Carbohydrate Research, 118 (1983) 111–117
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

AQUEOUS COMPLEXATION CONSTANTS OF CYCLOHEXAAMYLOSE WITH *p*-IODOANILINE AND *p*-IODOANILINIUM ION*

ROBERT I. GELB[†], LOWELL M. SCHWARTZ, AND DANIEL A. LAUFER[†]
Department of Chemistry, University of Massachusetts at Boston, Boston, Massachusetts 02125 (U.S.A.)

(Received November 22nd, 1982; accepted for publication, January 23rd, 1983)

ABSTRACT

Formation constants for binary complexes of cyclohexaamylose (Cy) with *p*-iodoaniline (B) and *p*-iodoanilinium cation (BH^+) were determined by means of pH potentiometric experiments at 10–45°. The temperature dependences of these constants yielded the following thermodynamic parameters: $\Delta H°_{CyB} = -8.2 \pm 0.5$ kcal · mol^{-1}, $\Delta S°_{CyB} = -12.1 \pm 1.8$ cal · mol^{-1} · K^{-1}; and $\Delta H°_{CyBH^+} = -6.3 \pm 0.7$ kcal · mol^{-1}, $\Delta S°_{CyBH^+} = -7.6 \pm 2.2$ cal · mol^{-1} · K^{-1}. Enthalpic and entropic contributions to the overall complexation of BH^+ are discussed in terms of partial desolvation of the cationic center upon binding. A ^{13}C-n.m.r. investigation confirmed a configuration of the Cy · BH^+ complex reported earlier.

INTRODUCTION

Cyclohexaamylose (Cy) complexes with a variety of substrate species, and these adducts have been extensively studied in recent years[1]. Several theories have been proposed to rationalize bonding in these complexes. Among these are (a) a predominantly hydrophobic interaction resulting from the release of constrained solvent molecules both within the unbound Cy cavity and surrounding the uncomplexed substrate[2,3], and (b) a predominantly dipolar, or induced dipolar (van der Waals), or both, interaction between the Cy cavity and the substrate, but not involving appreciable solvent interactions[4–6]. Because these bonding mechanisms appear to differ substantially, we sought some further basis for understanding the relative roles of these interactions in Cy complexations. Very few of the substrate species studied recently were cationic; rather, Cy complexes of anionic and neutral species have received almost exclusive attention[1]. Because of the different solvent-ordering properties of aqueous cationic, neutral, and anionic species, it seemed likely that the extent of solvent participation in these reactions might be clarified by

---

*This work was supported by a grant (GM 26004) from the National Institute of General Medical Sciences, National Institutes of Health.

[†] To whom correspondence may be addressed.

0008-6215/83/$ 03.00

a careful study of a cationic complex. Among the few cationic substrate species that indeed have been examined is the *p*-iodoanilinium ion[7] (BH^+). However, the main focus of that work was the structure of the complex rather than the bonding mechanism. Also, the Cy complex of *p*-iodoaniline (B) has been the subject both of experimental and theoretical treatments[2] relating to bonding, but the essential thermodynamic properties of the complexations, ΔH° and ΔS°, are not available for either the Cy · B or Cy · BH^+ complex. In light of correlations recently noted[5,6] between ΔH° and ΔS° for Cy complexations, as well as a correlation between ΔH° and the ^{13}C-n.m.r. spectral properties of complexed Cy, we sought to determine these properties for the Cy · B and Cy · BH^+ complexes

RESULTS

Methodology. — The temperature-dependent formation constants of Cy · BH^+ and Cy · B, which would in turn yield estimates of ΔH° and ΔS° for these complexations, were determined with a pH potentiometric method that has been reported in detail previously[8]. Briefly, this method relies on a perturbation of the pH of a buffer solution of BH^+ and B upon addition of Cy. The data of pH values *vs.* added Cy were fitted to a set of model equations including mass- and charge-conservation equations, equilibrium-constant expressions, and activity-coefficient correlations. A small correction to the measured pH was applied to compensate for a junction potential induced by the presence of Cy in the measurement solutions. In these calculations, we estimated ionic-activity coefficients from the Debye–Hückel correlation by use of the temperature-dependent coefficients given by Robinson and Stokes[9]. Ion-size parameters of 0.9, 0.8, and 1.6 nm were estimated for the H^+, BH^+, and Cy · BH^+ ions, respectively. The set of coupled, nonlinear equations was solved numerically by means of an interactive computer-program that seeks rms "best fit" values for K_{CyBH^+} and K_{CyB}, the formation constants for the Cy · BH^+ and Cy · B complexes, respectively, and K_a the acidic dissociation constant for BH^+. The calculation also yielded standard-error estimates for these parameters, which values appear in Table I. The rms "fit" of the experimental data to the model equations was always less than 0.001 pH unit, and the pattern of residuals, differences between experimental and calculated data, appeared to be random and free from systematic trend[10]. Consequently, we concluded that the proposed model equations featuring only binary Cy · BH^+ and Cy · B complexes accurately represent the physicochemical behavior under our experimental conditions, and that the equilibrium-constant values obtained represent reasonably accurate estimates of these parameters.

