BIOORGANIC CHEMISTRY 9, 299–304 (1980)

Acid Dissociation of Cyclohexaamylose and Cycloheptaamylose

ROBERT I. GELB, LOWELL M. SCHWARTZ, JOHN J. BRADSHAW, AND DANIEL A. LAUFER

Department of Chemistry, University of Massachusetts, Boston, Massachusetts 02125

Received June 29, 1979

Acid dissociation constants of aqueous cyclohexaamylose (6-Cy) and cycloheptaamylose (7-Cy) have been determined at 10–47 and 25–55°C, respectively, by pH potentiometry. Standard enthalpies and entropies of dissociation derived from the temperature dependences of these pK_a's are $\Delta H^0 = 8.4 \pm 0.3$ kcal mol^{-1}, $\Delta S^0 = -28. \pm 1$ cal mol^{-1} °K^{-1} for 6-Cy and $\Delta H^0 = 10.0 \pm 0.1$ kcal mol^{-1}, $\Delta S^0 = -22.4 \pm 0.3$ cal mol^{-1} °K^{-1} for 7-Cy. Intrinsic ^{13}C nmr resonance displacements of anionic 6- and 7-Cy were measured at 30°C in 5% D_2O (v/v). These results indicate that the dissociation of 6- and 7-Cy involves both C2 and C3 2^0-hydroxyl groups. The thermodynamic and nmr parameters are discussed in terms of interglucosyl hydrogen bonding.

The complexation reactions and structural features of cycloamyloses have been widely studied in recent years (*1–3*). One reason for this research is the catalytic role played by cycloamyloses and derivatives in certain ester hydrolysis reactions (*4, 5*). Rate enhancements up to $\sim 10^6$, rivaling the catalytic activity of many enzymes systems, have been observed (*6*). The currently accepted catalytic mechanism involves loss of a proton from the cycloamylose molecule early in the reaction sequence, and Bender (*7*) has used the kinetics of such a reaction to estimate pK_a for cyclohexaamylose to be 12.1 at 25°C and ionic strength 0.2 *M*. Another ^{13}C nmr study has shown that the C2 and C3 resonances of hexa- and heptaamylose are particularly sensitive to pH (*8*), which suggests that the acid dissociation involves both OH-3 and OH-2 on the 2^0-hydroxyl rim of the cycloamylose molecule. In view of the widespread interest in the catalytic and complexation properties of cycloamyloses at pH ranges approaching their pK_a's, we have undertaken to determine precise pK_a values of both cyclohexaamylose (to be denoted as 6-Cy) and cycloheptaamylose (to be denoted 7-Cy). The temperature dependence of pK_a leads to values of the thermodynamic functions ΔH^0 and ΔS^0 of the acid dissociation reactions, and these values along with ^{13}C nmr resonance changes observed upon dissociation yield structural information about the anionic forms of cycloamyloses. By measuring the intrinsic ^{13}C resonance displacements of anionic 6- and 7-Cy in aqueous solutions undergoing gradual pH change, we also seek to resolve some of the uncertainties in the ^{13}C nmr assignments of these cycloamyloses.

0045-2068/80/030299-06$02.00/0

PROCEDURES

In principle, pH measurements recorded during the titration of Cy with NaOH reagent should yield pK_a values by straightforward and well-known calculational procedures. However, the experiment is complicated by the extreme acid weakness of Cy. Data must be recorded near pH 12 or 13 and in this region both the linearity of glass electrode response and the conventional pH meter standardization procedure are questionable. We have dealt with these two problems by the following measures.

To check the linearity of glass electrode response, we performed a calibration experiment which involved titration of pure water with NaOH reagent up to $[OH^-]$ of ~0.1 *M*. The pH = $(-\log a_{H+})$ at any point along this titration is expected to be

$$pH = f \log \gamma_{OH^-}[OH^-] - pK_w + pH_{cal},$$

where f is a proportionality constant normally equal to unity when the potentiometric system is Nernstian, γ_{OH^-} is the molar activity coefficient of OH^-, and pH_{cal} is the meter calibration offset which is normally zero when properly calibrated with a standard buffer. We plotted observed pH vs $\log \gamma_{OH^-}[OH^-]$ along the course of this titration having calculated γ_{OH^-} from the Debye–Hückel correlation with OH^- ion size parameter a_i of 0.35 nm and calculated $[OH^-]$ assuming volume additivity. In these and subsequent calculations we employed the Debye–Hückel equation in the form

