

# Calorimetric study of binding of some disaccharides with crown ethers

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## Abstract

Isothermal titration calorimetry has been applied to the determination of the thermodynamic parameters of binding of  $\beta$ -lactose,  $\alpha,\alpha$ -trehalose and sucrose with 15-crown-5 and 18-crown-6 in water at 298.15 K. The formation of 1:1 molecular associates has been found for the systems studied except 18-crown-6 and  $\beta$ -lactose. The associates are preferentially or completely entropy stabilized. The most stable associate is formed between  $\alpha,\alpha$ -trehalose and 18-crown-6. The obtained values of thermodynamic parameters of binding are discussed from the point of view of solute–solvent interactions as well as conformational and structural peculiarities of the disaccharides (DS) and crown ethers (CE). © 2004 Elsevier B.V. All rights reserved.

*Keywords:* Disaccharide; Crown ether; Aqueous solution; Molecular associates; Titration calorimetry

## 1. Introduction

Non-covalent interactions between organic molecules in aqueous solutions have been the focus of increased study during the last years. Although these interactions are accompanied by small changes of energy, they often play an important role in a wide range of biological recognition processes involving saccharides, such as molecular transport, antigen–antibody interactions, enzymatic reactions, and cellular recognition [1–3].

Crown ethers recognize a wide variety of organic and inorganic molecules [4] and can serve as potential artificial receptors for saccharide recognition in water. Therefore, the study of saccharide–crown ether interactions is of interest. Our study has been carried out at low concentrations of the solutes. For more comprehensive understanding of the nature of interactions between saccharides and crown ethers and also of factors controlling the associate stability, we report thermodynamic parameters of binding between disaccharides of different structure and 15-crown-5 and 18-crown-6 in aqueous solutions.

## 2. Materials and methods

### 2.1. Materials

15-crown-5 (1,4,7,10,13-pentaoxacyclopentadecane) and 18-crown-6 (1,4,7,10,13,16-hexaoxacyclooctadecane) (Sigma) were used without further purification. Sucrose (pure) was re-crystallized from ethanol. The samples of  $\beta$ -lactose (Sigma) and sucrose were dried in vacuum at 343 K over several days before use. The water content of  $\alpha,\alpha$ -trehalose (dihydrate) (Sigma) was confirmed by thermogravimetry. Solutions were prepared by weight with doubly distilled deionized water.

### 2.2. Titration calorimetry

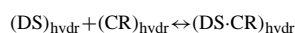
Calorimetric titrations were performed at 298.15 K using a differential, heat-conduction calorimeter which was connected to a computer for automated titration and data processing. The detailed description of the calorimeter, the principle of the measurement and the experimental procedures were reported earlier [5]. In a typical run, an aqueous solution of disaccharide (0.2–0.3 M) was introduced into an aqueous solution of crown ether (2–6 mM) placed in the sample cell ( $V \sim 40$  ml). About 20–30 injections, spaced at 1800 s, were made in each experiment. The reference cell

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was filled with water. The heats of dilution were measured separately, for which appropriate corrections were made. The parameters of titration (volume, number of injections, time intervals between them) were introduced into program controlling the titration process and data obtained. Areas for all injections were determined by computer integration, edited for heats of dilution and processed to arrive at final data for calculation of thermodynamic parameters of binding. The experimental titration data were treated using different binding models but the best fit of experimental and calculated curves as well as the inflection point at 1:1 stoichiometry testifies to the accuracy reliability of a 1:1 binding model.

For a 1:1 binding between disaccharides (DS) and crown ethers (CR)



the stability constant can be calculated as:

$$k = \frac{[DS \cdot CR]_i}{[DS]_i \cdot [CR]} \quad (1)$$

or

$$\frac{[DS \cdot CR]_i}{([DS]_0 - [DS \cdot CR]_i) \cdot ([CR]_0 - [DS \cdot CR]_i)} \quad (2)$$

where  $[DS \cdot CR]_i$ ,  $[DS]_i$ ,  $[CR]_i$  are the equilibrium concentrations of associate, disaccharide and crown ether, respectively;  $[DS]_0$ ,  $[CR]_0$  are the initial concentrations of disaccharide and crown ether after each injection.

Heat evolved (positive peaks, exothermic) or absorbed (negative peaks, endothermic) during the  $i$ th injection is given as:

$$\Delta Q_i = \pm \Delta H \cdot V_i \cdot [DS \cdot CR]_i \quad (3)$$

where  $V_i$  is the solution volume after each injection. Resolution of the system of Eqs. (2) and (3) allows calculation of enthalpy change and stability constant values by continuously changing  $K$  and  $\Delta H$  to minimize the function  $\Sigma((Q_i)_{exp} - (Q_i)_{calc})^2$ .

The stability constants and thermodynamic parameters of binding obtained are listed in Table 1. The data for binding of sucrose with 18-crown-6 were obtained earlier [6]. As an example, a typical plot of the processed data for the 18-crown-6- $\alpha,\alpha$ -trehalose-water system is shown in Fig. 1.

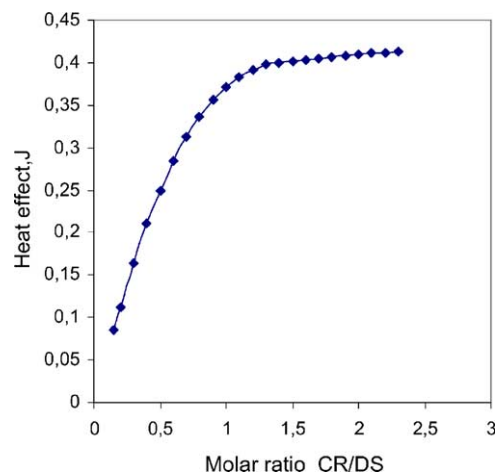


Fig. 1. Integral curve of titration of aqueous solution of 18-crown-6 (5.59 mM) by aqueous solution of  $\alpha,\alpha$ -trehalose (0.2204 M) at 298.15 K; calculated  $K = 1295 \text{ dm}^3 \text{ mol}^{-1}$  and  $\Delta H_{bind} = 2.08 \text{ kJ mol}^{-1}$ .

