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Inclusion Complexes of β-Cyclodextrin with Tranquilizing Drugs Phenothiazines in Aqueous Solution[1]

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The interaction of tranquilizing drugs phenothiazines with β-cyclodextrin in aqueous solution was studied by circular dichroism, ultraviolet (UV) absorption, and proton magnetic resonance spectroscopies. The induced circular dichroism bands and UV absorption changes were quantitatively investigated and stoichiometric ratio, which was found to be 1:1, and formation constants of inclusion complexes were obtained. In β-cyclodextrin-drug system, protons located in a cavity of β-cyclodextrin were found to be subjected to anisotropic shielding, while protons of phenyl and N-substituted groups in the drug shifted to low field, in which all peaks caused remarkable broadening. Formation constants were correlated well with partition coefficient of drug and also with the magnitude of proton magnetic resonance spectral broadening. These spectral changes strongly suggested that aromatic portion of drug was included into hydrophobic cavity of β-cyclodextrin, while N-substituents of drug interacted with the outside groups of β-cyclodextrin cavity.

Various compounds have been known to become optically active by the binding to cyclodextrins.[3–7] We recently reported[8] that antiinflammatory drugs fanamates show the induced circular dichroism (CD) by the formation of inclusion complexes with β-cyclodextrin. The present study deals with the interaction of tranquilizing drugs phenothiazines and their related compounds with β-cyclodextrin in aqueous solution. As the induced Cotton effects give useful information on the nature of inclusion complex, it is the aim of this work to show that these drugs actually form inclusion complexes with β-cyclodextrin and to elucidate the mode of inclusion and structure of complex from their induced CD bands. The induced CD bands and ultraviolet (UV) absorption changes observed were quantitatively investigated to obtain formation constants. The proton magnetic resonance (PMR) chemical shift changes due to the binding of these drugs to β-cyclodextrin were also investigated. Phenothiazine derivatives studied were listed in Table I.

Experimental

Material——The tranquilizing drugs phenothiazines were favored from Shionogi & Co., Ltd. and used without further purifications. β-Cyclodextrin was favored from Teijin Ltd. α- and β-cyclodextrins were recrystallized from water and dried with P_2O_5 *in vacuo*. Their specific rotatory powers were; $[\alpha]_D^{25}=152.0\pm0.5°$ for α-cyclodextrin and $[\alpha]_D^{25}=162.0\pm0.5°$ for β-cyclodextrin, respectively. All other materials were of analytical grade.

1) Main part of this study was presented at the 94th Annual Meeting of Pharmaceutical Society of Japan, Sendai, April 1974.
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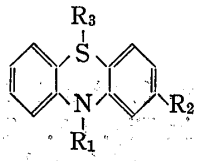
3) K. Sensse and F. Cramer, *Chem. Ber.*, **102**, 509 (1969).
4) J.R. Runyon, Ph. D. thesis, Iowa State University, Ames, Iowa, 1968.
5) A.L. Thakkar, P.B. Kuehn, J.H. Perrin, and W.L. Wilham, *J. Pharm. Sci.*, **61**, 1841 (1972).
6) K. Takeo and T. Kuge, *Die Stärke*, **24**, 281 (1972).
7) M. Otagiri, K. Ikeda, K. Uekama, O. Ito, and M. Hatano, *Chemistry Letters*, **1974**, 679.
8) K. Ikeda, K. Uekama, M. Otagiri, and M. Hatano, *J. Pharm. Sci.*, **63**, 1168 (1974).

Table I. Structure of the Phenothiazines Studied



Compound	R_1	R_2	R_3
Phenothiazine (I)	H	H	
Chlorpromazine (II)	$CH_2CH_2CH_2N(CH_3)_2$	Cl	
Promazine (III)	$CH_2CH_2CH_2N(CH_3)_2$	H	
Chlorpromazine sulfoxide (IV)	$CH_2CH_2CH_2N(CH_3)_2$	Cl	O
Methopromazine (V)	$CH_2CH_2CH_2N(CH_3)_2$	OCH_3	
Promethazine (VI)	$CH_2CH(CH_3)N(CH_3)_2$	H	
Levomepromazine (VII)	$CH_2CH(CH_3)CH_2N(CH_3)_2$	OCH_3	
Propericiazine (VIII)	$CH_2CH_2CH_2N$(piperidine)-OH	CN	
Prochlorperazine (IX)	$CH_2CH_2CH_2N$(piperazine)NCH_3	Cl	

