A Helical Polyelectrolyte Induced by Specific Interactions with Biomolecules in Water

Hisanari Onouchi,† Katsuhiro Maeda,† and Eiji Yashima*,†,‡

Department of Molecular Design and Engineering
Graduate School of Engineering, Nagoya University
Nagoya 464-8603, Japan
Form and Function, PRESTO, JST, Chikusa-ku
Nagoya 464-8603, Japan

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Nucleic acids are typical polyelectrolytes with a phosphate backbone and bind to proteins and a variety of oppositely charged biomolecules and drugs in aqueous solution where electrostatic interactions and/or directed hydrogen bonding play a central role.[1] Polyelectrolytes are completely different from small electrolytes; that is, a portion of the counterions are bound to polyelectrolytes of a sufficiently high charge density, so that polyelectrolytes can efficiently interact with small charged molecules in water.[2] In sharp contrast, small electrolytes exhibit only the dissociated free ions in water by hydration, therefore, the rationale design of charged synthetic receptors for biomolecular recognition in water still remains a very difficult problem to solve.[3]

We previously reported the induction of helicity in an achiral, stereoregular poly((4-carboxyphenyl)acetylene) with chiral amines in dimethyl sulfoxide (DMSO)[4] and some amino acids in water.[5] The complexes showed an induced circular dichroism (ICD) in the UV−visible region due to the predominantly one-handed helix formation. However, the polymer is not sensitive to amino acids and other important chiral biomolecules in the fields of biology and medicine. Here we show that a rationally designed polyelectrolyte, *cis*−*transoidal* poly((4-phosphonophenyl)acetylene) (poly-**1**; Figure 1),[6] bioinspired by the interaction motifs of nucleic acids (Figure 2), interacts with a variety of biomolecules and forms supramolecular assemblies with controlled helicity through electrostatic and hydrogen bonding interactions in water. Phosphodiesters of nucleic acids are proposed to bind the backbone of the polypeptide main chain as well as the terminal and side chain basic amino groups (A),[1,7] the 1,2- or 1,3-amino alcohol residues (B),[1b−d,8] and the 1,2-diols (C).[9] If these interactions would occur in water, we could detect and evaluate their specific interactions without derivatization using CD spectroscopy with poly-**1** as a sensitive probe.

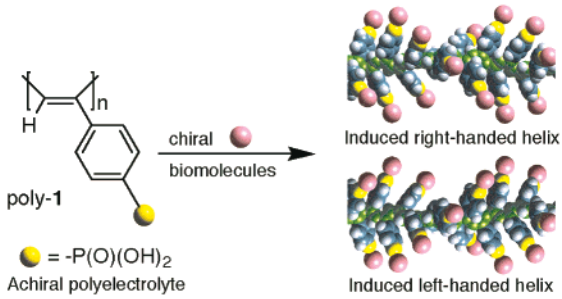

Figure 1. Schematic illustration of right- and left-handed helicity induction in achiral poly-**1** upon complexation with chiral biomolecules.

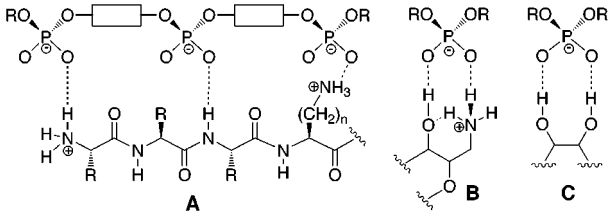

Figure 2. Possible interaction models of peptides (A), 1,3-amino alcohols (B),[8] and 1,2-diols (C)[9] with the phosphodiester backbone of the nucleic acids.[1]

Figure 3A shows the typical CD and absorption spectra of poly-**1** in the presence of a free amino acid, D-tryptophan, and L-tryptophan (Trp) in water at 25 °C. The complexes showed mirror images of the split-type ICDs. The assay of 19 of the common free L-amino acids produced ICDs with the sign reflecting the absolute configurations; thirteen neutral and two acidic L-amino acids gave the same ICD signs, while the secondary amino acid, L-proline, and the three basic amino acids, L-arginine, L-histidine, and L-lysine, gave the opposite ICD sign (negative second Cotton) as expected (see Supporting Information).[10] The magnitude of the ICD was influenced by the pH and the salt (NaCl) concentration because the phosphono group of poly-**1** has two acidic OH groups with different pK_a values (ca. 1.8 and 7.1), which is consistent with the ionic nature of the interaction. This is the first example of chirality assignments of all the common free L-amino acids in water.[11]

As expected, poly-**1** also complexes with the terminal amino group of the peptides and exhibits an ICD in water at 25 °C. It is worth noting that poly-**1** showed ICDs in the presence of Gly-L-Ala (the molar ellipticities of the second Cotton ($\Delta\epsilon_{second}$) of poly-**1** = 9.0 ($M^{-1}cm^{-1}$)) and Gly-Gly-L-Ala ($\Delta\epsilon_{second}$ = 8.3) at pH 5.0 with remote stereogenic centers from the terminal amino

† Nagoya University.
‡ PRESTO, JST.

