

Acid Dissociation of Cyclooctaamylose

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Acid dissociation constants of aqueous cyclooctaamylose (8-Cy) have been determined at 15–45°C by pH potentiometry. Standard enthalpies and entropies of dissociation are derived from the temperature dependences of these pK_a 's. These results are compared to corresponding measurements of aqueous cyclohexaamylose and cycloheptaamylose, and the observed trends are interpreted in terms of complexation of cycloamylose with hydroxide ion. ^{13}C -nmr spectral measurements are reported for 8-Cy in 99.8% D_2O solution, and assignments of the observed lines are made with the help of deuterium-induced differential isotopic shift experiments.

The complexations and structural features of the cycloamyloses have been widely studied in recent years. Interest in these compounds stems in part from their catalytic role in certain hydrolytic and oxidative reactions. The currently accepted hydrolysis reaction mechanism involves loss of a proton from the cycloamylose molecule early in the reaction sequence (1), and the acidic properties of the cycloamylose molecule may also have a role in oxidative catalysis.

In a previous communication (2) we reported aqueous acid dissociation pK_a values, thermodynamic dissociation parameters ΔH° and ΔS° , and ^{13}C -nmr line assignments for cyclohexaamylose (also known as α -cyclodextrin, but to be denoted as 6-Cy here) and cycloheptaamylose (β -cyclodextrin, 7-Cy). More recently, Vincendon (3) has also made ^{13}C and ^1H -nmr measurements on cycloamyloses and interpreted these in terms of hydrogen bonding and ring strain. We have now measured these same properties of cyclooctaamylose (γ -cyclodextrin, 8-Cy) and report the results in the present paper. On the basis of these results certain comparisons and correlations may be drawn between the members of the homologous series of cycloamyloses.

ACID DISSOCIATION THERMODYNAMIC PARAMETERS

The experimental and computation procedures used in the pK_a and thermodynamic parameter determinations are essentially the same as those described previously (2). The values found for pK_a of 8-Cy between 15° and 45° are shown in Table 1. A least-squares fit of van't Hoff equation to these data yielded ΔH° and ΔS° of the acid dissociation as shown in Table 2, where corresponding parameter values for 6-Cy and 7-Cy are shown for comparison purposes. We note that trends

TABLE 1
p*K*_a VALUES OF
CYCLOOCTAAMYLOSE AT
VARIOUS TEMPERATURES

T (°C)	p <i>K</i> _a
15	12.346 ± 0.004 ^a
25	12.083 ± 0.004
25	12.080 ± 0.005
35	11.734 ± 0.003
45	11.561 ± 0.006

^a Uncertainties quoted are standard error estimates based on statistical error in pH and titrant volume measurements and calculated as explained elsewhere (2).

in these parameter values with ring size continues in a regular way from 6-Cy to 8-Cy: the cycloamylose acid strength, ΔH° and ΔS° values all increase with increasing ring size. Because ΔH° becomes more positive, we see that the enhanced acidity is an entropically controlled phenomenon. Although we previously interpreted trends in acidic parameters primarily in terms of interglucosyl hydrogen bonding in neutral cycloamyloses (2), a more recent study of complexations of 6-Cy with a variety of substrates (4) leads to a more lucid interpretation. To utilize this interpretation, it is necessary to view the acid dissociation of Cy in an alternative way, as a complexation reaction between Cy and the substrate OH⁻ ion. This can be seen by writing the acid dissociation as



and formally adding this to



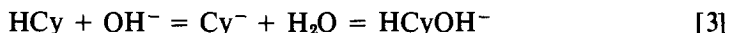
TABLE 2
ACID DISSOCIATION PARAMETERS FOR THE SERIES OF CYCLOAMYLOSES

	p <i>K</i> _a at 25°	ΔH° ^b (kcal mol ⁻¹)	ΔS° ^b (cal mol ⁻¹ K ⁻¹)
Cyclohexaamylose ^a	12.332	8.36 ± 0.34	-28.3 ± 1.4
Cycloheptaamylose ^a	12.202	9.98 ± 0.09	-22.4 ± 0.3
Cyclooctaamylose	12.081	11.22 ± 0.56	-17.6 ± 1.8

^a From Ref. (2).

