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Effect of the Sugar Moiety on Complex Formation of Cycloamyloses with *p*-Nitrophenyl Glycosides

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Circular diochroism (CD) spectra of four *p*-nitrophenyl glycosides and their cycloamylose complexes were investigated at various concentrations of cycloamylose and at temperatures ranging from 20 to 60°C. The CD spectra of *p*-nitrophenyl glycosides changed remarkably in the presence of cycloamyloses, and significant differences in spectral shape and intensity were observed between the cyclohexaamylose complex and the cycloheptaamylose complex. The difference CD spectra between the free guest and its complex indicates that the nitrophenyl group is included in the cycloamylose cavity but its disposition is different between the complexes with cyclohexaamylose and cycloheptaamylose. Values of enthalpy and entropy of the cyclohexaamylose complex are considerably larger than those of the corresponding cycloheptaamylose complex, although the free energy differs only slightly. It is suggested that the nitrophenyl group is more loosely bound to the cycloheptaamylose cavity than to the cyclohexaamylose cavity, and has much more flexibility in its disposition.

INTRODUCTION

A number of investigations have been carried out for the purpose of determining, on the basis of enzyme models, why cycloamyloses form inclusion complexes with a variety of guest substances (*1*). Recently, many crystal structures of cycloamylose complexes have been determined by the X-ray method, and the inclusion phenomena have been discussed on that basis (*2, 3*). But the definition of binding forces of the complex remains controversial, although several proposals have been made (*1*). Circular dichroism (CD) is a powerful tool for investigating the cycloamylose–guest interaction, since the guest chromophor perturbed by the asymmetric cycloamylose molecule produces the induced CD (*4*). We have shown that the thermodynamic parameters of the cycloamylose complex, as well as the geometry of the host–guest interaction, can be evaluated on the basis of the temperature-dependent CD spectra (*5*). In the present study, the CD spectra of cycloamylose complexes with *p*-nitrophenol and four *p*-nitrophenyl glycosides were measured at various temperatures and cycloamylose concentrations. Many spectroscopic studies of cyclohexaamylose–*p*-nitrophenol complexes have been reported (*6–10*), but only a few have been done for cycloheptaamylose complexes (*11, 12*). We will discuss the structure and binding force by comparison of thermodynamic parameters between the cyclohexaamylose complex and the cycloheptaamylose complex.

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MATERIALS AND METHODS

Cycloamyloses, *p*-nitrophenol, and *p*-nitrophenyl–β-D-glucoside were obtained from Tokyo Kasei Company, and other *p*-nitrophenyl glycosides were purchased from BDH Chemicals Ltd. Cycloamyloses were recrystallized three times from water, and dried *in vacuo* over phosphorous pentaoxide. *p*-Nitrophenyl glycosides were used without further purification. Solutions were prepared with deionized and distilled water. CD and difference CD spectra were recorded on a JASCO J-40A circular dichrograph with a J-DPZ data processor. The temperature was regulated by use of a Tokyo Rico TC-100 thermo-controller, and a water-jacketed, 1-mm-thick cylindrical cell was used.

The molecular ellipticity of the complex and thermodynamic parameters for the 1 : 1 complex formation were determined by the least-squares method from the equation (*5*)

$$\ln K_d = \frac{\Delta H}{RT_i} - \frac{\Delta S}{R}, \qquad [1]$$

$$K_d = \frac{ab_j\Delta\theta_m}{\Delta\theta_{ij}} - a - b_j + \frac{\Delta\theta_{ij}}{\Delta\theta_m}, \qquad [2]$$

where a and b_j are concentrations of the guest and cycloamylose, respectively, $\Delta\theta_{ij}$ is the observed difference CD at the temperature of T_i(K), and $\Delta\theta_m$ is the difference in the molecular ellipticity between the free guest and the complex.

