Bispyrene-Conjugated 2′-O-Methyloligonucleotide as a Highly Specific RNA-Recognition Probe**

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In designing antisense molecules, it is crucial to detect the regions of the target RNA where the antisense molecule can hybridize.[1] To date, several studies have focused on the detection of these regions. The studies include theoretical calculations,[2] enzymatic digestion,[3] and DNA arrays,[4] and all of them have achieved a partial success. However, none of these methods have the potential to be applied to cells in which various endogenous molecules interact with RNA in a variety of manners.

Solution-based fluorescence methods should be suitable to deduce the RNA structure in vitro, because these methods primarily function without disturbing the RNA structure. One of the most useful fluorescence methods is based on fluorescence resonance energy transfer (FRET), which provides information about the distance between two chromophores. A three-dimensional structure of a ribozyme was revealed by such methods.[5] A molecular beacon, which is based on FRET, was applied to the detection of RNA in living cells.[6] In another method, various types of pyrene-conjugated oligonucleotides have been used for the detection of both RNA and DNA sequences.[7] The methods were based on excimer formation upon the formation of hybrid molecules and could not discriminate RNA from DNA. As a final goal, we intend to elucidate the detailed structure of RNA in living cells, and have developed a novel bispyrene-conjugated 2′-O-methyloligonucleotide probe (OMUpy2; Figure 1). The probe is also expected to be feasible for use in analysis of intracellular RNA traffic and consequently to evaluate RNA functions.

OMUpy2 was synthesized and purified according to the reported procedure with slight modifications.[8] To evaluate whether OMUpy2 can detect single-stranded regions of native-folded RNA molecules, we used *E. coli* 5S-rRNA, which contains 120 nucleotides (nt). 5S-rRNA is a component of the 50S ribosome subunit and the secondary structure has been reported (Figure 2).[9] We chose the regions for probing with OMUpy2 as follows; OMUpy2-I was complementary to region I (38–47 nt), and OMUpy2-II and III were complementary to regions II (45–56 nt) and III (106–115 nt), respectively.

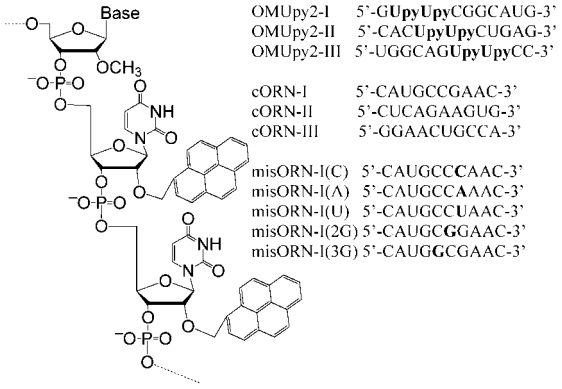

Figure 1. Structure of bispyrene-conjugated 2′-O-methyloligoribonucleotides and the sequence of OMUpy2 and target oligoRNA (ORN). Upy = 2′-O-(1-pyrenylmethyl)uridine. The mismatch bases are shown in bold.

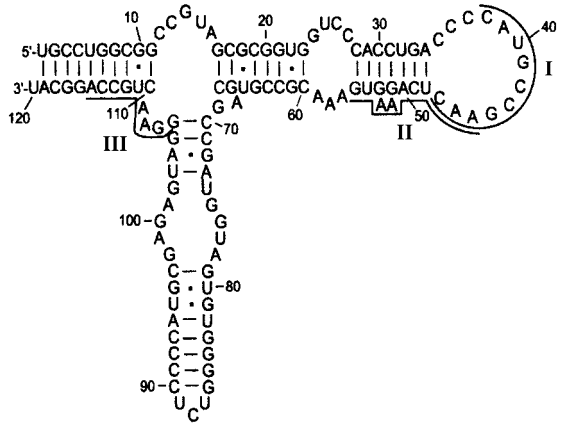

Figure 2. Secondary structure of 5S-rRNA and the region for probing by OMUpy2.[10]

The fluorescence characteristics of OMUpy2 in the absence and presence of the equimolar solution of its complementary oligodeoxyribonucleotide (cODN) or oligoribonucleotide (cORN) are shown in Figure 3. Compared with 1-pyrenylmethanol, the structured emission caused by the pyrene of the OMUpy2-I around 380 nm was largely quenched, and a weak, broad, and structureless emission around 480 nm was observed. It is likely that the monomeric pyrene emission was quenched by the intercalation of the pyrene between nucleobases.[8,10] On the other hand, when the OMUpy2-I was hybridized with cORN, the broad structureless fluorescence band around 480 nm was increased by 43.7-fold compared with the corresponding single-stranded OMUpy2-I, and the structured emission around 380 nm was substantially unchanged. The mechanism of the increment in fluorescence intensity around 480 nm is not known. However, it is rational to assume that two pyrenes would participate in the emission and that the pyrene would interact with each other, forming a defined complex upon hybridization. In most cases reported, the broad, strong, structureless emission around 480 nm is attributed to the pyrene excimer. As another explanation, we are verifying the possibility that the emission is from the ground state dimer of pyrenes. At this stage, we cannot