CD and UV Absorption Studies——The CD and UV spectra were recorded by a Jasco 20A recording spectropolarimeter and a Hitachi EPS-3T spectrometer, respectively. All measurements were carried out in 0.1M sodium phosphate buffer of pH 7.0 and solutions prepared were shielded from the light to prevent the photodecomposition of phenothiazine derivatives. The optical anisotropy factor, g value, which is proportional to the magnitude of the induced Cotton effects was calculated from the equation, $g=[\theta]/3300\times\varepsilon$, where $[\theta]$ is molar ellipticity (deg/meter·mole/liter) and ε is the molar absorptivity of phenothiazines in the presence of β-cyclodextrin at the maximum wavelength of CD spectra.

PMR Studies——PMR spectra were measured by a JEOL PS-100 spectrometer at the ambient probe temperature of $31\pm1°$. Tetramethylsilane was used as an external reference for D_2O and no correction was made for susceptibility of the capillary.

Determination of Formation Constants——The formation constants, K_c, for the phenothiazines-β-cyclodextrin complexes were determined spectrophotometrically. UV absorption changes and ellipticities of phenothiazines (constantly 2.5×10^{-5}M) in the presence of β-cyclodextrin (varied from 1.0 to 6.25×10^{-3}M) were measured at appropriate wavelength of UV absorption and induced CD bands, respectively. The K_c and ε values were determined according to conventional Scott's equation[9] (1),

$$\frac{a\cdot b}{d}=\frac{1}{K_c\cdot\varepsilon_c}+\frac{b}{\varepsilon_c} \qquad (1)$$

where a is the total concentration of phenothiazines, b is the total concentration of β-cyclodextrin, ε_c is the difference of the molar absorptivities for free and complexed phenothiazines, and d is the change in absorbance or ellipticity of the phenothiazines by the addition of β-cyclodextrin.

Determination of Partition Coefficients——Partition coefficients of phenothiazines were determined by shaking of 10 ml of aqueous phenothiazines solution (1×10^{-4}M in 0.1M sodium phosphate buffer of pH 5.0) and 10 ml of cyclohexane–*n*-octanol (9: 1) for one hour at 25°. Partition coefficient was defined as the ratio of the equilibrium concentration in organic phase to that in aqueous phase.

9) R.L. Scott, *Rec. Trav. Chim.*, **75**, 787 (1956).

Results and Discussion

Induced CD and UV Absorption Changes

In the presence of β-cyclodextrin, new CD band of phenothiazine (I) having two positive sign peaks (a and b) appeared as is seen in Fig. 1A. The UV absorption spectrum of phenothiazine was also cha nged in the presence of β-cyclodextrin (Fig. 1B). Figure 2 shows the CD and UV spectra of chlorpromazine (II)-β-cyclodextrin system as an example of N-substituted phenothiazine derivatives. As can be seen in Fig. 2A, the observed CD spectrum of this system is significantly large compared to that of phenothiazine (I) and shows several peaks with positive (*p*, *r*, *s* (shoulder), and *t*) and negative (*q* and *u*) sign in the absorption region of chlorpromazine, which is also distinct from phenothiazine (I). Figure 2B shows

