[Chem. Pharm. Bull. 26(4)1162—1167(1978)]

UDC 547.458.6.03 : 547.551.55.03

Inclusion Complexation of β-Cyclodextrin with Some Sulfonamides in Aqueous Solution[1]

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(Received September 8, 1977)

The interaction of β-cyclodextrin with some sulfonamides in aqueous solution has been investigated by circular dichroism, UV absorption, and solubility techniques. Solubility and spectral changes were quantitatively treated to obtain stoichiometric ratio, which was found to be 1 : 1, formation constants, and thermodynamic parameters for inclusion complexation. All results suggest that steric and hydrophobic factors of the drugs were largely responsible for this interaction.

Keywords——β-cyclodextrin; sulfonamides; inclusion complex; circular dichroism; formation constant; solubility method; thermodynamic parameters

In preceding papers, complexation of β-cyclodextrin (β-CyD) with various drugs, such as N-phenylanthranilic acids,[3] phenothiazines,[4] barbiturates,[5] cinnamates,[6] and prostaglandins[7] in aqueous solution have been reported. It has then been shown that the forces holding together these complexes seem to be van der Waals, hydrogen bonding, as well as hydrophobic interaction, and that the magnitude of these forces depends upon the geometry of the host and guest molecules. In these continuing investigations the interaction of β-CyD with some sulfonamides is examined by circular dichroism (CD) and ultra-violet (UV) spectra, and solubility technique. Effects of pH and temperature on these interactions are also investigated to gain further insight into the mechanism and geometry of the inclusion process.

Experimental

Materials——Sulfamerazine, carbutamide, and sulfamethomidine were donated by Sanwa Kagaku Kenkyusho Co., Ltd., Ono Pharmaceutical Industries Ltd., and Tanabe Seiyaku Co., Ltd., respectively. Other sulfonamides were obtained commercially and recrystallized from EtOH–water. α- and β-Cyclodextrins were the gift of Teijin Ltd. All other materials and solvents were of analytical reagent grade.

CD and UV Absorption Studies——The CD and UV spectral measurements were made on a Jasco J-40 AS spectropolarimeter and a Shimazu 200-type spectrophotometer, respectively. All solutions were prepared in 0.1 M sodium phosphate buffer (pH 7.0) at 25°. For the pH profile experiments (Fig. 4), the solution was adjusted to appropriate value by the addition of 0.1 M NaOH or 0.1 M HCl. The molar ellipticity, $[\theta]$, and molar absorptivity, ε, were calculated on the basis of total drug concentration. The formation constant, K, was calculated as previously described.[3]

1) Part of this work was presented at the 95th Annual Meeting of the Pharmaceutical Society of Japan, Nishinomiya, April 1975.
2) Location: *a) 5–1, Oe-honmachi, Kumamoto 862, Japan*; *b) 3–1, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan.*
3) K. Ikeda, K. Uekama, M. Otagiri, and M. Hatano, *J. Pharm. Sci.*, **63**, 1168 (1974); K. Ikeda, K. Uekama, and M. Otagiri, *Chem. Pharm. Bull.* (Tokyo), **23**, 201 (1975); M. Otagiri, J.H. Perrin, K. Uekama, K. Ikeda, and K. Takeo, *Pharm. Acta Helv.*, **51**, 343 (1976).
4) M. Otagiri, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **23**, 188 (1975).
5) M. Otagiri, T. Miyaji, K. Uekama, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **24**, 1146 (1976); T. Miyaji, Y. Kurono, K. Uekama, and K. Ikeda, *ibid.*, **24**, 1155 (1976).
6) K. Uekama, M. Otagiri, Y. Kanie, S. Tanaka, and K. Ikeda, *Chem. Pharm. Bull.* (Tokyo), **23**, 1421 (1975); S. Tanaka, K. Uekama, and K. Ikeda, *ibid.*, **24**, 2825 (1976).
7) K. Uekama, F. Hirayama, K. Ikeda, and K. Inaba, *J. Pharm. Sci.*, **66**, 706 (1977).

