# Complexation of Carboxylic Acids and Anions by Alpha and Beta Cyclodextrins

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**Abstract.** A pH potentiometric method is used to measure complex formation constants of aqueous alpha- and/or beta-cyclodextrin with several carboxylic acids and carboxylate anions: butyric acid/butyrate; valeric acid/valerate; hexanoic acid/hexanoate; octanoic acid/octanoate; decanoic acid/decanoate; cyclohexanecarboxylic acid/cyclohexanecarboxylate and benzoic acid/benzoate. Standard enthalpies and entropies of complex formation are calculated from the temperature dependencies of the equilibrium constants. These thermodynamic parameters of the alpha-cyclodextrin complexes largely conform to a correlation based on complexes with other substrate species previously reported. Both standard enthalpies and entropies of formation of beta-cyclodextrin complexes are found to be more positive than the corresponding complexes of alpha-cyclodextrin with the same substrates. These observations lead to insights into the bonding mechanism of cyclodextrin complexation.

**Key words.** Cyclodextrin, formation constant, carboxylic acid guests.

## 1. Introduction

Cyclohexaamylose ($\alpha$-cyclodextrin) and cycloheptaamylose ($\beta$-cyclodextrin) which we denote by 6-Cy and 7-Cy, respectively, form inclusion complexes with a wide variety of substrate molecules and ions [1, 2]. In spite of many reports of the structural [3, 4], thermodynamic and dynamic [5–9] properties of these complexes, the nature of the binding mechanism between host and substrate apparently remains unclear. The most frequently cited binding mechanisms [1] are (a) increased solvent–solvent interaction following inclusion of a hydrophobic solute substrate within the cycloamylose cavity, (b) the expulsion of 'high energy' water molecules from within the cycloamylose cavity upon inclusion of a substrate, and (c) dipolar or induced dipolar interactions between the cavity and the substrate. Also, it is generally assumed that complexations of the structurally similar cycloamyloses have similar bonding mechanisms and that differences in complex stabilities are simple consequences of differing cavity sizes, i.e. bulky substrate species unable to penetrate the 6-Cy cavity form more stable complexes with the larger 7-Cy molecule. However, we have noted [10] an apparent correlation between $\Delta H^0$ and $\Delta S^0$ for many 6-Cy complexes having substantially negative standard entropies of complexation. We concluded from this observation that there is a logical connection between the negative $\Delta S^0$ values and rigidity in the 6-Cy host induced by inclusion of the substrate. This hypothesis seems consistent with another observed correlation

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between both $\Delta H^0$ and $\Delta S^0$ and the displacement with complexation of the $^{13}C$ NMR resonance line of the anomeric carbon atom (C1) of 6-Cy [10]. However, there has been no report in the literature of similar correlations involving 7-Cy complexations.

The present study seeks to investigate comparative complexations of 6-Cy and 7-Cy with common substrates in hopes of obtaining further insights into cycloamylose/substrate binding mechanism. In particular our experiments were designed to yield complexation reaction thermodynamic parameters by measuring the temperature dependencies of complexation constants for both 6-Cy and 7-Cy with a series of alkanoic and related carboxylic acids. We measured complexation equilibrium constants by a pH potentiometric method described previously [11–13]. pH measurements were made on aqueous solutions initially containing a conjugate acid–base buffer pair, to be denoted by the generic symbols HB and $B^-$. As cycloamylose is added to such a solution, complexes may form with either or both buffer components and the buffer/$H^+$ equilibrium is shifted to an extent which depends on the difference in stabilities of the HB and $B^-$ complexes. Our experimental procedure involved adding weighed portions of solid cycloamylose to the buffer solution, waiting for thermal and chemical equilibrium after each addition, and measuring the pH. Then computerized analysis of the data provided equilibrium contants for all inclusion complexes that formed.

## 2. Experimental

Cyclohexaamylose and cycloheptaamylose reagents were obtained from Aldrich Chemical Company. The 6-Cy reagent was pretreated by exposure to the atmosphere for about one week in order to convert any under-hydrated crystals to the hexahydrate form. Samples of this aerated material were tested for hydration level by prolonged vacuum drying at 100°C. The 6-Cy was used only if this test confirmed the hexahydrate configuration. The 7-Cy reagent was twice recrystallized from water and air dried for one week to prepare the octahydrate. Carboxylic acid buffer solutions were prepared by dissolving weighed samples of reagent grade acids and then partial neutralization with standardized NaOH. The analytical concentrations of the buffers ranged from about 2 mM for butyric acid/butyrate anion to about 0.2 mM for decanoic acid/decanate.

pH measurements were made with an Orion Model 801 pH meter equipped with conventional glass and reference electrodes. The pH meter was standardized with 0.05 M phosphate buffers whose temperature dependent pH values were taken from Bates [14]. pH data were recorded only after thermal equilibration was achieved as indicated by stability to $\pm 0.001$ pH for ten minutes. In typical experiments 8 to 10 weighed portions of cycloamylose were added such that the final concentrations of cycloamylose reached 0.02 M for 6-Cy or 0.01 M for 7-Cy.