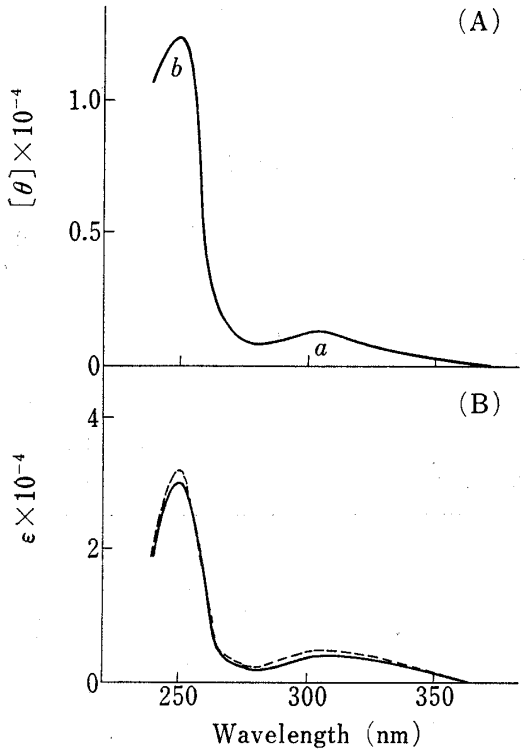


Fig. 1. Circular Dichroism (A) and Absorption Spectra (B) of Phenothiazine-β-cyclodextrin System

——: phenothiazine (2.5×10^{-6} M) alone, ——: phenothiazine (2.5×10^{-6}M)+β-cyclodextrin (5×10^{-5} M) solvent: 0.1M sodium phosphate buffer (pH 7.0)

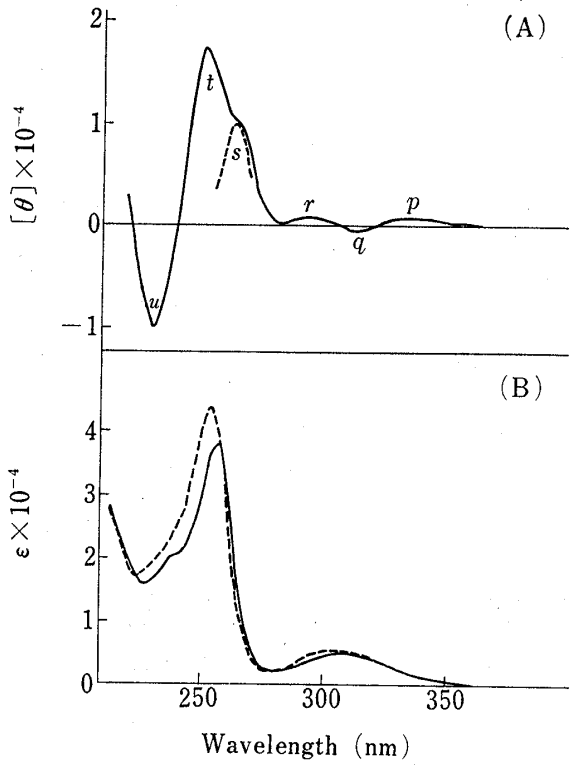


Fig. 2. Circular Dichroism (A) and Absorption Spectra (B) of Chlorpromazine-β-cyclodextrin System

——: chlorpromazine (2.5×10^{-5}M) alone, ——: chlorpromazine (2.5×10^{-5}M)+β-cyclodextrin (5×10^{-4}M) solvent: 0.1M sodium phosphate buffer (pH 7.0)

the effect of β-cyclodextrin on the UV absorption spectrum of chlorpromazine, where the absorption maximum of this drug shifted to longer wavelength accompanying some lowering and a shoulder appeared at 240 nm in the presence of β-cyclodextrin. Other N-substituted drugs (III—IX) also showed new CD bands and UV absorption changes in the presence of β-cyclodextrin, which have characteristics similar to that of chlorpromazine. Since intrinsic Cotton effects of β-cyclodextrin are observed below 220 nm, these CD bands above 220 nm can be attributed to the induced optical activity of phenothiazine derivatives by the formation of inclusion complexes with β-cyclodextrin. The fact that all the phenothiazines studied generated the induced Cotton effects, may indicate that phenothiazine ring in drugs is essential to generate the optical activity.

Table II summarizes λ_{max}, ε, $[\theta]$, and g values obtained for phenothiazine derivatives bound to β-cyclodextrin, where the differences mentioned above are quantitatively shown.

