molecular weight DNA ligands having two positive charges such as quinacrine and propidium. Gradually grown hypochromism in the spectra of 1 was observed a few hours later from the mixing with Cu$^{2+}$ in the absence of DNA. After that fine aggregates appeared slowly in the solution. This means that the metal-mediated dimer of 1 tends to aggregate by themselves in nature. Extremely high binding constant of 1 dimer with DNA seems to relate with this trend. That is, the force of the self-aggregation could be a positive cooperativity for binding with DNA in phase I. In this phase, DNA binding of 1 dimer should be regarded as a kind of the electrostatic interaction between polyelectrolytes.

The binding mode in each phases was studied by circular dichroism (CD). Figure 3 shows the CD spectra of 1 measured at several P/L ratios in phase II where 100 % of 1 bound to DNA as the dimer. It is apparent that 1 dimer provided strong induced CD (ICD) when bound DNA. All the spectra observed here were bisignate, which are typical shapes of exciton-type CD spectra. Interestingly, the shape was highly depended on the P/L ratio. The signs of the ICDs were reversed gradually on going from low to high P/L ratio. This exciton spectra should not be due to the exiton interaction between the dimers, because strong bisignate CD was also observed at high P/L ratio. The spectra, therefore, seem to be of the intradimer exciton interactions. That is, the 1 dimers predominantly observed at low and high P/L ratio are thought to have a pseudo-enantiomeric structures each other; their square coordination plane should be tetrahedrally distorted to take opposite propeller twist configurations. The configuration at low and high P/L ratios might be that of A and A, respectively. Phase II is a transitional process between the configurations (from A to A, in the present measurement procedure).

Considering the non-planer coordination in 1 dimer, intercalation is hardly conceivable. The study for elucidating the orientation of the dimer in the groove of double-stranded DNA remains to be solved.

REFERENCES