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Oligoresorcinols Fold into Double Helices in Water

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Double helical structures are frequently found in Nature and are deeply connected to the exquisite biological functions of biomacromolecules, such as nucleic acids.[1] Since the discovery of the double helix of DNA,[2] the double helical structures have attracted ever-increasing attention as synthetic targets. Nevertheless, double helices formed from synthetic strands are relatively rare when compared to synthetic single helices.[3] The metal-directed self-assembly is the most widely used strategy to construct doubly stranded helical complexes, or so-called helicates.[4] Some aromatic oligoamides as well as peptide analogues of nucleic acids give rise to double helical structures by utilizing hydrogen-bonding and aromatic−aromatic interactions.[5,6] Although plenty of supramolecular duplexes assembled with hydrogen bonding have been reported, most of their three-dimensional structures are not intertwined, but are characterized as ladder- or zipper-like linear conformations.[7] Thus, the recognition motifs utilized so far for constructing doubly stranded assemblies rely on the ligand−metal coordination, hydrogen bonding, or aromatic stacking. It should be emphasized that there have been no examples of wholly artificial double helices that are formed by the self-assembly process in water, in which biomacromolecules form single or double helices. Here we describe oligoresorcinols, a kind of oligophenol, as a new, simplest structural motif, which self-assemble into well-defined double helices resulting from interstrand aromatic stacking in water.[8] In addition, a bias in the helical twist sense was achieved by incorporating chiral substituents at both ends of the strands.

We synthesized three oligoresorcinols with different chain lengths, which are soluble in common organic solvents and in water.[9,10] The aggregation of the oligoresorcinol strands in water was first suggested by the ^1H NMR spectra of the nonamer (**9merH**, Figure 1A). In methanol, the NMR signals were sharp, and each signal was assigned to a single strand with a random coil conformation. These signals significantly shifted upfield, accompanied by considerable broadening with an increase in the content of water, suggesting the aggregate formation of **9merH**. The upfield shifts of the aromatic protons suggest parallel stacking of the aromatic rings, which is favored in water.[11] The absorption spectra of **9merH** also showed a noticeable hypochromic effect with the increasing amount of water, indicating a π-stacking interaction (Figure 1B). The chemical shifts (δ) and the maximum absorptivity (ϵ_{max}) at ca. 290 nm of **9merH** in water−methanol mixtures were plotted versus the water content (Figure 1C). These plots clearly showed a transition point at ca. 72 vol % of water. These results indicate that the molecular strands of **9merH** self-assemble above 72 vol % of water. Moreover, the aggregate dissociated with increasing temperature in the 72 vol % of water (Figure S3).[9]

