Synthesis of Symmetric Oligo-DNA Dimers and Their Formation of Polymeric Supramolecular Assembly

Yuichi Ohya,* Hiroshi Noro, Mamoru Komatsu, and Tatsuro Ouchi
Department of Applied Chemistry, Faculty of Engineering, Kansai University, Suita, Osaka 564

(Received February 5, 1996)

p-Hydroxybenzene was coupled with tetrathymidine or tetraadenosine through phosphodiester bonds to give symmetric oligo-DNA dimers, $(T4)_2Bz$ and $(A4)_2Bz$, as components for a polymeric supramolecular assembly. The equivalent mixture of them formed a high-molecular-weight aggregate in aqueous media via complemental hydrogen bonds.

In recent years, supramolecular assembly has become a topic in organic chemistry.[1] Several biomolecules are used for the non-covalent assembly systems. In natural products, nucleic acid bases are well known as the molecules which can bind complemental others by hydrogen bonds, and are often used as a specific recognition moiety to bind two or more molecules.[2] A natural photo-synthetic system has a higher ordered arrangement of porphyrins and other chromophores. Much attention is being devoted to arrange chromophores in higher order. Our object is to construct a or more dimensional polymeric supramolecular assembly using only hydrogen bond in aqueous media and to arrange chromophores in higher order. In this study, we designed symmetric oligo-DNA dimers as components for a polymeric supramolecular assembly. These molecules have a dihydroxybenzene group as chromophore model in their centers and 5'-terminals of two kinds of oligo-DNAs, tetrathymidine and tetraadenosine, are attached to two hydroxyl groups of the dihydroxybenzene via phosphodiesters.

Oligo-DNA dimers were synthesized in liquid phase by the phosphorotriester method reported previously[3] according to Scheme 1. 5'-Hydroxy group of the nucleotides was protected by dimethoxytrityl group (DMTr) which can be deprotected with trichloroacetic acid (TCA). Phosphate group was protected with 4-chlorophenyl group and β-cyanoethyl group, which have different stability in alkaline solution. The protected phosphate, was introduced to *p*-hydroxybenzene. Subsequently, the protected 5'-hydroxynucleotidetriesters were coupled with it by using 1-(mesitylene-2-sulfonyl)-3-nitro-1,2,4-triazole (MSNT). The obtained $(T)_2Bz$ and $(A)_2Bz$ were reacted with protected trithymidine or triadenosine, respectively. The obtained fully protected compounds were treated with 28%-NH_3aq./pyridine (8/1) at 60°C for 5 h to give the oligo-DNA dimers, $(A4)_2Bz$ and $(T4)_2Bz$. The purification of the products was carried out by silica-gel column chromatography and reverse phase HPLC until a single peak was given in the HPLC elution profile.

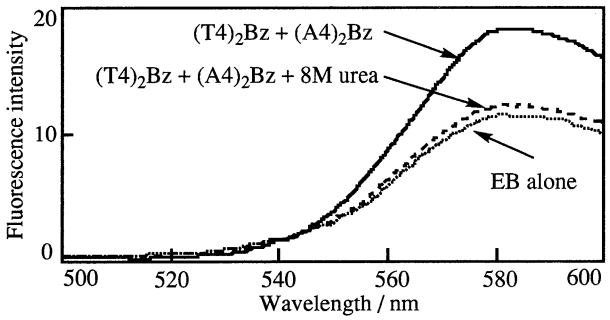
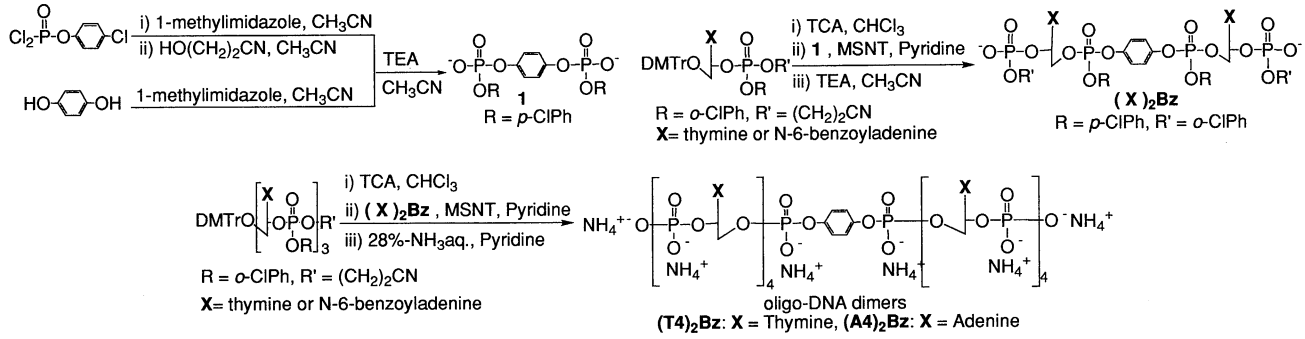


