Metal ion-directed outside binding of small DNA ligand

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ABSTRACT
DNA binding of the ligand bearing an oxine and a pyridinium group was regulated by coexisting Cu2+ over the binding constant range of three orders of magnitude. The ligands coordinated to Cu2+ to form a dimer and then cooperatively bound outside of DNA duplex to give the well-regulated 1-D structure along the DNA backbone.

INTRODUCTION
The techniques to regulate the binding of DNA ligands by certain signals are very useful in the development of DNA probes and gene regulatory systems. Such ligands have been synthesized by combining some functionalities such as signal responsive and DNA binding units (1).

The authors have designed a series of DNA ligands that are composed of two functionally different parts: one part carries the DNA binding function and the other has the ability to bind metal ions (2). The DNA binding characteristics of these synthetic ligands were regulated by metal ions coexisting in solution through metal complexation, because the metal complexation by the ligands should lead to a change in the net electric charge as well as a change in the entire conformation of the ligands. The ligand presented in this paper is an azo derivative, 1, containing an aromatic quaternary ammonium and an oxine group for binding with DNA and a metal ion, respectively (Figure 1). The ligand was designed so that the distance between the ammonium moieties would match that between the opposite phosphates on both backbones when the ligands form a metal-mediated dimer.

RESULTS AND DISCUSSION
We synthesized 1 through the usual azo coupling between the oxine and 3-aminopyridine and subsequent quaternarization of the aromatic amine using methyl iodide. The counter anion, iodide, was changed to chloride before use. The metal binding properties of 1 were studied using UV/vis spectrophotometry. In the Cu2+ titration experiment, the spectral changes in 1 clearly indicated that 1s form a dimer (12-Cu) through 2 : 1 complexation with a Cu2+ ion in the absence and in the presence of DNA (data not shown). Although it is known that Cu2+ can bind to the phosphates and the nucleobases (3), Cu2+, in the present system, strongly binds to the oxine moiety of 1 even in the presence of a large excess of DNA.

![Figure 1](https://example.com/figure1.png)

**Figure 1** The structure of 1 and possible equilibria with metal ion and DNA

![Figure 2](https://example.com/figure2.png)

**Figure 2** DNA binding behavior of 1 in the absence (-Cu, open circle) and presence (+Cu, closed circle) of Cu2+. Calf thymus DNA was titrated into the solution of 20 μM 1 containing 10 mM Hepes (pH 7.0), 0.1M NaCl, and for, +Cu, 10 μM Cu2+. (a) Absorbance changes with DNA addition. (b) Scatchard plots.
Figure 2 shows the DNA binding behavior of 1 in the absence and in the presence of a half mole of Cu²⁺. It is apparent that Cu²⁺ drastically affects the behavior. In the absence of Cu²⁺, the absorbance of 1 monotonously decreased with the increasing amount of sonicated calf thymus DNA. The binding constant of 1 with DNA was ca. 3.2 x 10⁴ 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 under the present conditions. On the other hand, the DNA binding behavior of 1,Cu was biphasic. As the DNA increased, the absorbance steeply declined and reached bottom at nearly equivalence point of the charge of 1 and DNA (phase I). Subsequently, the absorbance exhibited a slight increase with additional DNA (phase II). The spectral change in phase I was typical of self-stacking; 47% hypochromic effect and 8 nm blue shift were observed. In phase II, the spectral change was composed of a 25% hyperchromic effect and 18 nm red shift (data not shown). Isosbestic points were observed in each phase. These results mean that there are definitely two different binding modes for 1,Cu according to the P/L (nucleotide units of DNA/ligand, 1) ratio. That is, the dimer of 1 stoichiometrically bound to DNA in phase I and then changed its binding mode with increasing P/L in phase II. The binding constant of 1,Cu (in phase I) was ca. 2.4 x 10⁵ M⁻¹. The addition of one half mole of Cu²⁺ enhanced the binding constant of 1 almost thousand times. The binding constant of 1,Cu surpassed those of typical low molecular weight DNA ligands having two positive charges such as quinacrine and propidium (4).

Hydrodynamic techniques, linear flow dichroism and viscosity titration, were employed to study the binding mode and then to account for enormous enhancement of DNA binding of 1,Cu. Figure 3 shows the results of LD (LD = A₁₋₂) titration of 1,Cu with calf thymus DNA. LD is the reduced LD normalized by isotropic absorbance at the same wavelength (LD(λ) = LD(λ)/A₀(λ)). Magnitude of LD of the dimer (visible region) at low P/L was almost the same with that of nucleobases (UV region). It means that the orientation of the long axis of the dimer is nearly perpendicular to the helix axis of DNA at P/L = 2.0-7.5. From the value of the LD, the angle was estimated to be 78° (5). The angle gradually decreased with DNA addition and reached plateau, 62°, at P/L = 75-100. It coincides with the results of UV titration of 1,Cu in phase II shown in Figure 2.

Viscosity titrations revealed that 1,Cu scarcely changed the viscosity of the linear DNA solution (data not shown). Quinacrine, which was used as a typical intercalator (positive control), significantly raised the viscosity of the same solution. Although 1,Cu binds to DNA with its long axis nearly parallel to the nucleobases especially at low P/L, it seems not to insert into adjacent base pairs but to bind to outside of DNA.

These results suggest that 1,Cu binds to DNA in a self-stacked outside binding manner. Presumably, 1,Cu form well-regulated 1-D spiral aggregates like stairs along either groove of the DNA duplex so that the ammonium moieties on both ends of 1,Cu are closely located in the vicinity of the opposite phosphates on both backbones. Therefore, the extremely high binding constant of 1,Cu with DNA seems to be attributed to the effective synergism between the electrostatic interaction with the DNA backbones and the force of their self-aggregation. It should also be added that the formation of this highly stable structure was reversible; the aggregates could be easily decomposed by the addition of excess EDTA.

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REFERENCES