## 3. Calculational Procedures

Although we have described our data analysis method previously, a summary is as follows: The recorded data are pH vs. added weight of cycloamylose. Using a nonlinear regression technique these are fitted to a set of model equations consisting

of mass and charge conservation equations, equilibrium constant expressions, an activity coefficient correlation, and small correction terms. These latter include (a) an increase in the solution volume due to the addition of solid cycloamylose, and (b) an empirically determined correction to the pH reading (only a few thousandths of a pH unit) as a function of cycloamylose concentration which is presumably due to changes in the liquid junction potential at the reference electrode. The Debye–Hückel activity coefficient correlation utilized temperature-dependent parameters published by Robinson and Stokes [15] and the following ion-size parameter values in nm:

| | | | |
|---|---|---|---|
| hydronium | 0.9 | benzoate | 0.6 |
| hydroxide | 0.35 | cyclohexanecarboxylate | 0.6 |
| butyrate | 0.6 | 6-Cy binary complex ions | 1.2 |
| valerate | 0.65 | 7-Cy binary complex ions | 1.4 |
| hexanoate | 0.7 | 6-Cy ternary complex ions | 1.6 |
| octanoate | 0.8 | 7-Cy ternary complex ions | 1.8 |
| decanoate | 1.0 | | |

The calculational procedure admitted the possible existence of both binary and ternary complexes. The binary complexes will be denoted HBCy or BCy when the substrate is HB or $B^-$, respectively, and similarly the ternary complexes will be denoted $HBCy_2$ or $BCy_2$. The formation constant of these complexes are $K_{HBCy}$, $K_{BCy}$, $K_{HBCy_2}$ and $K_{BCy_2}$, where the ternary constants are understood to be stepwise formations from the corresponding binaries, e.g. $K_{BCy_2}$ refers to the reaction $BCy + Cy = BCy_2$. In the nonlinear regression, each equilibrium constant, including the buffer acid dissociation constant, is treated as an adjustable parameter. Thus a particular model equation may adjust as many as five parameters in seeking a fit to the data. With this large number it was necessary to adopt a procedure which can identify the minimum number of equilibrium constants required for an adequate fit. Without such a procedure the nonlinear regression routine could calculate specious results, perhaps yielding formation constants for non-existent complexes. For this purpose we calculated a 'joint parametric uncertainty interval' (JPUI) [16] for each parameter fitted by a regression calculation. This statistic measures the uncertainty of a particular parameter when fitted along with other parameters and takes into account the increased uncertainty due to statistical correlation among all parameters. We illustrate the procedure by considering the 7-Cy complexes with cyclohexanecarboxylic acid and anion at 40°. The experimental data consisted of 11 points and were fitted adequately with four adjustable parameters: the $HB/B^-$ dissociation constant, and complex formation constants for HBCy, BCy and $BCy_2$. The latter complex is weak, having a $K_{BCy_2}$ value of 8.6 and a standard error estimate of 0.32. The JPUI statistic for this parameter is 2.6 based on 11 data points and 4 fitted parameters [16]. This interval is symmetric about $K_{BCy_2}$ and so the JPU limits are 7.3 and 9.9. Because these limits do not include zero, we confirm the existence of the $BCy_2$ complex of 7-Cy with cyclohexanecarboxylate ion and enter this complex in Table II.

In contrast to this case the 7-Cy complexes with butyrate and valerate anions both yielded very low $K_{BCy}$ values. The calculated JPU limits in both cases included zero. Thus we concluded that these very weak complexes may be specious, omitted the equilibrium constants from the model equations, recalculated the nonlinear regressions and reported the final results in Table II.

Table I. Complex formation constants of cyclohexaamylose (6-Cy) with various carboxylic acid and carboxylate ion substrates