^b Uncertainties quoted are standard error estimates based on the scatter of points from the van't Hoff straight lines as fitted by least squares.

yielding



Because $\text{Cy}^- + \text{H}_2\text{O}$ is equivalent to HCyOH^- in these experiments, reaction [3] is seen to be the complex formation reaction of Cy with OH^- . The thermodynamic parameters for this complexation are easily calculated by combining the data in Table 2 with the well-known properties of reaction [2] (5). The formation constant K_f is K_a/K_w , and the thermodynamic parameters ΔH_f° and ΔS_f° at 25° similarly follow by adding the Cy acid dissociation ΔH° to $\Delta H_w^\circ = -13.36 \text{ kcal mol}^{-1}$ and ΔS° to $\Delta S_w^\circ = +19.3 \text{ cal mol}^{-1} \text{ K}^{-1}$. The results of these calculations are shown in Table 3. By comparison with corresponding entries in Table 2, we see that the increasing acidity observed from 6-Cy to 8-Cy translates into stronger complexes with OH^- and that this trend remains entropically controlled, because the ΔH_f° values become correspondingly more positive. In Ref. (4) we reported a correlation of ΔS_f° with ΔH_f° in the form

$$\Delta H_f^\circ = (403 \pm 24 \text{ K}) \Delta S_f^\circ - (1.2 \pm 0.4)10^3 \text{ cal mol}^{-1}$$

based on 20 different substrates complexing with 6-Cy and also showed that the 6-Cy $\cdot \text{OH}^-$ complex conformed quantitatively. We interpreted this correlation in terms of a mechanism involving essentially dipolar Cy-substrate interactions. Stronger complexes are characterized by more negative ΔH_f° values, i.e., stronger dipolar interactions, and also by more negative ΔS_f° values, i.e., enhanced cycloamylose molecular perturbation and rigidity. The fact that the data for 6-Cy $\cdot \text{OH}^-$ fitted this correlation seemed to confirm that our view of 6-Cy acid dissociation as the hydroxide complex was valid. We would not, however, expect 7-Cy $\cdot \text{OH}^-$ or 8-Cy $\cdot \text{OH}^-$ to conform to the 6-Cy correlation and, indeed, we see they do not. As the complexes of Cy with OH^- become stronger from 6-Cy to 8-Cy, the corresponding formation reaction become less exothermic and ΔS° for the complex formation reaction becomes more positive, the controlling thermodynamic factor. If we extend the hypothesis of essentially dipolar host-guest interaction to 7-Cy and 8-Cy, then these interactions become weaker from 6-Cy to 8-Cy and the substrate-induced rigidity of the cycloamylose also becomes less effective in this same order. At 25° this lesser rigidity overcompensates for the weaker ion-

TABLE 3
FORMATION PARAMETERS OF THE Cy $\cdot \text{OH}^-$ COMPLEX

	K_f^a at 25°	$\Delta H_f^{\circ b}$ (kcal mol $^{-1}$)	$\Delta S_f^{\circ c}$ (cal mol $^{-1} \text{ K}^{-1}$)
Cyclohexaamylose	46.2	-5.00	-9.0
Cycloheptaamylose	62.4	-3.38	-3.1
Cyclooctaamylose	82.4	-2.14	+1.7

^a $\text{p}K_f = 13.997 - \text{p}K_a$ from Table 2.

^b $\Delta H_f^\circ = \Delta H^\circ - 13.36 \text{ kcal mol}^{-1}$ from Table 2 and Ref. (5).

^c $\Delta S_f^\circ = \Delta S^\circ + 19.3 \text{ cal mol}^{-1} \text{ K}^{-1}$ from Table 2 and Ref. (5).

dipolar interactions, and thus $8\text{-Cy} \cdot \text{OH}^-$ is thermodynamically the most stable of the three complexes. As the ring size increases the conformational effect of the OH^- guest on the Cy rapidly decreases, and in the case of 8-Cy the observed entropy change on complexation is negligible. The standard error estimate for ΔS°_f in this case is at least $1.8 \text{ cal mol}^{-1} \text{ K}^{-1}$, as seen from Table 2; and thus ΔS°_f is within statistical uncertainty of zero. It should be emphasized that the conformational effect above refers to a difference in system ordering between 8-Cy and $8\text{-Cy} \cdot \text{OH}^-$ as compared to the difference in rigidity between 6-Cy and $6\text{-Cy} \cdot \text{OH}^-$ but does not imply any conformational trend from the species 6-Cy to the species 8-Cy.