RESULTS

CD spectra of cycloamylose complexes with *p*-nitrophenol are shown in Fig. 1. The cyclohexaamylose complex gives a positive-signed CD band centered at 340 nm while two negative CD peaks appear at 224 and 256 nm. On the other hand, the cycloheptaamylose complex has a positive CD peak at 328 nm and a negative one at 222 nm.

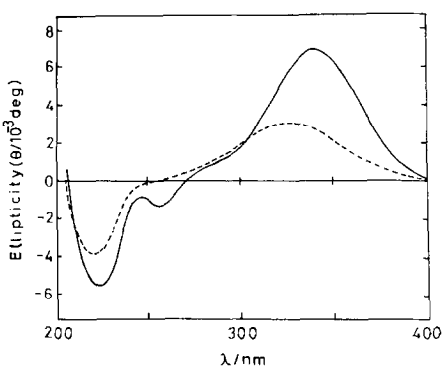

FIG. 1. CD spectra of *p*-nitrophenol complexes with cyclohexaamylose (——) and cycloheptaamylose (– – –) at 25°C. The concentrations of *p*-nitrophenol, cyclohexaamylose, and cycloheptaamylose are 1.12, 18.5, and 8.7 m*M*, respectively.

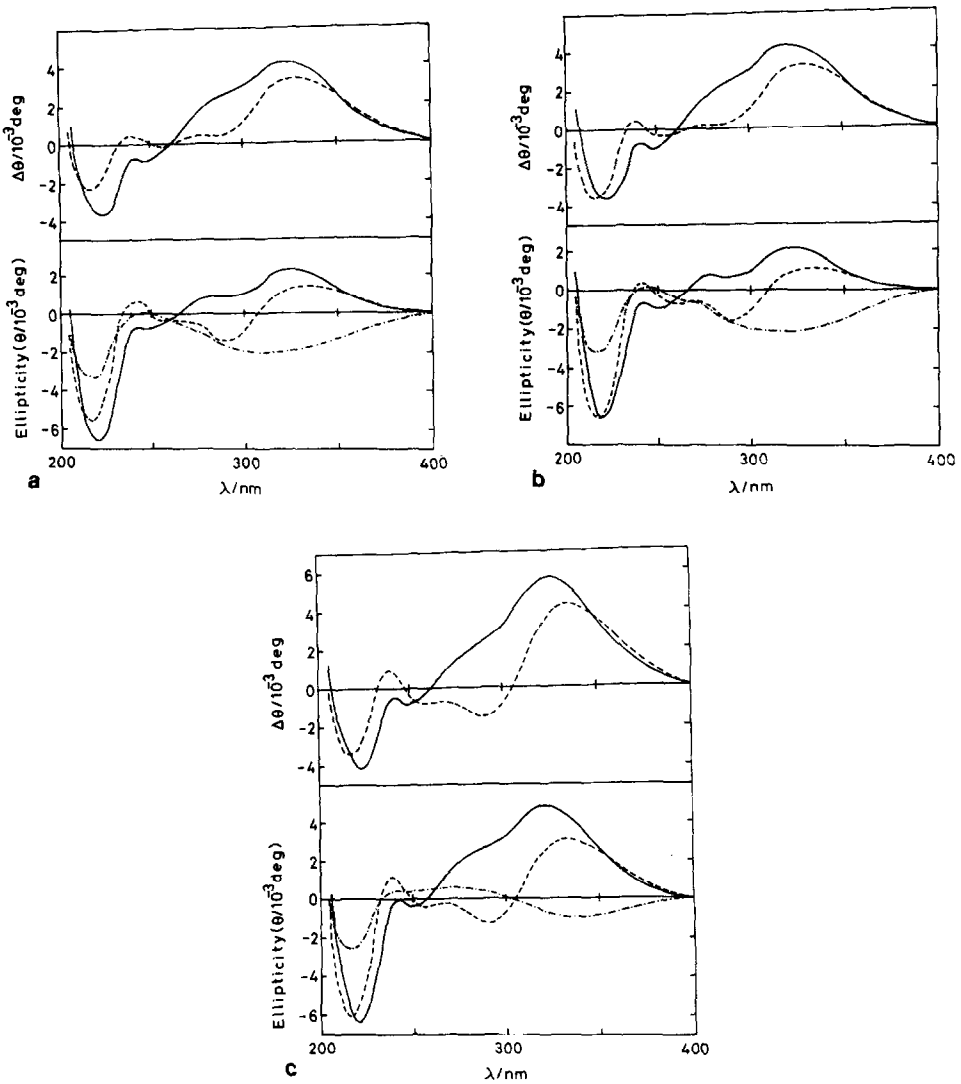

FIG. 2. CD spectra of *p*-nitrophenyl derivatives (– · – · –) of β-D-glucoside (*a*), β-D-galactoside (*b*), and β-D-xyloside (*c*), and CD and difference CD spectra of their complexes with cyclohexaamylose (——) and cycloheptaamylose (– – –) at 25°C. Concentrations of cyclohexaamylose, cycloheptaamylose, *p*-nitrophenyl-β-D-glucoside, *p*-nitrophenyl-β-D-galactoside, and *p*-nitrophenyl-β-D-xyloside are 18.5, 8.9, 1.03, 1.04, and 1.00 m*M*, respectively.

Figures 2–4 show CD and difference CD spectra of *p*-nitrophenyl glycoside complexes with cycloamyloses. *p*-Nitrophenyl derivatives of β-D-glucoside and β-D-galactoside give two negative-signed CD peaks, and a close resemblance is observed in their spectral shape. When they form complexes with cyclohexaamylose, the negative-signed CD band of the guest in the 250- to 400-nm region changes to the positive-signed one with a shoulder at 280 nm. The CD values of their cycloheptaamylose complexes are quite different from those of the cyclohexaamylose complexes, especially in the 250- to 400-nm region; a positive peak is observed at 335 nm and a negative one at 293 nm.