The 25° value of $1.1 \cdot 10^3$ for K_{CyBH^+} is in accord with an earlier estimate of $\sim 10^3$ (ref. 7). However, our value of $(2.8 \pm 0.2) \cdot 10^3$ for K_{CyB} is substantially at variance with the estimate of $\Delta G^\circ = -5.9$ kcal · mol^{-1} ($K_{CyB} = 2 \cdot 10^4$) quoted by Tabushi *et al.*[2]. The standard enthalpies and entropies of complexation, as shown in Table II, were calculated from Van 't Hoff plots of the temperature-dependent

TABLE I

EQUILIBRIUM CONSTANTS CALCULATED FOR THE SYSTEM OF *p*-IODOANILINE, *p*-IODOANILINIUM ION, AND CYCLOHEXAAMYLOSE

Temp. (°)	K_{CyB}[a] ($\times 10^{-3}$)	K_{CyBH^+}[a] ($\times 10^{-3}$)	pK_a[b]
10	(5.2 ± 0.3)[c]	(1.7 ± 0.1)[c]	4.017 ± 0.003[c]
20	(3.8 ± 0.3)	(1.5 ± 0.1)	3.840 ± 0.003
25	(2.8 ± 0.2)	(1.1 ± 0.1)	3.780 ± 0.003
30	(2.3 ± 0.1)	(1.02 ± 0.08)	3.700 ± 0.003
40	(1.4 ± 0.1)	(0.66 ± 0.07)	3.562 ± 0.003
45	(1.0 ± 0.1)	(0.52 ± 0.05)	3.502 ± 0.003

[a] Complex formation constants of cyclohexaamylose with substrates B or BH^+. [b] Acid dissociation constant of *p*-iodoanilinium ion. [c] Standard error estimates made by the nonlinear regression calculation and based on estimated standard deviations of 0.002 pH unit and 0.2 mg in the pH and gravimetric data, respectively.

equilibrium constants given in Table I. The results for B complexation differ substantially from the theoretical estimates[2] of -7.35 kcal · mol^{-1} and $+5.5$ cal · mol^{-1} · K^{-1} for $\Delta H°$ and $\Delta S°$, respectively, which are derived from a bonding model heavily weighted by hydrophobic interactions. Before interpreting the data reported in Table II, we will describe the results of the ^{13}C-n.m.r. spectrometric experiments.

^{13}C-N.m.r. spectrometric measurements. — Chemical shifts were recorded for seven solutions containing 5% D_2O (v/v aqueous), *p*-iodoanilinium ion (0–0.100 *F*), cyclohexaamylose (0–0.155 *F*), and hydrochloric acid (~0.1 *F*) at 30°. Intrinsic chemical shifts for the unbound Cy and BH^+, and for the Cy · BH^+ complex were calculated from these observations and from the potentiometrically determined K_{CyB} and K_{CyBH^+} values (30°) by a procedure described earlier[11] (see Table III, where δ_0 refers to the intrinsic shift of the unbound species and $\Delta\delta$ to the displacement, *i.e.*, the difference between the chemical shift in the complex and δ_0). A substantial displacement of the C-6 resonance in Cy and a major deshielding effect at C-1 of BH^+may be observed. These indicate that the substrate is deeply

TABLE II

$\Delta H°$ AND $\Delta S°$ VALUES FOR CYCLOHEXAAMYLOSE COMPLEXATION WITH *p*-IODOANILINE AND *p*-IODOANILINIUM ION, AND FOR ACID DISSOCIATION OF *p*-IODOANILINIUM ION

Reaction	$\Delta H°$ (*kcal · mol^{-1}*)	$\Delta S°$ (*cal · mol^{-1} · K^{-1}*)
$B + Cy = Cy \cdot B$	−8.2 ± 0.5[a]	−12 1 ± 1.8[a]
$BH^+ + Cy = Cy \cdot BH^+$	−6.3 ± 0.7	−7.6 ± 2.2
$BH^+ + H_2O = B + H_3O^+$	6.0 ± 0.1	3.0 ± 0.3

[a] Standard error estimates based on scatter of points about the least squares Van 't Hoff lines.

