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NMR Spectroscopic Study of the Complexation Behaviors of Deuterated Cyclodextrins and [60]Fullerene

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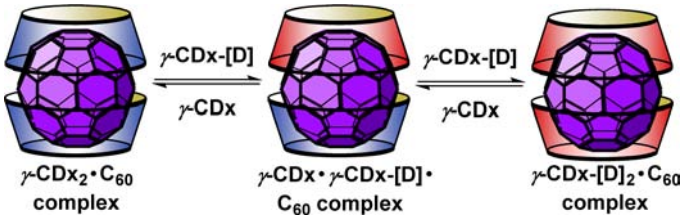
ABSTRACT



Cyclodextrins (CDxs) have been selectively deuterated using a Ru/C-catalyzed H−D exchange reaction in D_2O. The structures of the deuterated CDxs barely changed and their ^1H NMR spectra became very simple, which made it possible for the deuterated CDxs to be applied to the analysis of CDx complexes. Furthermore, the deuterated CDxs allowed for the existence of the equilibrium between free and complexed CDx to be confirmed, even at rt.

Cyclodextrins (CDxs) are cyclic oligomers of glucose that can form water-soluble complexes in their hydrophobic cavities with small molecules, such as water-insoluble or poorly soluble compounds.[1] For this reason, there has been significant interest in the use of CDxs as drug carrier systems in a number of different medical applications.[2,3] These CDx·guest molecule complexes generally give particularly complicated ^1H NMR spectra because (i) almost all of the proton peaks of the free CDx appear in the same region of the spectra as a dense collection of overlapping signals in the range 3.3−3.8 ppm; (ii) some of the proton peaks in chiral CDxs are intricately split because of the diastereotopic hydrogens; and (iii) the peaks of the CDx in the complex often overlap with the peaks of the free CDx. One strategy for avoiding the problems described above would involve the preparation of deuterium-labeled CDxs (CDxs-[D]), which could be used instead of the conventional H-containing glucose units of the CDx. Wennerström et al.[4] reported that the γ-CDx·C_{60} complex was relatively stable in the presence of some free γ-CDxs, whereas the pure γ-CDx·C_{60} complex without any free γ-CDx decomposed slowly. This result suggested that an equilibrium existed between the free and complexed γ-CDxs at rt. Although it

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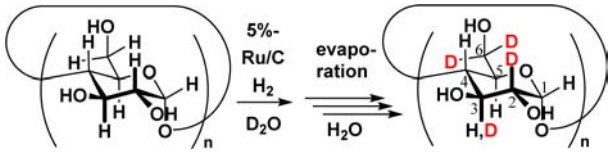
would be difficult to definitively prove the existence of this equilibrium process, we envisaged that the use of γ-CDx-[D] in this context would facilitate the development of a better understanding of this equilibrium process.

Sajiki et al.[5−7] reported the synthesis of deuterium-labeled sugars using a Ru/C-catalyzed H−D exchange reaction in D_2O. Furthermore, given that this H−D exchange reaction never proceeded at the α-position of protected-hydroxy groups, such as acetyl and methoxy groups, it could be successfully used for the preparation of a variety of different stereo- and regioselective multideuterium-labeled sugars.[6]

Here, we report the direct deuteration of three typical cyclodextrins using Sajiki's method (Scheme 1), as well as the deuterium positions of the CDxs. Furthermore, we have confirmed that no conformational changes occurred before or after the deuterium exchange reactions of the CDxs and the formation of a γ-CDx-[D] complex with C_{60}. Finally, we have demonstrated the existence of the equilibrium between the free and complexed γ-CDxs in the γ-CDx·C_{60} complex using the γ-CDx-[D], even at rt.

Scheme 1. Regioselective Deuterium Exchange Reaction of α-CDx (n = 6), β-CDx (n = 7), and γ-CDx (n = 8)



As shown in Figure S1, the ^{1}H NMR spectra of the CDxs-[D] became very simple after the H−D exchange reactions, with the splitting patterns of several peaks changing significantly. Although the H-2 and H-6 peaks (except for the H-6 peak of β-CDx) almost disappeared completely, the intensities of the H-3 peaks barely changed after the H−D exchange reactions (Table 1). The intensities of the H-1, H-4, and H-5 peaks also remained unchanged following the H−D exchange reaction.