| Temp. °C | Binary complexes | | Ternary complexes | | Fit $10^{-3}$ pH |
|---|---|---|---|---|---|
| | $K_{HBCy}$ | $K_{BCy}$ | $K_{HBCy_2}$ | $K_{BCy_2}$ | |
| | | | Butyric acid/butyrate ion | | |
| 10 | 226 ± 2.3, 10[a] | 17.2 ± 0.7, 3.2 | | | 0.5 |
| 25 | 135 ± 1.2, 5 | 15.4 ± 0.4, 1.8 | | | 0.5 |
| 40 | 88.3 ± 1.4, 6 | 13.6 ± 0.4, 2.6 | | | 0.6 |
| | | | Valeric acid/valerate ion | | |
| 10 | 1029 ± 7.0, 31 | 95.7 ± 1.2, 5.4 | | | 1.0 |
| 20 | 669 ± 1.3, 5 | 82.3 ± 0.2, 1.0 | | | 0.2 |
| 25 | 533 ± 2.4, 11 | 75.8 ± 0.6, 2.4 | | | 0.4 |
| 40 | 300 ± 2, 11 | 63.5 ± 0.2, 0.7 | | | 1.4 |
| 45 | 258 ± 1.5, 6 | 59.3 ± 0.8, 3.2 | | | 1.2 |
| | | | Hexanoic acid/hexanoate ion | | |
| 10 | 1706 ± 58, 258 | 297 ± 13, 58 | | | 2.5 |
| 25 | 810 ± 41, 167 | 211 ± 12, 48 | | | 3.9 |
| 40 | 441 ± 16, 64 | 162 ± 7, 27 | | | 1.8 |
| 45 | 388 ± 17, 61 | 154 ± 8, 29 | | | 2.7 |

| | Octanoic acid/octanoate ion | | | | |
|---|---|---|---|---|---|
| 10 | $(5.08 \pm 0.35, 1.8) \times 10^3$ | $(2.28 \pm 0.12, 0.60) \times 10^3$ | $(1.89 \pm 0.08, 0.43) \times 10^3$ | $51.4 \pm 2.7, 14$ | 1.0 |
| 30 | $(1.42 \pm 0.05, 0.23) \times 10^3$ | $622 \pm 26, 123$ | $415 \pm 5, 25$ | $24.4 \pm 1.1, 5.1$ | 1.7 |
| 45 | $567 \pm 1.2, 5.7$ | $257 \pm 8, 39$ | $143 \pm 9, 43$ | $12.0 \pm 2.0, 9.6$ | 1.4 |
| | Decanoic acid/decanoate ion[b] | | | | |
| 20 | $3.1 \times 10^3$ | $1.8 \times 10^3$ | $3.3 \times 10^3$ | $0.49 \times 10^3$ | 1.0 |
| 30 | $1.3 \times 10^3$ | $0.66 \times 10^3$ | $1.4 \times 10^3$ | $0.35 \times 10^3$ | 1.9 |
| 45 | $0.84 \times 10^3$ | $0.55 \times 10^3$ | $0.61 \times 10^3$ | $0.17 \times 10^3$ | 3.3 |
| | Cyclohexanecarboxylic acid/cyclohexanecarboxylate ion | | | | |
| 15 | $87.1 \pm 0.5, 1.4$ | | | | 1.8 |
| 25 | $50.2 \pm 0.3, 1.0$ | | | | 0.9 |
| 30 | $38.8 \pm 0.3, 0.8$ | | | | 1.7 |
| 40 | $23.9 \pm 0.2, 0.5$ | | | | 1.8 |
| 50 | $13.9 \pm 0.2, 0.6$ | | | | 2.7 |

[a] Two statistical uncertainties are quoted. The first is the standard error estimate, the second is one-half of the joint parametric uncertainty interval.

[b] No statistical uncertainties are cited for the decanoic acid/decanoate ion complex formation constants because of the inadequacies of the model equations used in the calculations.

## 4. Results and Discussion

We list in Tables I and II those equilibrium constant values which comprise the minimum, sets of adjustable parameters needed to adequately fit the experimental data of each experiment. A pair of statistical uncertainty estimates is quoted with most entries. Following the '$\pm$' sign the first of the pair is the standard error estimate and following a comma the second is one-half the JPUI estimate. The

Table II. Complex formation constants of cycloheptaamylose (7-Cy) with various carboxylic acid and carboxylate ion substrates

