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시클로 덱스트린과 그 메틸유도체의 구조분석

崔 姬 淑†
퍼듀대학 약학대학 약화학과
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Conformational Analysis of Cyclodextrins and Their Methylated Analogs

Hee-Sook Choi†
Department Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907
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요 약. α-와 β-시클로덱스트린과 그의 메틸유도체들을 Chemical shift(δ)와 Coupling constant(J)를 470 MHz ^{1}H NMR을 이용해서 수용액 안에서 분석하였다. 정확한 δ와 J값들을 얻기 위해서 Raccoon spin simulation program을 이용해서 실험 data를 분석하였다. 시클로덱스트린의 C_5-C_6 bond 주위의 rotamer의 분포를 J_{56a}와 J_{56b}값을 이용하여 계산하였다. 위 계산에 의해 α-와 β-시클로덱스트린에서는 *gg* conformer가 가장 많이 존재하였고 *tg* conformer가 가장 적게 존재했다. 그러나 그 메틸 유도체에서는 *gg* conformer가 더 많이 증가하였고 *gt* conformer가 가장 적게 존재함을 알았다.

ABSTRACT. The ^{1}H NMR chemical shifts and coupling constants for α-, permethyl-α-, β- and permethyl-β-cyclodextrins in neutral aqueous media were assigned based on the 470 MHz spectra. In order to obtain accurate chemical shifts and coupling constants the experimental spectra were analyzed with the Raccoon spin simulation program. The rotamer distribution around the C_5-C_6 bond of the cyclodextrins evaluated from the coupling constants of J_{56a} and J_{56b}. In our calculation of the α-, and β-cyclodextrin showed that *gg* conformers were most favorable form and *tg* conformers were least favorable form. It is very interesting to note the changes in J_{56a}, J_{56b} coupling constants of permethylated α- and β-cyclodextrins from unmodified one. The *gg* conformers were more incresed than unmodified one and instead of *tg* conformers *gt* conformers were least favorable one upon methylation.

INTRODUCTION

Cyclodextrins have been studied extensively in recent years as complexing agents for drugs in controlled drug delivery systems and as enzyme models for enzyme-substrate reactions.

The α-, β-, and γ-cyclodextrins are the most common natural cyclodextrins, consisting of six, seven, and eight glucopyranose units, respectively. Because of their different internal cavity diameters, each cyclodextrin shows a different degree of inclusion complex formation with different-sized guest molecules[1]. Cyclodextrins are water-soluble, since all of the free hydroxyl groups are on the outer surface of the ring; the internal cavity of the doughnut-shaped molecule is slightly apolar.

The natural cyclodextrins can be chemically modified for many different purposes. The hydroxyl groups of cyclodextrins are available as starting points of structural modification, and various funtional groups have been incorporated into the cyclodextrin molecules[2]. For example, methylated cyclodextrins[3,4], ethylated cyclodextrins[5], hydroxy-

†이화여자대학교 자연과학대학 화학과

propylated cyclodextrins[6,7], and polymeric cyclodextrins[8,9] are successfully being employed in the rational design of new drug carrier systems.

When methyl groups are introduced onto the hydroxyls of C_2', C_3', or C_6' hydrogen bondings are made impossible and the physicochemical properties of cyclodextrins are significantly altered. As an example, heptakis (2,6-di-o-methyl)-β-cyclodextrin is extremely soluble in both water and organic solvents, less hygroscopic than β-cyclodextrin, and highly surface active[10,11].

The ^1H NMR study of permethyl-α and β-cyclodextrin in chloroform was reported by casu *et al.* by 60 MHz was difficult to analyse due to low resolution[12].

In 1976, the proton chemical shifts and coupling constants for α-cyclodextrin were obtained at 100 and 220 MHz by D.J. Wood *et al.*[13]. From this study, they report that the C_1 chair form of α-cyclodextrin from the vicinal coupling constants.

In 1985, Johnson *et al.*[14] reported chemical shifts and coupling constants of permethyl-β-cyclodextrin using two-dimensional chemical shift correlation spectroscopy (COSY). Analysis of the vicinal coupling constant (J_{12}-J_{45}) by these workers revealed that the C_1 chair form of β-cyclodextrin was maintained in permethyl-β-cyclodextrin. The assignments of the 2- and 3-methoxy proton resonance values made by Johnson *et al.*[14], however, is reversed from the assignments made by Casu *et al.* by 60 MHz ^1H NMR[12].