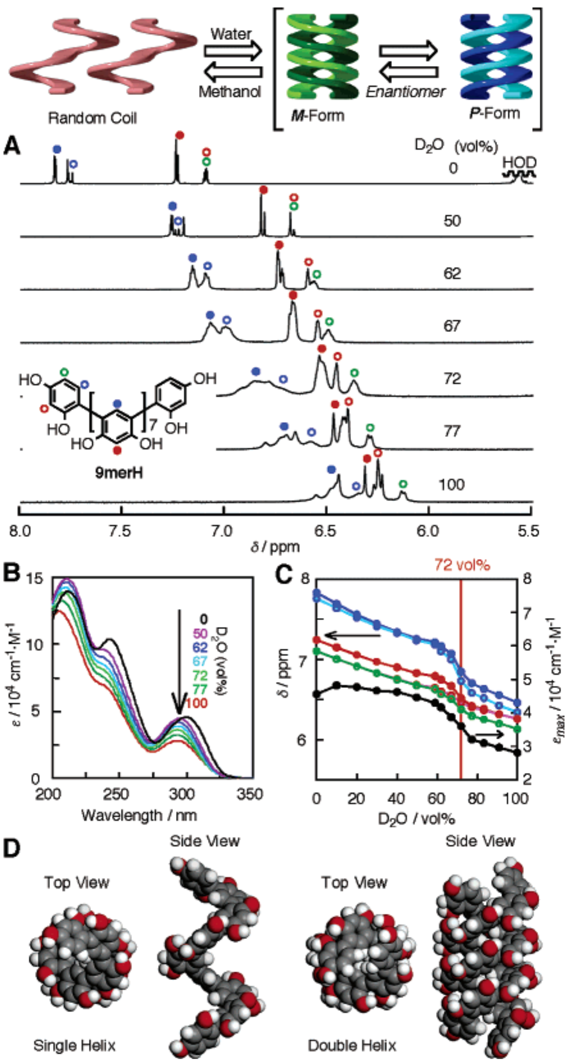

Figure 1. Double helix formation of **9merH**. (A) ^1H NMR and (B) absorption spectra of **9merH** in D_2O/CD_3OD (D_2O: 0−100 vol %) at 25 °C; [**9merH**] = 1 mM; pD = 6.2−6.4. 1,4-Dioxane in D_2O was used as the external standard for the chemical shifts. (C) Plots of chemical shifts of the marked signals and ϵ_{max} at ca. 290 nm of **9merH** in D_2O/CD_3OD at 25 °C. The chemical shifts of **9merH** were assigned on the basis of 2D COSY and NOESY spectra. (D) MM-calculated structures of the single (left) and double (right) helices of **9merH**. The parameter, "relative electric", was set to 4 as recommended for calculations in water.

Molecular mechanics (MM) calculations for **9merH** suggest a 5_1-helical conformation as an energy-minimized structure for the single-stranded **9merH** (Figure 1D); this structure is similar to that reported in the crystal structures of the *m*-phenylene oligomers and polymers.[9,12] However, the single helical conformation of **9merH** is not stable and changed to a rather random conformation after 1

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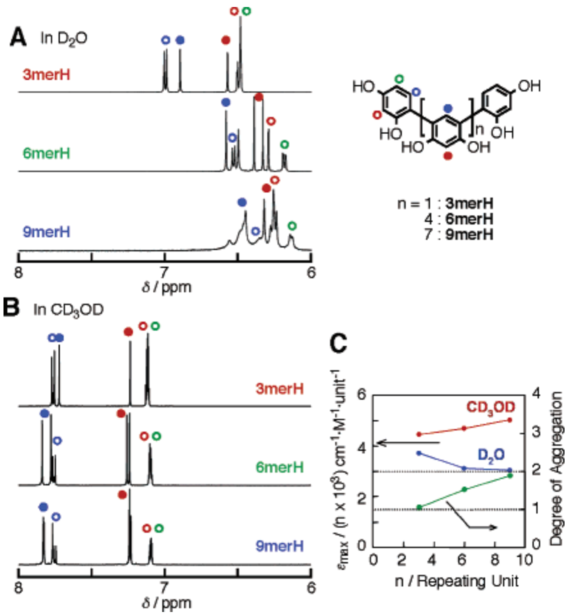

Figure 2. Chain-length-dependent spectral changes. ^{1}H NMR of oligoresorcinols (**3merH**, **6merH**, and **9merH**) in D_2O (A) and CD_3OD (B) at 25 °C. (C) Plots of ϵ_{max} at ca. 290 nm per repeating unit of oligoresorcinols in D_2O (blue) and CD_3OD (red) versus the repeating unit number ([oligomers (per repeating units)] = 9 mM) and plots of the degree of aggregation of the oligomers estimated by VPO measurements in H_2O at 40 °C (green, degree of aggregation = M_n(found)/M_n(calcd)); at that temperature, the double helical structure was retained, as confirmed by ^{1}H NMR (Figure S2).[9]

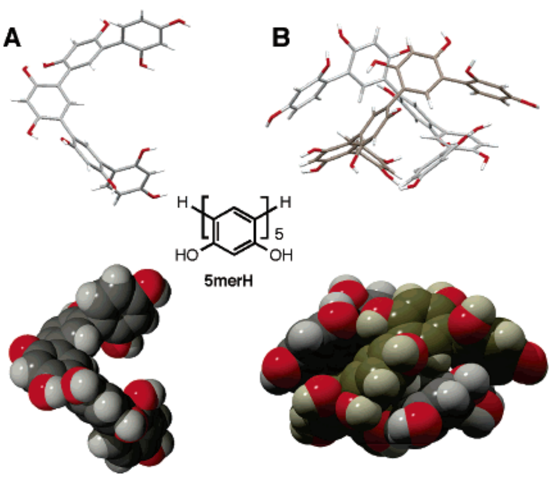

Figure 3. Capped sticks (top) and space-filling (bottom) drawings of the crystal structures of the single helix (A) and the double helix (B) of **5merH**. Carbon, gray (or brownish gray); hydrogen, white; oxygen, red. The structures were determined by X-ray diffraction study of two independent crystals grown from $CHCl_3$/CH_3CN (single helix) and H_2O (double helix). Solvent molecules are omitted for clarity. (A) Single helical structure of **5merH** with a pseudo-5_1-helical conformation. The crystal consists of an equimolar mixture of the right-handed and left-handed helices, and only the left-handed helix is shown. (B) Double helical structure of **5merH**. Each strand has almost identical structure. The crystal consists of an equimolar mixture of the right-handed and left-handed double helices, and only the right-handed double helix is shown.

ns molecular dynamics (MD) simulations at 300 K. On the other hand, the duplex of **9merH** adopting a double helical structure was energetically more stable than the single helix of **9merH** and retained the double helix even after 1 ns MD simulations at 300 K (Figure S8).