| Temp. °C | Binary complexes | | Ternary complex | Fit $10^{-3}$ pH |
|---|---|---|---|---|
| | $K_{HBCy}$ | $K_{BCy}$ | $K_{HBCy_2}$ | |
| | Butyric acid/butyrate ion | | | |
| 10 | $42.8 \pm 0.3, 1.1$[a] | | | 0.4 |
| 25 | $32.4 \pm 0.1, 0.9$ | | | 0.2 |
| 40 | $25.1 \pm 0.7, 2.5$ | | | 2.3 |
| | Valeric acid/valerate ion | | | |
| 10 | $118.6 \pm 2.0, 8.6$ | | | 1.5 |
| 20 | $106.3 \pm 1.5, 6.4$ | | | 0.9 |
| 25 | $90.3 \pm 2.2, 9.5$ | | | 3.2 |
| 40 | $68.6 \pm 1.5, 5.4$ | | | 3.0 |
| 45 | $59.6 \pm 2.5, 8.2$ | | | 6.3 |
| | Hexanoic acid/hexanoate ion | | | |
| 10 | $349 \pm 15, 76$ | $64.6 \pm 7, 37$ | | 0.9 |
| 25 | $293 \pm 5, 23$ | $67.6 \pm 1.8, 10$ | | 0.6 |
| 40 | $266 \pm 0.5, 2.1$ | $81.5 \pm 1.1, 4.7$ | | 1.2 |
| 45 | $228 \pm 7, 29$ | $63.5 \pm 3.4, 14$ | | 1.2 |
| | Octanoic acid/octanoate ion | | | |
| 10 | $1860 \pm 27, 160$ | $687 \pm 2, 11$ | $26 \pm 4, 25$ | 1.9 |
| 20 | $1651 \pm 3.7, 17$ | $683 \pm 8, 36$ | $32 \pm 2, 7$ | 0.7 |
| 30 | $1594 \pm 49, 224$ | $752 \pm 29, 130$ | $29 \pm 2, 7$ | 2.2 |
| 45 | $1023 \pm 28, 121$ | $517 \pm 21, 88$ | $22 \pm 2, 8$ | 4.1 |
| | Decanoic acid/decanoate ion | | | |
| 20 | $(10.9 \pm 1.3, 5.2) \times 10^3$ | $(7.5 \pm 0.9, 3.6) \times 10^3$ | $9.6 \pm 0.9, 3.6$ | 2.0 |
| 30 | $(8.1 \pm 0.1, 0.4) \times 10^3$ | $(6.0 \pm 0.1, 0.4) \times 10^3$ | $34 \pm 0.7, 2.5$ | 1.3 |
| 45 | $(4.9 \pm 0.3, 1.2) \times 10^3$ | $(3.9 \pm 0.2, 0.7) \times 10^3$ | $39 \pm 0.5, 2.0$ | 0.6 |
| | Cyclohexanecarboxylic acid/cyclohexanecarboxylate ion | | | |
| 15 | $5618 \pm 6, 23$ | $294 \pm 3, 12$ | $5.8 \pm 1, 4$ | 0.9 |
| 20 | $5143 \pm 35, 211$ | $262 \pm 0.3, 1.7$ | $9.0 \pm 1.1, 6.5$ | 1.2 |
| 30 | $3369 \pm 66, 281$ | $239 \pm 6, 26$ | $9.5 \pm 0.3, 1.2$ | 2.2 |
| 40 | $2824 \pm 77, 312$ | $253 \pm 8, 34$ | $8.6 \pm 0.3, 1.3$ | 2.3 |
| 50 | $2016 \pm 65, 262$ | $226 \pm 10, 40$ | $8.3 \pm 0.4, 1.6$ | 2.8 |
| | Benzoic acid/benzoate ion | | | |
| 15 | $678 \pm 1.6, 7.3$ | $37.9 \pm 1.0, 4.8$ | | 1.0 |
| 25 | $546 \pm 2, 7.9$ | $36.2 \pm 0.4, 1.5$ | | 0.3 |
| 35 | $452 \pm 8.5, 34$ | $37.8 \pm 1.4, 6$ | | 2.4 |
| 45 | $344 \pm 2.6, 10$ | $30.5 \pm 0.6, 2$ | | 0.8 |

[a] Two statistical uncertainties are quoted. The first is the standard error estimate, the second is one-half of the joint parametric uncertainty interval.

full JPUI estimate is symmetrical about the parameter value so the joint para-
metric uncertainty interval for a given parameter $K$ listed in Table I or II is
$(K - \mathrm{JPUI}/2) \leqslant K \leqslant (K + \mathrm{JPUI}/2)$. We also cite under the heading 'Fit' the
weighted rms residuals between experimental data and corresponding values pre-
dicted by the fitted model equations.

The entries in Table I for 6-Cy complexes with decanoic acid and its anion
require some comment. We report both binary and ternary complexes with both
substrates but do not quote uncertainty estimates. The values were calculated from
experimental data recorded from solutions containing 6-Cy concentrations ranging
from 0 to about 10 mM. Within this range the pH increased markedly, i.e. by about
1.0 pH unit, which reflected the fact that that decanoic acid complexes with
formation constants $K_{\mathrm{HBCy}}$ and $K_{\mathrm{HBCy}_2}$ are stronger than those of decanoate ion
with constants $K_{\mathrm{BCy}}$ and $K_{\mathrm{BCy}_2}$. At higher concentrations of 6-Cy, near 15 mM, we
observed that the pH decreases slightly as more 6-Cy is added. This behavior is
consistent with the existence of 6-Cy/decanoate ion complexes of higher stoichiome-
try such as the quaternary $\mathrm{BCy}_3$. However, if we attempt to include an equilibrium
expression for such a complex in the model equations, at least six adjustable
parameters must be fitted and, consequently, all the parameters have unacceptably
large uncertainties at the level of precision of these experiments. Indeed, the
five-parameter analysis required to achieve an acceptable fit to the low 6-Cy
concentration data here yields large parameter uncertainties in spite of rms fit values
as low as 0.001 pH. Consequently, we regard the 6-Cy/decanoic acid/decanoate
results as only indicating the existence of the four complexes listed in Table I and
of a higher order complex with decanoate ion at higher 6-Cy concentrations. The
parameter values given in Table I should be regarded as rough approximations
only.