### 3. Discussion

The binding constant values (Table 1) reflect that the studied disaccharides are able to form molecular associates with the crown ethers except 18-crown-6 and  $\beta$ -lactose. For this system no difference between  $(Q_i)_{exp}$  and  $(Q_i)_{dilat}$  was detected ( $\Delta Q_i \rightarrow 0$ ). Either molecular associates are not formed in this case or the enthalpy change for binding is zero. The most stable associate is between  $\alpha,\alpha$ -trehalose and 18-crown-6.

The obtained data indicate that the formation of associates is accompanied by small enthalpy changes and significant entropy contributions. Saccharides, especially disaccharides, are known to be extensively hydrated in aqueous solutions [7–9]. The same is true for crown ether aqueous solutions [10,11]. The water molecules either hydrogen bonded to accessible polar groups or ordered at the interface of apolar groups are released to the bulk solvent upon intermolecular binding; providing a significant favorable entropic contribution to stability of disaccharide-crown ether associate. Therefore, as can be seen from Table 1, the entropy contribution from dehydration of the reactants is a major factor governing the intermolecular associate formation in the systems studied. The associates of  $\alpha,\alpha$ -trehalose either with 15-crown-5 or 18-crown-6 are completely

Table 1

Associate stability constants ( $K$ ) and thermodynamic parameters for 1:1 associate formation of some disaccharides with 15-crown-5 and 18-crown-6 in water at 298.15 K

Disaccharide	15-Crown-5			18-Crown-6		
	$K$	$\Delta H_{bind}^a$	$\Delta S_{bind}^b$	$K$	$\Delta H_{bind}^a$	$\Delta S_{bind}^b$
$\beta$ -Lactose	$214 \pm 7$	$-1.00 \pm 0.16$	$41 \pm 2$	–	–	–
$\alpha,\alpha$ -Trehalose	$643 \pm 44$	$1.15 \pm 0.08$	$58 \pm 2$	$1295 \pm 155$	$2.08 \pm 0.09$	$66 \pm 3$
Sucrose	$364 \pm 12$	$-4.16 \pm 0.06$	$35 \pm 2$	$393 \pm 15^c$	$-4.85 \pm 0.04^c$	$32 \pm 1^c$

<sup>a</sup> Units:  $\text{kJ mol}^{-1}$ .

<sup>b</sup> Units:  $\text{J mol}^{-1} \text{ K}^{-1}$ .

<sup>c</sup> See Ref. [6].

entropy stabilized. For the other systems, the associates are preferentially entropy stabilized.

$\Delta H_{\text{bind}}$  values between disaccharides and crown ethers are small. Possible sources of negative enthalpy changes between these molecules include intermolecular hydrogen bonds and dispersion forces of attraction. It is likely that the interactions between disaccharide and crown ether are weak. However, the binding enthalpy is a summary value of contributions from different processes occurring in the solutions studied (the interactions mentioned above as well as dehydration processes, conformational changes, etc.). Taking into account large entropy changes accompanying the binding process, it is reasonable to suppose that the enthalpy contribution arising from extensive dehydration of the solutes is significant. Among the three disaccharides, sucrose is the most compatible with three-dimensional H-bonded structure of water. Therefore, it has the least dense hydration shell and the smaller value of the hydration number [7,8]. Perhaps, this explains the more negative enthalpy of binding obtained for sucrose associates with the crown ethers. In comparison with sucrose, the formation of associates of  $\alpha,\alpha$ -trehalose with both crown ethers is accompanied by spontaneous absorption of heat. The higher value of interaction strength with water and of hydration number for  $\alpha,\alpha$ -trehalose [7,8] leads to dominant endothermic effect of dehydration process over possible hydrogen bonding and van der Waals interactions between  $\alpha,\alpha$ -trehalose and crown molecules.

It is interesting to note that the thermodynamic parameters of binding of sucrose with 15-crown-5 and 18-crown-6 do not differ significantly. However, it is not true for  $\alpha,\alpha$ -trehalose and  $\beta$ -lactose. It is well known that for efficient binding, the structures of interacting particles must be complementary to each other according to form, size and positions of binding groups [1,3]. The crown ether molecules have different cavity sizes [4]. In aqueous solutions they have different conformations (the predominant centrisymmetrical  $D_{3d}$  for 18-crown-6 [10] and the non-symmetrical for 15-crown-5 [12]) and hence, different hydration energies [11]. On the other hand, stereochemical configurations of saccharide molecules play an important role in their selective binding by synthetic macrocycles [13,14]. Moreover, the disaccharide molecules are flexible and can exist in different conformations in aqueous solutions due to rotation of the monosaccharide moieties around the glycosidic oxygen [15,16]. The data obtained

by ultrasonic velocity measurements and NMR [7,17] indicate that  $\alpha,\alpha$ -trehalose molecules may exist in a more packed conformation in dilute aqueous solutions in comparison with sucrose. Probably, such packed conformation of  $\alpha,\alpha$ -trehalose molecule as well as suitable size and conformation of 18-crown-6 molecule results in more stable associate.  $\beta$ -Lactose forms 1:1 associate with 15-crown-5 but apparently does not associate with 18-crown-6. We suppose that the principle of complementarity or mutual fitting of the interacting particles is not kept in the latter case.

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