TABLE III

DISPLACEMENTS OF ^{13}C-N.M.R. RESONANCES IN CYCLOHEXAAMYLOSE AND *p*-IODOANILINIUM ION UPON COMPLEXATION

Atom	*Cyclohexaamylose*		*p-Iodoanilinium ion*	
	δ_0(*p.p.m.*)^a	$\Delta\delta$(*p.p.m.*)^b	δ_0(*p.p.m.*)^a	$\Delta\delta$(*p.p.m.*)^b
C-1	102.41	0.41	131.16	1.17
C-2	72.89	−0.11	140.22	−0.16
C-3	74.51	0.47	126.03	0.03
C-4	82.27	0.26	94.98	0.05
C-5	73.10	−0.10		
C-6	61.64	−0.31		
SE^c	0.01	0.02	0.01	0.02

^{a}Intrinsic chemical shift of unbound species. ^{b}Displacement of chemical shift upon complexation. ^{c}Standard error estimates are based on an uncertainty of 0.01 p.p.m. in the observed resonances.

included within the Cy cavity[5], and this is consistent with the structure of the complex proposed earlier by Wood *et al.*[7] that places the NH_3^+ group within the wide rim of Cy and the iodo substituent at the narrow rim. It is possible that the large deshielding of the Cy C-3 (0.47 p.p.m.) is indicative of a hydrogen-bonding interaction between the NH_3^+ and OH-3 groups.

DISCUSSION

Cy · B complexation. — A recently reported correlation between ΔH° and ΔS° for cyclohexaamylose complexations[5] of twenty substrates included such widely variate species as aliphatic and aromatic carboxylic acids, nitrophenols, cyanophenols, and their anions. Since we have found[6] that a number of commonly employed solvent molecules also form Cy complexes that fit the correlation, as do Cy complexes of perchlorate, iodide, and thiocyanate ions[12]. If these recent findings are included, the new least-squares, regression line becomes Eq. *1*,

$$\Delta H^\circ = (406 \pm 15)\Delta S^\circ - (1.2 \pm 0.2) \cdot 10^3 \text{ cal} \cdot \text{mol}^{-1} \qquad (1)$$

which is shown plotted in Fig. 1, and where the circles are drawn representing the complexes involved. The ΔH° *vs.* ΔS° value of the Cy · B complex is depicted in Fig. 1 as a square and it deviates somewhat from the correlation relating to a total of 31 electrically neutral or anionic substrate-species. Nevertheless, we concluded that bonding in the Cy · B complex is related primarily to dipolar and induced dipolar interactions between the substrate and the Cy cavity. The large negative ΔS° value of -12.1 ± 1.8 cal · mol^{-1} · K^{-1} for Cy · B formation seems to preclude any substantial contribution of solvent-release effects to the overall free energy of the reac-

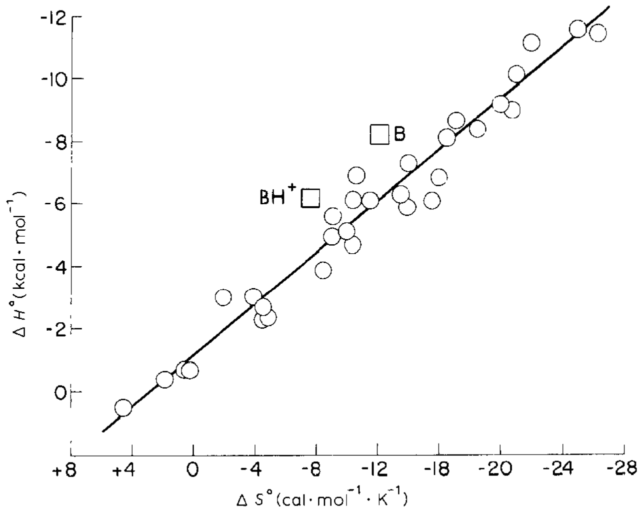

Fig. 1. Correlation of ΔH° *vs* ΔS° parameters for cyclohexaamylose complexations. The line drawn represents a least-squares fit ($\Delta H^\circ = 406\Delta S^\circ - 1.2 \times 10^3$ cal · mol^{-1}) to 31 substrate systems denoted by circles. The points denoted by squares refer to the *p*-iodoaniline complexes and are not included in this correlation.

tion. Instead, we associate the increased ordering in the complex primarily with torsional restriction of Cy resulting from the bonding interaction.