Standard enthalpies and entropies of complexation are calculated from the
temperature variations of the equilibrium constants from slopes of conventional
van't Hoff lines fitted by least-squares. These thermodynamic parameters are
shown in Table III with uncertainty estimates which are standard error estimates
based on the scatter of points about the least-squares lines. We have excluded the
relatively weak 7-Cy ternary complexes of octanoate, decanoate and cyclohexane-
carboxylate anions. While these complexes are detectable within our JPUI criterion,
in our experiments the complex formation constants obtained are not sufficiently
precise to warrant further analysis. The thermodynamic data of Table III are
plotted in Figure 1. 6-Cy complexes are plotted with solid triangles and 7-Cy
complexes with solid circles. The locations of individual complexes in Figure 1 are
indicated by labels which are listed in the first column of Table III. The solid line
drawn in the figure represents an empirical correlation between $\Delta H^0$ and $\Delta S^0$ for
6-Cy complexes with about 40 substrate species and which was reported previously
[10]. The equation of this correlation is

$$\Delta H^0 = (410 \pm 15)\Delta S^0 - (1.2 \pm 0.2) \times 10^3$$

with $\Delta H^0$ in cal mol$^{-1}$ and $\Delta S^0$ in cal mol$^{-1}$ K$^{-1}$.

We note that most 6-Cy complexes with carboxylic acids and carboxylate anions
seem to conform to the correlation line reported previously. We shall call these
'typical' 6-Cy complexes. However, a few 6-Cy complexes, such as those with valeric

Table III.   Standard enthalpies and entropies of complexation of cycloamyloses with carboxylic acids and carboxylate anions

| Label[a] | 6-Cy complexes | | 7-Cy complexes | |
|---|---|---|---|---|
| | $\Delta H^{0}$ [b] | $\Delta S^{0}$ [c] | $\Delta H^{0}$ [b] | $\Delta S^{0}$ [c] |
| | Butyric acid/butyrate ion | | | |
| Hbu | $-5.55 \pm 0.14$[d] | $-8.8 \pm 0.5$ | $-3.14 \pm 0.02$ | $-3.6 \pm 0.1$ |
| bu | $-1.39 \pm 0.1$ | $0.8 \pm 0.3$ | | |
| | Valeric acid/valerate ion | | | |
| Hva | $-7.13 \pm 0.09$ | $-11.4 \pm 0.3$ | $-3.6 \pm 0.3$ | $-3.2 \pm 1.0$ |
| va | $-2.42 \pm 0.05$ | $0.5 \pm 0.2$ | | |
| | Hexanoic acid/hexanoate ion | | | |
| Hhx | $-7.7 \pm 0.3$ | $-12.1 \pm 1.0$ | $-1.9 \pm 0.3$ | $4.7 \pm 1.2$ |
| hx | $-3.4 \pm 0.2$ | $-0.7 \pm 0.6$ | $0.5 \pm 0.8$ | $9.8 \pm 3.0$ |
| | Octanoic acid/octanoate ion | | | |
| Hoc | $-11.2 \pm 0.2$ | $-22.7 \pm 0.8$ | $-1.3 \pm 1.0$ | $8.3 \pm 3.5$ |
| oc | $-11.2 \pm 0.1$ | $-24.1 \pm 0.2$ | $-2.9 \pm 0.8$ | $4.5 \pm 2.7$ |
| Hoc2 | $-13.3 \pm 0.2$ | $-31.7 \pm 0.6$ | | |
| oc2 | $-7.4 \pm 0.8$ | $-18.4 \pm 2.6$ | | |
| | Decanoic acid/decanoate ion | | | |
| Hde | | | $-6.0 \pm 0.3$ | $-1.8 \pm 1.0$ |
| de | | | $-4.9 \pm 0.4$ | $1.0 \pm 1.4$ |
| | Cyclohexanecarboxylic acid/cyclohexanecarboxylate ion | | | |
| Hcc | $-9.7 \pm 0.2$ | $-24.8 \pm 0.6$ | $-5.5 \pm 0.4$ | $-1.9 \pm 1.2$ |
| cc | | | $-1.1 \pm 0.4$ | $7.4 \pm 1.2$ |
| | Benzoic acid/benzoate ion | | | |
| Hbz | $-10.15 \pm 0.15$[e] | $-20.9 \pm 0.5$[e] | $-4.1 \pm 0.3$ | $-1.0 \pm 0.9$ |
| bz | $-3.9 \pm 0.3$[e] | $-8.4 \pm 1.1$[e] | $-1.1 \pm 0.7$ | $3.4 \pm 2.3$ |
| | Ferrocenecarboxylate ion | | | |
| fc | | | $-2.8$[f] | $5$[f] |

