Assessment of molecular interaction in a cycluron-cyclodextrin inclusion complex

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Abstract. Thermodynamic parameters and the host-guest stoichiometry of inclusion complex cycluron with β-cyclodextrin in aqueous solution have been determined. The supramolecular structure has been investigated by isothermal titration nanocalorimetry and ¹H NMR spectroscopy at 298.15 K.

1. Introduction
Cycluron is a urea herbicide used to control broadleaf and grassy weeds in cereals and other crops. The inclusion of agrochemicals into cyclodextrins is used to improve the physical properties of many agrochemicals. The inclusion of the herbicides into cyclodextrins is an attempt to improve several physical properties: the low solubility of agrochemicals in water, reduced stability and keeping their efficiency given by the high toxicity.

Cyclodextrins (CDs), are cyclic oligosaccharides having hydrophilic out surfaces and a hydrophobic cavity. They are very good candidates as host molecules because of their ability to form inclusion compounds with a large variety of molecules by selectively incorporating such molecules on the basis of size considerations and selective interactions [1,2]. Accordingly, cyclodextrins are good candidates able to enhance the aqueous solubility, chemical reactivity and spectral properties of cycluron. Among this class of “host” molecules, β-cyclodextrin (β-CD) with seven glucose units in its molecule has been proved to be the optimal molecular partner for cycluron.

The purpose of our research is to investigate and characterize the complexation thermodynamics of β-CD with cycluron using isothermal titration calorimetry (ITC) and to relate the thermodynamic parameters with the structure, by measuring ¹H NMR spectra of the inclusion complex.

2. Materials and methods

2.1. Materials
The solutions were prepared as follows: 15 mM β-CD by dissolving 191.85 mg of β-CD in 10 mL of water and 0.75 mM cycluron by dissolving 7.44 mg in 50 mL of water. Both solutions were prepared using degassed water in a thermal bath at 35-40 °C. The weight of each sample has been measured with a precision of ± 10 μg.
2.2. Isothermal titration calorimetry

ITC is an extremely powerful, highly sensitive technique, able to characterize the weak interaction associated with \( \beta \)-cyclodextrin–cycluron complexation. An ITC experiment provides the complete thermodynamics of a binding reaction, including \( K, \Delta G, \Delta H, \Delta S \) and reaction stoichiometry (\( n \)).

The experiments were performed on a VP-ITC (2G) MicroCal (New Castle, Delaware, USA) calorimeter at 25°C. From a computer-controlled 250-µL Hamilton syringe a series of 40 injections into the 1.4 mL calorimeter cell has been performed. Each injection leads to heater compensation peak, which is integrated to obtain the reaction heat response. The 15 mM \( \beta \)-CD was injected into the stirred (150 rpm) sample cell containing 0.75 mM cycluron in aliquots of 5 µL at 300 s intervals. As compared to reaction heat the dilution contribution was found to be negligible.

2.3. \(^1\)H NMR

\(^1\)H NMR experiments were performed on a Brucker Avance III 500. The outcome used to characterize the \( \beta \)-cyclodextrin–cycluron complex were the chemical shifts of all the protons from the investigated macromolecular structure. The experimental data allows us to determine the reaction stoichiometry and to calculate the association constant \( K \) of the guest-host complex.

The stoichiometry of the complex was determined by the continuous variation method (Job plot).

2.4. Methods

The equilibrium in guest–host reaction is described by the following hypothetical scheme:

\[
A + nB \rightleftharpoons AB_n \equiv C; \quad K = \frac{C}{(AB^n)}
\]

where \( A, B, \) and \( C \) stand for the concentrations of cycluron, \( \beta \)-cyclodextrin and \( \beta \)-cyclodextrin–cycluron complex respectively. The implicit guess is that a cycluron molecule can bind one or two (\( n = 1 \) or 2) \( \beta \)-cyclodextrin molecules.

2.4.1. ITC

In an ITC experiment the reaction heat per mole of injected molecules after the \( i^{th} \) injection is given by:

\[
\frac{q_i}{vB_0} = \frac{\Delta H}{2} \left[ 1 + \frac{1 - r_i/n - (r_0 + r_i)/nKB_0}{\sqrt{(1 + r_i/n + (r_0 + r_i)/nKB_0)^2 - 4r_i/n}} \right]
\]

where \( r_0 = \frac{B_0}{A_0} \); \( r_i = \frac{B_i}{A_i} = \frac{A_0}{V} \), \( A_0 \) and \( B_0 \) are the initial concentrations of cycluron and of the \( \beta \)-CD respectively, \( \Delta H \) is the reaction enthalpy, \( V \) and \( v \) standing for the cell and injection volumes. Equation (1) will be used as fit function for the ITC data. The ITC experiments alowd us to obtain the the stoichiometry \( n \), the reaction enthalpy \( \Delta H \) and the equilibrium constant \( K \).

