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COMMUNICATION

Interaction of Cyclomaltononaose (δ-CD) with Several Drugs

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ABSTRACT

The effects of δ-cyclodextrin (δ-CD; cyclomaltononaose) on solubility of 14 drugs that are slightly soluble or insoluble in water were studied and compared with those of conventional cyclodextrins (CDs) such as α-cyclodextrin (α-CD), β-cyclodextrin (β-CD), and γ-cyclodextrin (γ-CD). In general, δ-CD had a weak complex-forming ability with the drugs examined in comparison with β-CD and γ-CD. However, in the case of digitoxin, δ-CD enhanced solubility of the guest molecules. To determine the mechanism of inclusion complex formation of δ-CD with digitoxin, the interaction of both drugs was investigated by the solubility method and spectroscopic methods such as ultraviolet (UV) and ^{1}H-NMR (nuclear magnetic resonance). The changes in chemical shift (^{1}H) and hypsochromic shift of UV suggested that digitoxin was partially included in the cavity of δ-CD.

Key Words: *Complex formation; Digitoxin; δ-CD; Solubilizer.*

INTRODUCTION

Large-ring cyclodextrins (CDs) composed of more than nine D-glucose units have not been well studied because of difficulties in purification and preparation in reasonable yields (1,2). The complex-forming ability of the large-ring CDs has been assumed to be negligible due to an expected unsuitable cavity size and increased flexibility of the macrocycles (3). Recently, we established an isolation and purification method for several kinds of

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large-ring CDs and obtained a relative large amount of δ-CD (cyclomaltononaose) composed of nine D-glucose units (4,5). The inclusion-complex-forming properties of δ-CD with nine different drugs was investigated previously; δ-CD was not found to have marked effects on their solubility in comparison with conventional α-, β-, and γ-CD (1). However, we found that the effects of δ-CD on the solubility of spironolactone and digitoxin, which have a steroidal framework, were greater than those of α-CD, but less than those of β- and γ-CD (1).

In the present study, we examined the effects of δ-CD on the solubility of seven relatively large and slightly water-soluble drugs to find compounds that interact strongly with δ-CD. In addition, digitoxin and six related drugs were also studied. The interaction between digitoxin and δ-CD was studied in detail by the solubility method and ^{1}H-NMR (nuclear magnetic resonance) spectroscopy.

METHODS

Materials

The δ-CD with a purity of more than 98% was prepared by the method described previously (6). All other chemicals and solvents were from commercial sources and were used without further purification. Milli-Q water was used in all of the experiments.

Solubility Studies for Poorly Water-Soluble Drugs

Excess amounts of drugs were added to aqueous solutions containing δ-CD (15 mg/ml). The solutions were sonicated three times for 10 min each time at 30-min intervals and then shaken at 25°C. After reaching equilibrium (about 24 hr), the solutions were filtered through 0.45-μm membrane filters. A portion of each sample was diluted and analyzed by spectrophotometry at the following wavelengths: reserpine at 268 nm, [2,2]-paracyclophane at 224 nm, perylene at 401 nm; pyrene at 334 nm; triphenylene at 257 nm, 1,8-naphthalic anhydride at 338 nm, naphthalene-1,4,5,8-tetracarboxylic dianhydride at 352 nm, digitoxin at 221 nm, gitoxin at 218 nm, digoxin at 219 nm, methyldigoxin at 219 nm, lanatoside C at 219 nm, G-strophanthin at 220 nm, and proscillaridin A at 298 nm. As a reference, the abilities of α-, β- and γ-CD to solubilize these drugs were also determined in the same way. All ultraviolet (UV) measurements were carried out on a Ubest-30 Double Beam spectrophotometer (Jasco Co., Ltd.).

Solubility Study of Digitoxin

Solubility measurements were carried out according to the methods of Higuchi and Connors (7). Excess amounts of digitoxin were added to aqueous solutions containing various concentrations of CDs, and the mixtures were shaken at 25°C ± 0.5°C. After reaching equilibrium (approximately 3 days), an aliquot of solution was filtered through a 0.45-μm Millipore membrane filter and analyzed by spectrophotometry.

Nuclear Magnetic Resonance Spectroscopic Study

The NMR spectroscopy was performed in D_2O using a JNM GX-400 NMR spectrometer (Jeol). The ^{1}H-chemical shifts are given relative to external tetramethylsilane within ±0.0005 ppm.