In 1986, Inoue *et al.*[15] reported the 500 MHz ^1H NMR spectra of α- and β-cyclodextrin and their permethyl analogs in acidic and basic conditions. However, no attempt was reported to calculate the accurate chemical shifts and coupling constants.

The glucose units in α-cyclodextrin behave as relatively rigid building blocks[16], with the main conformational freedom being rotation about the glucosidic C_1-04 and C_4-04 bonds and about the C_5-C_6 bond of pyranose can be discussed in terms of the relative constributions from the gauche-gauche (*gg*), gauch-trans (*gt*), and tran-gauche (*tg*) (*Fig.* 1).

In this paper, the ^1H NMR chemical shifts and coupling constants for α-, permethyl-α-, β- and permethyl-β-cyclodextrins in neutral aqueous media were assigned based on the 470 MHz spectra and by their extensive spin simulations. The population distribution of the rotamers around the C_5-C_6 bond of the cyclodextrins and their permethyl cyclodextrins were evaluated from the coupling constants.

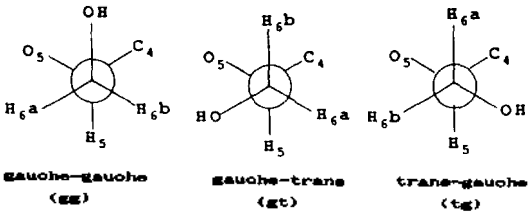


Fig. 1. Classically staggered conformations about the C_5-C_6 bond.

EXPERIMENT

Instrumentation

NMR spectroscopy. All structural studies of cyclodextrins by ^1H NMR were recorded on Nicolet NT-470 spectrometer with 16 *K* computer memory operating at 469.5 MHz. The spectra were measured with 20.5 μsec pulse width and 10 seconds repetition time. DMSO-d_6 (Aldrich Chemical Co.) was used as external reference with a signal at 3.03 ppm relative to TMS (at 0.0 ppm) for ^1H NMR spectra. A 5 mm sample tube was used.

In order to determine accurate chemical shifts and coupling constants of cyclodextrins, all 470 MHz ^1H NMR spectra spins (7 spins) were calculated by the Raccoon spin simulation program. Zenith data system Al Tec-286 personal computer and Hewlett-packard 7470A plotters were used. The concentration of NMR samples was 10 mM and 0.1 *M* deutrated phosphate buffer (pD 7.4) was used for the solvent.

Chemicals

α-cyclodextrin was obtained from Anspec, Ann arbor, MI, and β-cyclodextrin was obtained from Chemical Dynamic Corp. South Plainfield, NJ. Permethyl-α-cyclodextrin and permethyl-β-cyclodextrin were prepared from the method developed by Van Hooidnk *et al.*[17].

Methods

Preparation of Phosphate Buffer. Phosphate buffer was prepared by mixing precalculated amounts of monobasic and dibasic phosphate stock solutions.

To prepare the deuterated buffer, the regular buffer was lyophilized, exchanged once with 99.5% D_2O (Aldrich Chemical Co.) and redissolved to the original volume with 99.5% D_2O.

RESULTS AND DISCUSSION

The 470 MHz ^1H NMR spectrum of α-cyclodextrin, permethyl-α-cyclodextrin, β-cyclodextrin and permethyl-β-cyclodextrin were analyzed with the Raccoon spin simulation program. The experimental and simulated spectra are shown in *Fig.* 2, 3, 4 and 5 and the simulated results are summarized in *Table* 1 and 2.

In the α-cyclodextrin, the magnitudes of the vicinal coupling constants J_{12} through J_{45} are consistant with the C_1 chair conformation form for the glucose units. The 500 MHz ^1H NMR spectra of permethyl-α-cyclodextrin and permethyl-β-cylodextrin in pD 10 reported by Inoue *et al.*[15] are similar to our 470 MHz pD 7.4 spectra shown in *Fig.* 3 and 5, respectively. Based on our spin simulation results, however, the peak assignments made by these workers for H_3, H_4, H_5, and H_6 protons are not in agreement with our assignments (*Fig.* 3 and 5).