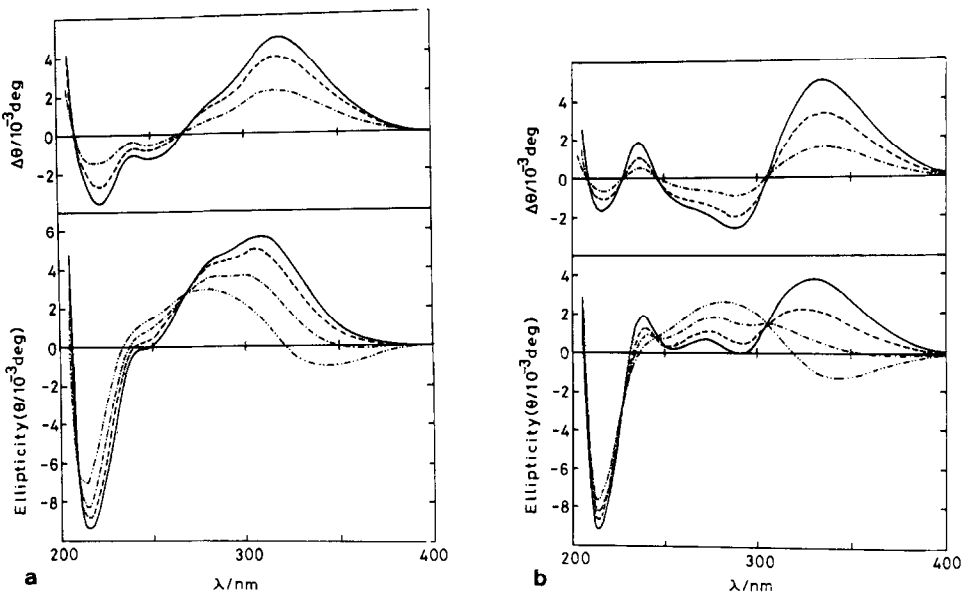

FIG. 3. CD spectra of *p*-nitrophenyl–*N*-acetyl-β-D-glucosaminide (– · – · –) and its complexes with cyclohexaamylose (*a*) and cycloheptaamylose (*b*) at 25°C, and at the guest concentrations of 0.85 m*M*. The concentrations of cyclohexaamylose are 18.5 (——), 10.2 (– – –), and 4.1 m*M* (– · – · –), and those of cycloheptaamylose are 8.9 (——), 5.0 (– – –), and 2.0 m*M* (– · – · –).

The CD of *p*-nitrophenyl–β-D-xyloside is relatively weaker than that of other *p*-nitrophenyl glycosides, and a positive CD peak appears in the 250- to 300-nm region. The CD spectra of its cycloamylose complexes resemble those of the *p*-nitrophenyl derivatives of β-D-glucoside and β-D-galactoside, but have relatively larger intensity. *p*-Nitrophenyl–*N*-acetyl-β-D-glucosaminide, being different from other *p*-nitrophenyl glycosides, has a weak and negative CD peak at 342 nm, a positive one at 285 nm, and a strong and negative one at 213 nm. Therefore, the CD curves of its cycloamylose complexes also differ from those of the complexes with other guests.

Difference CD curves of cyclohexaamylose complexes with *p*-nitrophenyl glycosides are quite similar to that of the cyclohexaamylose–*p*-nitrophenol complex. On the other hand, a remarkable difference in the spectral shape is observed in their cycloheptaamylose complexes. In the 250- to 300-nm region, the *p*-nitrophenyl derivatives of β-D-xyloside and *N*-acetyl-β-D-glucosaminide give a negative-signed CD band, which is not observed in the *p*-nitrophenol complex.

Figure 3 shows CD and difference CD curves of *p*-nitrophenyl–*N*-acetyl-β-D-glucosaminide complexes at different cycloamylose concentrations. The intensity of difference CD lowers with decreasing the cyclodextrin concentration, but there is no significant change in spectral shape. The increase of the temperature also causes the decrease of the intensity of difference CD. The intensity measured at 60°C is about a half of the intensity measured at 20°C. Figure 5 shows the temperature dependence of the CD intensity measured at 320 nm for the cyclohexaamylose complex and at 335 nm for the cycloheptaamylose complex.