RESULTS AND DISCUSSION

It has been demonstrated that δ-CD does not show any significant solubilization effect on several kinds of drugs except for spironolactone and digitoxin (1). To find compounds that have strong complex-forming ability with δ-CD, the interactions of δ-CD with seven poorly water-soluble compounds were studied by measuring changes in their solubilities. Table 1 summarizes the effects of CDs on the solubility of slightly soluble or insoluble compounds in water at 25°C. The δ-CD did not show any significant effects on solubility of these compounds, as previously reported (1). Although δ-CD has a larger cavity than α-, β- and γ-CD, the complex-forming ability is not necessarily strong for relatively large compounds in solution. The diameter of the δ-CD cavity had much less effect on the inclusion ability than expected. This was suggested previously to be due to the high flexibility of the ring of large-ring CDs and the behavior of complexed water molecules in the cavity of large-ring CDs (3). However, we reported that δ-CD had a strong interaction with a fullerene, C_{70}, which had relatively large molecular volume (8). Therefore, it is not possible to come to general conclusions from the available information.

Table 2 summarizes the effects of CDs on the solubility of digitoxin and six related drugs tested in this experiment. These drugs were chosen because δ-CD has specific effects on the solubility of digitoxin. δ-CD showed solubilization effects on the drugs tested, but was not superior to β- and γ-CD. To discuss the differences among the effects of δ-, β-, and γ-CD, inclusion complex forma-

Table 1

Effects of Cyclodextrins on Solubility of Slightly Soluble or Insoluble Drugs in Water at 25°C

Drug	Solubility in Water (mg/100 ml)	Solubility in 15 mg/ml CD Solution (mg/100 ml)							
		α-CD		β-CD		γ-CD		δ-CD	
Reserpine	0.058	0.066	(1.1)	0.493	(8.5)	0.179	(3.1)	0.097	(1.7)
[2.2]-Paracyclophane	0.045	0.131	(2.9)	3.345	(74.3)	2.217	(49.3)	0.094	(2.1)
Perylene	0.010	0.012	(1.2)	0.015	(1.5)	0.016	(1.6)	0.031	(3.1)
Pyrene	0.037	0.019	(0.5)	0.052	(1.4)	0.039	(1.1)	0.037	(1.0)
Triphenylene	0.002	0.004	(2.0)	0.020	(10.0)	0.015	(7.5)	0.014	(7.0)
1,8-Naphthalic anhydride	0.245	0.255	(1.0)	0.319	(1.3)	0.339	(1.4)	0.402	(1.6)
Naphthalene-1,4,5,8-tetracarboxylic dianhydride	11.31	11.95	(1.1)	11.98	(1.1)	13.20	(1.2)	16.64	(1.5)

The solubilization rates are shown in parentheses.

tion for digitoxin with each of these CDs in aqueous solution was studied using the solubility method. Figure 1 shows the phase solubility diagrams obtained for digitoxin with CDs in water. The β-CD and γ-CD systems showed typical B_S-type solubility curves. On the other hand, the solubility of digitoxin increased linearly as a function of δ-CD concentration, and the solubility curve was classified as being of type A_L. In contrast, no precipitation was observed for the δ-CD system. The apparent stability constant *K*, as a tentative measure of inclusion complexation, was estimated from the initial straight-line portion of the solubility plot. Stoichiometry of the complexes was then analyzed using the plateau portion of the solubility diagram and was found to be 1:3 (digitoxin: β-CD) and 1:4 (digitoxin: γ-CD). The magnitude of *K* and stoichiometry of the digitoxin/δ-CD system were consistent with previously reported values (9). The magnitude of *K* was found to decrease in the order γ-CD ($6.3 \times 10^4\ M^{-1}$) > β-CD ($3.3 \times 10^4\ M^{-1}$) > δ-CD ($1.7 \times 10^3\ M^{-1}$). This suggested that δ-CD had weak complex-forming ability with digitoxin in comparison with β-CD and γ-CD.

The ^{1}H-NMR measurement was carried out to examine the inclusion mode in aqueous solution. Table 3 summarizes a typical example of the effect of δ-CD on some ^{1}H-chemical shifts (18-methyl and 19-methyl) of digitoxin. The other proton signals were too weak to be analyzed quantitatively under the experimental conditions used. In the presence of δ-CD, both signals moved downfield, probably due to steric hindrance through inclusion complexation. Signals for δ-CD protons moved upfield due to a shielding effect. H-3 and H-5 protons located within the cavity were affected more than other protons. These NMR data indicated that the digitoxin molecule is bound loosely in the δ-CD cavity. The effect of δ-CD on the UV absorption spectrum of digitoxin was also

