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MODIFICATIONS OF THE SECONDARY HYDROXYL SIDE OF α-CYCLODEXTRIN AND NMR STUDIES OF THEM

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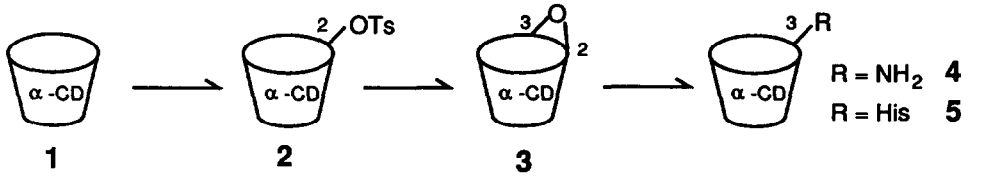
Some modified α-CD on secondary hydroxyl side were prepared and conformation and configuration of them were studied by COSY, TOCSY, and ROESY spectra. These spectra indicated that tosyl group of α-CD-2-OTs directed perpendicular to z-axis of α-CD. α-CD-3-NH_2 and α-CD-3-His had modified altrose residue with $^1C_4$ conformation. Catalytic activity of α-CD-3-His was over 4 times larger than that of α-CD-6-His.

The ability of making inclusion complex of cyclodextrin (CD) is valuable to construct artificial enzyme and CD can accelerate hydrolysis reactions by a similar mechanism as a natural enzyme.[1] But chemical modification of CD with some functional groups is needed for preparation of more excellent artificial enzyme. Since the secondary hydroxyl side of CD is more opened, a guest molecule can be included more easily from the secondary hydroxyl side than from the primary hydroxyl side. The chirality of CD is more apparent on the secondary hydroxyl side. From these reasons, it is interested to modify the secondary hydroxyl side of CD. Recently a few artificial enzymes were prepared by modifications of the secondary hydroxyl side of β-CD.[2, 3] But artificial enzyme using α-CD bearing a functional group on the secondary hydroxyl side has probably never been reported. So far identifications for modified CDs were almost taken by only one dimensional (1D) NMR. These assignments have some uncertainties and it is necessary to use 2D NMR. The combination of some kinds of 2D NMR provides many information for conformation and configuration of modified CD. We wish to describe preparations of some modified α-CD on the secondary hydroxyl side, and conformational and configurational studies of them with 2D NMR spectra (COSY, TOCSY (2D HOHAHA), and ROESY spectra). And catalytic activity of α-CD bearing a histamine on the secondary hydroxyl side was measured.

α-CD-2-OTs (**2**) was prepared by mono-tosylation of α-CD in 0.4 N alkaline aqueous solution at 0 °C for 5 hrs.[4] **2** was treated with 0.05 N alkaline aqueous solution at room temperature for 48 hrs followed by isolation with column chromatography on DIAION HP-20 to give **3**. **3** was reacted with 28 % aqueous ammonia or histamine in aqueous solution to give **4** or **5** respectively. They were purified with CM-Sephadex C-25 and Sephadex G-10.

The 1D ^1H NMR spectrum of **2** is too complicated to make assignment of its peaks. But 2D NMR



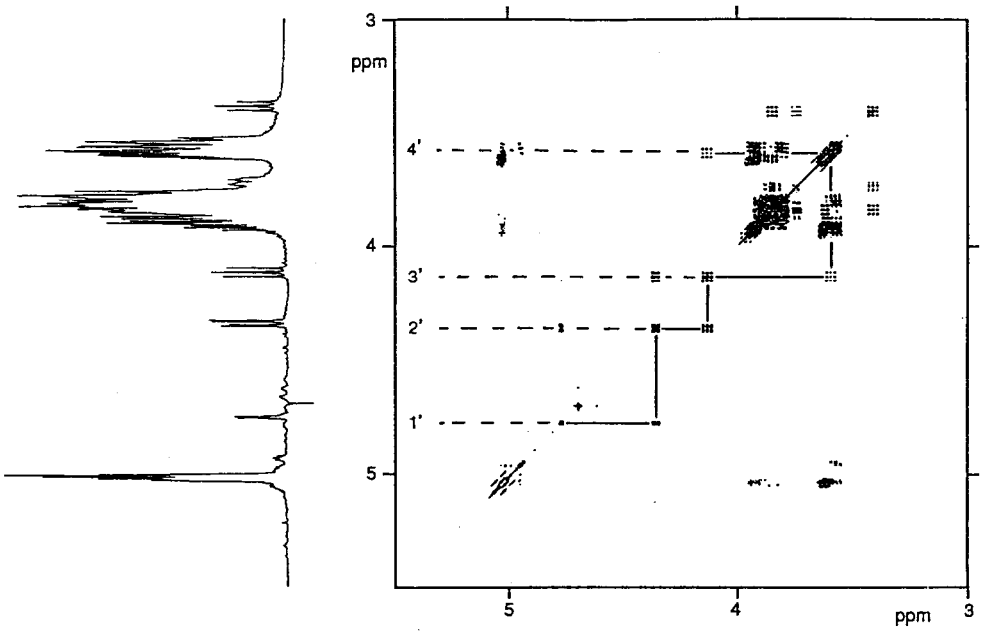


Fig. 1 COSY Spectrum of 2 in D_2O (Varian VXR 500 MHz).