Determination of Partition Coefficients and Solubilities——Partition coefficients were determined following the shaking of 10 ml aqueous solution of the drug [1.0×10^{-4} M in phosphate buffer (pH 7.0)] and 10 ml of chloroform for one hr at 25°. The partition coefficient was defined as the ratio of the equilibrium concentration in organic phase to that in aqueous solution. For solubility determination, the drug was shaken with distilled water at 25° for 48—52 hrs. After filtration, a sample was diluted with 0.1 M phosphate buffer (pH 7.0) and analyzed spectrophotometrically.

Results and Discussion

Induced CD and UV Absorption Changes

Since β-CyD has a large asymmetric cavity, various compounds have been shown to generate the extrinsic Cotton effects by the formation of inclusion complexes.[3-6] The induced CD of the complexes is generally characterized by their sign, magnitude, and wavelength of the location. The sign of the induced CD depends upon the spacial relationship between the asymmetric center and perturbed chromophore,[8] whereas the magnitude of the optical activities seems to depend upon the rigidity of the complex formed.

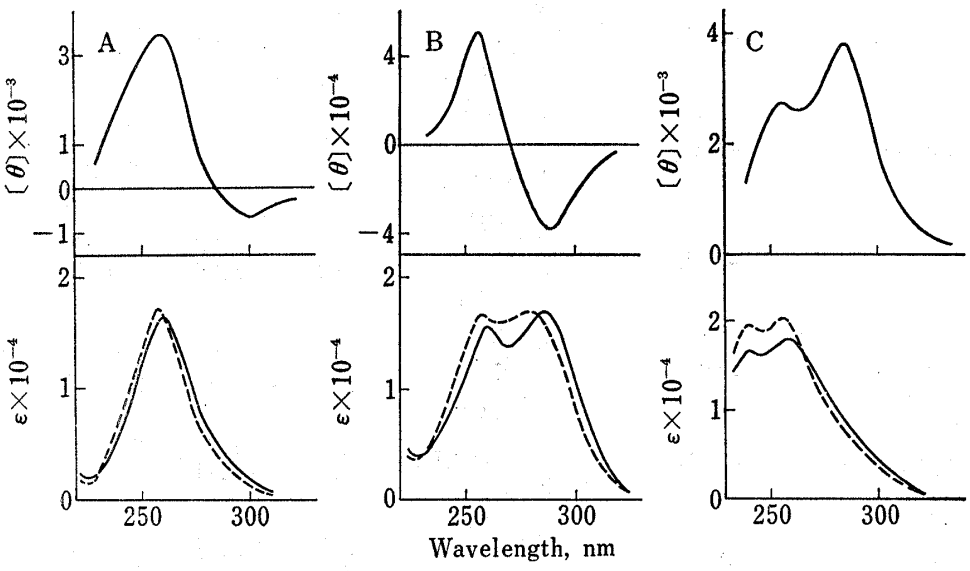


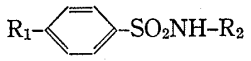
Fig. 1. The CD (Upper) and UV (Lower) Absorption Spectra of Some Sulfonamides (5.0×10^{-5} M) Following the Binding to β-CyD (5.0×10^{-3} M) in 0.1 M Phosphate Buffer (pH 7.0)

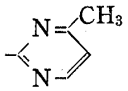
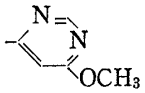
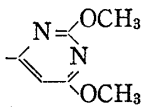
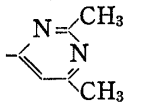
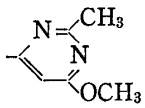
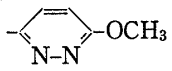
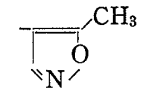
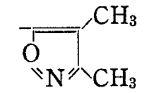
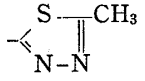
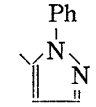
A: sulfanilamide, B: sulfathiazole, C: sulfadiazine.
——: in the presence of β-CyD, ----: in the absence of β-CyD.