Next, the chain-length dependence of the oligoresorcinols on their aggregation behavior was investigated by ^{1}H NMR and absorption spectroscopies in water and in methanol. In water, the ^{1}H NMR signals of **6merH** and **9merH** exhibited considerable upfield shifts of ca. 0.4−0.5 ppm compared to those of **3merH** (Figure 2A). In methanol, however, the ^{1}H NMR signals of the oligomers appeared at similar chemical shifts (Figure 2B). The absorption spectra of these oligomers showed a similar tendency (Figures S7).[9] The ϵ_{max} values per repeating unit at ca. 290 nm of the oligomers in methanol slightly increased, but significantly decreased in water as the chain length increased (for plots of the ϵ_{max} value versus the repeating unit, see Figure 2C). In addition, **6merH** was found to have a melting temperature of T_m = ca. 30 °C, while T_m = ca. 40 °C was observed for **9merH** (Figure S5).[9] These results suggest that **6merH** and **9merH** self-assemble in water and **3merH** exists as a single strand, whereas all the oligomers do not form aggregates in methanol.

To estimate the degree of aggregation of the oligomers in water, vapor pressure osmometer (VPO) measurements were conducted in water at 40 °C (Figure 2C).[9] The degree of aggregation (M_n(found)/M_n(calcd)) of **3merH** was nearly equal to unity, that is, **3merH** exists as a single strand in water, while those of **9merH** and **6merH** were 1.9 and 1.5, respectively (Figure S6), suggesting that **9merH** almost quantitatively self-assembles to form the double helices and **6merH** exists as a ca. 1:1 mixture of the single strand and the double helix under equilibrium in water.[5a,13] These VPO data are consistent with the above ^{1}H NMR and absorption spectral behaviors. The **9merH** maintained the double helical structure at concentrations as low as 0.1 mM, suggesting a dimerization constant of $>10^4$ M^{-1} at 25 °C in water (Figure S1).[9]

It should be stressed that **5merH** was found to crystallize in a single or a double helix depending on the solvent (Figure 3).[9] The single helix crystallized from $CHCl_3$/CH_3CN, whereas the single crystals of the double helix were grown from water. This is consistent with the fact that the double helix formation of the oligoresorcinols is mainly driven by the aromatic interactions in water. In the solid state, the double helical structure of **5merH** is stabilized by several $\pi-\pi$ and CH$-\pi$ interactions (Table S2).[9] This single-crystal structure is in good agreement with the hypochromicity of the absorption spectra and the upfield shifts of the ^{1}H NMR signals of the double helices of the oligoresorcinols in water.

The double helices of the oligoresorcinols formed in aqueous solution exist as equimolar mixtures of the right- and left-handed forms, that is, racemates. The introduction of an optically active group to a part of the oligoresorcinol derivatives may cause a bias in the screw sense of the double helices, resulting in an excess helical sense, because the helical oligomers bearing the chiral units become diastereomeric pairs (Figure 4). We then synthesized an undecamer bearing two (*S*)-2-methylbutyl groups at both ends of the strand (**11merHR2**).[9] We confirmed that **11merHR2** also formed the double helix in water as evidenced by the upfield shifts and significant broadening of its ^{1}H NMR signals together with a large hypochromicity of its absorption spectra, as in the cases of the achiral oligomers (Figure 4A,B). In methanol, **11merHR2** showed no CD in the backbone chromophore (200−350 nm) as expected from the fact that it takes a random-coil conformation in methanol. In sharp contrast, large Cotton effects were observed at 25 °C in water, in the region of the chromophore assigned to **11merHR2**, which is a strong evidence that **11merHR2** forms the double helix with an excess one-handed screw sense (Figure 4B). The fact that the specific rotation of **11merHR2** dramatically increased from +4.6° in methanol to +107° in water also supports the predominant one-handed double helix formation. The induced CD (ICD) reversibly changed in response to temperature (Figure

