Metallo-Regulation of DNA Triple Helix Formation through Cooperative Dimerization of Two Oligonucleotides

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The DNA binding affinity of oligonucleotide carrying iminodiacetic acid (IDA) group is regulated by metal ion through cooperative dimerization mediated by formation of 2:1 complex of IDA with metal ion.

Oligonucleotides can bind to double helix DNA by forming a triple helix.[1] This oligonucleotide-directed triple helix formation is one of the most useful method for sequence specific recognition of double helix DNA.[2] In the viewpoint of artificial control of gene expression, it is desirable that the DNA binding affinity of oligonucleotides can be regulated by some stimuli. We direct our attention to metal ions as this sort of stimuli and have already reported the synthetic DNA intercalators,[3] whose DNA binding affinity can be regulated by coexisting metal ions. On the other hand, many DNA binding proteins including transcription factors bind to dyad symmetrical sequences on DNA as a dimer.[4] In this system, DNA binding affinity of the proteins is increased by cooperative binding of two molecules of the same protein. Here, we report that the binding affinity of oligonucleotide to dyad symmetrical double-stranded sequence can be regulated by metal ion through cooperative dimerization of the two oligonucleotides.

In this study, we synthesized oligonucleotide **1** carrying iminodiacetic acid (IDA) group as metal chelating moiety (Figure 1).[5] It is well-known that IDA forms 1:2 complexes with various metal ions such as Cu(II), Fe(III), and lanthanoid ions.[6] The target duplex for **1**, 44mer, contains quasi-dyad symmetrical sequence composed of two 14 base purine tracts separated by 4 base pairs (Figure 2).[7] Thus, oligonucleotide **1** is expected to bind to the 44mer as a dimer in the presence of appropriate metal ions (Figure 3), and it should be possible to regulate the binding affinity of oligonucleotide **1** by coexisting metal ions.

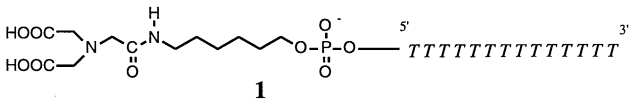

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Figure 1. Structure of IDA-modified oligonucleotide **1**.

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5'-CTGGACTTTTTTTTTTTTTTGACGAAAAAAAAAAAAAAACAGGTC-3'
3'-GACCTGAAAAAAAAAAAAAAACTGCTTTTTTTTTTTTTTTGTCCAG-5'
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44mer

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5'-CTGGACAAAAAAAAAAAAAAACAGGTC-3'
3'-GACCTGTTTTTTTTTTTTTTTGTCCAG-5'
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26mer

Figure 2. The sequences of target DNA duplexes used in the present study.

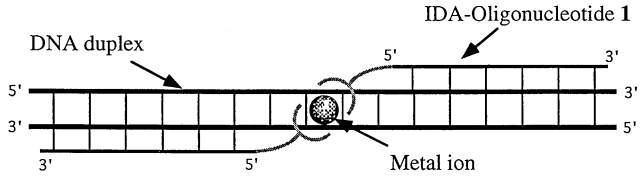

Figure 3. Schematic illustration of DNA triple helix formed by dimerized oligonucleotide **1** with DNA duplex (44mer) in the presence of metal ion.

The DNA binding behavior of **1** was studied by the melting experiments of the complexes (DNA triple helix). Lu(III) ion which has a high binding affinity to IDA was adopted as the regulator.[8] Figure 4 shows UV melting curves for complexes of **1** with the 44mer in the presence and absence of Lu(III) along with their differential curves. The melting curves of the transition at higher temperature were superimposable with that of the duplex 44mer (data not shown). Therefore, the transitions at lower temperature (Figure 4) are attributed to the triplex melting, i.e., dissociation of **1** from the duplex 44mer. The melting temperature of the triplex, Tm, increased by 10 degrees, from 21 to 31°C, by the addition of 0.5 equiv. (to **1**) of Lu(III). On the other hand, the melting curve of the triplex comprised of unmodified (IDA-lacking) oligonucleotide and the 44mer was not affected at all by the addition of Lu(III) (data not shown).

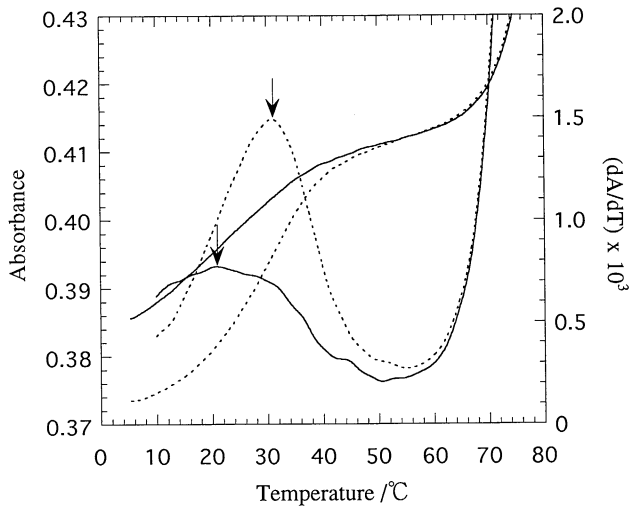

Figure 4. UV melting curves recorded at 260 nm for complexes of **1** with 44mer in the presence (dotted line) and absence (solid line) of Lu(III) and their differential curves. Melting experiments were done in a buffer containing 2 mol dm^{-3} NaCl and 1 mmol dm^{-3} HEPES (pH 7.5), and the solutions were heated from 0℃ to 80℃ at a rate of 0.5 deg min^{-1}. The concentrations of **1**, 44mer, and $LuCl_3$ were 1.0 μmol dm^{-3}, 0.5 μmol dm^{-3}, and 0.5 μmol dm^{-3}, respectively. The peak tops of differential curves (Tm) are indicated by arrows.