^{13}C -NMR ASSIGNMENTS OF CYCLOOCTAAMYLOSE

The ^{13}C -nmr resonances of 8-Cy in D_2O solution are compared with corresponding published assignments for 6-Cy and 7-Cy in Table 4. Here we see that, in common with 6- and 7-Cy, the 8-Cy resonances at 102.68, 81.50, and 61.30 ppm are relatively remote from neighboring peaks; and so we assign them, respectively, to C-1, C-4, and C-6. The remaining methine 8-Cy resonances at 73.35, 73.96, and 72.84 ppm are closely spaced. We shall provisionally assign these to C-2, C-3, and C-5, respectively, by comparison with the known assignments of 7-Cy (2), and will confirm these assignments by NaOH titration and by experiments featuring deuterium-induced differential isotope shift (DIS) measurements analogous to those developed by Pfeffer *et al.* (7).

The DIS method seeks to discriminate ^{13}C -nmr resonances on the basis of proximity to acidic hydrogens which are capable of exchanging with deuterium in

TABLE 4
 ^{13}C CHEMICAL SHIFTS OF 6-Cy, 7-Cy, AND 8-Cy IN 99.8%
 D_2O SOLUTION^a

Carbon	δ_{OD}		
	6-Cy ^b	7-Cy ^c	8-Cy
1	102.5	102.87	102.68
2	72.8	72.10	73.35
3	74.4	74.11	73.96
4	82.3	82.16	81.50
5	73.1	72.87	72.84
6	61.5	61.35	61.30
SE ^d	—	0.02	0.01

^a ppm downfield from external Me_4Si at 30°C .

^b Ref. (6).

^c Ref. (2).

^d Standard error estimates derived from repeated measurements with $\sim 0.01 F$ Cy solutions.

solution. Since in the cycloamyloses the C-2 and C-3 carbons are hydroxylated while the C-5 carbon is not, we would expect by this method to select effectively the C-5 resonance from the other two. Because the resonance lines encountered here are so broad (0.2–0.3 ppm), single-line splitting measurements in a coaxial tube system as suggested by Pfeiffer *et al.* (7) are imprecise. Therefore, we adopted a method whereby ^{13}C -nmr data were obtained from a sample tube containing cycloamylose in D_2O to which H_2O was added in small increments. These experiments were carried out on a Bruker HX-270 spectrometer equipped with a 10-mm sample tube for proton-decoupled ^{13}C observation at 67.89 MHz (80° tip angle, 0.3-sec recycle time, 14-kHz spectral width, 8192 data points, and 2K–5K transients). The initial concentrations of D_2O (99.8%), 7-Cy (0.016 *F*), or 8-Cy (0.050 *F*) were thus ultimately reduced fourfold upon addition of H_2O in six increments.

Each observed resonance δ_{obs} is the concentration-weighted average of intrinsic chemical shifts from deuterated δ_{OD} and protonated δ_{OH} cycloamylose species. We assumed that the ratio of deuterated to protonated Cy concentrations to be the same as the solvent D to H ratio and so write the weighted average as

$$\delta_{\text{obs}} = X_{\text{H}}(\delta_{\text{OH}} - \delta_{\text{OD}}) + \delta_{\text{OD}} \quad [4]$$

where X_{H} is the mole fraction of H. For each resonance a simple linear regression of the observed ^{13}C -nmr chemical shift versus mole fraction of H yields values for both δ_{OH} and δ_{OD} . We would expect the values of δ_{OH} and δ_{OD} for the γ -hydroxylated C-5 carbon to be nearly the same but the $\delta_{\text{OH}} - \delta_{\text{OD}}$ difference to be much greater for the β -hydroxylated C-2 and C-3 carbons. To test this methodology and also to check for possible displacement of the deuterium lock signal upon dilution with H_2O , we made these DIS measurements with 7-Cy as well as 8-Cy. The results of our calculations appear in Table 5.