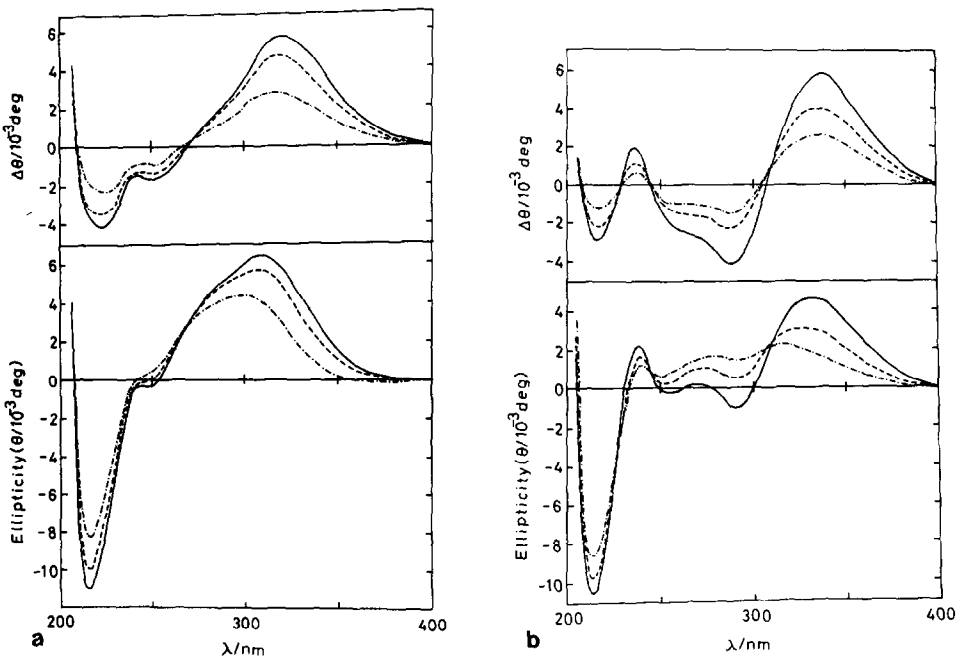

FIG. 4. CD and difference CD spectra of *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide complexes with cyclohexaamylose (*a*) and cycloheptaamylose (*b*) at 20 (——), 40 (– – –), and 60°C (–·–·–). The concentrations of *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide, cyclohexaamylose, and cycloheptaamylose are 0.96, 18.5, and 8.9 m*M*, respectively.

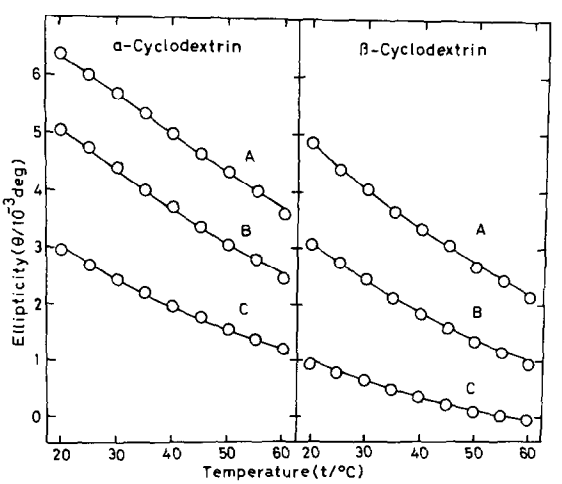

FIG. 5. Temperature-dependent CD intensity of *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide complexes measured at 320 nm (cyclohexaamylose) and 335 nm (cycloheptaamylose). The concentrations of cyclohexaamylose are 18.5 (A), 10.3 (B), and 4.1 m*M* (C), and those of cycloheptaamylose are 9.6 (A), 5.3 (B), and 2.1 m*M* (C), and the guest concentration is 0.96 m*M*. Solid lines are calculated intensity change on the basis of the 1 : 1 stoichiometry by using the parameters given in Table 1.

TABLE 1

OBSERVED AND CALCULATED ELLIPTICITY OF *p*-NITROPHENYL–*N*-ACETYL-β-D-GLUCOSAMINIDE COMPLEXES

t (°C)	θ_{obsd}	θ_{calcd}	θ_{obsd}	θ_{calcd}	θ_{obsd}	θ_{calcd}
	Cyclohexaamylose (320 nm)					
	[C][a] = 18.45 m*M*		[C] = 10.25 m*M*		[C] = 4.10 m*M*	
20	63.6	63.4	50.4	50.9	29.6	30.4
25	60.2	60.1	47.5	47.2	26.8	27.2
30	56.9	56.7	44.1	43.6	24.3	24.3
35	53.4	53.3	40.1	40.1	22.1	21.7
40	49.8	49.9	36.9	36.7	19.7	19.3
45	46.5	46.6	33.7	33.6	17.8	17.2
50	43.4	43.3	30.5	30.6	15.6	15.3
55	40.2	40.2	28.0	27.9	13.8	13.7
60	36.4	37.1	24.7	25.3	12.1	12.2
	Cycloheptaamylose (335 nm)					
	[C] = 9.62 m*M*		[C] = 5.35 m*M*		[C] = 2.14 m*M*	
20	48.4	48.2	30.5	30.4	9.3	10.0
25	43.9	44.2	27.4	27.0	7.8	8.1
30	40.5	40.4	24.5	23.9	6.4	6.4
35	36.7	36.8	21.3	21.1	5.1	4.8
40	33.5	33.4	18.4	18.5	3.6	3.5
45	30.6	30.2	15.9	16.1	2.4	2.2
50	26.8	27.2	13.5	13.9	1.0	1.1
55	24.3	24.4	11.6	11.9	0.3	0.2
60	21.6	21.9	9.6	10.2	−0.4	−0.7

Note. Results are expressed $\times 10^{-3}$ degrees.