Another control experiment was made in order to confirm that the observed enhancement of DNA binding affinity of oligonucleotide **1** was due to the cooperative binding of the two oligonucleotides, that is, the dimerization of IDA-oligonucleotide on the 44mer duplex. In this experiment, duplex 26mer was used as target DNA for **1** (Figure 2). The 26mer has

an isolated half-site for binding, so that the dimerization of **1** on the 26mer duplex is impossible. Figure 5 shows UV melting curves for complexes of **1** with the 26mer in the presence and absence of Lu(III) along with their differential curves. As expected, no significant change was observed by the addition of Lu(III). The observed slight stabilization on Lu(III) addition can be attributed to the increase of positive charge of IDA moiety through complexation with Lu(III).

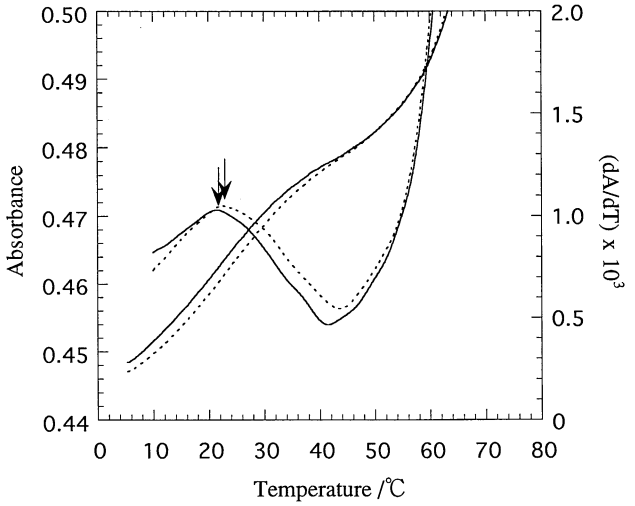

Figure 5. UV melting curves recorded at 260 nm for complexes of **1** with 26mer in the presence (dotted line) and absence (solid line) of Lu(III) and their differential curves. Experimental conditions were the same as those described in Figure 4 except for the concentration of DNA duplex, 26mer. The concentration of 26mer was 1.0 μmol dm^{-3}.

For detailed analysis of the triple helix formation, thermodynamic parameters ($\Delta H°$, $\Delta S°$) were calculated from the plots of Tm^{-1} vs. log C according to the conventional method assuming the two-state model.[9] The results are listed in Table 1. The values of $\Delta H°$ and $\Delta S°$ in the presence of Lu(III) are more negative than those in the absence of Lu(III). The larger enthalpy and entropy changes are the feature of the complex formation of the longer oligonucleotides. $\Delta G°$ values and equilibrium binding constants at 25℃ were also determined (Table 1). The binding constants K of **1** with 44mer increased about 7-fold on addition of Lu(III). So far, several efforts have been made in facilitating the triple helix formation by making use of the oligonucleotide covalently dimerized.[10] We believe that the increase in the affinity of IDA-modified oligonucleotide to the target duplex is fairly high considering the unique strategy of ours where two molecules of oligonucleotide are dictated to be collected and dimerized on the duplex reversibly.[11]

Table 1. Thermodynamic parameters[a] for triple helix formation of **1** with 44mer in the presence and absence of Lu(III)[b]

	$\Delta H°$ / kcal mol^{-1}	$\Delta S°$ / cal mol^{-1} deg^{-1}	$\Delta G°$ (25℃) / kcal mol^{-1}	K (25℃) / dm^3 mol^{-1}
none	-26.9	-63.6	-7.92	6.4×10^5
Lu(III)	-39.3	-101.4	-9.04	4.3×10^6

[a] These values refer to the reaction in which one molecule of **1** and one molecular purine tract in the 44mer form one molecule of triple helix.
[b] Concentration range studied: **1**, 0.2 - 5.0 μmol dm^{-3}; 44mer, 0.1 - 2.5 μmol dm^{-3}; Lu(III), 0.1 - 2.5 μmol dm^{-3}

In conclusion, the DNA binding of IDA-modified oligonucleotide was found to be regulated by metal ion through cooperative binding of the two oligonucleotides. We believe that this work proposes a new clue for artificially controlling the gene expression.

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References and Notes

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5 IDA-modified oligonucleotide **1** was prepared according to the published chemical synthesis (K. Matsumura, M. Endo, and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, **1994**, 2019) after small modifications in the purification step. Oligonucleotide **1** was purified by reversed-phase HPLC under conditions: Asahipak ODP-50 column, 4.6 i.d. x 150 (mm); flow rate, 1.0 ml min^{-1}; buffer A, 0.1M triethylammonium acetate (TEAA, pH 7.0); buffer B, acetonitril; linear gradient, 10-40% B in 30min.
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7 44mer was designed so that IDA moieties of two **1**s face each other when **1**s bind to purine tracts on 44mer. The length of the linker that connects two **1**s in IDA dimer (Figure 3) was estimated to be 4 base pairs long according to CPK model building. Thus, 4 null base pairs were introduced in the center of 44mer.
8 Cu(II) and Fe(III) ions are also known to have high binding affinity to IDA.[6] However, the melting curve for complex of **1** with 44mer was not affected by the presence of these metal ions under our experimental conditions.
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