Molecular Recognition of Nucleosides and Nucleotides Based on Circular Dichroism Induced by Helix Formation of Poly[(4-dihydroxyborophenyl)acetylene]

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(Received, October 12, 2000; CL-000929)

Poly[(4-dihydroxyborophenyl)acetylene] was found to form a predominantly one-handed helical conformation upon complexation with various nucleosides and (oligo)nucleotides, and the complexes exhibited characteristic, split-type induced circular dichroisms depending on the nucleobases.

The nucleosides and nucleotides represent some the most important classes of substances in nature and a number of receptor molecules capable of discriminating them have been prepared in order to understand the fundamental mechanism that occurs in biological events.[1] In these studies, the difference in their binding affinity was detected by spectroscopy, such as NMR, UV, and fluorescence, but little is known about the molecular recognition of nucleosides and nucleotides by circular dichroism (CD).[2] We now report that poly[(4-dihydroxyborophenyl) acetylene](poly-**1**) base-selectively interacts with various nucleosides (Chart 1), nucleotides, and oligonucleotides to form a predominant one-handed helix which exhibit characteristic induced CDs (ICDs) depending on the type of nucleobases.

Functional aromatic boronic acids have been used as receptors and chemosensors of carbohydrates[3] including nucleosides and nucleotides[4] because boronic acid residues can form a rather strong, reversible complex with diol-containing compounds.[5] We also reported that poly-**1** exhibited an ICD in the UV–vis region due to the prevailing one-handed helix formation of the polymer upon complexation with various kinds of chiral molecules including diols, amino alcohols, carbohydrates, and steroids in aqueous solution.[6] The Cotton effect signs can be used as a probe for the chirality assignments of the chiral molecules.

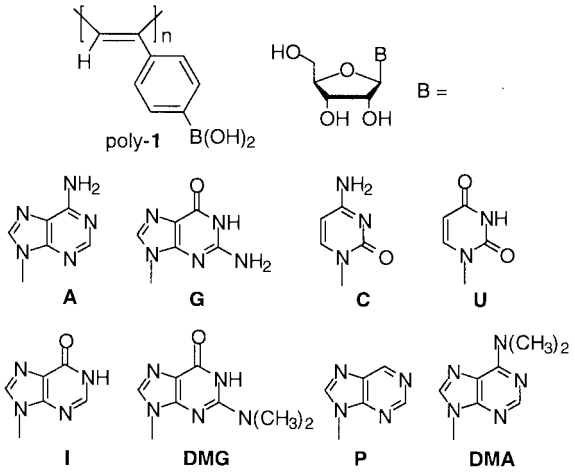

Chart 1. Structures of poly-**1** and ribonucleosides.

Figure 1 shows the CD spectra of poly-**1** in the presence of four ribonucleosides in 0.05 M Na_2HPO_4 buffer at pH 11.8.[7] The complexes exhibited intense, split-type ICDs in the UV–vis region, indicating that ribonucleosides can sufficiently interact with the boronic acid residues of poly-**1** to induce the helical conformation with a predominant screw sense,[6,8] through tetragonal complex formation in alkaline solution.[3–5] The complexes of phenylboronic acid, a model compound of poly-**1**, with four ribonucleosides showed weak Cotton effects at the shorter wavelength (< 300 nm) under the same conditions. Unexpectedly, the poly-**1**—**G** complex showed a completely different ICD pattern with almost mirror images, compared with those of the complexes with **A**, **C**, and **U**. We first assumed that the complexes of poly-**1** with these ribonucleosides should give the same ICDs irrespective of the ribonucleosides, because the helical chirality of poly-**1** may be induced by chiral D-ribofuranoside residues and it has generally been recognized that boronic acids including phenylboronic acid preferentially form a cyclic boronate complex with the 2,3-*cis*-diol group in ribonucleosides regardless of the nucleobases.[4,5] However, the present results indicate that the ribonucleoside bases play a critical role in the helix-sense control of poly-**1**.[9]

We then measured the CD spectra of poly-**1** in the presence of inosine (**I**) and *N*,*N*-dimethylguanosine (**DMG**) under identi-

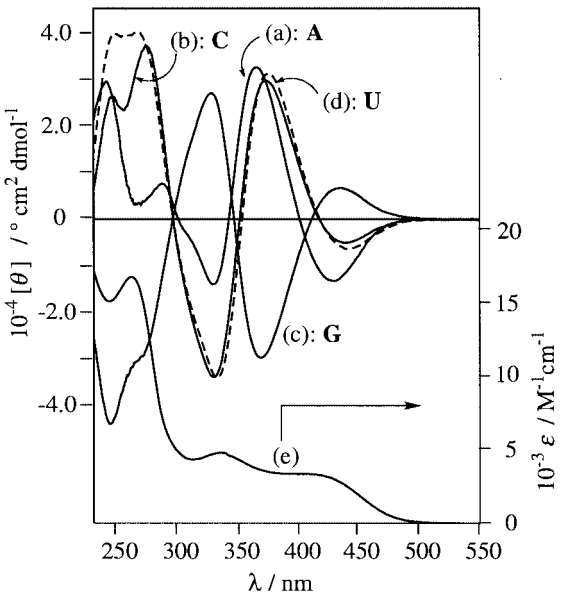

Figure 1. CD spectra of poly-**1** with **A** (a), **C** (b), **G** (c), and **U** (d); [poly-**1**] / [ribonucleosides] = 1.0. UV-visible spectrum of poly-**1**—**U** complex is also shown in (e).

cal conditions to determine the effect of the 2-amino group of **G**. The ICD patterns of the poly-**1**—**I** and —**DMG** complexes were almost mirror images to that of the poly-**1**—**G** complex, indicating that hydrophobic purine bases are not a critical factor, but the 2-amino group of **G** appears to play a major role in controlling the helix sense.[10] We measured the ^{1}H NMR spectrum of the poly-**1**—**G** complex to construct a model of the complex, but it was difficult due to the broadening of poly-**1** peaks in the presence of **G**.