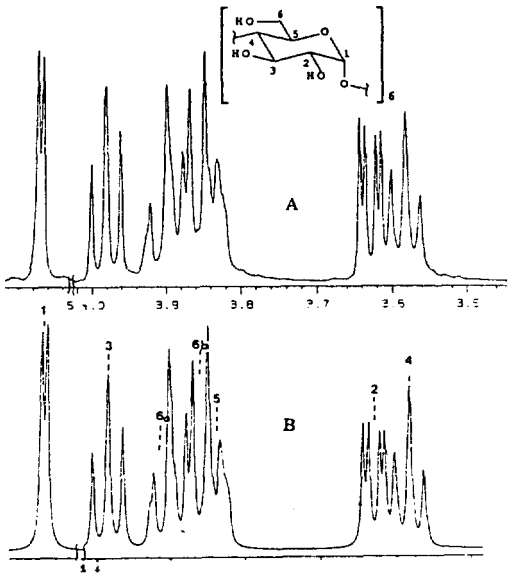


Fig. 2. 470 MHz ^1H NMR spectrum of (A) α-cyclodextrin and (B) its spin simulated spectrum.

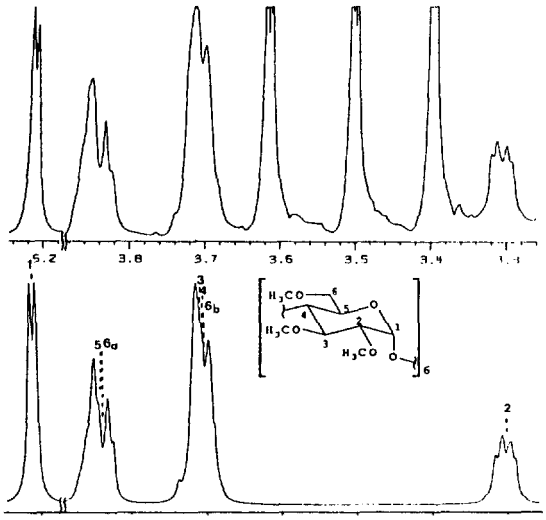


Fig. 3. 470 MHz ^1H NMR spectrum of permethyl-α-cyclodextrin (top) and its spin simulated spectrum (*bottom). *2, 3, 6-O-CH_3 protons are not included in the spin simulation.

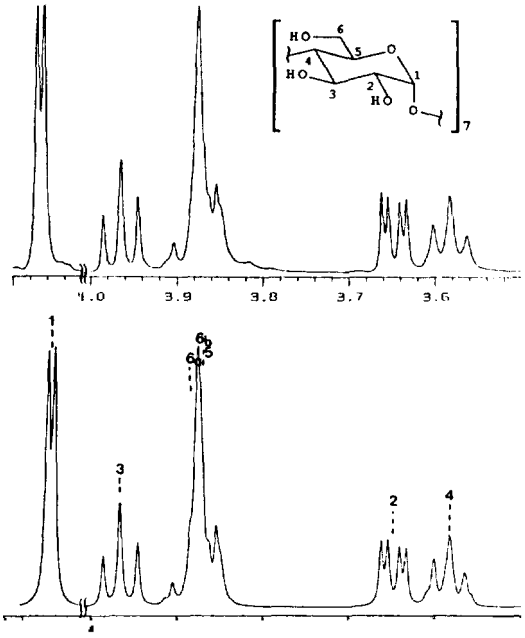


Fig. 4. 470 MHz ^1H NMR spectrum of β-cyclodextrin (top) and its spin simulated spectrum (bottom).

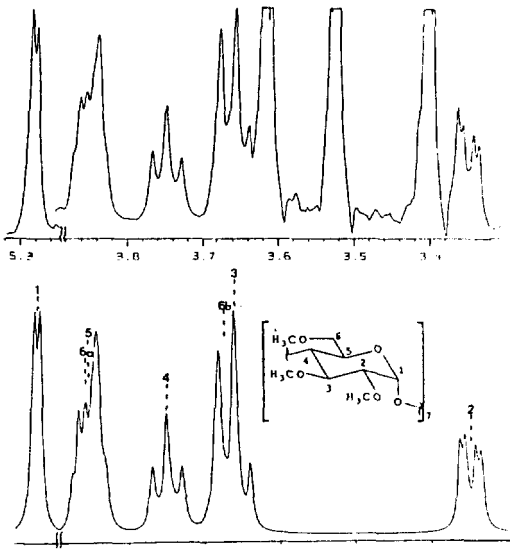


Fig. 5. 470 MHz ^{1}H NMR spectrum of permethyl-β-cyclodextrin (top) and its spin simulated spectrum (*bottom). *2, 3, 6-O-CH_3 protons are not included in the spin simulation.

Population distribution of the rotamers can then be evaluated from the coupling constants of J_{56a} and J_{56b}[18,19]. A single relationship between P_{gg} (The fractional population of the *gg* rotamer) and $\sum$ (the observed sum of J_{56a} and J_{56b}) can be expressed in terms of equation 1 which was derived by Hruska *et al.*[20].

