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Supramolecular Control of Unwinding and Rewinding of a Double Helix of Oligoresorcinol Using Cyclodextrin/Adamantane System

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Abstract: The double helix of the oligoresorcinol nonamer formed in water was unwound by β-cyclodextrin (β-CD), and the resulting single strands of the nonamer threaded the β-CD to form a twisted [3]-pseudorotaxane with a controlled helicity. Upon the addition of an adamantane, the single strand of the oligoresorcinol nonamer was expelled out of the β-CD wheels, thus regenerating the double helix. This supramolecularly controlled, reversible unwinding and rewinding of the double helix is unique and can be readily monitored by spectroscopic techniques.

Introduction

The control of the unwinding and rewinding of double-stranded DNA is indispensable for its replication, transcription, recombination, and repair processes.[1] The unwinding is catalyzed by helicases, a ubiquitous class of enzymes found in diverse species from *E. coli* to humans.[2] Most helicases form stable, ringlike hexameric assemblies, which thread onto single strands of DNA to form rotaxane-like supramolecular assemblies.[3] Inspired by such a controlled assembly of sophisticated supramolecular architectures in biological systems, chemists have developed synthetic molecular strands that fold into double helices through noncovalent bonds in solution.[4−7] The coordination-driven self-assembly has been widely employed to construct double-stranded helical complexes, so-called helicates.[4] Some synthetic aromatic strands[5] as well as peptide analogues of nucleic acids[6] fold into double helical assemblies by hydrogen-bonding and/or aromatic−aromatic interactions. A few helicates and aromatic double helices have been shown to reversibly transform between the single- and double-stranded helical states either electrically[7] or by the changing temperature[5a] or solvent composition.[5d] However, it remains a difficult but an attractive challenge to control the unwinding and rewinding of synthetic double helices with a controlled helicity, optimally in water, where biological polymers form single or double helices.

We have recently reported oligoresorcinols, a type of oligophenols that fold into a double helix by aromatic interactions in water.[5d] We now report a supramolecular approach to control the reversible unwinding and rewinding of the oligoresorcinol double helix in water using cyclodextrins (CDs) as receptors with a helicase-like activity together with an adamantane as its inhibitor. We anticipated that CDs, rigid, well-defined cylindrical macrocycles with a hydrophobic chiral cavity, would unwind the oligoresorcinol double helix to form a (pseudo)rotaxane, in which a single-stranded oligoresorcinol is threaded into the CD wheels; the subsequent addition of an adamantane will regenerate the double helix because the encapsulated single-stranded oligoresorcinol is expelled from the CD cavity (Figures 1A and 2C).[8]

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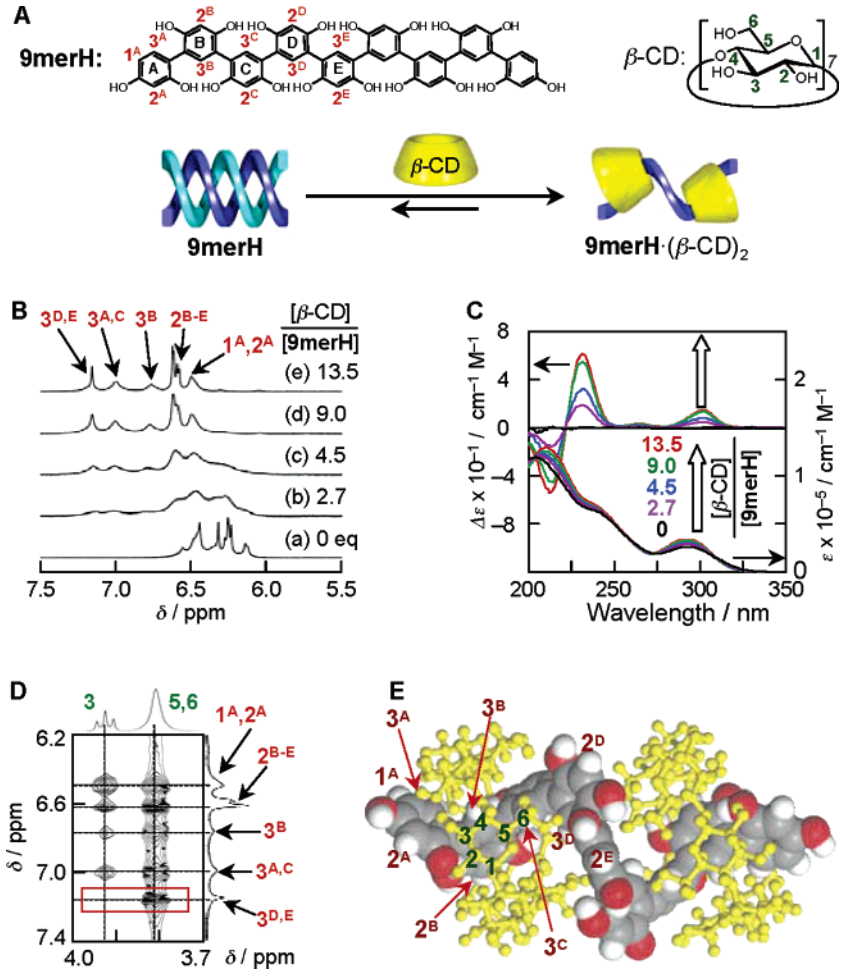

