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Pressure Perturbation Calorimetry of Solvation Changes in Cyclodextrin Complexes

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Abstract

Solvation changes occurring during cyclodextrin host:guest complex formation were investigated using the new calorimetric technique of Pressure Perturbation Calorimetry (PPC). This can determine the thermal expansion coefficient of molecules in solution. PPC was used to measure the change in heat (ΔQ) that occurs upon application of pressure to three different solutions: guest (1-adamantanecarboxylic acid or 1-adamantanamine), β-cyclodextrin, and β-cyclodextrin/guest mixture. ΔQ for the complex in solution was found to be smaller than anticipated from the sum of the heat changes of the separate components. Since ΔQ is directly related to thermal expansivity (α), the results imply that the complex expands less with temperature than expected. This reduction is most likely due to the removal of the solvation shell around the ligand and, to a lesser extent, expulsion of water molecules from the cyclodextrin cavity during complex formation.

Introduction

Cyclodextrins (CDs) are cyclic oligosaccharides of α-1,4-linked D-glucose units, formed by the breakdown of starch by *B. macerans* [1]. The three most readily available α-, β-, and γ-cyclodextrins consist of six, seven, and eight glucose units respectively. The cyclodextrins have a hollow truncated cone like structure in which the wider side is formed by the secondary 2- and 3-hydroxyl groups and the narrower side by the primary 6-hydroxyl groups. Of most interest is the possession of an internal hydrophobic cavity which can include a wide range of guest molecules via a variety of non covalent interactions. This ability of cyclodextrins to form inclusion complexes has led to them being used in a diverse range of applications [2], though the precise thermodynamic basis for complexation is not yet established [3].

Complexation of a guest molecule to a cyclodextrin in aqueous solution results in the removal and rearrangement of water molecules originally solvated to both the cyclodextrin and the guest molecule, and the cyclodextrin must release water molecules from its cavity to allow entry by the guest, which must also lose at least part of its solvation shell in the process [2–4].

The changes in solvation which occur during complexation of a ligand to a CD are difficult to observe directly. However, their effects can be estimated using a new calorimetric technique, Pressure Perturbation Calorimetry (PPC), which measures the thermal response of solutions when squeezed gently by relatively small pressure changes [5]. PPC makes possible the accurate measurement of volumetric properties of molecules in solution and can be used to calculate apparent molecular thermal expansion coefficients (α), which may also be related to solvation effects [6]. We have used this new technique here to provide information on solvation changes of ligand and cyclodextrin upon complexation. α can be determined calorimetrically by measuring the change in heat (ΔQ) that occurs in response to the application of pressure. Here we present preliminary results showing how the solvation of two different ligands, 1-adamantanecarboxylic acid and 1-adamantanamine, changes upon complexation to β-cyclodextrin.

Experimental section

Materials

β-cyclodextrin was purchased from Sigma, 1-adamantanecarboxylic acid and 1-adamantanamine were purchased from Aldrich and D-glucose, sodium dihydrogen orthophosphate dihydrate and di-sodium hydrogen orthophosphate were purchased from BDH. β-cyclodextrin was dried *in vacuo* before use and all other reagents were used without further purification. Solutions were prepared in 0.1 M phosphate buffer, pH 7.2, under conditions where approximately 95% of the β-cyclodextrin had ligand bound. The percentages of ligand and cyclodextrin bound in the complex were calculated from their complex formation association constants (K_a) and binding stoichiometries (N), which were determined by Isothermal Titration Calorimetry (ITC). All concentrations were determined by weight.

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Isothermal titration calorimetry (ITC)

Isothermal Titration Calorimetry (ITC) was used to characterise the binding of both 1-adamantanecarboxylic acid and 1-adamantanamine to β-cyclodextrin in solution, using a VP-ITC Microcal calorimeter [7]. In a typical experiment, a syringe is used to titrate a solution of ligand (3 mM) into the calorimetric cell containing a solution of β-cyclodextrin (0.15 mM), at 25 °C. As the substances bind, heat is released or absorbed in direct proportion to the amount of binding that occurs as the two interact. Measurement of this heat allows the determination of the association constant (K_a), reaction stoichiometry (N), enthalpy (ΔH) and entropy (ΔS^0) changes using standard data fitting algorithms.