Figure 1 shows the CD and UV spectra of some sulfonamides following the binding to β-CyD, where marked differences in sign and magnitude of the CD bands were noted. As listed in Table I, all the sulfonamides showed the induced CD and UV absorption changes with different spectral characteristics, suggesting a fundamentally different binding of each drug to β-CyD. By the binding to β-CyD, the UV peaks were generally shifted to longer wavelength with concomitant decrease in molar absorptivity. Similar UV spectral changes were observed when the drugs were dissolved in less polar solvents such as ethanol-buffer and dioxane-buffer mixtures. These results apparently indicate that the drug chromophores were located within the hydrophobic cavity of β-CyD. It is noteworthy that the drugs with sulfur-containing substituents such as sulfathiazole and sulfamethizole showed significantly large molar ellipticities and UV spectral changes. Similar results were previously reported

8) J.A. Schellman, *Accounts Chem. Res.*, **1**, 144 (1968).

TABLE I. CD and UV Spectra Data of Sulfonamides Bound to β-Cyclodextrin[a)]



Compound	R_1	R_2	CD λ_{max} (nm)	CD $[\theta]^{c)}$ ($\times 10^{-3}$)	UV[b)] λ_{max} (nm)	UV[b)] $\varepsilon^{d)}$ ($\times 10^{-4}$)
Sulfanilamide	$-NH_2$	-H	261 300	3.42 −0.63	260 (259)	1.66 (1.69)
Sulfapyridine	$-NH_2$		242 271 325	21.2 −15.2 3.88	245 (243) 266 (261) 315 (312)	1.42 (1.58) 1.62 (1.71) 0.75 (0.89)
Sulfadiazine	$-NH_2$		255 285	3.11 4.31	241 (241) 260 (258)	1.66 (1.95) 1.80 (2.02)
Sulfamerazine	$-NH_2$		240 293	4.27 1.68	242 (242) 262 (259)	1.58 (1.81) 1.77 (1.91)
Sulfamonomethoxine	$-NH_2$		250 276	6.04 −3.23	250 (250)[e)] 264 (263)	2.07 (2.22) 2.40 (2.56)
Sulfadimethoxine	$-NH_2$		245	3.00	255 (255)[e)] 268 (268)	2.21 (2.26) 2.65 (2.75)
Sulfisomidine	$-NH_2$		250 280	7.36 −5.96	260 (259) 279 (278)	2.11 (2.18) 2.16 (2.25)
Sulfamethomidine	$-NH_2$		246 269	10.5 −13.8	253 (252)[e)] 268 (266)	1.71 (1.82) 2.03 (2.05)
Sulfamethoxypyridazine	$-NH_2$		246 275	11.4 −9.11	245 (244) 264 (260)	1.61 (1.75) 1.82 (1.91)
Sulfisomezole	$-NH_2$		259 301	2.11 −0.49	258 (257)	1.65 (1.73)
Sulfisoxazole	$-NH_2$		254 292	7.43 −1.81	254 (253)	2.14 (2.23)
Sulfathiazole	$-NH_2$		257 291	52.9 −39.3	260 (257) 287 (280)	1.54 (1.67) 1.69 (1.71)
Sulfamethizole	$-NH_2$		259 295	15.8 −8.00	259 (259) 275 (272)	1.74 (1.95) 1.66 (1.84)
Sulfaphenazole	$-NH_2$		254 286	3.05 −0.86	250 (249)	2.11 (2.13)
Homosulfamine	$-CH_2NH_2$	-H	277	−0.69	222 (221)	1.05 (1.08)
Carbutamide	$-NH_2$	$-CONH(CH_2)_3CH_3$	258	0.71	255 (254)	1.63 (1.67)

a) Concentration of sulfonamides and β-cyclodextrin were 5×10^{-5} M and 5×10^{-3} M, respectively, in 0.1 M sodium phosphate buffer (pH 7.0).
b) Value in parenthesis was sulfonamides without β-CyD.
c) Apparent molar ellipticity.
d) Apparent molar absorptivity.
e) Shoulder.

