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MICROCALORIMETRIC TITRATION OF β-CYCLODEXTRINE WITH ADAMANTANE-1-CARBOXYLATE *

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ABSTRACT

The binding of adamantane-1-carboxylate (A^-) to β-cyclodextrine (C) has been studied at 298.15 K by a microcalorimetric titration technique. The results agree with a model where not only CA^- but also CA_2^{2-} is formed. Gibbs energies, enthalpies and entropies for the binding processes are reported.

INTRODUCTION

Cyclodextrines are cyclic oligosaccharides which form inclusion complexes with many types of compounds in water as well as in other solvents. The formation of such complexes has been much studied during recent years because of their importance in catalysis, in chromatography, in pharmaceutical applications, and as models for protein–ligand complexes; for references see, e.g., refs. 1–4. In most cases the cyclodextrine–ligand complexes are believed to be of the 1 : 1 type, but the formation of 1 : 2 and 2 : 1 compounds has also been observed [4].

Thermodynamic properties for the formation of cylcodextrine-inclusion complexes have been reported, in particular equilibrium constants, but also enthalpy and entropy values. Equilibrium constants are typically in the range of 10^2–10^5 mol^{-1}. It is then often possible to determine simultaneously both K and ΔH, and thus also ΔS, by calorimetric titrations. Several studies of this type have been reported; see, in particular, that by Eftink and coworkers [4].

Recently, significant progress has been made in micro-titration calorimetry [5], and it is now possible to determine titration curves precisely using

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less than 1 μmol of titrand. However, as in all microcalorimetric experiments, it is important in such work to watch out for systematic errors and for that reason it is desirable to have convenient and accurately known test reactions available for which the equilibrium constants and the enthalpy change are accurately known. As part of the ongoing methodological development work in our laboratory, we have searched for some time for suitable test reactions for use in biochemical ligand binding studies. In that connection we have studied several reactions with cyclodextrines, in particular the binding of adamantane-1-carboxylic acid to β-cyclodextrine. Results from that investigation will be reported here.

β-Cyclodextrine and adamantane-1-carboxylic acid are commercially available in sufficiently pure form, and are stable and relatively inexpensive compounds. Cromwell et al. [4] have recently performed extensive flow microcalorimetric titration studies of this and related binding reactions. As expected, the binding constant was shown to vary with the degree of ionization of the ligand. For a test reaction it is desirable to have few experimental variables and we therefore performed the work at pH 8.50 where practically all ligand molecules are in the ionic form. The results of Cromwell et al. [4] suggested that under these conditions only a 1:1 complex is formed and that the values for the binding constant and the enthalpy change were in a range suitable for a test reaction. However, from results of kinetic experiments, Breslow et al. [6] have concluded that, at relatively high concentrations, the cyclodextrine can bind two carboxylate ions.

EXPERIMENTAL

Materials

β-Cyclodextrine (C) was obtained from Sigma and was used without furhter purification. It has been reported that C is hydrated with 8–9 H_2O [4,7]. Most samples used in this work were equilibrated at 25°C above a saturated solution of $Ca(NO_3)_2$, giving a relative humidity of 51% [8]. Drying of these samples at 100°C and reduced pressure (3 Pa) showed that 10 ± 0.1 H_2O was bound to each C molecule. The molecular mass for C was therefore taken to be 1315.

Adamantane-1-carboxylic acid (HA) was obtained from Fluka and was used as received. Potentiometric titration with NaOH indicated a purity better than 99.8% by weight. The same results were obtained with samples which had been recrystallized from water or methanol, followed by sublimation.

Solutions used in the calorimetric measurements were prepared by dissolving C and HA, respectively, in 0.01 M sodium pyrophosphate buffer, pH 8.50. The pH of the carboxylate solution formed was adjusted to pH 8.50.

Calorimetry

A 1-ml calorimetric titration vessel made from stainless steel and fitted with a stirrer [9] was used with the LKB 4-channel microcalorimeter [10]. The vessel, which is the prototype of the LKB 2277-402 titration and perfusion vessel, was charged with 0.8–0.9 ml of cyclodextrine solution, concentration range 1–10 mmol l^{-1}. The carboxylate solution, concentration range 10–100 mmol l^{-1}, was contained in a 0.5-ml gas-tight Hamilton syringe (1750 LT) attached to a motor-driven micrometer screw. The permanently affixed needle of the syringe was soldered to a 1-m stainless steel tube, ID 0.5 mm.