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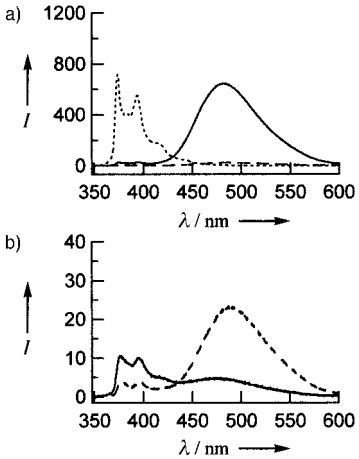

Figure 3. a) Fluorescence spectra of 1-pyrenylmethanol (••••) and OMUpy2-I (L), equimolar mixture of OMUpy2-I and cORN (——), b) fluorescence spectra of OMUpy2-I (- - - -) and equimolar mixture of OMUpy2-I and cODN (——). [OMUpy2-I] = [cORN] = [cODN] = 6 μM, λ_{ex} = 342 nm, 10 mM phosphate buffer (pH 7.0), 0.1M NaCl, 11 °C

conclude whether the emission around 480 nm is attributed to the excimer, to the dimer, or to both. The detailed features of the complex are now under investigation. When OMUpy2-I was hybridized with cODN, the emission around 480 nm could barely be observed, and the structured emission around 380 nm was slightly increased (Figure 3b). These results suggest that the pyrene remained intercalated between nucleobases when OMUpy2-I formed a hybrid with the cODN. Then, the fluorescence enhancement was further studied using several ORNs with a single-point mutation. When OMUpy2-1 was mixed with the ORNs containing the mismatch sequence whose mismatch positions were next to the UpyUpy core region, the fluorescence enhancement could scarcely be observed. As the mismatch positions were moved further away from the UpyUpy core regions, there was some fluorescence enhancement. These results clearly demonstrate that the firm hybrid formation of the UpyUpy core region is essential for fluorescence enhancement. OMUpy2-II and III showed very similar characteristics to OMUpy2-I. These results suggest that OMUpy2 increases the fluorescence intensity around 480 nm only when hybridized with the cORN and that the manner of probing is highly sequence specific.

OMUpy2 was then studied for its ability to detect single-stranded regions of naturally folded 5S-rRNA from *E. coli*. X-ray crystallographic analysis of 5S-rRNA[11] from *E. coli* suggested that region I of 5S-rRNA is in the region that gave an obscure diffraction pattern and that the regions II and III are in the firm-stem regions. Also, X-ray crystallography of the large ribosomal subunit from *Haloarcula marismortui* suggested that there were several non-Watson–Crick base pairs in region I of the 5S-rRNA.[12] When OMUpy2-I was added to 5S-rRNA, an emission around 480 nm was observed, which indicates that OMUpy2-I hybridized with 5S-rRNA. On the other hand, when OMUpy2-II and III were added to 5S-rRNA, the fluorescence did not change appreciably, which indicates that the OMUpy2-II and III did not hybridize with 5S-rRNA. These results (Table 1) suggest that region I is an accessible site of the antisense molecule, and that regions II and III are not accessible.

Table 1. Relative fluorescence intensity of OMUpy2 and the duplex with oligoRNA and oligoDNA and oligoRNA with single-point mutation and melting temperatures (260 nm)

Oligomer	Relative fluorescence intensity at 480 nm[a]	m.p. [°C][b]
OMUpy2-I	1.0	
+ cORN-I	45.7	48.2
+ cODN-I	0.3	45.1
+ misORN-I(C)	0.4	33.9
+ misORN-I(A)	0.9	33.9
+ misORN-I(U)	0.6	34.2
+ misORN-I(2G)	9.3	34.8
+ misORN-I(3G)	38.1	27.3
+ 5S-rRNA	32.3	
OMUpy2-II	3.5	
+ cORN-II	43.9	42.0
+ 5S-rRNA	4.0	
OMUpy2-III	0.4	
+ cORN-III	38.9	51.4
+ 5S-rRNA	0.4	

[a] Fluorescence spectra of OMUpy2 were obtained under the same conditions as shown in Figure 3. [b] Measurements were carried out at 260 nm for the equimolar mixture of OMUpy2 and oligonucleotide in 10 mM phosphate buffer (pH 7.0) with 0.1M NaCl.

In conclusion, bispyrene-conjugated 2′-O-methyloligoribonucleotide was a useful probe to detect the hybridization with complementary RNA and to evaluate the accessibility of the native folded RNA for oligonucleotides. As this probe is highly specific to RNA, it might be useful for a variety of purposes including the detection of endogenous RNAs in living cells.

Experimental Section

Synthesis of bispyrene-conjugated 2′-O-methyloligoribonucleotides: Synthesis of OMUpy2 was carried out by standard phosphoramidite chemistry using DMT-2′-O-methyloligoribonucleotide and 5′-DMT-Upy amidite (DMT = 4,4′-dimethoxytrityl).[9] OMUpy2 was purified by reverse-phase HPLC with a linear gradient of acetonitrile in 0.1M triethylammonium acetate (pH 7.0) at a flow rate of 0.8 ml min^{-1}.