[a] [C] indicates the concentration of cycloamylose.

These intensity changes are well fitted to the equilibrium formula of the 1 : 1 complex formation, as shown by the solid lines. The results for *p*-nitrophenol and other *p*-nitrophenyl glycosides are not shown, but they exhibit similar behavior. On the basis of these intensity changes, the molecular ellipticity of the complex and thermodynamic parameters were determined by the least-squares method. Observed and calculated ellipticity of the *p*-nitrophenyl–*N*-acetyl-β-D-glucosaminide complexes are listed in Table 1. Table 2 gives molecular ellipticity and dissociation constant data at each temperature which are determined by using Eq. [2]. These results show that the molecular ellipticity is independent of temperature in the 20–60°C range. The thermodynamic parameters are given in Table 3.

DISCUSSION

Since *p*-nitrophenol is optically inactive, the induced CD is observed only when it forms the complex with cycloamylose. On the other hand, *p*-nitrophenyl

TABLE 2

MOLECULAR ELLIPTICITY AND DISSOCIATION CONSTANT OF *p*-NITROPHENYL–*N*-ACETYL-β-D-GLUCOSAMINIDE COMPLEXES AT EACH TEMPERATURE

	Cyclohexaamylose		Cycloheptaamylose	
t (°C)	$[\theta_m]$ (10^4 deg · cm^2 · dmol^{-1})	K_d (10^{-2} mol)	$[\theta m]$ (10^4 deg · cm^2 · dmol^{-1})	K_d (10^{-2} mol)
20	10.1	0.82	13.6	1.22
25	9.8	0.89	12.5	1.22
30	9.7	1.01	12.9	1.35
35	9.7	1.18	12.1	1.49
40	9.5	1.33	12.9	1.82
45	9.4	1.51	13.8	2.20
50	9.8	1.87	13.0	2.34
55	9.6	2.08	12.9	2.94
60	9.6	2.44	12.8	2.81

glycosides have intrinsic optical activity. Therefore, the CD spectra of their cycloamylose complexes consist of two components, the intrinsic part and the induced part. If the conformation of the guest molecule does not change with complex formation, the induced part should be observed as the difference in CD between the guest and the complex. From the X-ray analysis of *p*-nitrophenyl–β-D-xyloside (*13*), and *p*-nitrophenyl–*N*-acetyl-β-D-glucosaminide (*14*), the conformation involving the glycosidic linkage is nearly the same in both molecules; and the flexibility of the linkage is restricted by steric hindrance. The effect of the conformational change of the primary hydroxyl group may also be small, since cycloamyloses have many primary hydroxyl groups which are in equilibrium between *gauche–gauche* and *gauche–trans* conformations (*7*).

TABLE 3

MOLECULAR ELLIPTICITY AND THERMODYNAMIC PARAMETERS OF CYCLOAMYLOSE COMPLEXES WITH *p*-NITROPHENOL AND *p*-NITROPHENYL GLYCOSIDES[a,b]