Figure 1. (A) Schematic illustration of the unwinding of the double helix of **9merH** by β-CD and the subsequent formation of **9merH**·(β-CD)$_2$. (B) ^{1}H NMR and (C) absorption and circular dichroism spectral changes in a solution of **9merH** in D_2O (pD = 5.3−6.8) at 25 °C with increasing amounts of β-CD; [**9merH**] = 1 mM, [β-CD]/[**9merH**] = 0−13.5. (D) Partial NOESY spectrum (500 MHz, mixing time = 0.5 s) of **9merH** with β-CD in D_2O at 25 °C; [**9merH**] = 2 mM, [β-CD] = 18 mM. (E) The energy-minimized model of **9merH**·(β-CD)$_2$.

Results and Discussion

Unwinding of a Double Helix of Oligoresorcinol by CDs. We first investigated the encapsulation of the molecular strand of the oligoresorcinol nonamer (**9merH**)[5d] with a series of CDs (α-, β-, and γ-CDs) using ^{1}H NMR and absorption spectroscopies (Figure 1). The **9merH** folds into a double helix in water, as evidenced by the characteristic upfield shifts of the aromatic protons with considerable peak broadening.[5d] The addition of an excess β-CD to a solution of the double helix of **9merH** in D_2O caused considerable downfield shifts and sharpening of the ^{1}H NMR signals with a hyperchromic effect in its absorption spectrum, indicating that the β-CD unwound the double helix into single strands by encapsulation, resulting in a pseudorotaxane formation (Figures 1B and 1C). The stoichiometric ratio of the threaded single strands of **9merH** to β-CD was found to be 1:2 using a Job plot (Supporting Information), denoting the formation of the [3]pseudorotaxane **9merH**·(β-CD)$_2$. Noticeably, upon the addition of β-CD, a distinct induced circular dichroism was observed in the absorption region of the **9merH** (200−350 nm), where β-CD shows virtually no Cotton effects (Figure 1C). The induced circular dichroism provided convincing evidence that the **9merH** strand adopts a predominantly one-handed helical conformation induced by the encapsulating β-CD wheels. γ-CD also induced similar changes in the ^{1}H NMR and absorption spectra of the **9merH**, and the appearance of the identical Cotton effects (Supporting Information), suggesting the unwinding of the **9merH** double helix and the formation of the [3]pseudorotaxane **9merH**·(γ-CD)$_2$ with a controlled helicity. On the other hand, spectral changes only slightly took place with the addition of α-CD (Supporting Information), indicating that the single strand of the **9merH** selectively forms [3]pseudorotaxanes with β- and γ-CDs in water.