Pressure perturbation calorimetry (PPC)

Measurements were made using a MicroCal VP-DSC microcalorimeter equipped with a PPC accessory [5], which applies or releases pressure simultaneously to both sample and reference cells. In a typical PPC experiment, the sample cell is filled with a solution of the test compounds dissolved in buffer and the reference cell with identical buffer. Controlled pressure pulses (ΔP) of up to 5 atmospheres nitrogen gas are applied, causing both solutions inside the cells to be either compressed or decompressed. As the applied pressure is altered, heat is released or absorbed by the solutions due to pressure-volume changes taking place inside the calorimetric cells. The difference in heat released or absorbed (ΔQ) by the sample and reference cell is measured and used to calculate the thermal expansion coefficient (α). The values of ΔQ are quite small since the solutions in the sample and reference cells are identical except for the presence of the relatively small amount of solute in the sample cell. The heat changes obtained by integration of the calorimetric response occurring during compression and decompression are normally of equal magnitude but of opposite sign.

ΔQ is related to the differences in thermal expansivity of the solvent and solute in the following way [5, 6],

$$\Delta = -T\Delta P g_S \overline{V}_S(\alpha_S - \alpha_0), \qquad (1)$$

where, T is the temperature, g_S is the total mass of solute in the sample cell, $\overline{V}_S$ is the partial specific volume of the solute, and $\alpha = (1/V)(\partial V/\partial T)_P$ is the coefficient of thermal expansion of the solute α_S or the solvent α_0 respectively.

Results and discussion

Before considering the PPC data it was necessary first to establish the extent of host:guest complexation in the cyclodextrin/ligand mixtures and to determine optimal solution conditions. Isothermal Titration Calorimetry data obtained for the binding of 1-adamantanecarboxylic acid (1-AC) and 1-adamantanamine (1-AA) to β-cyclodextrin (β-CD) are shown in Figures 1 and 2. Both ligands show exothermic 1:1 complexation with β-CD, with binding affinities of order