All calorimetric measurements were performed at 298.15 K. The charged titration vessel was inserted stepwise [9] into the calorimeter using four equilibrium positions. The residence time in each position was about 5 min. Before the titration experiments started, the instrument was allowed to equilibrate further for $\geqslant 30$ min. During this period the titration needle of the syringe was inserted. Time and volume for the stepwise injections of the carboxylate solution were regulated by a microprocessor which also was used for integration of the titration curves. Aliquots of 14–20 μl were injected in each step and time intervals between injections were $\leqslant 1$ h. For each titrations experiment 13–15 injections were made. In separate experiments small correction values for enthalpies of dilution of carboxylate solutions were determined. No significant dilution effect was found for the cyclodextrine solutions.

The instrument was calibrated electrically by use of a heater positioned in the sample cup [9].

Calculations

From the corrected results for the calorimetric titration, values for the thermodynamic parameters were calculated by use of the minimization program KALORI developed by Karlsson and Kullberg [11]. This program is designed to treat data from calorimetric titrations where complexes of the type XL_n ($n = 1$–4) are formed. The program allows equilibrium constants and enthalpy values to be varied simultaneously. Calculations were performed using a VAX 11/780 computer.

RESULTS AND DISCUSSION

Figures 1 and 2 summarize experimental results and calculations from two typical series of titrations where the concentrations of C and A^- were significantly different in the equilibrium mixtures. The points in the figures show the corrected heat quantities evolved following each injection. The

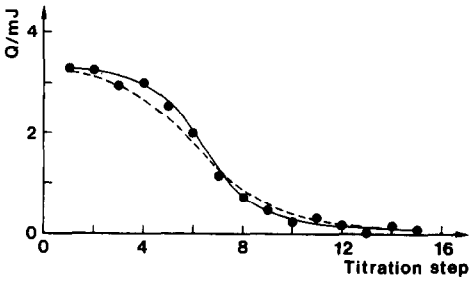

Fig. 1. Results of a calorimetric titration performed with 0.97 μmol of C. In each step 16 μl of A^-, concentration 9.8 mmol l^{-1}, was injected. Cf. the text.

titration curves, which are in close agreement with the experimental points, were calculated by use of the KALORI program assuming the following two-step model:

$$C + A^- \rightleftarrows CA^- \quad (1)$$

$$CA^- + A^+ \rightleftarrows CA_2^{2-} \quad (2)$$

The dashed curves were calculated assuming that contributions from the second binding step could be neglected. The results clearly suggest that a significant amount of CA_2^{2-} is formed under the experimental conditions used here. However, in case the titrations are not continued beyond the steep part of the titration curves, or if the experiments are conducted in very dilute solutions, the contributions from the second binding step will hardly be noticeable.

Results from 15 titration experiments were treated according to the two-step model. A few of these experiments were performed with cyclo-

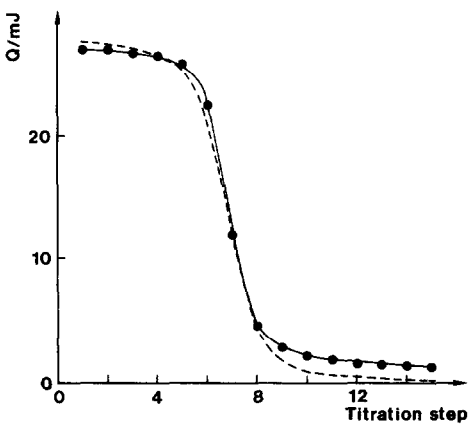

Fig. 2. Results of a calorimetric titration performed with 8.00 μmol of C. In each step 14 μl of A^-, concentration 89.7 mmol l^{-1}, was injected. Cf. the text.

TABLE 1

Thermodynamic values for the binding of adamantane-1-carboxylate to β-cyclodextrine at 298.15 K

Reaction step	K_c' (1 mol^{-1})	$\Delta H^{0\prime}$ (kJ mol^{-1})	$\Delta G^{0\prime}$ (kJ mol^{-1})	$\Delta S^{0\prime}$ (J mol^{-1} K^{-1})
1	32400 ± 2400	−21.48 ± 0.04	−25.74 ± 0.2	14.29 ± 0.3
2	36 ± 12	−7.7 ± 1.4	−8.9 ± 0.8	4.0 ± 2.0

dextrine samples which had not been equilibrated at 51% humidity. No difference in results between these and the equilibrated samples could be seen. The thermodynamic quantities derived are summarized in Table 1. Uncertainties are estimates.

The results reported by Cromwell et al. [4] were interpreted using the 1 : 1 model, $\Delta G^{0\prime} = -24.5 \pm 0.2$ kJ mol^{-1}, $\Delta H^{0\prime} = -20.3 \pm 0.2$ kJ mol^{-1}. These values are similar to the present $\Delta G^{0\prime}$ and $\Delta H^{0\prime}$ values for the first step, but the differences are significant.

The binding process studied here can be of some use as a test reaction in connection with calorimetric studies, e.g. protein–ligand binding reactions. However, we feel that for more general use in this field, a clean 1 : 1 process would be more suitable.

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