$$-\log \gamma_i = \frac{A(I)^{1/2}}{1 + a_i B(I)^{1/2}},$$

which is appropriate to the range of ionic strengths in these experiments. The temperature-dependent values of A and B were taken from the Robinson and Stokes (9) tabulation. The plot of pH vs $\log \gamma_{OH^-}[OH^-]$ was linear up to ~0.1 *M* $[OH^-]$ and the slope f was unity within the estimated ±0.002 precision of our pH measurements. Therefore, we concluded that as long as we restricted our pH measurements to the appropriate range during the course of Cy titrations we could rely on proper Nernstian response of our electrodes, i.e., the so-called "salt" or "alkaline" errors would be negligible.

The second problem is that of finding a reliable buffer standard near pH 12. This difficulty was avoided by a calculational method described elsewhere (*10*). Briefly, the method regards the meter calibration pH_{cal} as an adjustable parameter along with pK_a and optimal values for both parameters are found which yielded the best fit of the model equations to the titration data. These equations included Debye–Hückel activity coefficient correlations for all ionic species with ion size parameters of 0.9, 0.35, and 1.6 nm for H^+, OH^-, and Cy^-, respectively. In the experiments, we took precautions to use the same glass and reference electrodes for all runs and flushed the solutions continuously with N_2 to exclude CO_2. Titration solutions were typically 0.02–0.05 *F* in the cycloamylose and the ionic strength varied between 0 and 0.1 *M*.

^{13}C nmr spectra were recorded of about eight 6-Cy and 7-Cy solutions in 5%

D_2O (v/v) and containing 0 to 0.1 *F* NaOH. With this gradual change of solution pH we were able to follow the displacement of each resonance line without ambiguity even in cases where crossover was observed. These measurements were made with a Varian CFT-20 spectrometer. Intrinsic chemical shifts were calculated for the six cycloamylose resonances of 6-Cy and 7-Cy and their anions as described previously (*11*).

RESULTS AND DISCUSSION

The results of our calculations of pK_a's, thermodynamic functions ΔH^0, ΔS^0, and the intrinsic ^{13}C chemical shifts and their displacements in the anions are given for both 6- and 7-Cy in Tables 1, 2, and 3, respectively. The calculational methods employed in deriving these values are detailed in Refs. (*10*) and (*11*). The uncertainties given in these tables represent standard error estimates and are discussed in the same references. In the present work, ΔH^0 and ΔS^0 values are derived from a weighted linear least-squares regression of pK_a's vs $1/T$. The scatter of values around the regression line was random, and so we conclude that ΔH^0 and ΔS^0 are essentially constant in the temperature range of our measurements.

We note from Table 2 that 7-Cy has a larger ΔH^0 value but a smaller negative ΔS^0 value with respect to 6-Cy. To help interpret these results we cite the acid dissociation of the isomers adenosine and 9-β-D-xylofuranosyladenine, *cis* and

TABLE 1

pK_a VALUES OF CYCLOHEXAAMYLOSE AND CYCLOHEPTAAMYLOSE AT VARIED TEMPERATURES AND THEIR UNCERTAINTIES

T (°C)	pK_a	SE
	Cyclohexaamylose	
10	12.649	±0.011
15	12.565	±0.014
25	12.332	±0.011
30	12.263	±0.012
40	12.083	±0.014
47	11.882	±0.014
	Cycloheptaamylose	
25	12.203	±0.006
25	12.201	±0.004
30	12.091	±0.008
30	12.088	±0.009
40	11.911	±0.004
50	11.675	±0.005
55	11.295	±0.010

TABLE 2

ΔH^0 AND ΔS^0 VALUES AND THEIR UNCERTAINTIES FOR THE ACID DISSOCIATION OF CYCLOHEXAAMYLOSE AND CYCLOHEPTAAMYLOSE[a]

	ΔH^0 (kcal mol^{-1})	ΔS^0 (cal mol^{-1} °K^{-1})
Cyclohexaamylose	8.36 ± 0.34	−28.3 ± 1.4
Cycloheptaamylose	9.98 ± 0.09	−22.4 ± 0.3

[a] Uncertainties represent SE estimates obtained by propagation of variance techniques applied to the weighted least-squares analysis of pK_a vs $1/T$ data.