The H-2 peaks almost disappeared in all of the CDxs-[D] following the H−D exchange reactions. The D contents at H-2 for all of the CDxs evaluated in the current study were 90% (Table 1). As a result of the H−D exchange reaction at H-2, the proton peaks for H-1 and H-3, which were adjacent to H-2, changed from being a doublet and a triplet to being a singlet and a doublet, respectively.

The intensities of the overlapping H-5 and H-6 peaks at ∼3.7 ppm were apparently decreased (Figure S1B, S1D, and S1F). Given that two protons per glucose unit disappeared from the spectra, based on the peak intensities, it was envisaged that the H−D exchange reaction involved the exchange of one H-5 proton as well as one H-6 proton or the exchange of two H-6 protons. In the former case, the remaining H-6 proton would appear as a singlet, whereas, in the latter case, the remaining H-5 proton would appear as a doublet because of its coupling with the H-4 proton at ∼3.4 ppm. In practice, however, the peak at ∼3.7 ppm appeared as a doublet in the spectra of the α- and γ-CDxs, which indicated that the H-5 position remained unchanged and that the H-6 protons had been deuterated. In contrast, ∼60% of the H-6 protons in β-CDx had been deuterated during the H−D exchange process (Table 1). None of the H-5 protons were deuterated because the double−doublet peak of the H-4 protons resulting from the coupling of these protons with the H-5 and H-3 protons remained unchanged.

Table 1. D Content (%) of α-CDx, β-CDx, and γ-CDx Following the H−D Exchange Reactions

	D content (%)						
compd	H-1	H-2	H-3	H-4	H-5	H-6 (2H)[a]	total
α-CDx	0	90	30	0	0	90	43
β-CDx	0	90	0	0	0	60	30
γ-CDx	0	90	10	0	0	100	43

[a] There are two protons in the H-6 position.

The peak intensities of the H-3 protons at ∼3.8 ppm were decreased slightly in the α- and γ-CDxs (Figure S1B, S1D, and S1F). The H-3 protons were positioned to a hydroxy group, and it is known that the H−D exchange reactions of the H-3 protons in α-D-methylglucoside are as fast as those at H-2 and faster than those at H-6.[6] It was therefore expected that the D content at the H-3 position would be high but was lower than that at the H-2 and H-6 positions. These results suggested that the deuteration of the H-3 protons, which were inside the CDx cavities, was being suppressed by steric hindrance. The H-3 and H-6 positions of β-CDx had lower D content than those of α-CDx and γ-CDx. Although the reason for the lower reactivity of β-CDx remains unclear at this stage, the high rigidity of β-CDx, resulting from it possessing the strongest intramolecular H-bonding interactions of the three CDxs,[8] may have inhibited its interaction with the Ru/C catalyst and ultimately slowed down the rate of the H−D exchange reaction.

Although α-D-methylglucoside was reported to undergo the H−D exchange reaction at its H-2, H-3, H-4, and H-6 positions,[6] none of the CDxs were deuterated at their H-4 positions. This result, however, was not surprising, because none of these CDxs contained a hydroxy moiety at their H-4 position. Furthermore, this result was in agreement with previous reports pertaining to the H−D exchange reactions of similar systems, which have demonstrated that Ru/C-catalyzed H−D exchange reactions never proceed at a position α to a protected-hydroxy group, such as an acetoxy or methoxy group.[6,7]

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The deuterated numbers of the CDxs-[D] were calculated by high-resolution ESI$^-$MS using the distribution of isotopic peaks in the mass spectra (Figures S2−S4). As shown in Figure S5, the deuterated numbers of α-CDx-[D] and γ-CDx-[D] were spread over a narrower range than those of the β-CDx-[D]. Furthermore, the results revealed that the total D content for α-CDx-[D] and γ-CDx-[D] (α-CDx-[D]: 46%, γ-CDx-[D]: 42%) was much higher than that for β-CDx-[D] (β-CDx-[D]: 30%), and these results were consistent with those of the average deuterated numbers based on the results of the D content in Table 1 (α-CDx-[D]: 43%, β-CDx-[D]: 30%, γ-CDx-[D]: 43%).