Cy · BH⁺ Complexation. — As an ancillary result of experiments on Cy · BH$^+$complex formation, the standard enthalpy and entropy of the acidic dissociation reaction of BH$^+$ were determined. Of particular interest is the value of ΔS° of +3.0 cal · mol^{-1} · K^{-1} which is typical of substituted anilinium ions[13]. This entropy of dissociation is considerably more positive than those of electrically neutral acids, and this behavior is readily interpreted in terms of solvent ordering about both the solvated hydronium and anilinium ions. The dissociation process simply exchanges ordered solvent from BH$^+$ to H_3O^+, leaving the net-configuration entropy changed very little. That water molecules are highly ordered in the region of the NH_3^+ group will be useful in the following discussion.

The values of ΔH° and ΔS° for the Cy · BH$^+$ complex formation determined (see Table II) represent a deviation from the correlations noted in the previous section. The observed ΔS° value is too positive for the observed ΔH° value. This observation, along with a number of other factors detailed below, leads to an interesting but admittedly speculative interpretation of these observations. Let us imagine that the complexation reaction consists of two distinct, stepwise processes. The first of these involves insertion of the iodo end of the substrate into the Cy cavity, in accord with the structural analysis given by Wood *et al.*[7]. The key feature of this first-stage complexation is the insertion of the electrically neutral *p*-iodophenyl moiety into the Cy cavity without substantial disruption of the solvation sphere of the charged ammonium group. This process is then followed by a second stage, in which a portion of the ammonium solvation sphere is expelled. Presumably, this second-stage process would have little effect on the Cy-host structure, but would contribute to the observed ΔH° and ΔS° values for the overall complexation. In particular, ΔH_2° and ΔS_2° values for the second-stage process would both be expected to have more positive values, a consequence of the breakdown of the tight solvation sphere or "ice" structure which supposedly surrounds the cationic species.

In an attempt to separate the two steps of the complexation process, we invoked a correlation (2) that we had noted between $\Delta\delta^{\mathrm{Cy1}}$ and ΔH° for twenty Cy-complexation reactions[5].

$$\Delta\delta^{\mathrm{Cy1}} = (-0.038 \pm 0.004)\Delta H^\circ + (0.09 \pm 0.03) \tag{2}$$

The value $\Delta\delta^{\mathrm{Cy1}}$ (in p.p.m.) represents the displacement on complexation of the intrinsic chemical shift of the ^{13}C-n.m.r. resonance of C-1 of Cy. This correlation is derived from a wide variety of chemically differing substrate-species included in the Cy cavity, and might reasonably be expected to describe the aforementioned first-stage complexation. In particular, charged substrates, such as benzoate and *p*-nitrophenolate ions, have adduct structures wherein the charged groups are external to the Cy cavity and conform to the correlation (2) within statistical scatter. Therefore, *2* may be used to estimate the value of $\Delta H_1^\circ \cong -8.4$ kcal · mol^{-1} for the first stage of the complexation process from the observed value of $\Delta\delta^{\mathrm{Cy1}}$ 0.41 shown in Table III. The ΔH° to ΔS° correlation (Eq. *1*) then leads to $\Delta S_1^\circ \cong -18$ cal · mol^{-1} · K^{-1} for the initial stage of the complexation. Finally, these estimates yield $\Delta H_2^\circ \approx 2.1$ kcal · mol^{-1} and $\Delta S_2^\circ \approx 10$ cal · mol · K^{-1} for the second-stage desolvation of the cationic center.

