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STRUCTUAL ELUCIDATION OF THE INCLUSION COMPLEXES OF TOLBUTAMIDE WITH α- AND β-CYCLODEXTRINS IN AQUEOUS SOLUTION

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Inclusion complexation of tolbutamide (TBA) with α- and β-cyclodextrins (α-CyD, β-CyD) were studied by solubility measurement, carbon 13 nuclear magnetic resonance (^{13}C NMR), and circular dichroism (CD) spectroscopies. The results of ^{13}C spin-lattice relaxation times, ^{13}C chemical shift changes, and induced CD spectra suggested that the orientation of TBA in α-CyD cavity is fundamentally different from that in β-CyD cavity in aqueous solution.

The spatial relationship between guest and host molecules is essential for the inclusion complexation as has been frequently pointed out. Extensive studies on inclusion complexes of cyclodextrins (CyDs) with various organic molecules have been reported.[1-4] These studies were, however, based on mainly the kinetics and solubility methods, and do not provide direct elucidation of the spatial effects on the inclusion complexation. The present study deals with the structual elucidation of the inclusion complexes of tolbutamide (TBA) with α- and β-cyclodextrins (α-CyD, β-CyD) in aqueous solution by means of carbon 13 nuclear magnetic resonance (^{13}C NMR) and circular dichroism (CD) spectroscopies. TBA was chosen as an adequate guest molecule toward the CyD cavities, because their stability constants were large among the sulfonylureas.

The NMR spectra were taken on a Jeol PFT-100 spectrometer operating at 25.03 MHz, interfaced with Jeol EC-100 computer. The NMR spectra of degassed samples (160 mg/ml TBA, 200 mg/ml CyDs, and their mixtures in 2 N NaOD solution) were obtained in 10-mm spinning tube at ambient temperature (about 32 °C) using a deuterium lock. The ^{13}C chemical shifts were referenced to external tetramethylsilane with accuracy of ± 0.03 ppm. The ^{13}C spin-lattice relaxation time (T_1) measurements (accuracy of ± 10 %) were carried out by inversion-recovery-technique, as reported previously.[5] The CD spectra were measured by a Jasco J-40 AS recording spectropolarimeter in 0.1 M phosphate buffer

(pH 7.0) at 25 °C, and were expressed in terms of molar ellipticity, $[\theta]$. The solubility measurements were in the same manner as previous paper.[6]

Figure 1 shows the solubilities of TBA as a function of CyD concentration in water, where a different solubilizing effect was noted between α- and β-CyDs. The plateau observed in β-CyD system indicates that the complex of limited solubility is formed.[7] The apparent stability constants (K) were calculated on the basis of 1:1[8] from the initial straight line portions of phase solubility diagrams, and were 70 and 320 M^{-1} for α- and β-CyD complexes, respectively.

Figure 2 shows the induced CD spectra of TBA following the binding to CyDs. With α-CyD TBA showed a negative peak at 232 nm in the UV absorption region of TBA. In sharp contrast, TBA—β-CyD showed a positive peak at 224 nm, suggesting a different binding from that of α-CyD. Harata and Uedaira[9] have theoretically shown that the transition of the guest molecule in β-CyD cavity with a transition dipole moment parpendicular to z-axis of β-CyD gives a negative CD band and the transition having dipole moment parallel to z-axis gives a positive CD band. Thus, the structure of TBA—β-CyD complex is assumed to be an axial inclusion, while that of TBA—α-CyD complex may be fundamentally different from that of β-CyD complex, as expected from NMR data described below.

To obtain further elucidation on the spatial relationship between host and guest

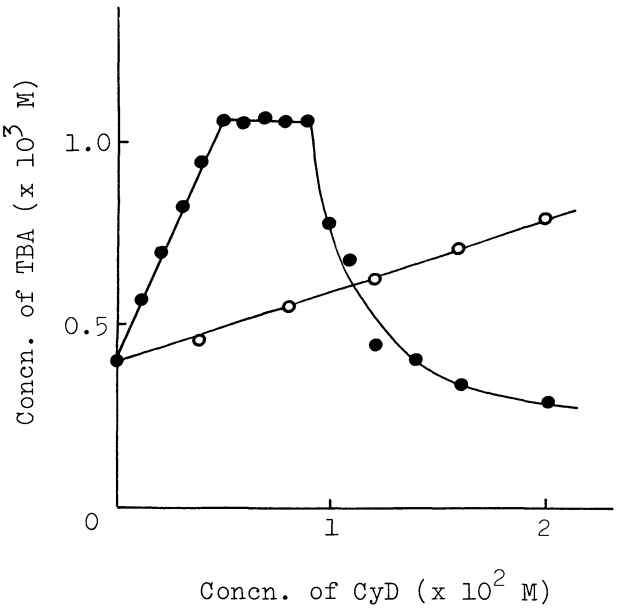


Fig. 1. Phase-solubility diagrams of TBA-CyD systems in water at 25 °C.
o: α-CyD, ●: β-CyD