To determine whether the H−D reactions had led to conformational changes in the CDxs-[D], we carefully investigated the chemical shifts of the remaining protons in the ^{1}H NMR spectra. The chemical shifts of the remaining protons hardly changed in all of the CDxs-[D], especially the α- and γ-CDxs-[D] (Table 2). These results indicated that (i) the H−D exchange reactions occurred stereoselectively and (ii) the structures and conformations of all of the CDxs-[D] were maintained in water.

Table 2. Chemical Shifts (ppm) of α-CDx, β-CDx, and γ-CDx Before and After the H−D Exchange Reactions

	chemical shift (ppm)					
compd	H-1	H-2	H-3	H-4	H-5	H-6
α-CDx	4.895	3.474	3.825	3.427	–[a]	–[a]
α-CDx-[D]	4.898	–[b]	3.828	3.430	3.679	–[b]
Δδ in α-CDx[c]	+0.003	–	0.003	+0.003	–	–
β-CDx	4.904	3.483	3.800	3.418	3.689	3.712
β-CDx-[D]	4.911	–[b]	3.806	3.428	3.691	3.714
Δδ in β-CDx[c]	+0.007	–	+0.006	+0.010	+0.002	+0.002
γ-CDx	4.955	3.499	3.781	3.436	–[a]	–[a]
γ-CDx-[D]	4.954	–[b]	3.780	3.436	3.702	–[b]
Δδ in γ-CDx[c]	−0.001	–	−0.001	0.000	–	–

[a] These chemical shifts cannot be determined because the resonance peaks overlapped with the other peaks in the spectrum and complicated the splitting patterns. [b] These peaks disappeared following the deuteration process. [c] A plus sign (+) denotes a shift to a lower magnetic field, whereas a minus sign (−) denotes a shift to a higher magnetic field following the deuteration process.

To investigate whether the deuteriums of CDxs-[D] prevented the guest molecules from accessing their cavities, a γ-CDxs-[D]·C_{60} complex was prepared using a mechanochemical high-speed vibration milling apparatus according to Komatsu's method.[8] The UV−vis absorption spectrum of the γ-CDx-[D]·C_{60} complex was very similar to that of the γ-CDx·C_{60} complex (Figure 1), clearly indicating that the γ-CDx-[D] also formed a 2:1 complex with C_{60}. The solubility of the γ-CDx-[D]·C_{60} complex (2.02 mmol L^{-1}) was found to be similar to that of the C_{60}·γ-CDx complex (2.21 mmol L^{-1}),[9] based on the absorbance at 332 nm. Furthermore, we demonstrated that the ^{1}H NMR spectrum of the γ-CDx-[D]·C_{60} complex

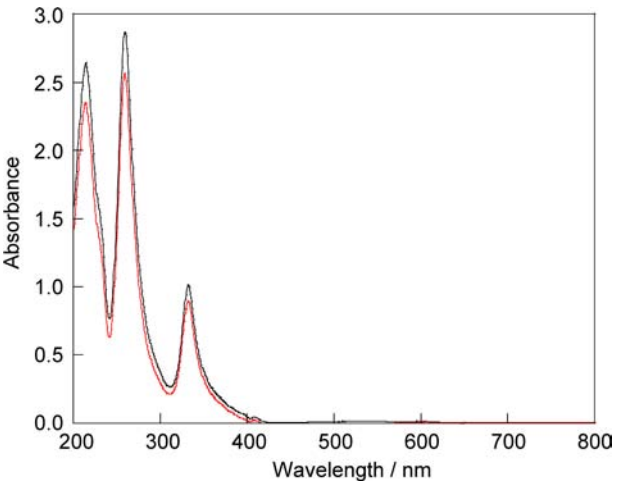

Figure 1. UV−vis absorption spectra of (A) γ-CDx·C_{60} complex (black line) and (B) γ-CDx-[D]·C_{60} complex (red line): D_2O, 25 °C, 1 mm cell, [γ-CDx·C_{60} complex] = 2.21 mmol L^{-1}, [γ-CDx-[D]·C_{60} complex] = 2.02 mmol L^{-1}.