for phenothiazines-β-CyD[4] and thiobarbiturates-β-CyD[5] complexes, indicating a particularly favorable inclusion of the sulfur-containing guest molecules toward β-CyD cavity. In contrast to β-CyD, α-CyD showed no induced CD with the sulfonamides studied, smaller cavity apparently allowing little penetration of these guest molecules.

Formation Constants of Inclusion Complexes

The induced CD, UV absorption, and solubility changes due to complex formation between β-CyD and sulfonamides were quantitatively investigated to obtain stoichiometric relationship, formation constants, and thermodynamic parameters. Figure 2 shows a continuous variation plots of the molar ellipticity changes for sulfathiazole-β-CyD system, as an example, which indicates 1:1 complex formation. Similar stoichiometric relationship can be expected for β-CyD complexes of the other sulfonamides. Figure 3 shows a solubility

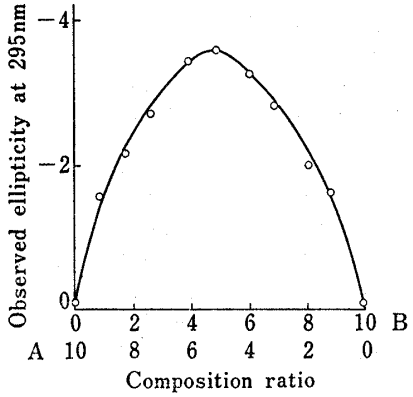


Fig. 2. Continuous Variation Plots for Sulfathiazole-β-CyD System in 0.1 M Phosphate Buffer (pH 7.0)

A: sulfathiazole (1.0×10^{-3} M),
B: β-CyD (1.0×10^{-3} M).

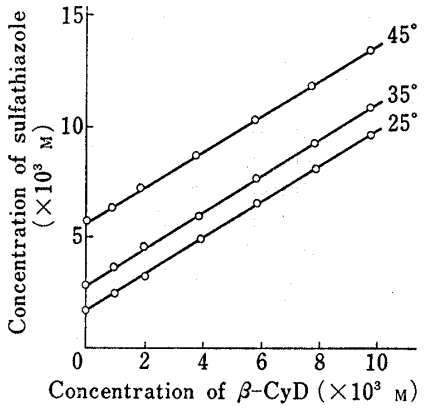


Fig. 3. Solubilizing Effect of β-CyD on Sulfathiazole in Water

TABLE II. Formation Constants of β-Cyclodextrin-Sulfonamides Complexes and Physical Parameters of Sulfonamides at 25°

Sulfonamide	Formation constant (M^{-1})		Partition[a] coefficient	pK_a[b]
	UV method	Solubility method		
Sulfanilamide	350	600	0.16	10.4
Sulfapyridine	480	500	0.74	8.56
Sulfadiazine	140	340	0.58	6.37
Sulfamerazine	170	150	2.53	6.85
Sulfamonomethoxine	250	310	6.84	6.05
Sulfadimethoxine	110	180	36.8	5.98
Sulfisomidine	130	130	0.42	7.47
Sulfamethomidine	150	220	3.26	7.06
Sulfisomezole	600	760	9.05	5.72
Sulfisoxazole	320	460	6.52	4.79
Sulfathiazole	1650	1800	1.02	7.23
Sulfamethizole	550	1070	3.07	5.22
Sulfaphenazole	110	230	38.9	5.87
Carbutamide	300	200	9.41	5.75

a) See text.
b) Dissociation of amide proton. A. Agren, R. Elofsson, and S.O. Nilsson, *Acta Pharm. Suecica*, 8, 475 (1971); M. Yoshioka, K. Hamamoto, and T. Kubota, *Yakugaku Zasshi*, 84, 90 (1964).