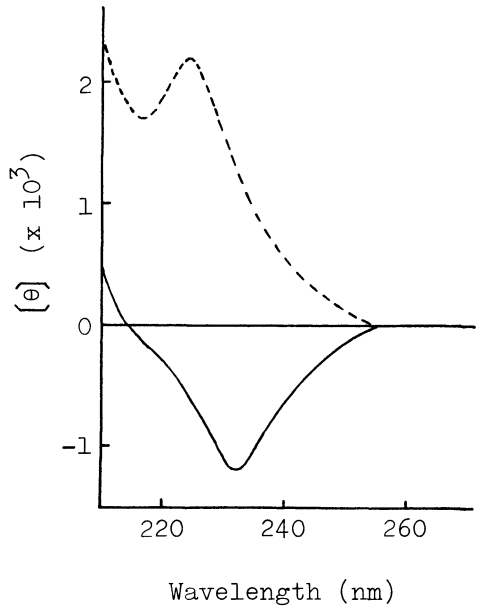
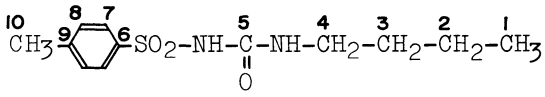


Fig. 2. Induced CD spectra of TBA (1.5×10^{-4} M) following the binding to α- and β-CyDs (1×10^{-2} M) in 0.1 M phosphate buffer (pH 7.0).
——: α-CyD, ----: β-CyD

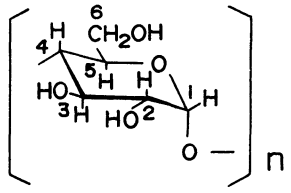
Table I. ^{13}C Chemical Shifts and Relaxation Times (T_1) of TBA in the Absence and in the Presence of α- and β-CyDs

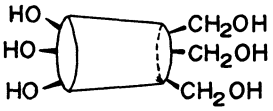


Carbon	Chemical Shift (ppm)			T_1 (sec)		
	without CyD, δ_o	with α-CyD, δ	with β-CyD, δ	without CyD	with α-CyD	with β-CyD
1	14.97	15.25 (0.28)	15.04 (0.07)	3.5	1.2	1.6
2	21.09	21.47 (0.38)	21.09 (0.00)	2.4	0.60	0.72
3	33.11	33.42 (0.31)	33.08 (-0.03)	1.5	0.40	0.43
4	41.58	41.70 (0.12)	41.52 (-0.06)	0.80	0.25	0.24
5	163.76	163.84 (0.08)	163.82 (0.06)	13.0	6.0	5.7
6	141.82	141.81 (-0.01)	141.94 (0.12)	11.0	5.8	5.2
7	130.87	130.98 (0.11)	130.85 (-0.02)	1.1	0.47	0.31
8	127.87	127.86 (-0.01)	127.87 (0.00)	1.1	0.45	0.33
9	143.96	144.14 (0.18)	144.08 (0.12)	6.0	4.1	2.3
10	22.36	22.40 (0.04)	22.48 (0.12)	2.4	1.1	0.93

The difference in chemical shifts between that of the free TBA and TBA in the presence of α- or β-CyD, $\delta - \delta_o$, was in parenthesis.

Table II. Effect of TBA on the ^{13}C NMR Chemical Shifts (ppm) of α- and β-CyDs





Carbon[a]	α-CyD (n = 6)			β-CyD (n = 7)		
	without TBA, δ_o	with TBA, δ	$\delta - \delta_o$	without TBA, δ_o	with TBA, δ	$\delta - \delta_o$
1	104.36	104.39	0.03	104.85	104.56	-0.29
2	74.59	74.47	-0.12	75.01	74.74	-0.27
3	76.29	76.22	-0.07	75.83	75.71	-0.12
4	83.72	83.55	-0.17	83.69	83.20	-0.49
5	73.86	73.91	0.05	73.59	73.71	0.12
6	62.47	61.91	-0.56	62.13	61.84	-0.29

a) Assignment following ref. 10.

molecules, ^{13}C NMR measurements were carried out, and the results are shown in Table I and II. Upon inclusion in CyDs, all the T_1 values of TBA decreased by a factor of about 3, indicating that the molecular motion of TBA reduced as a consequence of the coupling of its motion to CyDs.[11] By the binding to CyDs, most of TBA carbons showed a downfield shift, while that of CyDs carbons showed a shift to opposite direction,[12,13] indicating that CyDs interact with both of the aromatic and alkyl groups of TBA. However, preferable inclusion of alkyl chain ($C_1 \sim C_4$) by smaller α-CyD cavity and that of phenyl moiety ($C_6 \sim C_{10}$) by larger β-CyD cavity were rather noted from the magnitude of chemical shift changes in Table I. In TBA—β-CyD, the downfield shifts for carbons along a long molecular axis (C_6, C_9, and C_{10}) suggest an axial inclusion of phenyl moiety as expected in Fig. 2. In contrast, aromatic C_7 and C_9 signals showed a shift to downfield in TBA—α-CyD, indicating a partial inclusion of phenyl moiety because of the smaller size of α-CyD cavity. Table II summarizes the effects of TBA on ^{13}C chemical shifts of CyDs, where the magnitude of upfield shift for most of β-CyD carbons were larger than that of α-CyD. This may indicate that TBA forms more rigid complex with β-CyD having a larger K value. It is noteworthy that upfield shifts of C_4 and C_6 was significant among the α-CyD carbons, suggesting that TBA rather interacts at smaller entrance site of α-CyD cavity. As mentioned above, different inclusion behaviors between α- and β-CyDs may be substantially responsible for the generation of opposite CD sign (Fig. 2).

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12) The downfield shift of TBA following the binding to CyDs may result from a hydrophobic dissociation of TBA molecules, since the shorter the alkyl chain (for example, N-n-Ethyl-N'-tosylurea), the smaller the magnitude of downfield shift was observed. In contrast, the upfield shift for CyD carbons may be the reflection of hydrophobic association between TBA and CyDs. (see refs. 5 and 13)
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