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(6) Poly-**1** was prepared by the polymerization of diethyl (4-ethynylphenyl)-phosphonate with $[Rh(nbd)Cl]_2$ (nbd = norbornadiene), followed by hydrolysis of the ester groups (86% yield). The molecular weight (M_n) was 2.36×10^5 as determined by size exclusion chromatography (see Supporting Information).

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(10) The ICD intensities increased with the increasing concentration of the amino acids. The CD titration data were then used to estimate the binding constants. Sodium acetate buffer (pH 3.8) was used to maintain the pH. Plots of the CD intensities of the second Cotton of poly-**1** as a function of concentrations of L-Ala and L-Trp gave a saturation binding isotherm at 25 °C. The Hill plot analysis of the data resulted in apparent binding constants (K) of 9.8 (Ala) and 137 M^{-1} (Trp) with the Hill coefficient (cooperativity factor) of 1.0 and 1.1, respectively. The CD titrations with aminosugars and carbohydrates were performed in imidazole−HCl (pH 6.6) and sodium borate/HCl (pH 8.6) buffers, respectively. For the Hill plot analysis, see Supporting Information and the following: Connors, K. A. *Binding Constants*; John Wiley: New York, 1987.

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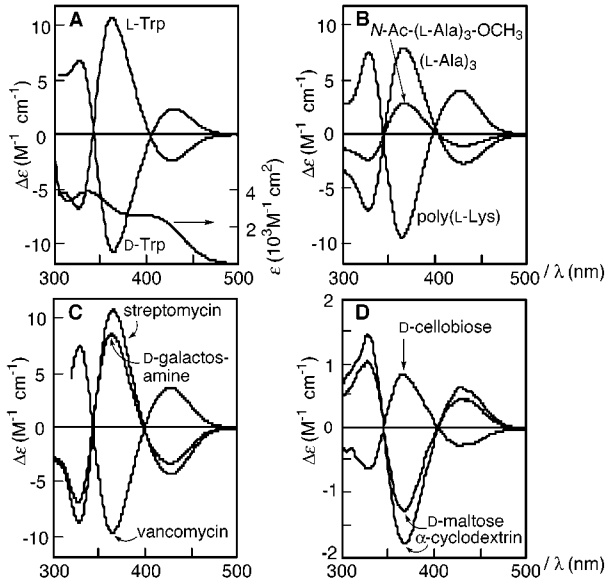

Figure 3. CD and absorption spectra of poly-**1** with biomolecules in water at 25 °C. Shown are the CD spectra of the poly-**1** with D- and L-Trp at pH 4.5 (A), $(L\text{-Ala})_3$ at pH 4.9, *N*-acetyl$(L\text{-Ala})_3OCH_3$ at pH 3.4, and poly(L-Lys) at pH 11.9 (B), D-galactosamine at pH 7.6, streptomycin at pH 12, and vancomycin at pH 8.9 (C), and D-cellobiose at pH 3.7, D-maltose at pH 3.8, and α-cyclodextrin at pH 3.9 (D). The concentration of poly-**1** is 1.0 mg/mL (5.5 mM monomer units) and the molar ratio to poly-**1** is 5 for the amino acids and aminoglycosides, 10 for the peptides, and 100 for the sugar units of the carbohydrates. The absorption spectrum of poly-**1** in the presence of L-Trp is also shown in A. The lower limits of detection of these biomolecules with concentrated poly-**1** solution (65 μg, 5 μg/μL) are 0.8 (L-Trp), 2 $(L\text{-Ala})_3$, 10 (*N*-acetyl-$(L\text{-Ala})_3OCH_3$), 0.8 (poly(L-Lys)), 2.6 (D-galactosamine), 0.07 (streptomycin), 0.09 (vancomycin), 61 (D-cellobiose), 31 (D-maltose), and 35 μg (α-cyclodextrin).