Further evidence for the rotaxane formation was obtained from diffusion-ordered ^{1}H NMR spectroscopy (DOSY) experiments (Table 1).[9] Due to the fast exchange on the ^{1}H NMR time scale, the measured diffusion coefficients are weighted averages among those of the [3]pseudorotaxanes **9merH**·CD_2, the double helix of **9merH**, and the free CD. The diffusion coefficients of the **9merH** and α-CD only slightly changed after mixing in D_2O, while those of the β- and γ-CDs and **9merH** in D_2O significantly decreased upon complexation (Table 1). These DOSY measurement results are consistent with the facts that the [3]pseudorotaxanes (**9merH**·(β-CD)$_2$ and **9merH**·(γ-CD)$_2$) are larger than the double helix of **9merH**, as indicated by the molecular mechanics calculations (Supporting Information).

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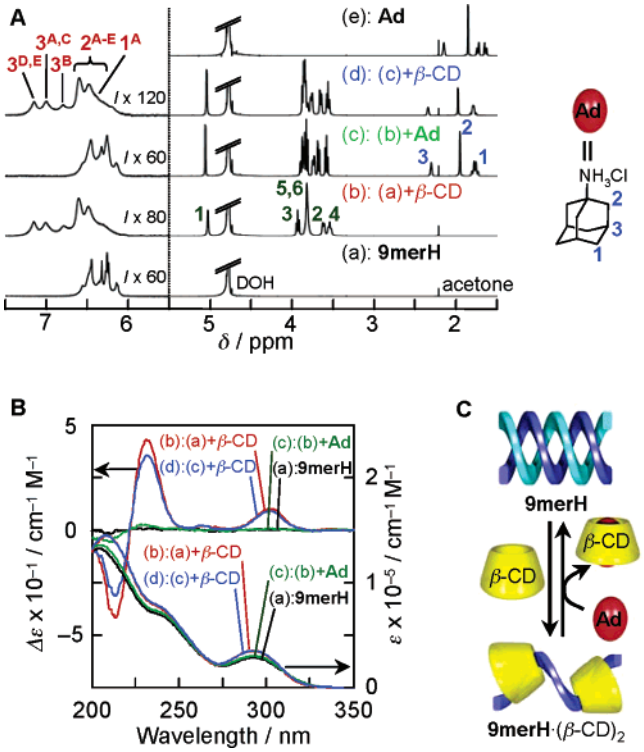

Figure 2. (A) ^{1}H NMR and (B) absorption and circular dichroism spectral changes in a solution of **9merH** in D_2O (pD = 5.8−6.0) at 25 °C (a), upon the addition of β-CD (b), the subsequent addition of **Ad** (c), and the further addition of β-CD (d); [**9merH**] = 0.625 mM, [β-CD] = 5 (b,c) and 10 mM (d), [**Ad**] = 5 mM (c−e). (C) Schematic illustration of the unwinding and rewinding of the double helix.

Table 1. Diffusion Coefficients (D) of **9merH**, α-, β-, and γ-CDs and Their Complexes[a]

system	concn (mM)	D (10^{-10} m^2 s^{-1}) **9merH**	D (10^{-10} m^2 s^{-1}) CD	D (10^{-10} m^2 s^{-1}) water
9merH	2	1.98		18.8
α-CD	18		2.75	19.7
9merH/α-CD	2/18	1.90	2.66	19.5
β-CD	18		2.54	19.8
9merH/β-CD	2/18	1.66	2.25	19.6
γ-CD	18		2.41	19.5
9merH/γ-CD	2/18	1.59	2.13	19.4

[a] Measured in D_2O at 25 °C.

