Design of an artificial restriction enzyme having simultaneous DNA cleavage activity

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ABSTRACT
We designed dimeric distamycin linked hydroxamic acid (DDHA)-metal complexes and investigated double strand simultaneous cleavage. Compared as distamycin linked hydroxamic acid (DHA)-metal complex, DDHA-metal complex cleaved DNA at the same level of DHA-metal complex without double strand simultaneous cleavage.

INTRODUCTION
Sequence-specific DNA cleavage by small functionalized molecule has attached much attention in connection with the design of an artificial restriction enzyme. Recently we have demonstrated that several types of DHA-metal complexes sequence-selectively cleave DNA by cooperative molecular recognition between distamycin and hydroxamic acid-metal complex. Since Lown et al. demonstrated that a cross-linked lexitropsin has a binding enhancement of ca. 1000 times compared with that of the monomer, the dimerization of DHA via methylene linker is expected to recognize DNA with more powerful binding affinity. We herein report the synthesis of DDHA 5 and the DNA cleavage properties of DDHA-metal complexes.

RESULTS and DISCUSSION
The synthesis of DDHA 5 was shown in scheme 1. Tetrapyrrole derivative 2 was prepared from 1,1’-(1,4-alkanediy1)bis(pyrrole) 1 according to the previously reported method. 2 was hydrogenolysis and condensed with carboxylic acid using DCC/HOBt in THF to give pyrrole triamide 3. Hydrogenation of 3 followed by the alkylation using pentafluorophenyl ester gave the methyl ester 4 in 84 % yield. Finally, 4 was converted into DDHA 5 using NH₂OH.HCl/t-BuOK in MeOH. This reaction was quantitatively proceeded, but the yield was 18 % because 5 was hardly purified. Introduction of hydroxamic acid into 5 was confirmed by FeCl₃ test and HRMS.

We examined the DNA cleavage activities of DHA and DDHA 5 toward Col E1 plasmid DNA by monitoring the scission states between supercoiled (form I), nicked circular (form II) and linear (form III). DDHA 5 has two
metal complexes by which the double strand simultaneous cleavage was expected. However, 1 % agarose gel electrophoresis showed that DDHA have the same intensity of DHA cleavage activity (Figure 1, Lane 4-7 vs Lane 9-12). And also, no linear (form III) in gel indicated that DDHA do not cleave double strand. These results suggested that two DHA moieties of DDHA were not effectively placed in the DNA minor groove because the length of linker (tetramethylene chain) was not enough long to fold two moieties.

Figure 1. DNA Cleavage Activities of DHA/DDHA-Fe$^{2+}$ Complex

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<th>Fe$^{2+}$ (10 μM)</th>
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A solution of supercoiled (form I) Col E1 plasmid DNA (0.3 μg), FeSO$_4$ (10 μM) and DHA/DDHA in 40 mM Tris-HCl (pH 8.0) was incubated at 37 °C for 1 hr. (Lane 1) intact, (lane 2) incubated DNA without DHA/DDHA; (lane 3 and 8) incubated DNA without Fe$^{2+}$.

In conclusion, we demonstrated that DDHA-metal complex can cleave DNA as well as DHA-metal complex. The double strand simultaneous cleavage will be realized by the optimization of the length of DDHA linker. More precise analyses for sequence-specific DNA cleavage including phosphorous hydrolysis are in progress.

REFERENCES