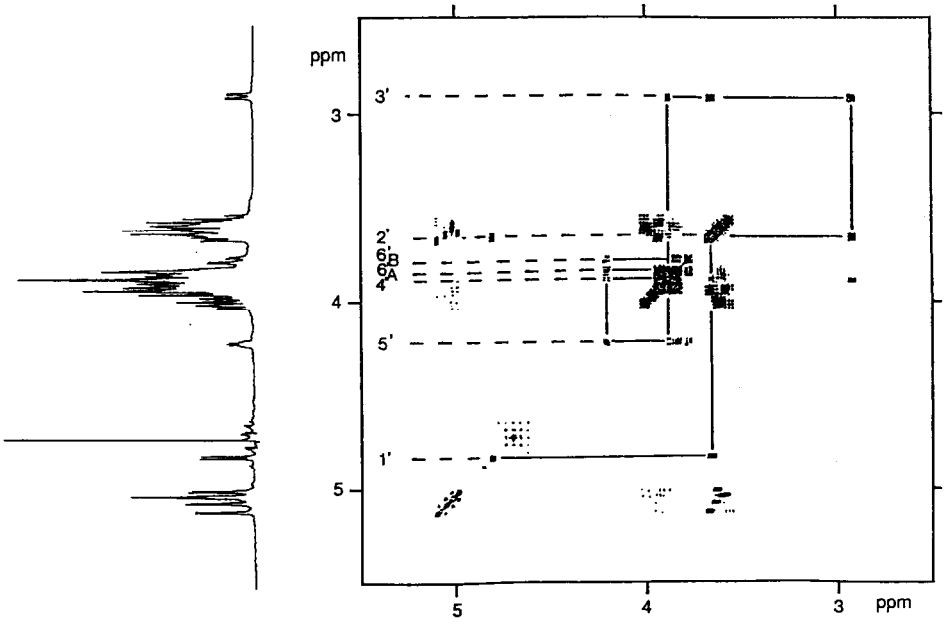


Fig. 2 COSY Spectrum of 4 in D_2O (Varian VXR 500 MHz).

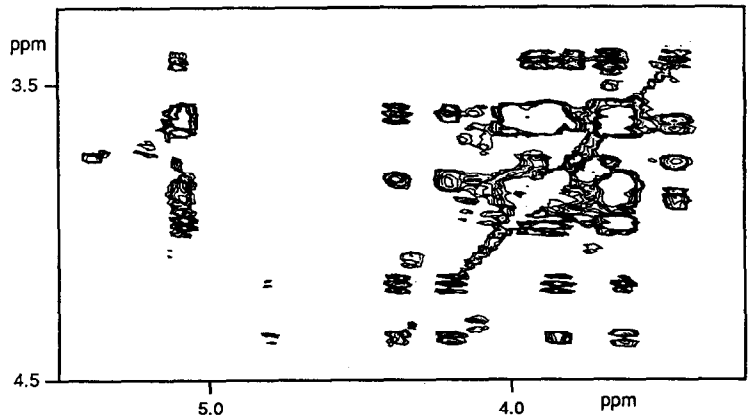


Fig. 3 TOCSY (2D HOHAHA) spectrum of **2** in D_2O at 21 °C with mixing time of 140 ms (Varian UNITY 400 MHz).

spectra could give many information for the assignment of signals of tosylated α-CD. COSY spectrum of 2 is shown in Fig. 1. Peaks around 5 ppm are for anomeric protons and a doublet at 4.76 ppm ($J_{1',2'}$ = 3.4 Hz) is for the up-field shift of $H_{1'}$. By tracing the coupling patterns from this peak, signals for the protons of tosylated glucose unit could be assigned. The result is shown in Fig. 1. This spectrum verifies that tosyl group is linked at C-2 position. A double-doblet at 4.12 ppm ($J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = 8.7 Hz) were assigned for the down field shift of $H_{3'}$. Peaks of $H_{5'}$ and $H_{6'}$ of tosylated glucose unit could not be assigned by only COSY spectrum. TOCSY spectrum is useful method to extract the spin system of a pyranose unit from overlapping spectrum region. NOESY spectrum is usually used to find pairs of protons that are close in space. But in some cases ROESY spectrum is superior to NOESY spectrum.[7] ROESY spectrum is better than NOESY spectrum, when the correlation time (τ_c) approaches the inverse in the Larmor frequency (ω) of the protons, that is, in the case of medium-size molecules. And in ROESY spectrum, NOE cross-peak and J cross-peak can be easily differentiated. TOCSY spectrum (Fig. 3) shows that peaks at 4.76, 4.35, 4.12 ppm belong to the same spin system, but a peak at 3.39 ppm belongs to other spin system. ROESY spectrum of 2 had a closs peak at (4.76 ppm, 3.39 ppm). Thus the triplet at 3.39 ppm is not the peak for a proton of tosylated glucose unit, but for the up-field shift of H_4 in the adjacent un-tosylated glucose unit. This shifted peak is probably due to the anisotropic ring current effect from the aromatic ring of tosyl group and this indicates that tosyl group might direct perpendicular to z-axis of α-CD. This result was supported by negative induced circular dichroism band at about 260 nm. The determination of the more detailed conformation of α-CD-2-OTs (**2**) is now in progress.