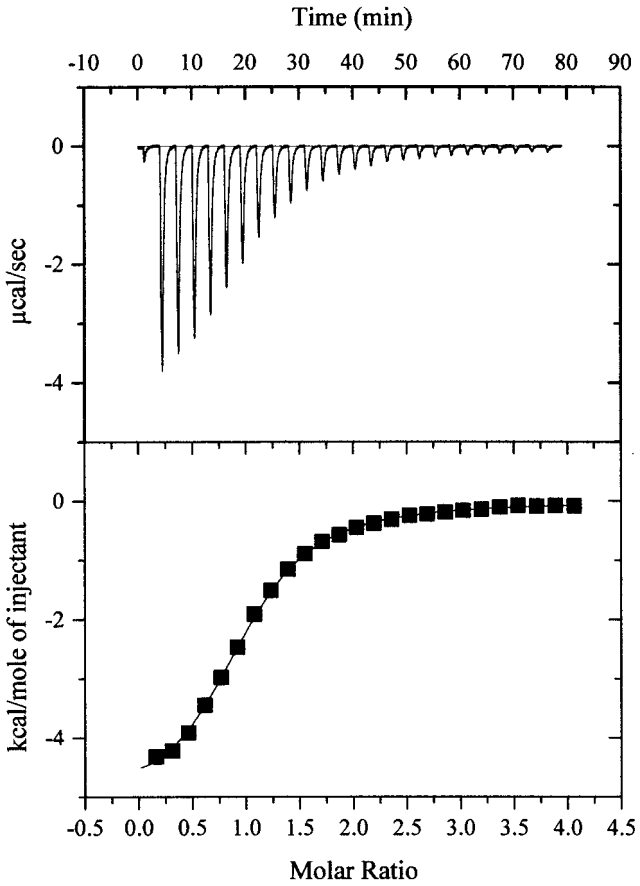

Figure 1. Typical ITC data obtained for the binding of 1-adamantanecarboxylic acid to β-cyclodextrin, in 0.1 M phosphate buffer, pH 7.2. The upper panel corresponds to the exothermic heat released upon injection of 10 μl aliquots of 1-adamantanecarboxylic acid (3 mM) into the β-cyclodextrin solution (0.15 mM, cell volume 1.4 ml). The lower panel shows the integrated heat data, giving a differential binding curve which was fit to a standard single-site binding model yielding the following parameters: stoichiometry of binding, N = 0.97, binding affinity $K_a = 4.28 \times 10^4$ M^{-1} and enthalpy of binding, $\Delta H = -21.78$ kJ/mol.

10^4 M^{-1}, or greater. This guarantees that in the mixtures used for PPC experiments, the extent of cyclodextrin complexation is at least 95%. The actual extent of complexation under PPC conditions was calculated from these measured affinities and used in subsequent calculations.

Typical PPC data obtained for the binding of 1-AC and 1-AA to β-CD as well as some control experiments using non-binding glucose in place of β-cyclodextrin are given in Table 1. From Equation (1), the change in heat (ΔQ) from each component of the complex upon application of pressure, is directly proportional to its thermal expansion coefficient (α). In the particular case of cyclodextrin (CD) ligand (L) binding:

$$CD + L \rightleftharpoons CD : L,$$

comparison of the heat change (ΔQ) from three different solutions: (a) β-cyclodextrin alone; (b) ligand alone; and (c) β-cyclodextrin/ligand complex at the same total concentrations, provides information on any changes in solvation occurring around the ligand and cyclodextrin upon complex-

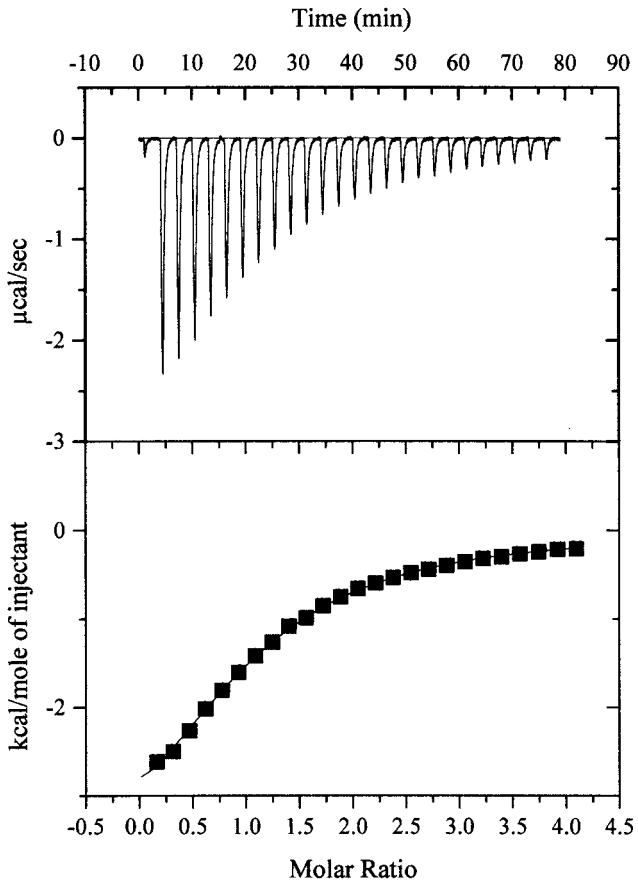

Figure 2. Typical ITC data for the binding of 1-adamantanamine (1-AA) acid to β-cyclodextrin under the same conditions as Figure 1, giving N = 0.97, $K_a = 9400M^{-1}$ and $\Delta H = -19.20$ kJ/mol.