trans with respect to the furanosyl OH-2′ and OH-3′ groups. The comparative thermodynamic parameter values are ΔH^0 of 9.7 ± 0.7 and 8.4 ± 0.2 kcal mol^{-1} and ΔS^0 of −24. ± 0.7 and −28.3 ± 0.8 cal mol^{-1} °K^{-1} for the *cis* and *trans* isomers, respectively (*12*). The larger ΔH^0 value associated with the adenosine seems to reflect the difficulty in removing a proton from the internally hydrogen-bonded structure compared with the *trans* isomer where internal hydrogen bonding is not possible. Similarly, the less negative ΔS^0 value of the *cis* isomer reflects a smaller change in solvent interaction due to bonding with the vicinal OH group. Another example of the same phenomenon is provided by the primary dissociation of malonic acid where the internally bonded ring structure of the monoanion results in a ΔS^0 value of about −13 cal mol^{-1} °K^{-1} compared with about −23 cal mol^{-1} °K^{-1} for most carboxylic acids (*13, 14*). Thus, the more positive ΔH^0 value for the dissociation of 7-Cy implies more effective internal hydrogen bonding in 7-Cy, presumably resulting in part from interglucosyl bonding between adjacent hydroxyl groups in the more flexible heptaamylose framework. The internal hydrogen bonding also tends to diminish hydrogen bonding with solvating water, and this might explain our observation that 7-Cy is

TABLE 3

INTRINSIC CHEMICAL SHIFTS AND THEIR DISPLACEMENTS IN THE ANIONS OF CYCLOHEXAAMYLOSE AND CYCLOHEPTAAMYLOSE[a]

	Cyclohexaamylose		Cycloheptaamylose	
Carbon	$\delta^{(6-Cy)a}$	$\Delta\delta^{(6-Cy-)}$	$\delta^{(7-Cy)}$	$\Delta\delta^{(7-Cy-)}$
1	102.40	0.42	102.83	0.52
2	72.88	0.45	73.19	0.65
3	74.46	0.41	74.21	0.45
4	82.26	0.25	82.16	0.33
5	73.04	0.17	72.88	0.07
6	61.59	0.17	61.45	0.17
SE	±0.01	±0.04	±0.01	±0.03

[a] $\delta \equiv$ ppm downfield from external TMS. $\Delta\delta^{(Cy-)} \equiv \delta^{(Cy-)} - \delta^{(Cy)}$.

about five times less soluble in water than 6-Cy at 25°C. Similarly, the less negative ΔS^0 value for 7-Cy conforms to the behavior expected for an intramolecularly bound anion. We therefore conclude that the degree of interglucosyl hydrogen bonding is greater in 7-Cy than in 6-Cy. The extent to which such bonding occurs in 6-Cy is difficult to evaluate, because the most direct comparison should be with glucose. However, this comparison is questionable, because OH-1 is the site of acid dissociation in glucose (*15*). Nevertheless, we note that the values of $\Delta H^0 = 7.7 \pm 0.3$ kcal mol^{-1} and $\Delta S^0 = -31.3 \pm 1.4$ cal mol °K^{-1} for the dissociation of glucose (*15*) are substantially different from those reported here for 6- and 7-Cy. Since the monomer by definition has no interglucosyl bonding, we attribute these differences to this phenomenon. It may be noteworthy that intramolecular hydrogen bonding between contiguous residues of 6-Cy and 7-Cy has also been detected in DMSO solutions by temperature dependent ^{1}H nmr measurements (*16, 17*).

^{13}C nmr displacements for the anions of both 6-Cy and 7-Cy are all in the downfield direction reflecting deshielding of the anions due to increased solvent interaction (*18, 19*). However, the data in Table 3 differ somewhat from those obtained in a previous study which compared ^{13}C nmr resonances at pD 7 to those at pD 14 in D_2O solvent (*8*). The displacements measured under those conditions are uniformly much larger than what we have observed. We ascribe this difference primarily to the effect of the high ionic strength on anionic Cy species at pD 14, which presumably causes additional deshielding of the anions by direct ion–ion interactions. In addition, our assignment of 7-Cy resonances reverses those of C2, C5 nuclei previously given by Smith *et al.* (*8*). We assign the peak at 73.19 ppm (73.1 ppm in Ref. (*8*)) to C2 because this carbon bears an acidic OH group and is likely to be pH sensitive. On the other hand, the resonance at 72.88 ppm (72.9 ppm in Ref. (*8*), formerly assigned to C2) is now assigned to C5, which does not bear an acidic OH and is not likely to be pH sensitive. The titrimetric behavior of 6-Cy resonances confirms previously published assignments of Smith *et al.* (*8*).