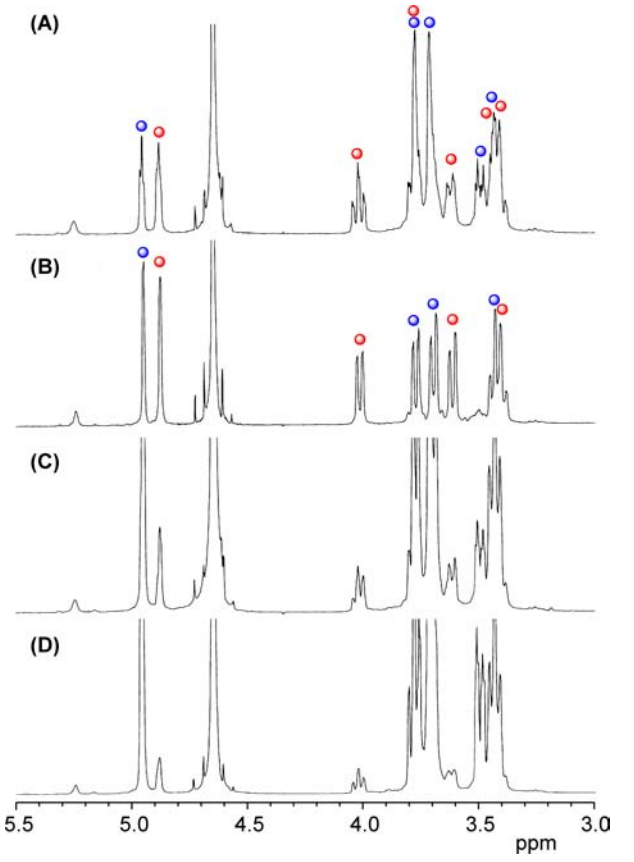

Figure 2. Partial ^{1}H NMR spectra of (A) γ-CDx·C_{60} complex, (B) γ-CDx-[D]·C_{60} complex, (C) γ-CDx·C_{60} complex after the addition of γ-CDx-[D] ([γ-CDx] = 2.79 mmol L^{-1}, [γ-CDx-[D]] = 4.33 mmol L^{-1}), and (D) γ-CDx-[D]·C_{60} complex after the addition of γ-CDx ([γ-CDx] = 4.32 mmol L^{-1}, [γ-CDx-[D]] = 3.00 mmol L^{-1}): D_2O, 25 °C, 400 MHz, blue dot: free γ-CDx or γ-CDx-[D], red dot: γ-CDx·C_{60} complex or γ-CDx-[D]·C_{60} complex.

Table 3. Chemical Shifts (ppm) of the γ-CDx·C_{60} Complexes Before and After the H−D Exchange Reactions

	chemical shift (ppm)					
compd	H-1	H-2	H-3	H-4	H-5	H-6
γ-CDx·C_{60} complex	4.885	–[a]	4.027	3.417	–[a]	3.778
γ-CDx-[D]·C_{60} complex	4.885	–[b]	4.015	3.410	3.646	–[b]
$\Delta\delta$[c]	0.000	–	−0.012	−0.007	–	–

[a] These chemical shifts could not be determined because the resonance peaks overlapped with other peaks in the spectrum for the free γ-CDx. [b] These peaks disappeared following the deuteration process. [c] A plus sign (+) denotes a shift to a lower magnetic field, whereas a minus sign (−) denotes a shift to a higher magnetic field.