ation. For the purpose of comparison, the double difference may be written as follows:

$$\Delta\Delta Q = \Delta Q(\text{CD:L}) - \Delta Q(\text{CD}) - \Delta Q(\text{L}). \qquad (2)$$

From Table 1 it is clear that the magnitude of the heat change from the β-cyclodextrin/ligand complex, ΔQ(CD:L), is less than one would expect from the sum of the heat changes from the individual components, $\Delta Q(\text{L}) + \Delta Q(\text{CD}) \cdot \Delta\Delta Q \approx 26\ \mu$J for the β-CD/1-AC complex and $\Delta\Delta Q \approx 36\ \mu$J for the β-CD/1-AA complex, under conditions where more than 95% of the cyclodextrin has ligand bound. $\Delta\Delta Q$ for the non-binding controls are close to zero, within experimental error.

Since ΔQ is directly related to the thermal expansion coefficient (α) and the heat change from the cyclodextrin/ligand complex is less than the heat change from the sum of the separate components, the results suggest that the complex expands less with temperature than would be expected from the sum of the individual components (as is the case in the non-binding controls). There are several possible explanations for this. First, if cyclodextrins are reasonably flexible in solution [3], this flexibility might be restricted by complexation [8, 9]. The tight binding of 1-adamantanecarboxylic acid and 1-adamantanamine inside the β-cyclodextrin cavity could result in the complex having a more rigid conformation and consequently a lower thermal expansivity. However

Table 1. Pressure perturbation calorimetry data for cyclodextrin-ligand complexation

Sample*	$\Delta Q/\mu$J	($\Delta Q/\mu$J
β-CD	–71.49 (5.54)	
1-AC	–20.89 (5.78)	
β-CD/1-AC	–66.37 (5.62)	26.01 (9.78)
Glucose	–18.50 (5.78)	
1-AC	–32.15 (5.78)	
Glucose/1-AC	–54.37 (5.55)	–3.72 (9.88)
β-CD	–68.23 (5.54)	
1-AA	–19.34 (6.07)	
β-CD/1-AA	–51.56 (5.62)	36.01 (9.96)
Glucose	–18.50 (5.78)	
1-AA	–30.8 (6.07)	
Glucose/1-AA	–46.76 (5.72)	2.58 (10.15)

* Experimental data determined at 25 °C in 0.1 M phosphate buffer, pH 7.2, using a Microcal PPC system with approximately 72 psi (5 atm) pressure pulses, each experiment comprising multiple pulses (>60). Concentrations used: β-cyclodextrin (3 mM), 1-adamantanecarboxylic acid and 1-adamantanamine (4.5 mM), and glucose (3 mM). Data are means of multiple determinations, corrected for buffer/buffer controls and with standard deviations given in brackets.

the magnitude of the thermal expansivity changes observed here, amounting to more than 25–30% of the total ΔQ, seems much too large to be accounted for by flexibility changes alone.

A second more likely cause of the reduction in thermal expansivity is due to changes in solvation occurring around the ligand and inside the cyclodextrin cavity during complexation. The apparent thermal expansion properties of a small molecule in solution is dominated by its solvation sphere [6]. The excluded volume of a small molecule is defined by covalent interactions and van der Waals radii which show very little response to changes in temperature, because covalent bond vibrational excitation energies lie well above normal experimental temperature ranges. However the solvation shell around the molecule is much more sensitive to temperature changes because it involves weaker non-covalent forces [6]. Consequently, in the case of the small molecules which we are studying here, volumetric changes upon binding will most likely reflect changes in solvation.

We have shown, using pressure perturbation techniques, that the thermal expansivities of cyclodextrin complexes are significantly different from the individual components. Subsequent work will establish the absolute volumetric parameters and allow estimation of the number of solvation molecules involved and the volumetric properties of the solvation shells from which they are displaced.

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