Table 2

Effects of Cyclodextrins on Solubility of Digitoxin and Related Drugs in Water at 25°C

Drug	Solubility in Water (mg/100 ml)	Solubility in 15 mg/ml CD Solution (mg/100 ml)							
		α-CD		β-CD		γ-CD		δ-CD	
Digitoxin	0.80	5.32	(6.7)	34.54	(43.2)	516.20	(645.3)	27.33	(34.2)
Gitoxin	0.470	0.958	(2.0)	38.320	(81.5)	40.520	(86.2)	1.838	(3.9)
Digoxin	2.64	14.89	(5.6)	500.80	(189.7)	469.10	(177.7)	9.66	(3.7)
Methyldigoxin	3.11	15.25	(4.9)	656.20	(211.0)	623.00	(200.3)	10.90	(3.5)
Lanatoside C	4.05	15.57	(3.8)	694.00	(171.4)	673.3	(166.2)	14.59	(3.6)
G-Strophanthin	1083	1961	(1.8)	1755	(1.6)	1833	(1.7)	1323	(1.2)
Proscillaridin A	21.92	46.22	(2.1)	7.72	(0.4)	616.60	(28.1)	142.8	(6.5)

The solubilization rates are shown in parentheses.

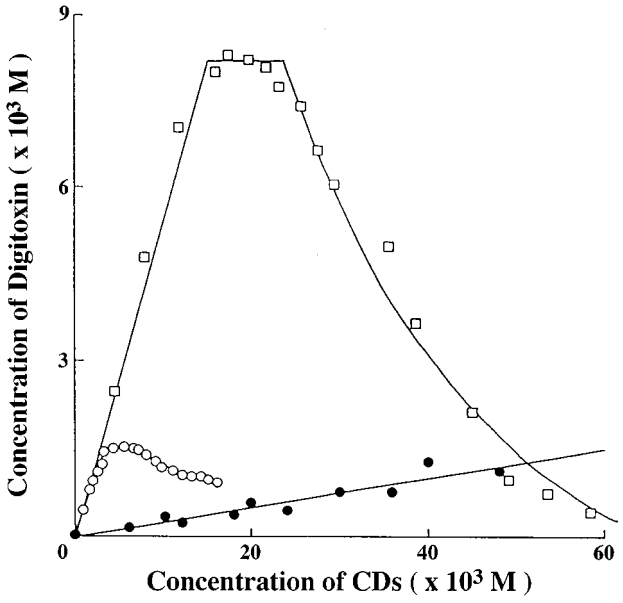
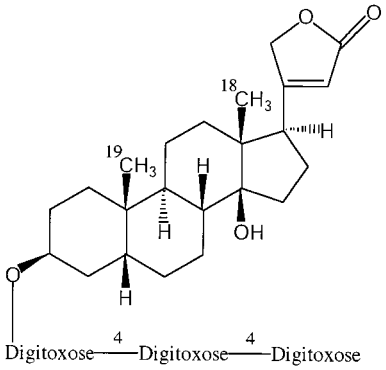


Figure 1. Phase solubility diagrams of digitoxin-CD system in water at 25°C: ○, β-CD; □, γ-CD; ●, δ-CD.

Table 3

Effects of δ-Cyclodextrin on ^{1}H-Chemical Shifts of Digitoxin Protons in D_2O at 50°C[a]



	Chemical Shift (ppm)[c]		
Proton[b]	Without δ-CD (δ_0)	With δ-CD (δ)	$\delta-\delta_0$
18-Methyl	0.862	0.911	0.049
19-Methyl	0.921	0.955	0.034

[a] Concentrations of digitoxin and δ-CD were 13.0×10^{-5} M and 13.0×10^{-3} M, respectively.
[b] Assigned according to H. W. Voigtländer and G. Balsam, Arch. Pharm., 301(3), 208–219 (1968).
[c] Results are ppm downfield from external Me_4Si.

studied; the absorption maximum of digitoxin showed a 3-nm hypsochromic shift and a decrease of intensity due to addition of δ-CD. The UV spectrum changes similar to the above results were that observed in a 50% ethanol solution of digitoxin. These results indicated that digitoxin interacts weakly with the hydrophobic cavity of δ-CD (10).

CONCLUSIONS

The effects of δ-CD on the solubility of 14 drugs slightly soluble or insoluble in water were lower than those of conventional cyclodextrins such as β-CD and γ-CD. This result might be attributable to weaker complex-forming ability of δ-CD in comparison with β-CD and γ-CD.

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