Figure 1. Fluorescence spectra of ethidium bromide (EB) in water. EB alone (dotted line), in the presence of mixture of $(T4)_2Bz$ and $(A4)_2Bz$ (solid line) and the addition of 8M-urea (dashed line). $[(T4)_2Bz] = [(A4)_2Bz] = 3.441\times10^{-5}$ mol/l, [EB] $= 1.70\times10^{-5}$mol/l. After incubation for overnight, fluorescence spectra of EB was measured (excitation at 310 nm) at 15°C.

The formation of hydrogen bonds based on complemental oligo-DNA dimers in aqueous media was investigated by fluorometry. Fig. 1 shows the fluorescence spectra of ethidium bromide (EB) with or without the equivalent mixture of $(T4)_2Bz$ and $(A4)_2Bz$ in water. EB shows the increase in fluorescence intensity when it enters the cavity between nucleic acid bases pairs. Therefore, the increase in fluorescence intensity of EB in the presence of the mixture of $(T4)_2Bz$ and $(A4)_2Bz$ means the formation of complemental hydrogen bonds between them. This was also supported the fact that the increase in fluorescence intensity of EB was very small in the case of the addition of 8M urea solution. We measured CD spectra of the oligo-DNA dimers before and after mixing. Fig. 2 shows the CD spectra pattern for equivalent mixture of $(A4)_2Bz$ and $(T4)_2Bz$ and additive CD spectra pattern of each oligo-DNA dimers alone in buffer solution. The CD spectra pattern of the mixture showed negative band at 250nm and positive band at 260nm, which were diferent from the additive CD spectra pattern. These results suggest that



Scheme 1.

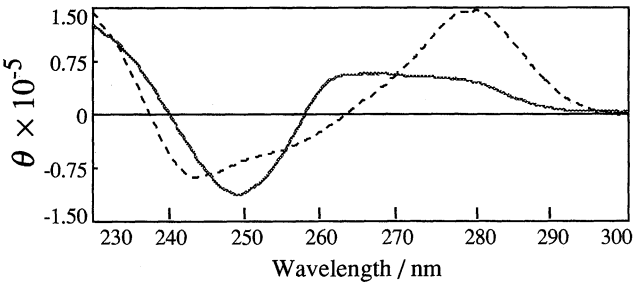


Figure 2. CD spectra for equivalent mixture of $(A4)_2Bz$ and $(T4)_2Bz$ (solid line) and additive CD spectra of the each oligo-DNA dimer (dotted line) in 50mM Tris•HCl-0.2M NaCl buffer solution (pH 7.5) at 15°C. $[(A4)_2Bz] = [(T4)_2Bz] = [(A4)_2Bz + (T4)_2Bz] = 1.1250 \times 10^{-4}$ mol/l.

$(T4)_2Bz$ and $(A4)_2Bz$ interacted with each other and form double helical conformation by complemental hydrogen bonds.