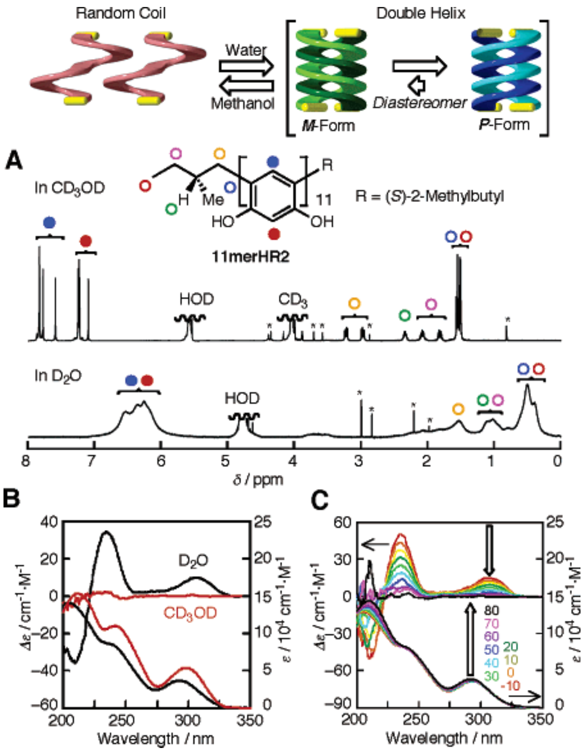

Figure 4. Double helix formation of **11merHR2** with an excess one-handed screw sense. (A) ^{1}H NMR spectra of **11merHR2** in CD$_3$OD (top) and D$_2$O (bottom) at 25 °C; [**11merHR2**] = 1 mM. The asterisks denote protons from the solvents or impurities. (B) CD and absorption spectra of **11merHR2** in D$_2$O (black) and CD$_3$OD (red) at 25 °C. (C) Changes in the CD and absorption spectra of **11merHR2** in D$_2$O upon heating. The predominant helix sense of the double helices of **11merHR2** in the schematic illustration (top) is tentative.

4C); it gradually decreased with increasing temperature and completely disappeared at 80 °C. The ICD unambiguously indicates a bias in the twist sense of the double helix of **11merHR2** in water.

Thus, the oligoresorcinols consisting of a very simple repeating unit self-assemble into double helices with the aid of aromatic interactions in water. The double helix formation is sensitive to the chain length, solvent composition, and temperature. Furthermore, a bias in the twist sense of the double helices can be achieved by incorporating chiral substituents at both ends of the strands. We believe that this simple structural motif will be used to design and develop water-soluble, more complex supramolecular assemblies and novel chiral materials based on double helical structures.

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Supporting Information Available: The experimental procedures for the synthesis and characterization of **3merH**, **5merH**, **6merH**, **9merH**, and **11merHR2**, the crystallographic data for the single and double helices of **5merH**, and the MM and MD calculation studies for **9merH** and **11merHR2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) The double helix formation of oligoresorcinols in water was in fact serendipitously discovered during our program to develop synthetic helical polymers and oligomers based on poly- and oligo-*m*-phenylenes. Katagiri, H.; Miyagawa, T.; Furusho, Y.; Yashima, E. *Angew. Chem., Int. Ed.* **2006**, *45*, 1741−1744. However, we now recognize that a key feature that allows the formation of the double helix instead of a single helix may be the directly linked *m*-phenylene repeating units; lack of spacers between the phenyl rings may prevent efficient $\pi-\pi$ interaction within the single strand due to the steric congestion, so that insertion of spacers, such as an ethynyl residue (ref 3b), will result in a foldamer which prefers to fold by itself.
(9) See Supporting Information for the details of the synthesis and characterization of **3merH**, **5merH**, **6merH**, **9merH**, and **11merHR2**, the crystallographic data for the single and double helices of **5merH**, and the MM and MD calculation studies for **9merH** and **11merHR2**.
(10) The solutions of the oligomers at a concentration of 1 mM had pD values of ca. 6−7, which indicate that almost no proton was dissociated under the conditions; the pDs ($-\log[D^+]$) were calculated by the equation, pD = pH reading + 0.40. Glasoe, P. K.; Long, F. A. *J. Phys. Chem.* **1960**, *64*, 188−190.
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(13) There may be a possibility that **6merH** exists as fast interconvertible different kinds of aggregates rather than the single and double helices at high temperatures, and this possibility could not be completely excluded at the present time.

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