Circular Dichroism Spectra of the Inclusion Complexes of Phlorizin in Cyclodextrins

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Abstract. Two aromatic rings of a phlorizin molecule form inclusion complexes with β-CD and γ-CD. Induced circular dichroism spectra of these complexes have been measured to estimate the orientation of the two aromatic rings in the hydrophobic space of CDs. Apparent complex formation constants have been also estimated for each complex. It is concluded that phlorizin forms a stronger inclusion complex with β-CD than with γ-CD.

Key words. Cyclodextrin, inclusion complex, phlorizin, circular dichroism.

1. Introduction

Phlorizin (Phz), shown in Figure 1, is known as a glucose transport inhibitor in the kidney [1, 2]. To obtain information about the mechanism of the inhibition reaction, the molecular interaction with cyclodextrins has been studied. Here, cyclodextrins are regarded as an extreme model of glycoside spiral chains at the brush border membrane. In a previous report, it was suggested that Phz made a loose molecular complex with β-CD or γ-CD mainly based on the results of ^{1}H and ^{13}C NMR spectroscopy [3].

It is well known that optically active molecules show changes in circular dichroism spectra upon the formation of inclusion complexes with CDs [4–6]. These changes are induced by the entrance of the guest molecules into the optically active environment. Up to now, the induced circular dichroism spectra (ICD) of some substituted benzenes and naphthalenes have been calculated [7–9] according to the Kirkwood–Tinoco equation [10]. Comparing the calculated values of those aromatic rings with the observed ones, the relation of the molecular axis of the CD to the electronic transition moment of the guest molecules is generally deduced as follows: in the case that the electronic transition moment of the guest molecule is polarized parallel to the molecular axis of the CD, a positive circular dichroism is induced. For the situation in which the electronic transition moment of the guest molecule is perpendicular to the axis of the CD, a negative circular dichroism is induced.

In this report, changes in the circular dichroism spectra of the aqueous solutions of Phz upon the addition of CDs have been measured at room temperature in order to estimate the orientation of the two aromatic rings in the inner space of CDs.
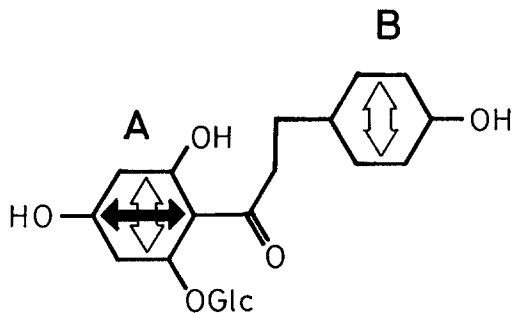
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Fig. 1.   The two aromatic rings of the Phz molecule (A and B) with the three electronic transition moments (black: CT band, long axis; white: α band, short axis).

Complex formation constants have also been estimated for both β- and γ-CD complexes.

2. Experimental

Circular dichroism spectra were obtained with a J-20 Optical Rotatory Dispersion Spectrometer (JASCO) at room temperature with aqueous solutions. UV absorption spectra were obtained with an MPS-2000 spectrometer (Shimazu Co.).

Phlorizin (Sigma Chemical Co.) was recrystallized from water. α-, β-, and γ-CD (Nakarai Chemicals LTD.) were used without any purification.

3. Results and Discussions

3.1. UV AND CIRCULAR DICHROISM SPECTRA OF PHZ

Only one absorption peak of Phz is observed at 284 nm at wavelengths longer than 230 nm as shown in Figure 2 (bottom). Since the B aromatic ring of Phz (see Figure 1) has only one hydroxyl group, the p band of the B aromatic ring is considered to be at a wavelength shorter than 240 nm [11]. The 284 nm absorption band can be assigned to a CT band [12, 13] of the A aromatic ring of Phz. Weak α bands of the two aromatic rings are observed as a shoulder on the strong CT band.

In the circular dichroism spectrum of the Phz solution in the same wavelength range as above, three peaks are observed at 278 nm ($[\theta] = 5000$ deg cm^2 dmol^{-1}), 305 nm ($[\theta] = -4200$) and 333 nm ($[\theta] = 1200$). The 274 nm band corresponds to the strong CT band of the UV absorption spectrum and the other two bands correspond to the shoulder part of the CT band. With regard to the order of the two α bands, the α band of the A aromatic ring is expected to be at the longer wavelength than that of the B aromatic ring, because the α band of benzene is known to shift to the red upon substitutions of the aromatic ring.