TABLE II. Induced CD and UV Absorption Bands by the Binding of Phenothiazines to β-Cyclodextrin[a)]

Compound	CD						UV	
	λ_{max} (nm)		$[\theta]^{b)}$ ($\times 10^{-4}$)		$g^{c)}$ ($\times 10^{4}$)		λ_{max} (nm)	$\varepsilon^{b)}$ ($\times 10^{-4}$)
Phenothiazine (I)	250	308	0.12	0.01	0.08	0.02	250	3.10
Chlorpromazine (II)	230	311	−1.00	−0.03	1.88	0.20	257	3.75
	252	335	1.65	0.04	2.98	0.55	305	0.45
	292		0.05		0.44			
Promazine (III)	235	295	−0.20	0.03	0.45	0.27	254	2.79
	250	322	0.70	0.03	0.83	0.41	303	0.36
	275		−0.06		0.67			
Chlorpromazine	220	280	0.68	0.08	0.94	0.29	240	2.04
Sulfoxide (IV)	245	330	0.24	−0.14	0.20	0.81	274	1.15
							298	0.80
							341	0.71
Methopromazine (V)	225	295	−0.80	0.80	1.12	6.39	252	2.41
	240	330	2.90	−0.25	1.12	6.39	305	0.42
	257		−1.40		1.96			
Promethazine (VI)	233	320	−0.50	0.08	1.12	6.39	252	2.59
	251		0.99		1.17		300	0.35
Levomepromazine (VII)	242	307	1.15	0.50	1.67	3.53	253	2.39
	260	343	−0.75	0.10	1.23	3.75	305	0.45
Propericiazine (VIII)	228	312	3.45	0.30	4.17	2.94	234	2.65
	254	347	−1.10	−0.35	1.99	4.08	270	3.04
	273		1.90		1.96			
Prochlorperazine (IX)	233	317	−0.55	−0.55	1.54	0.45	258	2.50
	255	345	1.15	0.03	2.49	0.64	310	0.35
	295		0.03		0.35			

a) Concentrations of phenothiazine derivatives and β-cyclodextrin were of 5×10^{-5}M and 1×10^{-3}M, respectively. *b*) molar ellipticity (deg·cm²·dmole⁻¹), *c*) optical anisotropy factor (see text), *d*) apparent molar absorption coefficient.

The spectral differences between I and II—IX may be ascribed to that N-substituted moiety in the latter compounds. The g values for N-substituted phenothiazine systems were significantly large compared to that for phenothiazine system. It is also noted that the induced CD spectra of N-substituted phenothiazine were resemble to that for dye-polypeptide system.[10,11] These observations suggest that not only phenothiazine ring but also aminoalkyl side chain in drugs participates in the complex formation with β-cyclodextrin as will be proposed later, and consequently the rotational strength of N-substituted phenothiazine system increased.

In contrast to β-cyclodextrin, α-cyclodextrin showed no appreciable complex formation with all compounds studied, suggesting that the cavity size of α-cyclodextrin is not large enough to include the bulky phenothiazine moiety.

Formation Constants of Inclusion Complexes

Figure 3 shows a continuous variation diagram of the ellipticity change at 250 nm for chlorpromazine-β-cyclodextrin system, which indicates 1:1 complex formation. Similar stoichiometric relationship can be expected for β-cyclodextrin complexes of the other phenothiazines. Then, the induced optical activities and UV absorption changes were quantitatively treated to obtain formation constants, K_c. Figure 4 shows Scott's plots for chlorpromazine-

10) R.E. Ballard, A.J. McCaffery, and S.F. Mason, *Biopolymers*, **4**, 97 (1966).
11) M. Hatano, M. Yoneyama, Y. Sato, and Y. Kawamura, *Biopolymers*, **12**, 2423 (1973).

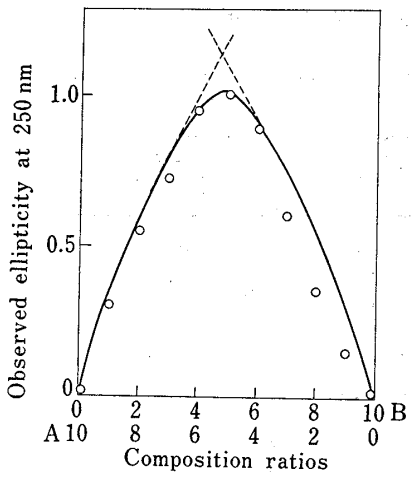


Fig. 3. Continuous Variation Plots for β-Cyclodextrin (A)-Chlorpromazine (B) System

solvent: 0.1M sodium phosphate buffer (pH 7.0)