Poly-**1** also complexed with ribonucleotides, particularly, the poly-**1** complexed with adenosine 5'-monophosphate (**AMP**) showed an ICD as intense as that of the poly-**1**—**A** complex ($[\theta] = 2.86 \times 10^4$ at 368 nm), while the complexes with other ribonucleotides showed weak ($[\theta] \times 10^{-4}$ (λ nm) = –0.13 (369) for **GMP** and 0.42 (368) for **CMP**) or no ICD for **UMP**; again, the poly-**1**—**GMP** complex showed the opposite Cotton effect signs among the four 5'-ribonucleotides used in this study.

It is worth noting that poly-**1** exhibited a rather intense ICD in the presence of 2'-deoxyadenosine (**dA**) ($[\theta] = 0.75 \times 10^4$ at 368 nm), while the complexes with the other 2'-deoxyribonucleosides exhibited weak ($[\theta]$ = 1,600 at 366 nm for 2'-deoxycytidine) and almost no ICD (2'-deoxyguanosine and thymidine). This is surprising because phenylboronic acids preferentially form a cyclic boronate complex with the 2,3-*cis*-diol group in ribonucleosides regardless of the nucleobases. Actually, the mixture of **dA** and phenylboronic acid (1:1) did not show any ICD, indicating that poly-**1** may also form a weak complex probably with the 3,5-diol of 2'-deoxyribonucleosides with a sequence of boronic acid residues of poly-**1**; this is the advantage of a stereoregular polymer receptor for molecular recognition over small molecular receptors. Therefore, 2'-deoxyribonucleoside 5'-monophosphates showed very weak ($[\theta] = 0.2 \times 10^3$ at 370 nm for **dAMP**) and no ICD (**dCMP**, **dGMP**, and **TMP**), and 2',3'-*O*-isopropylidene adenosine (**IPA**) also exhibited no ICD in the presence of poly-**1**.

As expected, poly-**1** can base-selectively form a complex with ribonucleotide dimers (cytidylyl(3'→5')adenosine (**CpA**), **CpG**, **CpC**, and **CpU**) at pH 10.0 and the poly-**1**—**CpG** complex exhibited a reversed Cotton effect compared with those of the other dimers.

In summary, we have found that poly-**1** bearing boronic acid residues can form complexes with various nucleosides and (oligo)nucleotides and the complexes exhibited characteristic, split-type ICD depending on the nucleobases, although the mechanism of the base-selective ICD changes is not presently clear. Poly-**1** provides a new promising probe for a sensory system of ribonucleoside and ribonucleotide base recognition, and will be applicable to the base-selective, molecular recognition of RNA with a different terminal base.

The authors are deeply grateful to Dr. T. Wada (Osaka University) and Dr. K. Matsuura (Nagoya University) for fruitful discussions. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture, Japan.

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7 *Cis-transoidal*, stereoregular poly-**1** (M_n = 37000) was prepared according to the previously reported method.[6] Nucleosides and nucleotides were purchased from Sigma and dinucleotides from ICN Pharmaceuticals, Inc. CD spectra were measured in a 0.02 cm quartz cell with a poly-**1** concentration of 1.0 mg/mL using a JASCO J-725 spectropolarimeter at ambient temperature (ca. 22–25 °C). The concentration of poly-**1** is calculated based on monomer units.

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9 The influence of pH on the ICD was also investigated. The pH was controlled with aqueous NaOH and HCl. The ICD intensities of the poly-**1**—**A** and —**C** complexes did not significantly change in the pH region of 9 to 12. However, the ICD intensities of the poly-**1**—**G** and —**U** complexes increased with decreasing pH (> ca. 9). These pH profiles can be explained by considering the protonation of the bases of **G** and **U** at the lower pH, where the electrostatic repulsion with boronate anions should be reduced.

10 CD titration experiments were then carried out. The CD intensities increased with an increase in the concentration of **G**, **C**, **U**, **I**, and **DMG** and reached an almost constant value at around [ribonucleoside]/[poly-**1**] = 0.5 (**C**, **DMG**) and 1 (**G**, **U**, **I**) while the ICD pattern of poly-**1** significantly changed with the increasing concentration of **A**; the signs of the Cotton effect were inverted with isosbestic-like points at around [**A**]/[poly-**1**] = 0.6–0.65. **A** has an amino group at the base, which may contribute to this unusual behavior. 1-Purinyl-β-D-ribofuranoside (**P**) and *N*,*N*-dimethyladenosine (**DMA**) were then employed to evaluate the effect of the amino group on the CD titrations. The poly-**1**—**P** and —**DMA** complexes exhibited no reversal of ICD during the titrations ([**P** or **DMA**] / [poly-**1**] = 0 — 1.0); the 6-amino group of **A** may also participate in the complexation with poly-**1** in a high concentration range ([**A**] / [poly-**1**] > 0.65).