The structure of the [3]pseudorotaxane **9merH**·(β-CD)$_2$ was further characterized by the 2D NOESY experiments (Figure 1D). All signals in the ^{1}H NMR spectrum were assigned on the basis of the integral values and the *g*COSY spectra (Supporting Information). Several NOE cross-peaks were observed between the aromatic protons of the **9merH** and the 3-H, 5-H, and 6-H protons of β-CD in D_2O at 25 °C. While the 5-H and 6-H protons located on the narrower rim of β-CD showed NOE cross-peaks with all the protons of the **9merH**, the 3-H protons on the wider rim exhibited no cross-peaks with the 3^D and 3^E protons of the middle resorcinol rings but showed cross-peaks with the other protons. This suggests that the wider rim bearing the 3-H protons is located close to both ends of the **9merH** strand; that is, the **9merH** strand penetrates from the narrower rim of β-CD and forms the [3]pseudorotaxane **9merH**·(β-CD)$_2$ in a head-to-head fashion (Figure 1E).[10] The head-to-head arrangement of the γ-CD wheels in the [3]pseudorotaxane **9merH**·(γ-CD)$_2$ was also deduced from the NOESY experiments (Supporting Information).[10]

Table 2. Thermodynamic Data for the Unwinding/Rewinding Processes

	K K_1[a] (M^{-3})	K K_2 (M^{-1})	K K_3[d] (M^{-1})	$\Delta G_{298}(3)$[e] (kcal mol^{-1})
β-CD	$(1.3 \pm 0.7) \times 10^7$	$(8.9 \pm 1.1) \times 10^{3}$[b]	6.0×10^8	−12
γ-CD	$(2.3 \pm 1.2) \times 10^5$	4.0 ± 0.5[c]	1.1×10^{-3}	+4.0

[a] Determined by UV titration using a single-point analysis (see Supporting Information). [b] Taken from ref 8g. [c] Determined by ^{1}H NMR titration using a nonlinear curve-fitting method. [d] Calculated by $K_3 = K_2^4/K_1$. [e] Calculated by $\Delta G_{298}(3) = -RT \ln K_3$.

Rewinding of a Double Helix of Oligoresorcinol. 1-Aminoadamantane hydrochloride (**Ad**), which forms a strong inclusion complex with β-CD,[8e−h] was then added to the [3]-pseudorotaxane **9merH**·(β-CD)$_2$ solution for the purpose of inhibiting the helicase-like activity of β-CD (Figure 2). Upon the addition of an equimolar amount of **Ad** to β-CD, the ^{1}H NMR signals of the **9merH** shifted upfield and became almost the same broadened signals as observed for the original double helix of the **9merH**, and the methylene and methine signals of **Ad** significantly shifted downfield compared to those of the free **Ad** in D_2O (Figure 2A, (c) and (e)). In addition, the absorption spectrum showed a hypochromic effect that became identical to that of the double helix of the **9merH** in water,[5d] and the induced circular dichroism completely disappeared (Figure 2B, (c)). These results clearly indicated that the **9merH** strand of the [3] pseudorotaxane **9merH**·(β-CD)$_2$ was expelled out of the β-CD wheels by the action of **Ad** and subsequently formed the double helix as a racemic mixture (Figure 2C). The further addition of β-CD brought about the unwinding of the double helix of the **9merH**, as evidenced by the downfield shifts of the ^{1}H NMR signals of the **9merH** moiety (Figure 2A, (d)), the hyperchromic shift in the absorption spectrum, and the reappearance of the identical induced circular dichroism (Figure 2B, (d)). In contrast to β-CD, the γ-CD wheels of the **9merH**·(γ-CD)$_2$ were only slightly removed by the addition of **Ad** probably due to the lower affinity of **Ad** to γ-CD, as indicated by the absorption, circular dichroism, and ^{1}H NMR spectra (Supporting Information).[8e−h]

To gain further insight into the unwinding/rewinding switching processes, we attempted to estimate the equilibrium constants (K's) for the complexation of the **9merH** with the β- or γ-CDs using absorption spectroscopy. However, the equilibria involved in the switching processes are highly complicated, and therefore, we evaluated the unwinding/rewinding processes based on the following two assumptions: (1) the **9merH** predominantly exists as a double helix and the single-stranded species may be neglected during the switching processes, because the **9merH** forms a double helix with a significantly high self-association constant over 10^4 M^{-1} in water, as previously reported;[5d] (2) the [3]pseudorotaxane **9merH**·CD$_2$ is formed from the **9merH** and CD in one step, since the formation of an intermediate [2]-pseudorotaxane may be negligible in water due to its large hydrophobic area exposed to water. Thus, the equilibrium