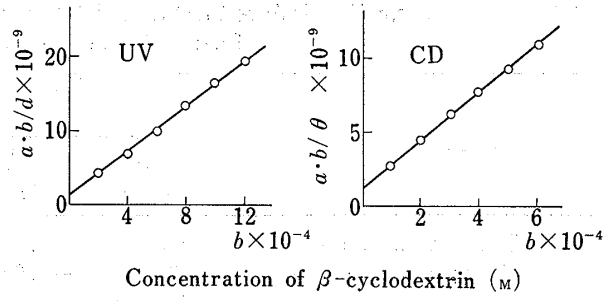


Fig. 4. Scott's Plots for Interaction between Chlorpromazine and β-Cyclodextrin

solvent: 0.1M sodium phosphate buffer (pH 7.0)

β-cyclodextrin system. Table III summarizes K_c values along with partition coefficients of the corresponding drugs. In all cases K_c values obtained by CD method fairly agreed with those obtained by UV method. Formation constants obtained are significantly larger than those for phenothiazine-low molecule complexation.[12] As is seen in Table III, the compounds with larger partition coefficients showed larger formation constants, which shows predominant role of hydrophobic nature of the guest molecule on the formation of inclusion complex.

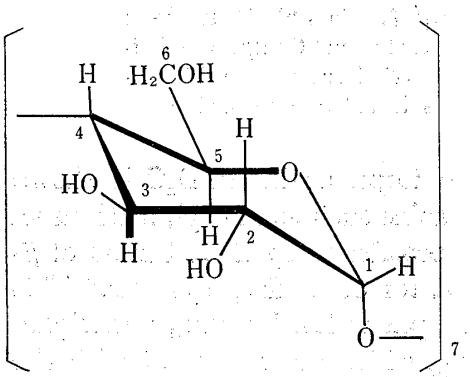
TABLE III. Formation Constants of β-Cyclodextrin Complexes with Tranquilizing Drugs Phenothiazines, and Their Partition Coefficients at 25°

Drug	Partition coefficient	Formation constant ($\times 10^{-3}$ M^{-1})	
		UV method	CD method
Chlorpromazine	10.9	12	7.9
Promazine	1.32	5.6	3.8
Chlorpromazine sulfoxide	0.04	0.5	
Methopromazine	1.38	13	8.0
Promethazine	3.21	2.1	1.4
Levomepromazine	5.97	25	16
Propericiazine	0.11	5.3	
Prochlorperazine	29.0	34	25

PMR Study on Inclusion Complexes

The chemical shift changes following the interaction between β-cyclodextrin and phenothiazines were examined by high resolution PMR. It is conceivable that when an aromatic moiety of guest molecule is included in a cavity of β-cyclodextrin, protons located within a cavity of β-cyclodextrin (H-3, H-5, and possibly, H-6) are susceptible to anisotropic shielding of aromatic moiety, and protons located on the exterior of the cavity (H-1, H-2, and H-4) are relatively unaffected. Alternatively, if association takes place at the exterior of the cavity, H-1, H-2, and H-4 must be effectively changed.[13,14]

12) S. Egawa, *Arch. Pract. Pharm.*, **21**, 183 (1961).
13) P.V. Demarco and A.L. Thakkar, *Chem. Commun.*, **1970**, 2.
14) A.L. Thakkar and P.V. Demarco, *J. Pharm. Sci.*, **60**, 652 (1971).