Physical Measurements: The fluorescence measurements were performed on a fluorescence spectrophotometer (RF-5300PC, Shimadzu Co.) with an excitation wavelength of 342 nm. No attempt was made to eliminate dissolved oxygen in the buffer solution (10 mM phosphate buffer with 0.1M NaCl) for the fluorescence measurements. UV–melting curves of the duplexes were acquired from an equimolar mixture of OMUpy2 and oligonucleotide with an increase in temperature from 0 to 90 °C, at a rate of 1.0 °C min^{-1}.

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Self-Assembly Using Organometalloligands as Spacers in the Controlled Formation of Isomeric 1D and 2D Supramolecular Quinonoid Networks**

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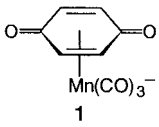
Metal-coordination-directed self-assembly of polymers and discrete nanostructures is a very active research area.[1] The construction of predictable architectures having useful electronic, catalytic, or host–guest properties is the driving force behind this work. To date, the vast majority of reported compounds have metal centers connected by simple organic molecules that serve as multifunctional ligands or spacers.[1] We recently demonstrated, however, that the π-bonded organometallic complex ion [(η^4-benzoquinone)$Mn(CO)_3$]$^-$ (**1**) can serve as a bifunctional ligand ("organometalloligand") by coordinating through the quinone oxygen atoms in the presence of divalent transition-metal ions.[2a] M^{2+} ions (M = Mn, Cd, Zn) and **1** were found to self-assemble into crystalline organometallic quinonoid polymers, whose dimensionality and geometry can be controlled by the choice of added metal ion.



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Herein, we report that the organometalloligand [(η^4-benzoquinone)$Mn(CO)_3$]$^-$ reacts with Mn^{2+} ions to form two isomeric coordination networks. Figure 1 illustrates the two architectures possible when **1** links to the Mn^{2+} centers. Either a 2D pseudoplanar quinonoid array (Figure 1a) or 1D "string" arrays (Figure 1b) can be formed. A representation

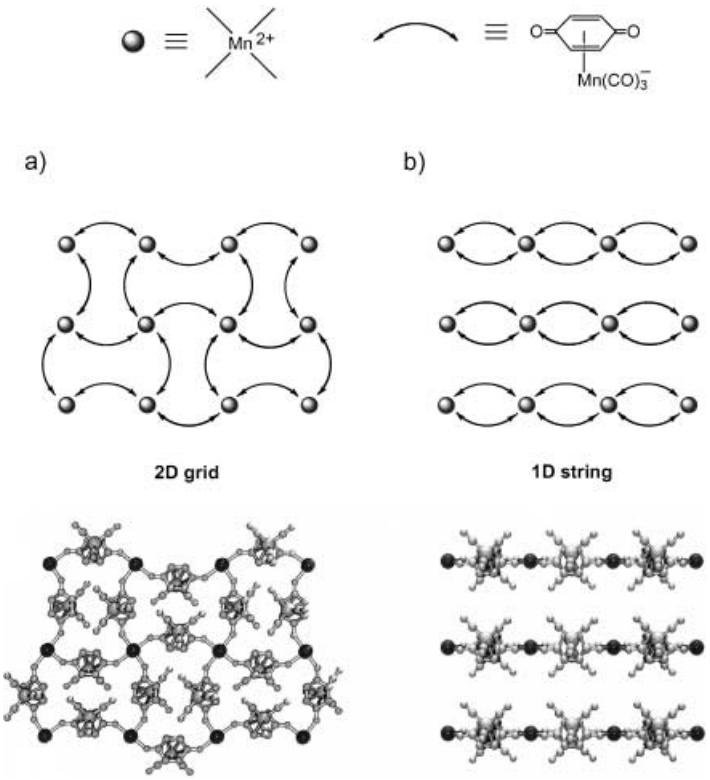

Figure 1. Representation of the self-assembly of Mn^{2+} ions with the η^4-quinone complex **1** to form neutral a) 2D grid and b) 1D string networks.

of these two networks with {$Mn(CO)_3$} moieties attached to the constituent quinone molecules is also given in Figure 1. One would expect that bifunctional spacer molecules could be used to join together the planes in Figure 1a, or the strings in Figure 1b. Indeed, we recently demonstrated that **1**, Mn^{2+} ions, and 4,4′-bipyridine self-assemble in dimethyl sulfoxide (DMSO) to afford a polymer (**2**), which contains a 2D planar network linked by bipyridine spacers.[2a] We now report that self-assembly of the same components, but with the Mn^{2+} ions maintained at a very low concentration, results in the formation of the isomeric polymer **3**, consisting of the 1D strings linked by bipyridine spacers. It is noteworthy that polymers **2** and **3** both contain rectangular grid networks. Typically, the isomer distribution found in supramolecular

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