	λ (nm)	$[\theta_m]$ (10^4 deg · cm^2 · dmol^{-1})	K_d(298 K) (10^{-2} mol)	ΔG(298 K) (kcal · mol^{-1})	ΔH (kcal · mol^{-1})	ΔS (cal · K^{-1} · mol^{-1})
Cyclohexaamylose						
p-Nitrophenol	342	9.4(0.1)	0.49	−3.15	−7.62(0.14)	−15.0(0.4)
Glucoside	325	6.0(0.1)	1.36	−2.55	−5.82(0.09)	−11.0(0.3)
Galactoside	325	5.9(0.1)	1.33	−2.56	−5.40(0.08)	−9.5(0.2)
Xyloside	325	9.3(0.1)	0.91	−2.78	−6.06(0.08)	−11.0(0.2)
Glucosaminide	320	9.8(0.1)	0.88	−2.81	−5.78(0.07)	−10.0(0.2)
Cycloheptaamylose						
p-Nitrophenol	330	4.8(0.1)	0.53	−3.10	−3.81(0.14)	−2.4(0.4)
Glucoside	333	10.5(0.7)	2.69	−2.10	−3.67(0.09)	−5.1(0.3)
Galactoside	333	7.2(0.3)	1.68	−2.42	−4.04(0.07)	−5.4(0.2)
Xyloside	338	13.8(0.8)	1.67	−2.42	−4.13(0.13)	−5.7(0.4)
Glucosaminide	335	12.9(0.3)	1.27	−2.59	−4.42(0.07)	−6.2(0.2)

[a] Letters in parentheses are standard deviations estimated by the equation given in Ref. (*5*).
[b] 1 cal = 4.184 J.

It has been confirmed from X-ray (*15*) and spectroscopic (*7, 8*) studies that *p*-nitrophenol is included in the cyclohexaamylose cavity with its orientation parallel to the molecular axis of the cyclohexaamylose. The nitro group is found at the primary hydroxyl side, while the phenolic hydroxyl group protrudes from the secondary hydroxyl side. In the cyclohexaamylose complexes with *p*-nitrophenyl glycosides, the difference CD spectra are quite similar to that of the *p*-nitrophenol complex in spite of the variety of sugar moieties. This suggests that the nitrophenyl group is included with a similar orientation in the cyclohexaamylose cavity.

The observed molecular ellipticity of the cyclohexaamylose–*p*-nitrophenol complex is about twice as large as that of the cycloheptaamylose complex (Table 1). Bergeron and McPhie (*11*) suggested that such higher intensity seen in the cyclohexaamylose complex is due to the stronger binding, since the intensity of induced CD will decrease with the third power of the distance between the transition-dipole-moment of the guest chromophor and the perturbing dipoles of cycloamylose. Theoretically, however, the molecular ellipticity of the cycloheptaamylose complex was predicted to be nearly the same as that of the cyclohexaamylose complex, if the guest chromophor is included in the same manner (*16*). It is more plausible that the weak CD of the cycloheptaamylose complex is ascribed to the geometry of inclusion. The rotational strength of the induced CD is approximately expressed as $R_{0a} \approx A_{0a}(1 + 3\cos 2\theta)\mu_{0a}^2$ (*4, 16*) if the transition-dipole-moment (μ_{0a}) of the chromophor is located at the center of the cavity. Since transition-dipole-moment of the 340-nm band is parallel to the long axis of *p*-nitrophenol (*12*), decrease of the CD intensity can be expected by tilting the guest molecule against the axis of cycloheptaamylose. In the crystalline complex of cycloheptaamylose–*p*-nitroacetanilide (*17*), the nitrophenyl group is positioned at a similar place to that found in the cyclohexaamylose–*p*-nitrophenol complex (*15*); but the nitrophenyl plane is tilted by about 30° against the cycloheptaamylose axis. If the *p*-nitrophenol molecule is included in the same geometry, the CD intensity will decrease to 62.5% of the value which is expected from the parallel inclusion.