of sulfathiazole as a function of β-CyD concentration in water. Since solubilizing effects of β-CyD on the other drugs were similar to that of sulfathiazole, formation constants were calculated on the basis of 1:1 from the initial straight line portion of solubility diagram. Formation constants were also determined by UV method[9] and summarized in Table II. The K values obtained by two method are in good agreement, in which stereospecific nature of the substituent apparently is responsible for the magnitude of K values. Only one ring of the guest molecule seems to be included within the cavity of β-CyD, and probably the nature of the substituents and the resultant geometry determine which ring enter the cavity. Although there is no regular behavior of the molecule in Table II, NMR study suggested that in most cases the phenyl ring of the drugs was predominantly included within the cavity of β-CyD.[10]

Table III. Thermodynamic Parameters[a] for Complexation of β-Cyclodextrin with Some Sulfonamides at 25°

Sulfonamide	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (e.u.)
Sulfapyridine	−3.68	−13.7	−33.6
Sulfadiazine	−3.45	−6.57	−10.5
Sulfamerazine	−2.97	−3.19	−0.74
Sulfamonomethoxine	−3.40	−0.67	9.16
Sulfadimethoxine	−3.08	−3.03	0.17
Sulfamethizole	−4.14	−8.54	−14.8
Sulfathiazole	−4.45	−10.1	−18.9

a) Accuracy of ±5%.

Thermodynamic parameters for the complex formation determined by temperature dependence of K values are shown in Table III, where the favorable enthalpy change could more than compensate for the unfavorable entropy change. In some cases a positive value of ΔS was obtained, which may indicate that a number of water molecules are set free in the complexation. In other cases a negative value of ΔS was obtained, indicating that more water molecules were bound by the product than reactants. It appears that ΔS becomes more positive with increasing the hydrophobic tendency of the drugs as expected from their partition coefficients. Figure 4 shows a pH profile of log K for sulfathiazole-β-CyD system, where a considerably high degree of interaction was observed around pH 5.5. This is expected because the attraction due to hydrophobic bonding between unionized species of the drug[11] and β-CyD became great enough around this pH, and then followed by decrease in K value with increasing fraction of ionized species of the drug, as pH increase or decrease.

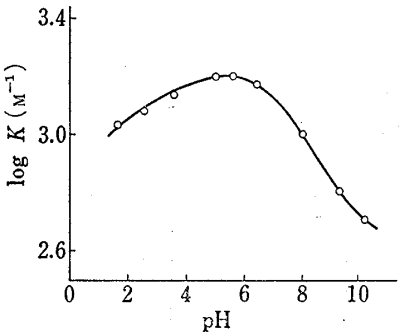


Fig. 4. The pH Profile for Formation Constant of Sulfathiazole-β-CyD Complex at 25°

9) R.L. Scott, *Rec. Trav. Chim.*, **75**, 787 (1956).
10) In the presence of β-CyD, phenyl signals (^{1}H-NMR) of the sulfonamides showed significantly higher-field shift with spectral broadning, but no appreciable changes in heterocyclic substituents of the drugs were observed in 0.1 M HCl (D_2O solvent).
11) The pK values of sulfathiazole are 7.12 and 2.3 for amide proton and amino group, respectively (P.H. Bell and R.O. Robin, Jr., *J. Am. Chem. Soc.*, **64**, 2905 (1942)).

As mentioned above, all the results suggest a predominantly hydrophobic interaction, however, no linear correlations between formation constants and partition coefficients were generally found, which may be due to the heterogeneous character of the group of drugs studied. It therefore seems to be rather difficult to draw any definite conclusions from present data concerning the degree of hydrophobic bonding in inclusion complexation.