The formation of the supramolecular assembly based on the complemental hydrogen bonds between $(T4)_2Bz$ and $(A4)_2Bz$ were determined by the elution behavior of size exclusion chromatography (SEC) (Fig. 3). The equivalent mixture solution of $(T4)_2Bz$ and $(A4)_2Bz$ showed an elution profile which has higher-molecular-weight shoulder fractions, however, such

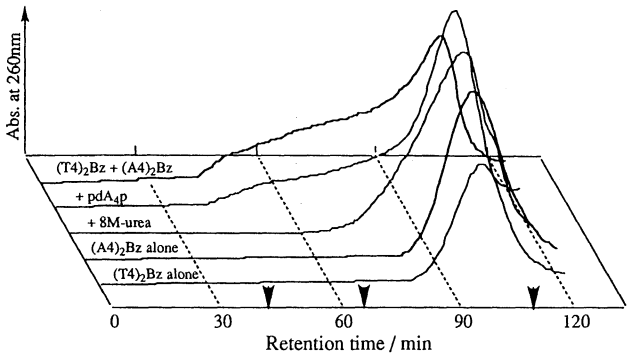


Figure 3. Elution profiles of SEC on Sephadex G-100 (water) for $(T4)_2Bz$ and $(A4)_2Bz$, equivalent mixture of $(T4)_2Bz$ and $(A4)_2Bz$, in the presence of the tetraadenosine (pdA_4p) or 8M-urea at 15°C. Arrows indicate the positions of standard (pullulan, MW = 1.0×10^5, 4.8×10^4, 5.8×10^3), respectively.

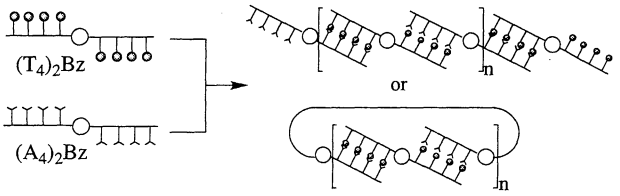


Figure 4. Schematic presentation of polymeric supramolecular assemblies for oligo-DNA dimers.

fraction was not shown in the cases of $(T4)_2Bz$ or $(A4)_2Bz$ alone. When two equivalent tetraadenosines (pdA4p) or 8M urea solution was added as inhibitor, the higher molecular weight fraction decreased or disappeared. These results mean that the oligo-DNA dimers form a high-molecular-weight aggregate through complemental hydrogen bonds of the oligo-DNA dimers. Thus, the quivalent mixture of $(A4)_2Bz$ and $(T4)_2Bz$ could form linear of cyclic supramolecular assembly in aqueous media (Fig. 4). If a stable certain-membered cyclic assembly is exist, there should be a specific peak in SEC profile. There was no peak having a specific molecular weight in SEC elution profile of the mixture (Fig. 3). By using chromophores for the center group of oligo-DNA dimers, the arrangement of chromophores with ordered spacing like a natural photo-synthetic system can be obtained. This system can also be easily extended to higher order (2 or 3-dimensional) polymeric supramolecular assemblies by using triangle- or tetrahedron-type oligo-DNA conjugates.

References and Notes

1 a) P. Tecilla, R. P. Dixon, G. Slobodkin, D. S. Alavi, D. H. Waldeck,and Hamilton, *J. Am. Chem. Soc.*, **112**, 9498 (1990). b) N. Armalori, V. Balzani, F. Barigelleti, L. D. Cola, J. P. Sauvage, and C. Hemmert, *J. Am. Chem. Soc.*, **113**, 4033 (1991). c) C. Fouquey, J. M. Lehn, and A. M. Levelut, *Adv. Mater.*, **2**, 254 (1990).

2 a) A.Harriman, Y. Kubo, and J. L. Sessler, *J. Am. Chem. Soc.*, **114**, 388 (1992). b) J. L. Sessler, B. Wang, and A. Harriman, *J. Am. Chem. Soc.*, **115**, 10418 (1995). c) P. R. Selvin, T. M. Rana, and J. E. Hearst, *J. Am. Chem. Soc.*, **116**, 6029 (1994).

3 K. Itakura, N. Katagiri, C. P. Bahl, R. H. Wightman, and S. A. Narang, *J. Am. Chem. Soc.*, **97**, 7327(1975). K. Itakura, N. Katagiri, C. P. Bahl, R. H. Wightman, and S. A. Narang, *J. Am. Chem. Soc.*, **97**, 7332(1975).