The difference CD curve of the cycloheptaamylose–*p*-nitrophenyl glycoside complex is remarkably different from the CD curve of the *p*-nitrophenol complex, suggesting that the position and/or orientation of the nitrophenyl group in the cycloheptaamylose cavity differs from those of *p*-nitrophenol. This is readily interpreted on the basis of the assumption that the guest disposition in the crystalline complex is retained in aqueous solution. The included *p*-nitrophenyl group contacts the interior surface of the cycloheptaamylose ring, while the glycoside moiety may be located outside the cavity. In the cyclohexaamylose complex, the nitrophenyl group is so rigidly fixed in the cavity (*15*) that the effect of the sugar moiety on the binding geometry seems to be less important. But, in the cycloheptaamylose cavity, the included nitrophenyl group is considered to be more flexible in its disposition. Therefore, the sugar moiety of the guest molecule may affect considerably the geometry of inclusion by having contact—possibly hydrogen-bonding contact—with the hydroxyl groups of cycloheptaamylose.

For some cycloamylose complexes, the formation of a 2:1 complex has been

suggested (*5, 18, 19*). In the present work, however, the CD intensity change is well explained on the basis of the 1 : 1 stoichiometry (Table 1 and Fig. 5). The free energy of complex formation is found in a relatively small region, which is from -2.4 to -3.2 kcal $\cdot$ mol^{-1}, although the cyclohexaamylose complex gives slightly larger value than the cycloheptaamylose complex. The value of enthalpy and entropy of the cyclohexaamylose–*p*-nitrophenol complex are larger than those of the *p*-nitrophenyl glycoside complexes. On the other hand, in the cycloheptaamylose complexes an opposite tendency is observed. The entropy value of the cycloheptaamylose–*p*-nitrophenol complex is less than half of the corresponding value of *p*-nitrophenyl glycoside complexes. This suggests that the *p*-nitrophenyl glycosides are more fixed in the cycloheptaamylose cavity by the interaction between the sugar moiety of the guest and the hydroxyl groups of cycloheptaamylose.

Several speculations have been made to interpret the inclusion phenomena of cycloamyloses. The X-ray study of cyclohexaamylose and its crystalline complexes pointed out the importance of the strain energy of the host molecule (*2*). The theoretical estimation of thermodynamic parameters has indicated that most of the binding energy is expressed in terms of the solvation energy and van der Waals interaction energy between the host and guest (*20, 21*). The present investigation indicates that the binding force is closely related to the size of the included guest moiety and the width of the cycloamylose cavity. This is also consistent with the fact that the cycloheptaamylose complexes with naphthalene derivatives give larger enthalpy and entropy values than those of the *p*-nitrophenyl glycoside complexes (*5*). Since the naphthalene ring is bulkier than the nitrophenyl group, it may have closer contact with cycloheptaamylose. The comparison between the cyclohexaamylose complex and the cycloheptaamylose complex indicates that the tight packing of the guest molecule produces the large binding enthalpy, but does not necessarily increase the free energy. It is obvious from Table 3 that tight packing reduces the conformational freedom of cycloamylose as well as the translational and rotational freedom of the guest molecule, thus increasing the unfavorable entropy change.

Although it is impossible to discuss in detail each contribution of interactions involved in the complex formation, these results suggest the importance of van der Waals interactions. Since the dispersion force is a short range force, the cyclohexaamylose molecule with a smaller cavity may give larger dispersion energy. The contribution of the strain energy of the host molecule is controversial in the cyclohexaamylose complex (*2, 20, 21*), while crystallographic studies of cycloheptaamylose complexes showed no significant conformational change of macrocyclic ring between the complexed state (*17*) and the uncomplexed state (*22*). Therefore, the contribution of the conformational energy may be small, at least in the cycloheptaamylose complex. In the crystalline state, the uncomplexed cycloheptaamylose molecule includes a larger number of "high energy" water molecules (*22*) than the uncomplexed cyclohexaamylose (*2*). If the major part of binding energy is derived from the release of "high energy" water, cycloheptaamylose should give larger enthalpy of complex formation, in contradiction to the present results. Therefore, the strain energy and the "high energy" water

seem to be minor components of the binding energy in this case, and the van der Waals energy may be most important.

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