2.4.2. \(^1\)H NMR

In the \(^1\)H NMR experiments, the overall concentration of the two species was kept constant (\( A_t + B_t = M \)) and the ratio \( \rho = B_t / M \) was varied from 0 to 1. \( A_t \) and \( B_t \) are the total concentrations of the host and guest respectively. This was accomplished by using equimolar solutions of \( A \) and \( B \) and mixing them to constant volume to the desired ratio \( \rho \). Taking into account that: \( A_t = A + C \), \( B_t = B + nC \) we have: \( C = K(\rho M - C)(M - \rho M - nC)^n \).

The absolute concentration of \( C \) will reach a maximum at \( dC/d\rho = 0 \), at \( \rho = 1/(1+n) \). For 1:1 stoichiometry (\( n = 1 \)) we have \( \rho = 0.5 \).

The absence of new peaks that could be assigned to the complex suggested that complexation is a dynamic process, the included guest being in a fast exchange between the free and bound states.
In this case the observed chemical shift $\delta_o$ for a certain proton ($X = A$ or $B$) is given by:

$$\delta_o = \frac{X\delta_j + C\delta_c}{X_j} \Rightarrow X_j\Delta\delta_o = C\Delta\delta_c$$

(2)

where $\Delta\delta_j = \delta_j - \delta_j$, $\Delta\delta_c = \delta_c - \delta_j$, $\delta_j$ and $\delta_c$ standing the chemical shifts corresponding to free $X$ and to the $\beta$-cyclodextrin–cycluron complex ($C$) respectively. The quantity $\Delta\delta_oX_j$, given by the changes in the chemical shift of H-3 and H-5 protons of the $\beta$-cyclodextrin and also of the protons from the methylene an methyl groups of cycluron, was plotted against $\rho$ (figure 4). As fitting function the following $\rho$ - dependence of the complex concentration:

$$C = \frac{M}{2} \left\{ 1 + \frac{1}{K_M} \left[ \left( 1 + \frac{1}{K_M} \right)^2 - 4\rho(1-\rho) \right]^{1/2} \right\}$$

(3)

has been used. All the curves presents a maximum at $\rho = 0.5$ indicating the existence of a 1:1 stoichiometry within the range of the investigated concentrations. Knowing the association constant $K$ from the ITC measurements, the four data sets have been used for finding the chemical shifts of the investigated protons in the $\beta$-cyclodextrin-cycluron complex.

3. Results

The equilibrium constant obtained from ITC measurement was $K = 4753$ M$^{-1}$ which corresponds to a Gibbs free energy $\Delta G = -21$ KJ/mol. The reaction enthalpy of the host-guest inclusion complex is $\Delta H = -8.92$ kJ/mol. This negative value indicates that the formation of host-guest inclusion complex is a weak exothermic process. The entropic effect of the host-guest interaction ($\Delta S = 40.5$ JK$^{-1}$/mol) is positive being due to the balance between the negative contribution brought by the complex formation, and positive contribution induced by the release of water molecules from the $\beta$-cyclodextrin cavity.

![Figure 2. Calorimetric titration of $\beta$-CD solution in cycluron solution. (a) Raw data obtained from 40 automatic injection of 5 µL aliquots of 15 mM $\beta$-CD solution into 0.75 mM cycluron solution 25°C. The heat of dilution has been subtracted. (b) Integration plot of the data calculated from the raw data. The solid line correspond the best fit curve.](image-url)

The main NMR results are given in table 1 and consist in the predicted chemical shifts for several protons from the host-guest inclusion complex.
4. Conclusions
The inclusion process between cycluron and β-CD has been investigated systematically by isothermal calorimetry and $^1$H NMR in aqueous solution at 298.15 K. The microcalorimetry allowed us to obtain the reaction enthalpy, stoichiometry, association constant, Gibbs free energy changes and the entropy effect of the inclusion process based on the directly calorimetric data utilizing non-linear fitting procedure. $^1$H NMR spectra showed that the inclusion phenomenon take place since the most marked modifications of the chemical shift parameters occur for protons that were oriented toward the cavity. The stoichiometry of inclusion complex of β-CD with cycluron is 1:1 revealing the recognition function of the host and guest molecules.

References
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