Metal Ion-Directed Cooperative Triple Helix Formation of Glutamic Acid−Oligonucleotide Conjugate

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In chromosome DNAs, there are many repetitive sequences, such as the dyad symmetrical and tandem repeat sequences, which play important roles in organisms.[1] For example, many kinds of transcription regulatory factors recognize the dyad symmetrical sequences. Certain kinds of tandem repeat sequences are also known to be associated with certain genetic diseases. Also, the sequence of TTAGGG in humans is repeated dozens or even thousands of times at each chromosome end, called telomeres. Our attention is directed to the specific recognition of such repetitive sequences on DNA in a smart manner, that is, cooperative action between the units of the DNA binding ligands such as small molecules,[2] peptides,[3] proteins,[4] and oligonucleotides (ODNs).[5] We have already reported the specific recognition of the C2 symmetrical sequence on double-stranded DNA (ds-DNA) by ODN conjugated with iminodiacetic acid (IDA) through the triple-helix formation, in which each IDA moiety of the two conjugates coordinates to a rare earth metal to give the dyad symmetric dimer on the targeted palindrome sequence.[6]

The aims of this study are the generalization of such a strategy for the metal-assisted cooperative recognition of the C2 symmetrical sequences through triple-helix formation and the quantitative assessment of the cooperativity. Here, we synthesized the conjugate (**GluODN**) between ODN and glutamic acid (Glu).

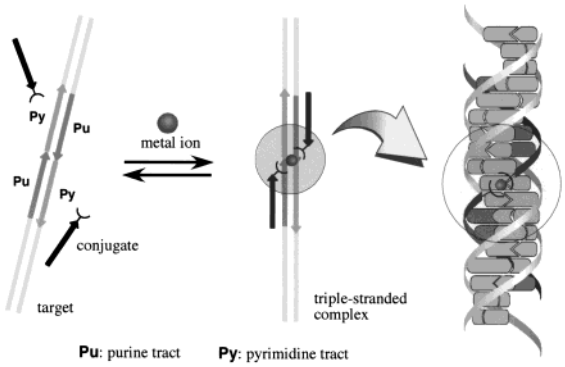

Figure 1. Schematic illustration of metal ion-directed cooperative triple-helix formation of the conjugates on the palindrome sequence.

Now, the metal ion used here was Cu^{2+}, which is the more common ion in organisms and is related to several neurological diseases such as Alzheimer's disease and ALS (amyotrophic lateral sclerosis).[7] It is well-known that amino acids form a two-to-one complex with Cu^{2+} under the appropriate conditions.[8] Therefore, Cu^{2+}, as a cooperative factor, would gather two **GluODN**s into a dimer on the complementary C2 symmetrical sequence of ds-DNA. This is also regarded as a novel methodology for recognizing the Pu−Py mixed-sequences (Figure 1).[9]

ODNs used in this study were prepared using a fully automated DNA synthesizer (Beckman, Oligo 1000M). The synthesized ODNs are indicated below.

f44	5′ CTGGAC**CTCTTCTCTTCTTT**ACGT**AAAGAAGAGAAGAG**CAGGTC 3′ GACCTG**GAGAAGAGAAGAAA**TGCA**TTTCTTCTCTTCTC**GTCCAG
h20	5′ CGACGT**AAAGAAGAGAAGAG** 3′ GCTGCA**TTTCTTCTCTTCTC**
c14	5′ TTTCTTCTCTTCTC
aminoODN	5′ H_2N-TTTCTTCTCTTCTC

GluODN was synthesized by the coupling between the activated ester of Glu (*N*-α-Fmoc-L-glutamic acid γ-succimide ester) and the 5′-aminohexyl-linked ODN (**aminoODN**) by following the procedures previously published (Figure 2).[10] **f44** is the target duplex that contains the quasi-dyad symmetrical sequence composed of two 14-base purine tracts (shown in bold letters) separated by 4 base pairs. On the other hand, **h20** has an isolated half-site for binding, so that the dimerization of **GluODN** on the **h20** duplex is impossible.

The circular dichroism spectrum of the solution of **GluODN**/**f44** indicated the typical features of the triple-stranded complex (ts-complex) of DNA in the presence of a half mole of Cu^{2+} toward **GluODN** under the appropriate conditions (data not shown).[11] The stability of the ts-complex containing **GluODN** and the Cu^{2+} effect on it were assessed by melting experiments using a UV−vis spectrophotometer equipped with a programmable temperature controller (Figure 3). A half mole of Cu^{2+}

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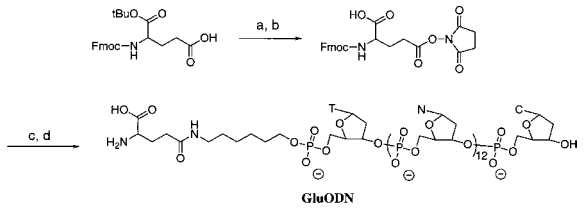


Figure 2. Synthesis of **GluODN**: (a) NHS, DCC, DMAP, dioxane; (b) TFA; (c) **aminoODN**, DMSO/water; and (d) 10% NEt_2H/DMF.

Table 1. Thermodynamic Parameters[a] for the Triple-Helix Formation of **GluODN** with a Binding Site on **f44** in the Presence and the Absence of Cu^{2+}

	ΔH/ kcal mol^{-1}	ΔS/ cal mol^{-1}	ΔG(298 K)/ kcal mol^{-1}	K(298 K)/ M^{-1}	T_m/K
none	-89.5 ± 0.4	-271.8 ± 1.4	-8.50	1.72×10^6	296.1
$+ Cu^{2+}$	-71.0 ± 1.0	-204.8 ± 3.3	-9.97	2.05×10^7	304.5

[a] The parameters, which were for bimolecular interaction between **GluODN** and a binding site on **f44**, were refined by fitting the experimental data with theoretical curves,[12] both of which were shown in Figure 3.