3.2. NO α-CD COMPLEX

In the presence of α-CD, no changes are observed in the UV and circular dichroism spectra of Phz. These facts suggest that Phz does not form an inclusion complex
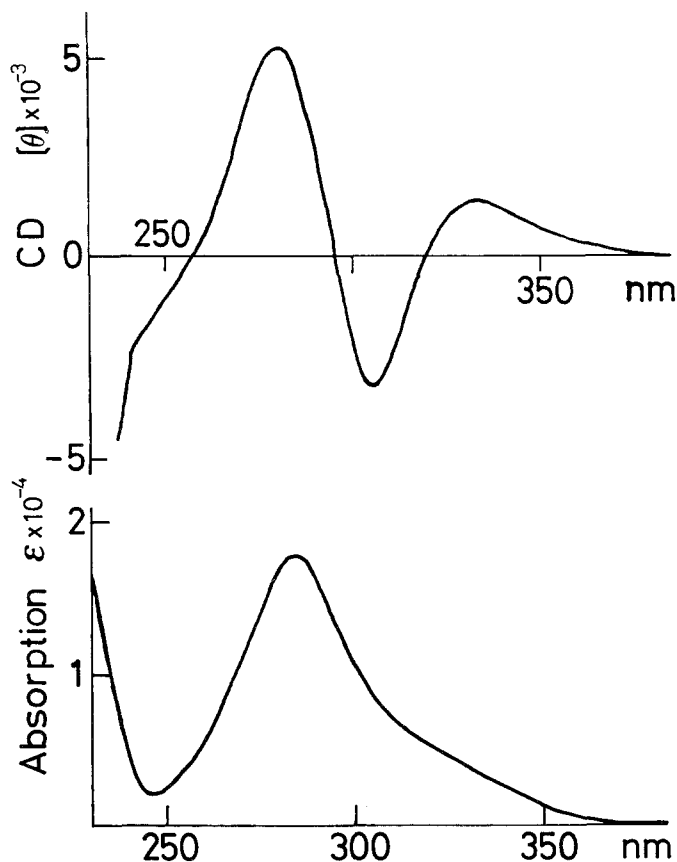
Fig. 2.   Circular dichroism spectrum (upper) and UV absorption spectrum (bottom) of the Phz aqueous solution at room temperature.

with α-CD. This result is consistent with the results obtained form ^{1}H and ^{13}C NMR spectroscopic data and solubility experiments [3].

3.3. β-CD COMPLEX

Upon the addition of β-CD to the Phz solution, negative ICD are observed at both the CT band of the A ring and the α band of the B ring, as shown in Figure 3. The small change in the α band of the A ring may be explained by the existence of the positive ICD of this band subtracted from the stronger negative ICD of the neighboring band. No peak shift in the UV absorption spectra and the circular dichroism spectra may indicate that there is no close proximity of two aromatic rings. In Figure 1, the direction of the three electronic transition moments of the two aromatic rings are also shown. Applying our experimental results to the relations described above between the sign of the ICD and the electronic transition moment, the orientations of the aromatic rings of Phz in the cavity of β-CD are estimated as shown in Figure 4a. The long axis of the A ring is almost perpendic-
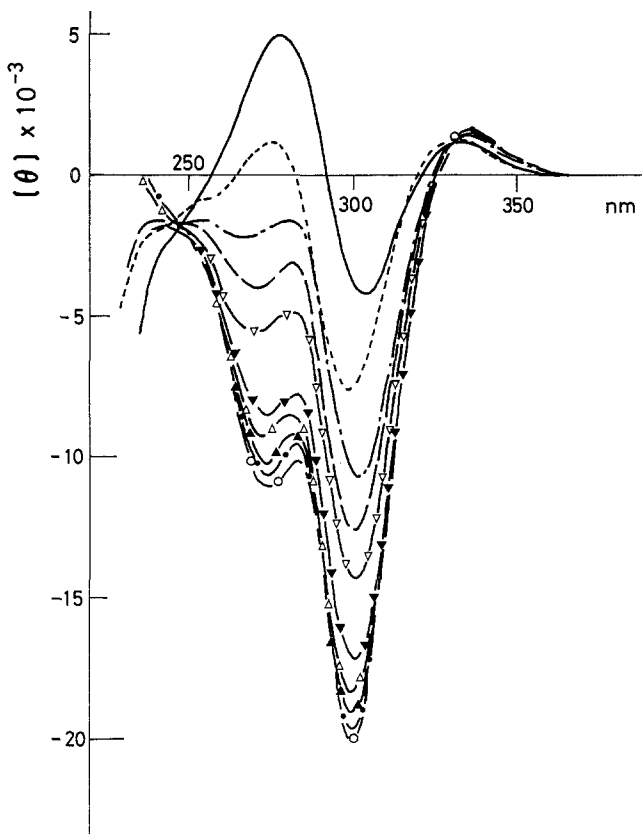
Fig. 3. Circular dichroism spectra of Phz at various mixing ratios with β-CD. The concentration of Phz is 0.848×10^{-3} M. Molecular ratios of β-CD to Phz are 0.0 (——), 0.5(- - - - -), 1.0(— -- —), 1.5(——) 2.0(— $\triangledown$ —), 3.0(— $\blacktriangledown$ —), 4.0(— $\triangle$ —), 5.0(— $\blacktriangle$ —), 6.0(— $\bullet$ —), and 7.0(— $\bigcirc$ —). Molecular ellipticities are normalized with Phz solution.