[a] Labels identifying complexes on the plot in Figure 1. The leading character $H$ in a label indicates an acid substrate, the absence of $H$ indicates an anion substrate. All complexes are binary except the ternaries Hoc2 and oc2.
[b] Standard enthalpy of complexation, kcal mol$^{-1}$.
[c] Standard entropy of complexation, cal mol$^{-1}$ K$^{-1}$.
[d] Uncertainties are standard error estimates calculated from the scatter of data points about the van 't Hoff line.
[e] From Reference 17.
[f] From Reference 19. Measured in 0.6 M ionic strength medium.

and hexanoic acids and their anions, seem to deviate somewhat from the correlation, lying below the line. $\Delta H^{0}$ is abnormally negative by about 1 kcal mol$^{-1}$ or $\Delta S^{0}$ is abnormally positive by about 3 cal mol$^{-1}$ K$^{-1}$ for these complexes. This behavior could be explained by (a) the expulsion of water from the rim of the 6-Cy cavity upon complexation or (b) from solvent–solvent interaction of water molecules released from the vicinity of the hydrocarbon tail of the substrate upon inclusion by
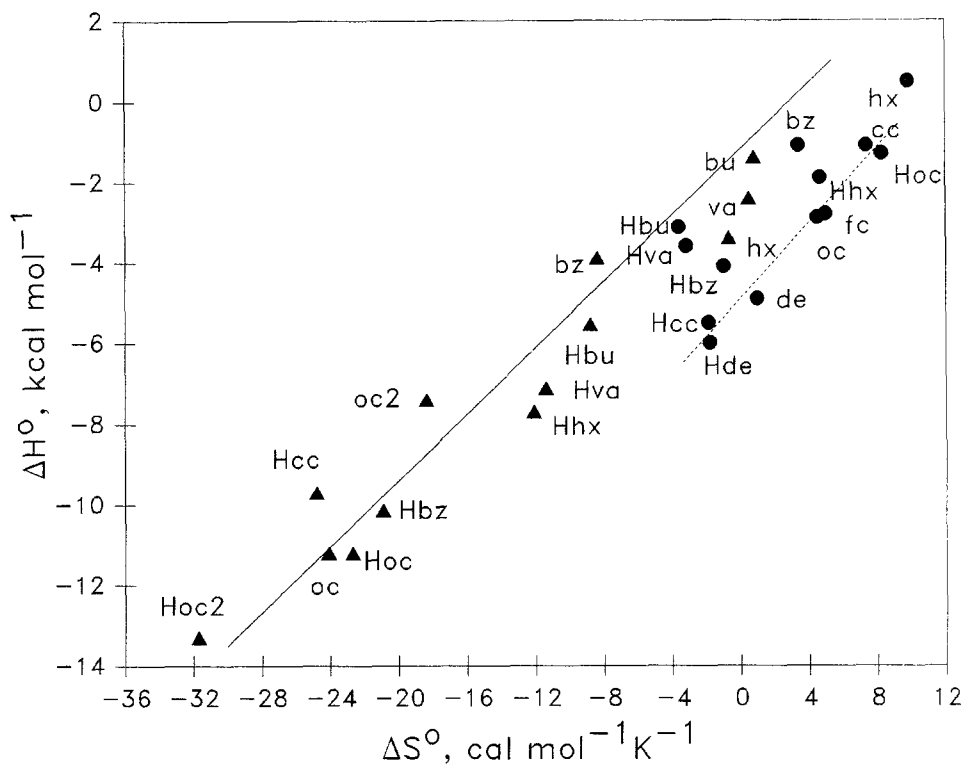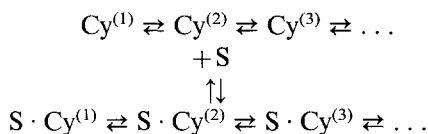
Fig. 1. Standard enthalpies vs. standard entropies of formation of carboxylic acid and carboxylate ion complexes. Solid triangles and solid circles indicate 6-Cy and 7-Cy complexes, respectively. Substrate species are indicated by labels listed in Table III. Carboxylic acid substrate labels are prefixed by $H$. The solid line is a correlation between $\Delta H^0$ and $\Delta S^0$ of formation of 6-Cy complexes with various substrate species reported previously [10]. The dashed line is an apparent correlation between $\Delta H^0$ and $\Delta S^0$ of complexation of 7-Cy with bulky substrates observed in the present study.