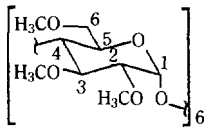
$$P_{gg}=(13-\sum)/10 \quad (1)$$

P_{tg} and P_{gt} can then be calculated from the following equations:

$$P_{tg}=J_{56a}/10-0.15 \quad (2)$$
$$P_{gt}=J_{56b}/10-0.15 \quad (3)$$

The rotamer distribution around the C_5-C_6 bond of the cyclodextrins and their permethyl analogs are summarized in *Table* 3. The magnitudes of all J_{56a} entries for α-cyclodextrin lie in the range 1.8~2.0 Hz while those for J_{56b} lie in the range 4.3 Hz. Thus, one may conclude that one of the trans conformers, *gt* or *tg*, can be excluded as a significant contributor. The most likely trans conformer *tg* is excluded due to unfavorable parallel 1, 3 interactions between oxygen atoms in C_4 and C_6[21].

Table 1. 470 MHz ^{1}H NMR computer-simulated spectra data for α-cyclodextrin and permethyl-α-cyclodextrin

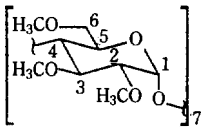


Protons	α-CDX	Permethyl-α-CDX
	Chemical shift (ppm)	
1	5.0510	5.2110
2	3.6300	3.3050
3	3.9820	3.7130
4	3.5830	3.7060
5	3.8370	3.8530
6a	3.9070	3.8390
6b	3.8630	3.7050
	Coupling constant (Hz)	
J_{12}	3.4	3.4
J_{15}	−0.7	−0.5
J_{23}	10.1	9.7
J_{34}	9.2	9.2
J_{45}	9.5	9.5
J_{46a}	−0.6	−1.5
J_{46b}	−0.5	−1.0
J_{56a}	2.0	3.7
J_{56b}	4.4	2.0
J_{6a6b}	−11.5	−10.5

In our calculation of the α-cyclodextrin showed that *gg* conformers were most favorable form and *tg* conformers were least favorable form. In crystalline pyranosides[22], formation of *gg* and *gt* but not *tg*, has been observed. It is very interesting to note the changes in J_{56a}, J_{56b} coupling constants of permethyl-α-cyclodextrin from that of α-cyclodextrin. The *gg* conformers were most favorable one and instead of *tg* conformers *gt* conformers were least favorable one upon methylation.

As was indicated in the α- and permethyl-α-cyclodextrin case, the magnitude of the vicinal coupling constants J_{12} through J_{45} of the β-cyclodextrins are consistant with the C_1 chair conformation form for the glucose units. The rotamer distribution around the C_5-C_6 bond of the β- and permethyl-β-cyclodextrin are summarized in *Table* 3. The *gg* conformers were most favorable in β- and per-

Table 2. 470 MHz ^{1}H NMR computer-simulated spectra data for β-cyclodextrin and permethyl-β-cyclodextrin



Protons	β-CDX	Permethyl-β-CDX
	Chemical shift (ppm)	
1	5.0690	5.2820
2	3.6484	3.3480
3	3.9640	3.6840
4	3.5840	3.7465
5	3.8640	3.8580
6a	3.8870	3.8450
6b	3.8640	3.6585
	Coupling constant (Hz)	
J_{12}	3.5	3.5
J_{15}	−0.6	−1.5
J_{23}	9.8	9.7
J_{34}	9.2	9.2
J_{45}	9.4	9.5
J_{46a}	−0.7	−1.0
J_{46b}	−0.7	−0.5
J_{56a}	1.9	3.5
J_{56b}	4.7	2.0
J_{6a6b}	−12.4	−10.0

Table 3. Rotamer distribution around the C_5-C_6 bond of the cyclodextrins

	α-CDX	PM-α-CDX	β-CDX	PM-β-CDX
J_{56a}	2.0	3.7	1.9	3.5
J_{56b}	4.4	2.0	4.7	2.0
$\sum$	6.4	5.7	6.6	5.5
P_{gt} (%)	66	73	64	75
P_{tg} (%)	5	22	4	20
P_{tt} (%)	29	5	32	5

methyl-β-cyclodextrin. The *tg* conformers were excluded in β-cyclodextrin and *gt* conformers were excluded in permethyl-β-cyclodextrin and these results were consistant with α-cyclodextrins.

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