ular to the molecular axis of β-CD. The plane of the A ring is expected to be parallel to the molecular axis of β-CD. On the other hand, the short axis of the B ring is perpendicular to the molecular axis of β-CD.

Although no information about the glucose ring of Phz can be directly obtained from the circular dichroism experiment, inclusion of the glucose ring can hardly be expected because the inner space of β-CD is hydrophobic and the inclusion of the A aromatic ring is preferred.

3.4. γ-CD COMPLEX

Upon the addition of γ-CD to the Phz solution, negative and positive ICDs were detected at the CT band of the A ring and the α band of the B ring, respectively (Figure 5). No band shift in the UV and circular dichroism spectra was obtained as in the case of β-CD. A small negative ICD is detected at the α band of the A ring.
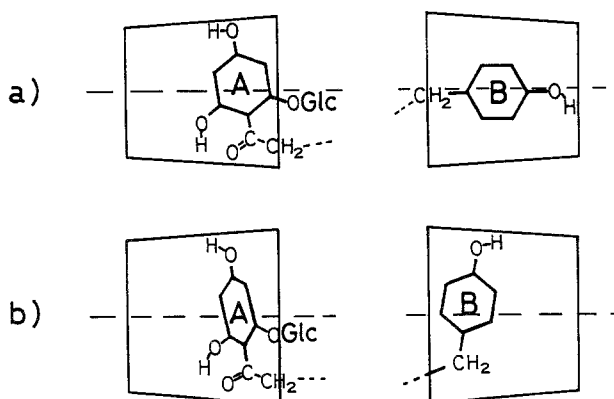
Fig. 4. Estimated orientations of the A and B aromatic rings in the inner space of β-CD (a) and γ-CD (b).

From these facts, the following three points are concluded. First, the A and B rings of Phz enter separately into the inner space of two different γ-CD molecules. Second, the long axis of the A ring is perpendicular to the molecular axis of γ-CD, as shown in Figure 4b. However, the plane of the A ring is considered perpendicular to the molecular axis of γ-CD. Third, the short axis of the B ring is parallel to the molecular axis of γ-CD. Whether the plane of the B ring is perpendicular or parallel to the axis of γ-CD is not clearly explained from these observations. The third point is clearly different from the case of β-CD.
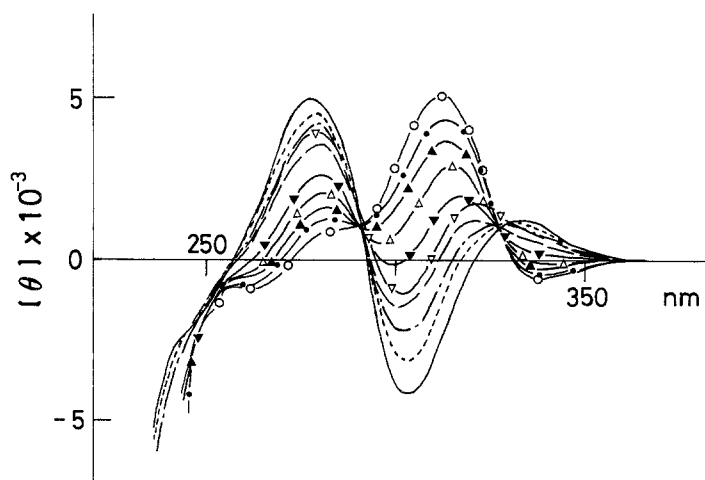