^1H NMR spectrum of **3** was free from aromatic protons. The spectrum showed that a singlet at 5.24 ppm for the down-field shift of $H_{1'}$ and a doublet at 3.44 ppm ($J_{2',3'}$ = 3.7 Hz) for the up-field shift of $H_{2'}$. In glucose-manno-epoxide, $J_{1,2}$ is normally close to 0 Hz, while in allo-epoxide $J_{1,2}$ is 2.5-4.5 Hz.[2] This implies that **3** has manno-epoxide.

COSY spectrum of **4** is shown in Fig. 2. This spectrum indicates amino group is connected at C-3 position. TOCSY spectrum also supported that these shifted peaks were for the protons of amidated pyranose unit. ^1H NMR spectrum of **4** showed a doublet at 4.79 ppm for the up-field shift of $H_{1'}$. The coupling constant $J_{1',2'}$ is 6.6 Hz, whereas the coupling constants $J_{1,2}$ of the other glucose units are 2.9-3.9 Hz. This means relationship between $H_{1'}$ and $H_{2'}$ is trans. A double-doublet at 2.88 ppm is for the up-field shift of $H_{3'}$. The coupling constants, $J_{2',3'}$ and $J_{3',4'}$ are 10.4 Hz and 3.8 Hz respectively. This indicates that both $H_{2'}$ and $H_{3'}$ are axial

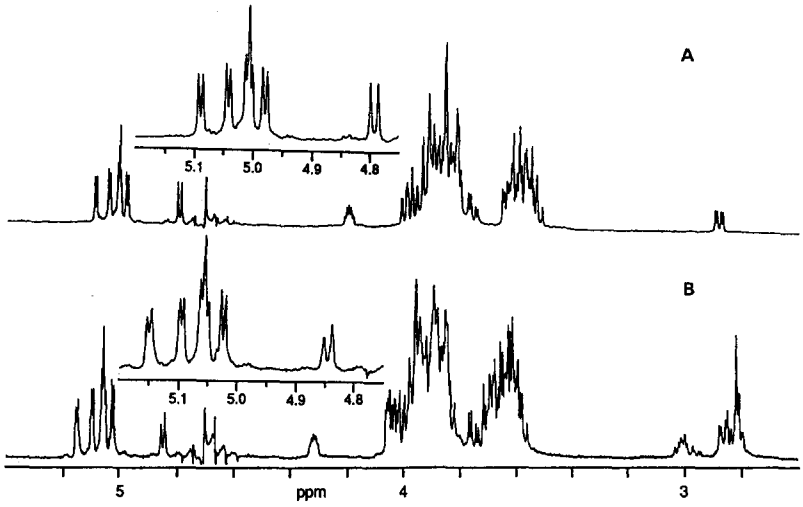


Fig. 4 1H NMR spectra of **4** (A) and **5** (B) in D_2O (Varian VXR 500MHz).

conformation and $H_{4'}$ is equatorial. These suggests that one of glucose units with $^4C_1$ conformation was changed to altrose unit with $^1C_4$ conformation in the processes of modifications. TOCSY and COSY spectra indicates that a peak at 4.19 ppm is for the down-field shift for $H_{5'}$ of amidated pyranose unit. Both 1D NMR spectrum and 2D NMR spectra of **5** are quite similar to those of **4** except peaks for histaminyl group. Only 1D ^1H NMR spectra are shown in Fig. 4. Thus histaminyl group is linked at C-3 position and histamine-appended residue is altrose unit with $^1C_4$ conformation. Some one suspected that pyranose unit having a large functional group at C-3 position could not flip from $^4C_1$ to $^1C_4$, but our results shows that pyranose unit bearing a histamine can also flip as same as amidated pyranose unit already reported.[5,6]

The rate of hydrolysis of p-nitrophenyl acetate by **5** was measured under the condition of large excess of substrate at 25 °C in pH 8.0 phosphate buffer. k_{cat} of **5** is 2.18×10^{-3} s^{-1}. It is over 4 times larger than that of α-CD bearing a histamine at C-6 position. K_m of the former is 3.03×10^{-3} mol dm^{-3} and nearly equal to the latter.

These results suggest that modification of α-CD on secondary hydroxyl side is significant for creating more excellent artificial enzyme and combination of 2D NMR spectra is powerful for investigate the conformation and configuration of modified CD on secondary hydroxyl side.

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