the cycloamylose cavity. On the other hand, a few 6-Cy complexes, such as those of cyclohexanecarboxylic acid and the ternary complexes of octanoic acid and octanoate ion, seem to be 'abnormal' and lie above the correlation line. These observations can be explained by abnormally negative contributions to the complexation $\Delta S^0$ caused by internal constraint on the cyclohexanecarboxylic acid substrate or by loss of mobility of the flexible octanoate chain.

In any case the deviations of these abnormal substrates from the general correlation line are minor and probably represent specific properties of the substrates. However, we believe that the principal player in the bonding interaction is not the substrate but the cycloamylose and its complement of associated solvent molecules. We arrive at this conclusion because the magnitudes of $\Delta H^0$ and $\Delta S^0$ of formation of most cycloamylose complexes are too large to reflect bonding and ordering effects, respectively, of the substrates. For example, the total enthalpy and entropy of ternary 6-Cy complexation of octanoic acid, i.e. for the reaction $HB + 2\,Cy = HBCy_2$, are $-25.5$ kcal mol$^{-1}$ and $-54.4$ cal mol$^{-1}$ K$^{-1}$, respectively.

It is very difficult to reconcile these large enthalpic and entropic changes with binding and loss of mobility of simple occlusion of octanoic acid in two 6-Cy cavities. These large values must reflect changes in a much larger molecular ensemble, which must be the cycloamylose ring together with its hydrogen-bonded solvent molecules. In order to understand this phenomenon, we envisage that uncomplexed cycloamylose solute is an equilibrium mixture of conformational isomers which we denote by $Cy^{(i)}$, i.e. several species $Cy^{(1)}$, $Cy^{(2)}$, $Cy^{(3)}$, etc. These isomers differ from one another by the conformation of the cycloamylose ring itself and/or by the arrangement of hydrogen-bonded water molecules. Similarly we view the inclusion complex $S \cdot Cy$ with substrate S as an equilibrium mixture of conformational isomers $S \cdot Cy^{(j)}$. The overall complexation reaction $S + Cy = S \cdot Cy$ thus involves both equilibrium mixtures and may be regarded as:

$$Cy^{(1)} \rightleftarrows Cy^{(2)} \rightleftarrows Cy^{(3)} \rightleftarrows \ldots$$
$$+ S$$
$$\uparrow\downarrow$$
$$S \cdot Cy^{(1)} \rightleftarrows S \cdot Cy^{(2)} \rightleftarrows S \cdot Cy^{(3)} \rightleftarrows \ldots$$

We imagine a mechanism by which the substrate complexes preferentially with one of the uncomplexed isomers forming one of the complexed isomers. Then the mixture of uncomplexed isomers shifts to replace the one which has been depleted by reaction with S and the complex distributes itself into the mixture $S \cdot Cy^{(j)}$. The large magnitudes of the observed overall $\Delta H^0$ and $\Delta S^0$ must reflect the perturbation of the mixture $Cy^{(i)}$ and/or the distribution into the mixture $S \cdot Cy^{(j)}$.

This is not to claim that the identity of the substrate has no effect on the bonding. We notice in Figure 1 that the positions of the 6-Cy complexes with bulkier substrates lie lower on the correlation line, i.e. with more negative $\Delta H^0$ and more negative $\Delta S^0$ values, while the smaller substrate species have less negative (more positive) $\Delta H^0$ and $\Delta S^0$ values. This behavior implies that the bulkier substrates enhance interglucosyl hydrogen bonding and rigidity of the cycloamylose structure more than do the smaller substrates. We also notice that some of the 7-Cy complexes (solid circles in Figure 1) also fall very near or slightly below or to the right of the 6-Cy correlation line. The 7-Cy complex with the smallest substrate, butyric acid (labeled Hbu), is closest to the 6-Cy line while the 7-Cy complexes with the most bulky substrates: decanoic (Hde), octanoic (Hoc), hexanoic (Hhx) and hexanecarboxylic (Hcc) acids together with their anions and ferrocenecarboxylate ion (fc) lie much lower. The complexes of these bulky substrates appear to fall close to a straight line, drawn as a dashed line in figure 1, which is roughly parallel to but displaced to the right of or lower than the 6-Cy correlation line. The fact that the thermodynamic parameters of these latter 7-Cy complexations appear to be correlated by a line having the same slope as the 6-Cy line is a strong indication that the same complexation mechanisms are at work in complexations with both cycloamyloses. The displacement of the line of 7-Cy complexes of bulky substrates from the 6-Cy line is consistent with the hypothesis that these 7-Cy complexes involve an additional binding mechanism with a positive entropic and/or negative enthalpic contribution. One possibility is that bulky substrates expel water molecules from the 7-Cy cavity. This effect is entropically positive and would displace the 7-Cy line to the right of the 6-Cy line. Also if the intramolecular