(10) Although the NOESY data suggest the rotaxane formation in a head-to-head fashion, the other two possible location patterns including the head-to-tail and tail-to-tail fashions could not be completely excluded.

constants defined by eqs 4−6 in the equilibria 1−3 were then roughly estimated.

$$(\mathbf{9merH})_2 + 4 \times \mathrm{CD} \overset{K_1}{\rightleftharpoons} 2 \times \mathbf{9merH}\cdot\mathrm{CD}_2 \quad (1)$$

$$\mathrm{CD} + \mathbf{Ad} \overset{K_2}{\rightleftharpoons} \mathrm{CD}\cdot\mathbf{Ad} \quad (2)$$

$$2 \times \mathbf{9merH}\cdot\mathrm{CD}_2 + 4 \times \mathbf{Ad} \overset{K_3}{\rightleftharpoons} (\mathbf{9merH})_2 + 4 \times \mathrm{CD}\cdot\mathbf{Ad} \quad (3)$$

$$K_1 = [\mathbf{9merH}\cdot\mathrm{CD}_2]^2/[(\mathbf{9merH})_2][\mathrm{CD}]^4 \quad (4)$$

$$K_2 = [\mathrm{CD}\cdot\mathbf{Ad}]/[\mathrm{CD}][\mathbf{Ad}] \quad (5)$$

$$K_3 = [(\mathbf{9merH})^2][\mathrm{CD}\cdot\mathbf{Ad}]^4/[\mathbf{9merH}\cdot\mathrm{CD}_2][\mathbf{Ad}]^4 \quad (6)$$

$$K_3 = {K_2}^4/K_1 \quad (7)$$

The equilibrium constants (K_1) for the [3] pseudorotaxane formation of the **9merH** with β- and γ-CDs were estimated by single-point determinations at each point of the UV titration curves (Table 2 and Supporting Information). While the association constant (K_2) for the complexation of **Ad** with β-CD was reported to be $K_2 = 9.4 \times 10^3$ M^{-1},[8g,h] the K_2 for γ-CD was not available in the literature but determined to be 4.0 M^{-1} by ^{1}H NMR titration (Supporting Information). From the K_1 and K_2 values, the equilibrium constants for the rewinding process (K_3) were calculated by eq 7 (${K_2}^4/K_1$) and estimated to be 6.0×10^8 M^{-1} and 1.1×10^{-3} M^{-1} for the β-CD and γ-CD, respectively, corresponding to $\Delta G_{298}(3) = -RT \ln K_3 = -12$ kcal mol^{-1} and $+4.0$ kcal mol^{-1} for the β-CD and γ-CD, respectively. Thus, the data rationally explains the unwinding/rewinding switching processes: (1) the unwinding equilibrium is favorably shifted to the pseudorotaxane formation in the presence of the β- and γ-CDs; (2) the rewinding process is highly dependent on the binding affinity of **Ad** to the CDs, and **Ad** expels the **9merH** strand from the β-CD wheels, whereas the γ-CD wheels are only slightly removed by **Ad**.

Conclusions

In summary, the β-CD has been shown to have a unique activity of unwinding the double helix of the **9merH** by encapsulating the single strands in its chiral cavity, resulting in the formation of [3]pseudorotaxane with a controlled helicity due to an excess of the one-handed helical conformation of the oligoresorcinol strand. In addition, this helicase-like activity can be regulated by the adamantane-based inhibitor. We believe that this supramolecularly controlled reversible unwinding and rewinding of the double helix offers an intriguing potential for the design of dynamic supramolecular devices.[11]

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Supporting Information Available: Full experimental details and the characterizations of the **9merH**·(β-CD)$_2$ and **9merH**·(γ-CD)$_2$. This material is available free of charge via the Internet at http://pubs.acs.org.

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