β-Cyclodextrin

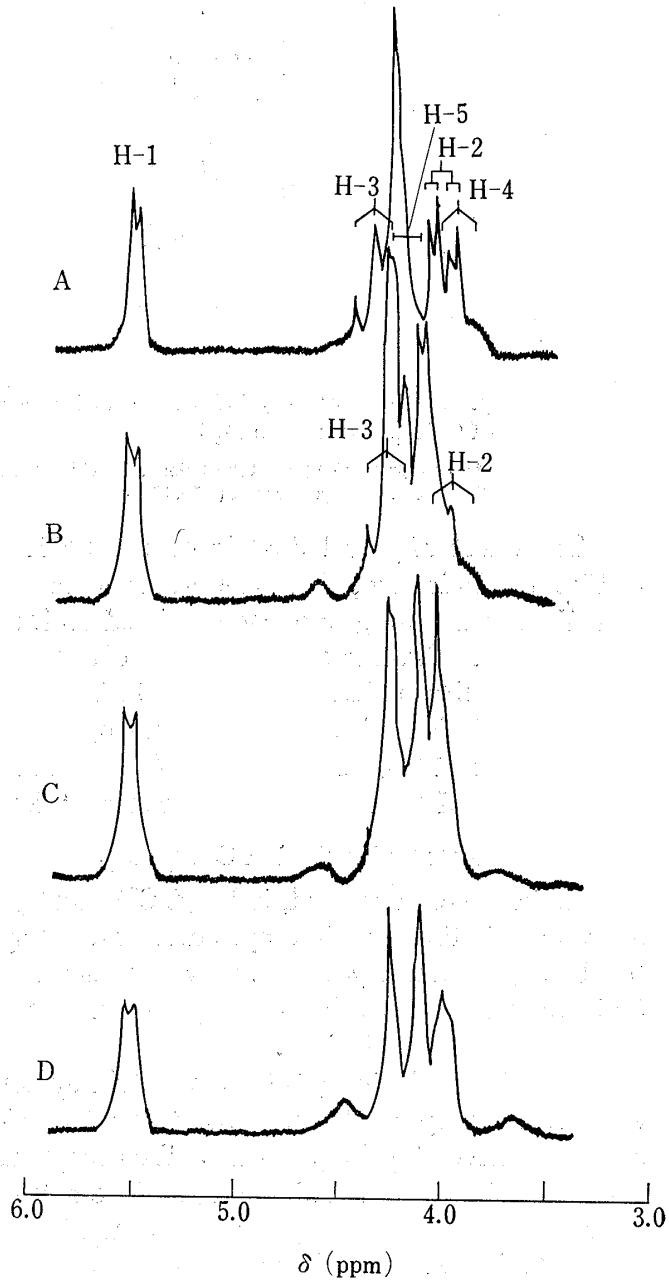


Fig. 5. PMR Spectra of β-Cyclodextrin containing Various Amounts of Chlorpromazine

molar ratio, chlorpromazine: β-cyclodextrin; (A) 0.00 (B) 0.50 (C) 1.00 (D) 2.00

Figure 5 shows the effect of chlorpromazine on the PMR spectrum of β-cyclodextrin in D_2O. It is apparent that lower field triplet assigned to H-3 signal progressively shifted to higher field and broadened by the increasing amounts of chlorpromazine. The expected anisotropic shielding of H-5 signal could not directly observed because this multiplet signal was remarkably broadened and superimposed in the spectral region of δ 4.5—3.8 ppm. On the other hand, no anisotropic shielding was detected for the exterior protons such as H-1 and H-2, but appreciable broadening was also exhibited for these protons. These chemical shift behaviors of β-cyclodextrin protons indicate that the aromatic moiety of chlorpromazine was included within a cavity of β-cyclodextrin. However, the fact that interior and exterior proton signals showed appreciable broadening suggests that the association with chlorpromazine took place tightly even at the outside of β-cyclodextrin cavity.

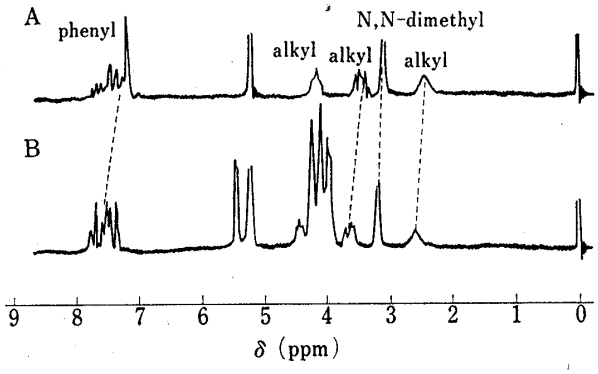


Fig. 6. Effect of β-Cyclodextrin on PMR Spectra of Chlorpromazine in D_2O

A: chlorpromazine (3.0×10^{-2}M) alone, B: chlorpromazine (3.0×10^{-2}M)+β-cyclodextrin (1.5×10^{-2}M)