(Figure 2B) consisted of very simple peaks compared with that of the γ-CDx·C_{60} complex (Figure 2A). The chemical shifts of the remaining protons hardly changed in the γ-CDx-[D]·C_{60} complex (Table 3), indicating that the deuteriums of the CDxs-[D] did not prevent the C_{60} from entering the cavity because most of the deuteriums of the CDxs-[D] existed outside of the cavity.

In Figure 2A and 2B, the differences in the peaks of the γ-CDx in the γ-CDx·C_{60} complex to those of free γ-CDx implied that (i) the complexation−decomplexation exchange rate was slower than the NMR time scale (Scheme 2) or (ii) the anticipated equilibrium between the complexation−decomplexation exchange was absent. To determine which explanation was correct, the solution of the pristine γ-CDx·C_{60} complex in Figure 2A was added to a solution containing a 1.5-fold excess of γ-CDx-[D]. The ^{1}H NMR spectrum of this mixture is shown in Figure 2C. The triplet peak corresponding to the H-4 proton in the pristine γ-CDx·C_{60} complex at 4.0 ppm was replaced primarily by a doublet peak. In contrast, when the solution of the γ-CDx-[D]·C_{60} complex in Figure 2B was added to a solution containing a 1.5-fold excess of pristine γ-CDx, the doublet peak corresponding to the H-4 proton in the γ-CDx-[D]·C_{60} complex at 4.0 ppm was replaced primarily by a triplet peak (Figure 2D). These spectral changes were observed immediately after the mixing of these solutions and clearly indicated that the C_{60}-complexed pristine γ-CDx and γ-CDx-[D] were immediately replaced by the added γ-CDx-[D] and pristine γ-CDx, respectively, even at rt, as shown in Scheme 2. That is, we had successfully

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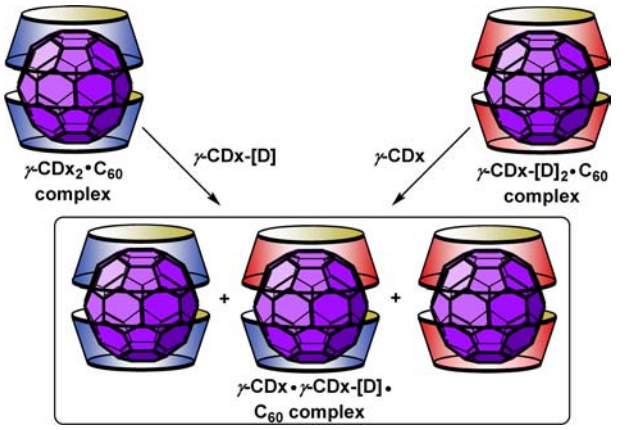
Scheme 2. Exchange Reaction between Pristine γ-CDx and γ-CDx-[D]



demonstrated the existence of an equilibrium between a free and complexed γ-CDx and the γ-CDx$_2$·C_{60}, γ-CDx·γ-CDx-[D]·C_{60}, and γ-CDx-[D]$_2$·C_{60} complexes coexisted at equilibrium.

In summary, we have successfully synthesized multi-deuterium-labeled α-CDx, β-CDx, and γ-CDx in a regioselective manner. Using ^{1}H NMR spectroscopy, we have demonstrated that the resulting CDxs-[D] maintained their chiralities, structures, and conformations. Furthermore, γ-CDx-[D] formed a complex with C_{60} at a concentration similar to that of pristine γ-CDx. Using γ-CDx-[D], we have proven the existence of an equilibrium between free and complexed γ-CDx. Given that the binding abilities of the CDxs-[D] were the same as those of the pristine CDxs, the CDxs-[D] could be used as useful deuterium-labeled compounds for pristine CDxs, which have been employed as carriers for a variety of water-insoluble drugs. It is envisaged that the addition of CDx-[D] will be used routinely in the future to evaluate the equilibrium of CDx-based rotaxane or pseudorotaxane complexes.

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Supporting Information Available. Experimental details and Figures S1−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.