We note that carbons C-1 and C-4 which are γ -hydroxylated have negligible DIS

TABLE 5

^{13}C -nmr INTRINSIC CHEMICAL SHIFTS^a OF 7-Cy AND 8-Cy IN D_2O AND THEIR DISPLACEMENTS^b IN H_2O SOLUTION AT 30°C

Carbon	7-Cy		8-Cy	
	δ_{OD}	$\Delta\delta$	δ_{OD}	$\Delta\delta$
1	102.87 ± 0.00	0.00 ± 0.01	102.68 ± 0.00	0.00 ± 0.01
2	73.10 ± 0.00	0.14 ± 0.02	73.35 ± 0.01	0.12 ± 0.01
3	74.11 ± 0.01	0.14 ± 0.01	73.96 ± 0.01	0.14 ± 0.02
4	82.16 ± 0.01	0.00 ± 0.01	81.50 ± 0.00	0.00 ± 0.01
5	72.87 ± 0.01	0.02 ± 0.03	72.84 ± 0.00	0.01 ± 0.00
6	61.35 ± 0.01	0.11 ± 0.02	61.30 ± 0.01	0.11 ± 0.02

^a δ_{OD} , ppm downfield from external Me_4Si .

^b $\Delta\delta = \delta_{\text{OH}} - \delta_{\text{OD}}$, ppm.

^c Quoted uncertainties are standard error estimates based on the scatter of points about the least-squares line (Eq. [4]).

displacements in both 7-Cy and 8-Cy molecules. The C-6 (β -hydroxylated) resonance, however, is displaced by 0.11 ± 0.02 ppm in both molecules. Thus we conclude that there is a negligible displacement of the deuterium lock resonance due to varying H_2O/D_2O composition. Further, the 7-Cy resonance at 72.87 ppm, previously assigned to the unhydroxylated carbon C-5, is essentially unchanged (0.02 ± 0.03 ppm), whereas the resonances at 73.10 and 74.11 ppm, which have been assigned to C-2 and C-3, respectively, of 7-Cy (2), are displaced by 0.14 ± 0.02 and 0.14 ± 0.01 ppm, respectively. These latter two are consistent within experimental error with Pfeffer's estimates of both β - and γ -DIS effects. The corresponding behavior of the 8-Cy resonances is clear. The resonance at 72.84 ppm is unchanged and so must be C-5, while those at 73.35 and 73.96 ppm must be C-2 and C-3. Since the C-2 resonance is upfield from C-3 in both 6-Cy and 7-Cy, we assume the same is true in 8-Cy. Thus, we confirm the provisional assignments made above as well as those recently reported by Vincendon (3).

The displacements of ^{13}C -nmr peaks of 8-Cy upon reaction with NaOH are shown in Table 6. Results for the 6-Cy and 7-Cy homologs are included for comparison. In these experiments, ^{13}C -nmr measurements were made on 0.030 to 0.054 *F* 8-Cy solutions reacted with 0.00 to 0.11 *F* NaOH. The computational methods employed in deriving the listed parameters are described in Refs. (2) and (8). The behavior of 8-Cy observed here further confirms the assignments based on the DIS effect. The resonances of both C-2 and C-3 hydroxylated carbons are substantially displaced upon acid dissociation, in contrast to those of the unhydroxylated C-5 resonances. It is also interesting to note that the chemical shifts of hydroxylated C-6 peaks show lesser displacement, suggesting that the 2° OH-2 and OH-3 rather than the 1° OH-6 groups are involved in the ionization process. Finally, the large displacements of resonances assigned to C-1 and C-4 bridgehead

TABLE 6

INTRINSIC ^{13}C -nmr RESONANCES^a OF 6-, 7-, AND 8-Cy AND THEIR DISPLACEMENTS^b UPON ACID DISSOCIATION IN 5% D_2O

Carbon	6-Cy ^c		7-Cy ^c		8-Cy	
	δ	$\Delta\delta$	δ	$\Delta\delta$	δ	$\Delta\delta$
1	102.40	0.42	102.83	0.52	102.66	0.53
2	72.88	0.45	73.19	0.65	73.44	0.53
3	74.46	0.41	74.21	0.45	74.08	0.27
4	82.26	0.25	82.16	0.33	81.49	0.46
5	73.04	0.17	72.88	0.07	72.84	0.09
6	61.59	0.17	61.45	0.17	61.39	0.12
SE ^d	± 0.01	± 0.04	± 0.01	± 0.03	± 0.01	± 0.02

^a δ in ppm downfield from external Me_4Si at 30°C.

^b $\Delta\delta = \delta(Cy^-) - \delta(HCy)$.

^c Ref. (2).

^d Standard error estimates calculated as denoted in Refs. (2) and (8).

carbons in the Cy homologs may be ascribed to conformational changes resulting from the ionization reaction.

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