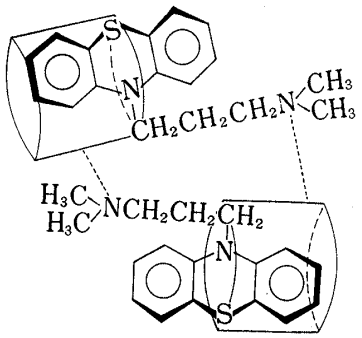


Fig. 7. Possible Structure of Inclusion Complex of β-Cyclodextrin with N-Substituted Phenothiazine

The effect of β-cyclodextrin on the PMR spectrum of chlorpromazine in D_2O is shown in Fig. 6. As is seen, all of the proton signals of chlorpromazine such as phenyl, methylene, and dimethylamino groups were shifted to lower field and broadened by the addition of β-cyclodextrin. Similar chemical shift changes were observed for other drug systems. This obviously indicate that N-substituents in drugs, besides the phenothiazine ring, participate in the association with β-cyclodextrin by hydrogen bonding, which are consistent with the results obtained from CD spectra. A parallel relationship between the magnitude of spectral broadening and that of formation constant for inclusion complex was also observed.

Possible Structure of Inclusion Complex

As has been discussed, induced CD bands, and PMR data suggest the mode of inclusion as well as binding sites in β-cyclodextrin-drug system. That the intensity of the induced CD band in the shorter wavelength was larger than that in longer wavelength for all systems indicates that orientation of phenothiazine moiety in the cavity of β-cyclodextrin may be same for all phenothiazines.

For phenothiazine (I)-β-cyclodextrin system the magnitude of induced optical activity was considerably small compared to that of N-substituted phenothiazines and the location of two positive ellipticity bands well corresponds to that of UV absorption spectrum (Fig. 1). These facts indicate that dipole-dipole interaction between phenothiazine (I) and β-cyclodextrin seems to be important for generation of the induced CD band. In contrast, CD characteristics of N-substituted phenothiazine systems were clearly distinct from that of phenothiazine (I) system, suggesting more complicate mechanism to generate the CD bands.

The complicated ellipticities observed for N-substituted phenothiazine-β-cyclodextrin systems may be explained by Davydov splitting pattern. In the shorter wavelength in Fig. 2A the large positive CD band with a definite shoulder at 265 nm seems to result from the superimposition of two positive bands; band *t* at 250 nm and band *s* at 265 nm. Band *t* may be corresponded to the band *b* of intact phenothiazine in Fig. 1A. And the other positive band *s* at 265 nm seems to be a set with negative band *u* as Davydov splitting. In the longer wavelength region in Fig. 2A negative band *q* and either positive band *p* or *r* could be assigned to other set of Davydov splitting, where the rest of the positive bands (*r* or *p*) must be corresponded to band *a* in Fig. 1A. It is particularly interesting that the pattern of these CD bands (*s*—*u*, *p* or *r*—*q*) with opposite sign and nearly equal in magnitude is closely resemble to that of dye-polypeptide system.[10,11] Since the splitting pattern observed for dye-polypeptide system is arised from dimeric dye bound to polypeptide, CD bands such as *s*—*u* and *p* or *r*—*q* observed in the present system could be ascribed to ellipticities of the dimeric inclusion compounds. Therefore two sets of bands (*s*—*u* and *p* or *r*—*q*) are attributed to a dimeric form of inclusion complexes and other bands (*t*, *p* or *r*) are attributed to monomer.

Reffering to above observations, the dimeric structure of β-cyclodextrin complex with N-substituted phenothiazine was proposed in Fig. 7, where two inclusion complexes of 1:1 are associated. In the primary 1:1 complex, the aromatic portion of phenothiazine moiety is included within the cavity of β-cyclodextrin, while aminoalkyl side chain of the drug interacts with the outside of the cavity of β-cyclodextrin through hydrogen bonding.

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