(0.5 μM) toward **GluODN** (1.0 μM) substantially stabilized the ts-structure; the difference between the melting temperatures in the presence and in the absence of Cu^{2+} was ca. 8 °C. On the other hand, the melting temperatures obtained by the control systems, **c14**/**f44** and **GluODN**/**h20**, were scarcely changed by the addition of Cu^{2+}. The ionic strength under all conditions is essentially the same, because the salts (1.0 mM HEPES and 50 mM $MgCl_2$) exist in large excess. Increasing concentration of Cu^{2+} up to 1.0 and 1.5 μM hardly affects the melting curve obtained from the **GluODN**/**f44** system at 0.5 μM Cu^{2+}. Therefore, these results clearly indicate that the stabilization of the ts-complex observed here is due to the cooperative action of the two molecules of **GluODN**s for binding to **f44** in the presence of Cu^{2+}, which plays a critical role as an allosteric effector.

The thermodynamic parameters for the binding of **GluODN** with each binding site on target **f44** could be estimated by curve fitting to the theoretical equations assuming the two-state model where the melting processes should be an all-or-none transition.[12] The parameters refined by the nonlinear least squares are summarized in Table 1. The ts-complex seems to be stabilized by an entropic factor in the presence of Cu^{2+}. Considering the binding constant between Cu^{2+} and the amino acids in the literature, a quantitative one-to-two complexation is not conceivable under the experimental conditions (especially, pH and the concentration of each component).[8] Therefore, dimerization of **GluODN**s in the bulk solution (prior to binding with **f44**) is not likely. Cupric ion presumably binds to the Glu moiety in the one-to-one complex of **GluODN**/**f44** with the assistance of a negative electrostatic field in the microenvironment in the vicinity of the ODN's backbone and then facilitates the subsequent accommodation of the second **GluODN** to the neighboring residual half-binding site on **f44**. As a result, the addition of Cu^{2+} made the apparent binding constant of **GluODN** at 298 K increase more than 10 times.

However, the thermodynamic parameters estimated from the UV-melting experiments, ΔH, ΔS, and K, represent a combination of parameters corresponding to the first binding of **GluODN** to an isolated complementary site on **f44** and those corresponding to subsequent binding to a neighboring site in the cooperative mode. To assess the cooperativity, we carried out the quantitative treatment of the results according to Weber's method.[13] The cooperativity, ω, which is also regarded as the equilibrium

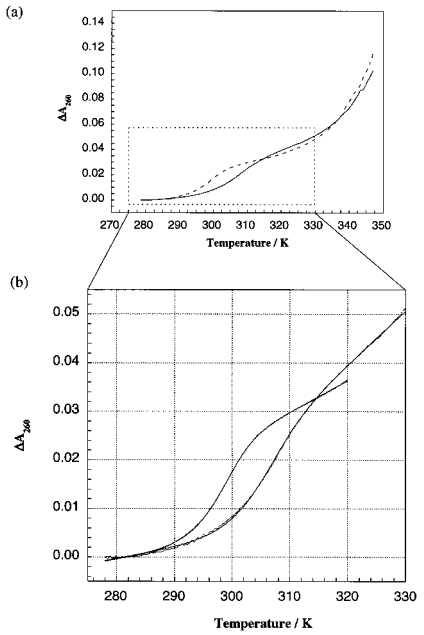
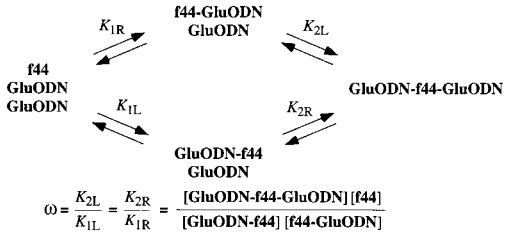


Figure 3. UV melting curves recorded at 260 nm for the complexes of **GluODN** with **f44** in the presence and the absence of Cu^{2+}. Melting experiments were carried out in a buffer solution containing 50 mM $MgCl_2$ and 1.0 mM HEPES (pH 7.0), and the solutions were heated at a rate of 0.5 deg min^{-1}. The concentrations of **GluODN**, **f44**, and Cu^{2+} were 1.0, 0.5, and 0.5 μM, respectively. (a) Both curves showed biphasic features. The transitions at lower temperatures are the meltings of the triplex to the duplex. The solid and broken curves indicate the melting behavior in the presence and the absence of Cu^{2+}, respectively. (b) Curve fitting to the theoretical equation. The points and the curves are the experimental data and theoretical curves, respectively.

constant for a kind of disproportionation reaction, is defined as follows:



where K_{1R} and K_{1L} are equilibrium constants for binding of the first **GluODN** on the right and on the left binding sites on **f44**, respectively. K_{2R} and K_{2L} are similar constants for the second ones on the residual half sites. According to a previously proposed procedure, the ω value was estimated to be ca. 165 at 298 K.[5b] This means that the first binding of **GluODN** on **f44** magnifies the binding constant of the second one by 165 times in the presence of a half mole of Cu^{2+} and the difference in the free energy of the first and the second binding is ca. 3.0 kcal mol^{-1}.

Cooperative binding by the units having a DNA binding ability should result in a higher sequence specificity as well as a greater sensitivity to concentration changes. When using the conjugates modified at both ends, the strategy presented here, metal-directed cooperative recognition, would be extendable to other repetitive sequences such as tandem repeats and the telomere.

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Supporting Information Available: Synthesis scheme, 400 MHz ^{1}H NMR data, and the MALDI-TOF mass spectrum for **GluODN** as well as the procedure of UV-melting experiments (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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