Fig. 5. Circular dichroism spectra of Phz at various mixing ratios with γ-CD in aqueous solution. The concentration of Phz is 0.848×10^{-3} M. Molecular ratios of γ-CD to Phz are 0.0(——), 0.5(- - - - -), 1.0(— - —), 1.5(— —), 2.0(— $\bigtriangledown$ —), 3.0(— $\blacktriangledown$ —), 5.0(— $\triangle$ —), 7.0(— $\blacktriangle$ —), 10(— $\bullet$ —), and 15(— $\bigcirc$ —). Molecular ellipticities are normalized with Phz solution.

3.5. COMPLEX FORMATION CONSTANT

Figures 3 and 5 show that the increases in the molecular ratio of CDs to Phz cause the increases of the ICDs, so that the complex formation and dissociation are in equilibrium in both the cases of β-CD and γ-CD. Both aromatic rings of Phz enter into the inner space of the CDs independently. Estimation of the complex formation constants is complicated by the existence of two possible sites in one Phz molecule for complex formation. However, ignoring the effect of the second step of the complex formation, it is possible to estimate the apparent complex formation constants for equilibrium reactions at low concentration of CDs.

When two molecules M and L form a complex $M \cdot L$ in an equilibrium reaction (1), the complex formation constant is expressed by Equation (2), using c_M, c_L, and x as the initial concentration of two molecules M and L, and the concentration of the complex, respectively.

$$M + L \rightleftharpoons M \cdot L \tag{1}$$

$$K = \frac{x}{(c_M - x)(c_L - x)} \tag{2}$$

In the case that only the molecule M and the complex $M \cdot L$ have optical activity, the ellipticity of the solution is shown by Equation (3),

$$\theta = [\theta_M](c_M - x) + [\theta_{ML}]x \tag{3}$$

where $[\theta_M]$ and $[\theta_{ML}]$ denote the molecular ellipticities of molecule M and complex $M \cdot L$, respectively. From Equations (2) and (3), Equation (4) is obtained [14].

$$\frac{c_L}{\Delta\theta} = \frac{c_M + c_L - x}{\Delta\theta_{ML}} + \frac{1}{\Delta\theta_{ML}K} \tag{4}$$

where

$$\Delta\theta = \frac{\theta}{c_M} - [\theta_M] \tag{5}$$

$$\Delta\theta_{ML} = [\theta_{ML}] - [\theta_M] \tag{6}$$

The apparent complex formation constants for the A-ring complex and the B-ring complex can be estimated using the ICDs of the CT band of the A ring and the α band of the B ring, respectively. They are estimated approximately first from plots of $c_L/\Delta\theta$ versus $c_M + c_L$, and second, from the plots of $c_L/\Delta\theta$ versus $c_M + c_L - x$, where x is estimated by Equation (6) using the previously estimated K. The best K values are obtained by repeating these plots until convergent values are obtained. The results for β-CD and γ-CD are shown in Table I, in which estimated values of the induced circular dichroism are also shown. These values may be considered to contain 10% of technical error.

The complex formation constants show that both the A and B aromatic rings of Phz form complexes more easily in β-CD than in γ-CD. This fact shows that β-CD has a more adequate size of hydrophobic space for the two aromatic rings of Phz to enter into than γ-CD [15]. This result is consistent with the solubility tests and the suppression phenomena of the H-D exchange reaction reported previously [3].

Table I.   Complex formation constants and ICDs.

	$K_{apparent}$ (M^{-1})		$[\theta_{ML}]_{apparent}$ (deg(dm^{-1})(mol/dl)$^{-1}$)	
	A-complex	B-complex	CT band of A-complex	α band of B-complex
β-CD	1100	970	-19000	-19000
γ-CD	400	420	-4900	$+11000$

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