group of the achiral glycine units, and the complexes showed as intense CDs as those with L-Ala-Gly ($\Delta\epsilon_{second} = 6.2$), $(L\text{-Ala})_2$ ($\Delta\epsilon_{second} = 9.8$), and $(L\text{-Ala})_3$ ($\Delta\epsilon_{second} = 8.0$) (Figure 3B). These results imply that the neutral amide residues of the peptide backbone also contribute to the helicity induction as well as the terminal amino group. We then measured the CD of poly-**1** in the presence of *N*-acetyl-$(L\text{-Ala})_3$-OCH_3 having no terminal amino group; the complex still showed a relatively strong ICD ($\Delta\epsilon_{second} = 2.9$) (Figure 3B) compared with that of $(L\text{-Ala})_3$, indicating that the phosphono group interacts with the peptide backbone by hydrogen bonding even in water as schematically illustrated in motif A of Figure 2. However, the multivalent hydrogen bonding must be necessary for effective interactions because *N*-acetyl-L-Ala-OCH_3 did not show any ICD. The binding affinity of $(L\text{-Ala})_3$ to poly-**1** ($K = 157$) was much greater than that of *N*-acetyl-$(L\text{-Ala})_3$-OCH_3 (5.2) by a factor of 30. Similar hydrogen bond interactions have been seen in nature where the phosphate groups of nucleic acids form hydrogen bonds with the main chain NH of peptides, although the positively charged side chains mainly contribute to the protein−nucleic acid interactions.[7] The negatively charged poly-**1** can interact with the basic polypeptide, poly(L-Lys) (Figure 3B), and the positively charged protein surfaces derived from the lysine and arginine side chains (bovine serum albumin ($\Delta\epsilon_{second} = -2.5$, pH 6.4), insulin ($\Delta\epsilon_{second} = -1.7$, pH 6.2), lysozyme ($\Delta\epsilon_{second} = -3.3$, pH 12), and a DNA polymerase I from *E. coli* lysogen ($\Delta\epsilon_{second} = -1.0$, pH 3.6)); all the complexes showed the same ICD signs with a negative second Cotton to form supramolecular assemblies with the induced helicity on poly-**1**.

Aminoglycoside antibiotics have been shown to interact with the negatively charged RNA through the 1,2- or 1,3-hydroxyamine moieties (motif B in Figure 2) in water,[1b−d,8] but their model studies are limited to organic media. Our sensory system can be applied to aminoglycoside sensing in water due to the ion condensation effect of the polyelectrolyte. Among structurally similar 2-aminoglycosides, D-glucosamine ($\Delta\epsilon_{second} = 1.4$, $K = 6.2$) and D-galactosamine ($\Delta\epsilon_{second} = 8.7$, $K = 14$) (Figure 3C) showed ICDs at neutral pH, while D-mannosamine with the same 2-amino group as D-glucosamine but the opposite configuration exhibited no ICD. Examination of these results indicates that the remote hydroxy groups also play a role in the interactions with a sequence of the phosphonate residues of poly-**1**. We then applied poly-**1** to sensing commercially available aminoglycoside antibiotics, streptomycin and vancomycin (Figure 3C), and found that these antibiotics showed a high affinity to poly-**1**, thus showing intense ICDs with an opposite CD sign from each other. It requires only 70−85 ng (10^{-9}) [less than 120 pmol (10^{-12})] of the antibiotics for the detection by CD in water.

On the other hand, carbohydrates have no positively charged amino groups and their interactions with charged phosphonate residues through hydrogen bonding in water have not yet been clearly confirmed, although the 1,2-diols-phosphonate interaction (motif C in Figure 2) has been proposed based on the model sugar receptor studies in organic solvents.[9a] Our system is quite sensitive for such weak interactions and we observed relatively weak, but apparent ICDs at 25 °C in the presence of various D-carbohydrates at pH 3−12 including glucose ($\Delta\epsilon_{second} = -0.3$), galactose ($\Delta\epsilon_{second} = 0.2$), mannose ($\Delta\epsilon_{second} = -0.4$), maltooligosaccharides, and cyclodextrins. Interestingly, the CD signs are dependent on the stereochemistry of the carbohydrates; disaccharides, cellobiose ($\Delta\epsilon_{second} = 0.8$, $K = 2.2$) and maltose ($\Delta\epsilon_{second} = -1.3$, $K = 1.8$), consisting of two D-glucose units with the β- and α-linkages, respectively, exhibited opposite CD signs from each other (Figure 3D). Moreover, the complex of poly-**1** with α-cyclodextrin ($\Delta\epsilon_{second} = -1.8$) (Figure 3D) showed a more intense CD than that with the corresponding linear oligomer, maltohexaose ($\Delta\epsilon_{second} = -0.5$). The permethylated β-cyclodextrin showed no ICD. These results clearly indicate that the phosphonate residues interact with carbohydrates through hydrogen bonding even in water.

The general applicability of our detection method to biomolecules in water relevant to nucleic acid−biomolecule recognition processes was demonstrated by measuring the CD of a particular chromophoric polyelectrolyte. Our concept can be extended to construct a specific sensory system for a target biomolecule by just tuning the functional group of the polyelectrolyte. The advantage of the present polyelectrolyte is not only its long-wavelength absorption, but also response to many kinds of chiral molecules on a micro- or nanoscale without derivatization in water. Furthermore, the binding affinity of biomolecules to particular functional groups or molecules and at the same time the stereochemistry information including the absolute configuration can be obtained.

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Supporting Information Available: Experimental procedures, CD data, and the Hill plots (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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