While these estimates are not likely to be highly accurate because of uncertainties in the data, the correlation, and the underlying assumptions, they do yield positive values for both enthalpy and entropy changes of solvent release, as is expected, and so this analysis appears to be reasonable. Although this two-stage mechanism is admittedly speculative, the calculations are given here for two reasons. The first of these is to illustrate that, even in this case where solvent interactions with a cationic site seem to play a significant role in the complexation, these interactions constitute only a minor part of ΔG° for the overall reaction. The complexation appears to be primarily controlled by the substantial decreases in enthalpy ($\Delta H_1^\circ \cong -8$ kcal · mol^{-1}) and entropy ($\Delta S_1^\circ \cong -18$ cal · mol^{-1} · K^{-1}), respectively, which is connected with dipolar-induced dipolar interactions between the Cy host and *p*-iodoanilinium guest, and by the conformational constraint of the macrocyclic system upon binding. Secondly, the aforementioned calculations serve as a useful hypothesis for further study of the solvent's role in Cy complexations. In particular, similar factors may operate in Cy · B, but relevant ^{13}C-n.m.r. experiments are precluded by solubility limitations.

EXPERIMENTAL

Cyclohexaamylose samples (Aldrich Chemical Co., Inc., Milwaukee, WI 53233) were aerated for several days before use. Prepared in this way, the cycloamylose consisted of the hexahydrate form as determined by heating (~110°) to constant weight in a vacuum oven. All other materials were reagent grade. pH-Measurements employed conventional glass and either external calomel or silver–silver chloride reference electrodes, and were standardized with 0.05M potassium hydrogenphosphthalate buffer at the measurement temperature. Electrodes were always equilibrated at the temperature of measurement until no thermal drift (<0.001 pH value) was detectable for a period of 20–30 min, a time period comparable to the duration of subsequent experiments. Readings were obtained after equilibration periods of 2–3 min subsequent to addition of cyclohexaamylose or substrate samples.

^{13}C-N.m.r. measurements were performed with a Bruker HX-270 instrument operating at 67.89 MHz for ^{13}C detection. Instrument parameters of 30° tip-angle, 1.3-s recycle time, and 2–10 k transients were used to observe solutions contained in 10-mm sample tubes that were thermostated at 30 ± 1°.

ACKNOWLEDGMENTS

The experiments with the Bruker HX-270 instrument were performed at the NMR facilities of the Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts. The NMR facility is supported by Grant 00995 from the Division of Research of the National Institutes of Health and by the National Science Foundation under Contract C-76D.

REFERENCES

1 W. Saenger, *Angew. Chem., Int. Ed Engl.*, 19 (1980) 344–362

2 I. Tabushi, Y. Kiosuke, T. Sugimoto, and K. Yamamura, *J. Am Chem. Soc.*, 100 (1978) 916–919.

3 M. Komiyama and M L. Bender, *J. Am. Chem. Soc.*, 100 (1978) 2259–2260.

4 R. J. Bergeron, M. A. Channing, G. J. Gibeily, and D. M. Pillor, *J. Am. Chem. Soc.*, 99 (1977) 5146–5151.

5 R. I. Gelb, L. M. Schwartz, B. Cardelino, H. S. Fuhrman, R. F. Johnson, and D. A. Laufer, *J Am. Chem. Soc.*, 103 (1981) 1750–1757.

6 R. I. Gelb, L. M. Schwartz, M. Radeos, R. B. Edmonds, and D. A. Laufer, *J. Am. Chem. Soc.*, 104 (1982) 6283–6288.

7 D. J. Wood, F. E. Hruska, and W. Saenger, *J. Am. Chem. Soc.*, 99 (1977) 1735–1740.

8 R. I. Gelb, L. M. Schwartz, R. F. Johnson and D. A. Laufer, *J. Am Chem. Soc.*, 101 (1978) 1869–1874.

9 R. A. Robinson and R. H. Stokes, *Electrolyte Solutions*, 2nd edn., Butterworths, London, 1965.

10 L. M. Schwartz and R. I Gelb, *Anal. Chem*, 50 (1978) 1571–1576.

11 R I. Gelb, L. M. Schwartz, and D. A. Laufer, *J. Am Chem Soc.*, 100 (1978) 5875–5879.

12 R I Gelb, L M Schwartz, M. Radeos, and D. A Laufer, *J. Phys Chem.*, 87 (1983) in press.

13 J. W. Larson, and L. G. Hepler, in J. F. Coetzee and C. D. Ritchie (Eds.), *Solute–Solvent Interactions*, Marcel Dekker, New York, 1969.