In general, our results agree with the earlier data (*8*) in that the comparable and relatively large displacements $\Delta\delta^{(Cy2)}$ and $\Delta\delta^{(Cy3)}$ in both 6-Cy and 7-Cy indicate involvement of both OH-2 and OH-3 in the dissociation process. The large C1 resonance displacements may be ascribed to conformational and electric field sensitivity of this anomeric carbon. In contrast to C2 and C3, the small displacements of C6 resonances confirm that the 1^0-hydroxyl groups are not substantially dissociated.

ACKNOWLEDGMENTS

We are indebted to Professor Elkan R. Blout, Department of Biological Chemistry, Harvard Medical School, for generously providing access to the CFT-20 NMR spectrometer. The support of the National Institute of General Medical Sciences, U.S. Public Health Service (GM 26004) is gratefully acknowledged.

REFERENCES

1. R. I. GELB, L. M. SCHWARTZ, R. F. JOHNSON, AND D. A. LAUFER, *J. Amer. Chem. Soc.* **101,** 1869 (1979) (other articles of this series are cited therein).
2. R. J. BERGERON, M. A. CHANNING, AND K. A. MCGOVERN, *J. Amer. Chem. Soc.* **100,** 2878 (1978).
3. D. J. WOOD, F. E. HRUSKA, AND W. SAENGER, *J. Amer. Chem. Soc.* **99,** 1735 (1977).
4. D. W. GRIFFITHS AND M. L. BENDER, *Advan. Catal.* **23,** 209 (1973).
5. M. L. BENDER AND M. KOMIYAMA, "Bioorganic Chemistry" (E. E. van Tameln, Ed.,) Vol. I, Chap. 2. Academic Press, New York, 1977.
6. M. F. CZARNIECKI AND R. BRESLOW, *J. Amer. Chem. Soc.* **100,** 7771 (1978).
7. R. L. VANETTEN, G. A. CLOWES, J. F. SEBASTIAN, AND M. L. BENDER, *J. Amer. Chem. Soc.* **89,** 3253 (1967).
8. P. COLSON, H. J. JENNINGS, AND I. C. P. SMITH, *J. Amer. Chem. Soc.* **96,** 8081 (1974).
9. R. A. ROBINSON AND R. H. STOKES, "Electrolyte Solutions," 2nd ed. Butterworths, London, 1965.
10. L. M. SCHWARTZ AND R. I. GELB, *Anal. Chem.* **50,** 1571 (1978).
11. R. I. GELB, L. M. SCHWARTZ, AND D. A. LAUFER, *J. Amer. Chem. Soc.* **100,** 5875 (1978).
12. J. J. CHRISTENSEN, J. H. RYTTING, AND R. M. IZATT, *J. Amer. Chem. Soc.* **88,** 5105 (1966).
13. S. N. DAS AND D. J. G. IVES, *Proc. Chem. Soc. London,* 373 (1961).
14. J. W. LARSON AND L. G. HEPLER, "Solute–Solvent Interactions" (J. F. Coetzee and C. D. Ritchie, Eds.), pp. 9–10. Dekker, New York, 1969.
15. R. M. IZATT, J. H. RYTTING, L. D. HANSEN, AND J. J. CHRISTENSEN, *J. Amer. Chem. Soc.* **88,** 2641 (1966).
16. B. CASU, M. REGGIANI, G. G. GALLO, AND A. VIGEVANI, *Tetrahedron* **22,** 3061 (1966).
17. M. ST-JACQUES, P. R. SUNDARARAJAN, K. J. TAYLOR, AND R. H. MARCHESSAULT, *J. Amer. Chem. Soc.* **98,** 4386 (1976).
18. G. E. MACIEL, J. W. MCIVER, JR., N. S. OSTLUND, AND J. A. POPLE, *J. Amer. Chem. Soc.* **92,** 1 (1970).
19. R. E. LONDON, T. E. WALKER, V. H. KOLLMAN, AND N. A. MATWIYOFF, *J. Amer. Chem. Soc.* **100,** 3723 (1978).