interactions of the expelled solvent molecules are stronger than the hydrogen bonding interactions between the solvent and the 7-Cy before complexation occurs, this effect would displace the 7-Cy line downward. The fact that all bulky substrates fall on the same displaced line implies that all such substrates expel the same amount of water. However, we notice that 7-Cy complexes with valeric acid (Hva), benzoic acid (Hbz), benzoate ion (bz) and hexanoate ion (hx) fall between the 6-Cy line and the 7-Cy line with bulky substrates. In these cases we would hypothesize that these intermediate-sized substrates expel some water from the 7-Cy cavity but not the full complement of water expelled by the bulkier substrates.

Finally we note that for each case in which we have obtained thermodynamic data for both 6-Cy and 7-Cy complexes with the same substrate species, both the $\Delta H^0$ value and the $\Delta S^0$ value for the 7-Cy complexation are more positive than those of the corresponding 6-Cy complexation. In fact the difference between corresponding parameters tends to increase in a more-or-less regular way as the size of the substrate species increases. This effect is shown by the bar graph in Figure 2. In this graph, the heights of the open bars and cross-hatched bars represent the differences in $\Delta H^0$ and $\Delta S^0$ values, respectively, between 7-Cy and 6-Cy complexations. The various complexes are arranged horizontally roughly in order of increasing bar height and we see that the smallest substrates have the shortest bars and the most bulky substrates have the longest bars. An explanation of these effects follows directly from our interpretation of the $\Delta H^0$ vs. $\Delta S^0$ correlations themselves. In both 6-Cy and 7-Cy complexes the most important binding mechanism is the enhanced intermolecular interactions of the host upon inclusion of the substrate. As stated above, the magnitude of this enhancement increases as the size of the substrate species increases for either 6-Cy or 7-Cy. But all the data illustrated in Figure 2 indicates that the magnitude of the effect *decreases* as the size of the macrocyclic
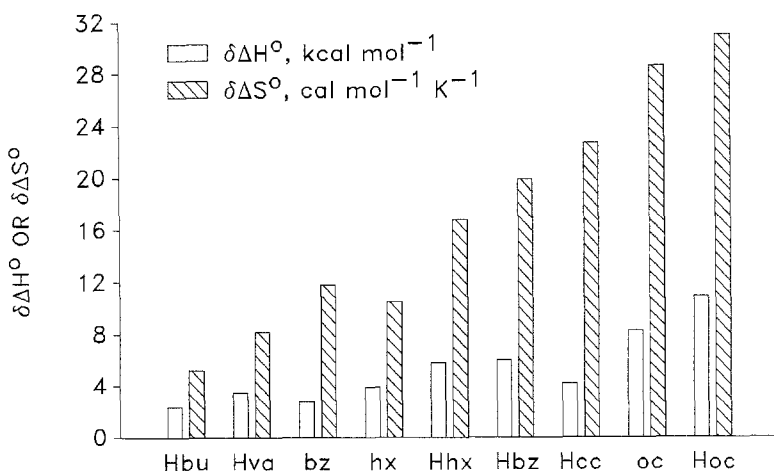


Fig. 2. The differences $\delta \Delta H^0$ and $\delta \Delta S^0$ of enthalpies and entropies of formation, respectively, between 7-Cy and 6-Cy complexes with the same substrate species. The substrate species are indicated below the pair of bars by labels defined in Table III. For any given substrate the thermoydnamic parameters for the 7-Cy complexation are always more positive than those for the 6-Cy complexation.

cavity increases from the smaller 6-Cy to the larger 7-Cy. Thus, the effect depends primarily on the size of substrate with respect to the internal size of the cycloamylose cavity.

In summary the present study seems to indicate that thermodynamic properties of 6-Cy and 7-Cy complexes reflect the structural and solvent interactions of the cycloamyloses themselves as well as their interactions with the substrate species. In particular the relationship between complexation enthalpies and entropies seems to be dominated by the cycloamyloses and this observation is contrary to the common perception that the host molecule plays a passive role in the complexation reaction. It seems likely that future attempts at theoretical descriptions of their inclusion complexes must take account of the active roles played by the host molecules in these reactions.

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