Thermodynamic analysis of ferulate complexation with α-, β- and γ-cyclodextrins

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ABSTRACT

Isothermal titration calorimetry (ITC) was used to characterize the thermodynamics of the complexation processes of α-, β- and γ-cyclodextrin (CD) with ferulate (FER) in aqueous solutions. The equilibrium constants of ferulate complexation with CDs (K_c, in $dm^3 mol^{-1}$) at pH 9.0 and 25.0 °C were: 176.5 ± 5.0 (β-CD), 53.2 ± 3.4 (α-CD) and 19.4 ± 0.4 (γ-CD). Although FER–β-CD is the tightest complex of the three studied, its binding reaction is also the least exothermic and the only one that is entropically favored. Calculated binding enthalpies, based on the buried surface area upon complexation, are close to those determined by ITC except for the FER–β-CD complex which is more than two times more exothermic. According to these results and those obtained by molecular docking simulations, it is proposed that ferulate binds to the hydrophobic cavity of β-CD, displacing more water molecules than in the other two CD complexes.

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1. Introduction

Ferulic acid/ferulate (FER) is a polyphenol that is employed as a precursor for the synthesis of vanillin [1,2]. Given its antioxidant capacity and antimicrobial properties [3], it is also used in the food industry as a flavoring in a wide range of products and as an ingredient in the formulation of functional foods [4]. However, its use is limited by its low solubility and instability in aqueous systems. To overcome these drawbacks, it can be complexed with natural cyclodextrins (CDs), which are cyclic molecules with six (α-CD), seven (β-CD) and eight (γ-CD) glucopyranose units linked by α-(1,4) bonds [5] that are generally recognized as safe (GRAS) [6]. Structurally, CDs have a truncated cone shape with a hydrophobic cavity and a hydrophilic exterior, and these gives them the ability to increase the solubility and stability of complexed compounds [7]. The thermodynamics of the inclusion of certain guest molecules in the CD cavity does not follow a common pattern given the different contributions of non-covalent interactions, such as hydrogen bonding, van der Waals forces, electrostatic interactions and the hydrophobic effect, which create various thermodynamic profiles. Regarding the energetics of the formation of the FER–CD complexes, only the complexation free energies (calculated from the equilibrium complexation constants, K_c) have been determined to date, whereas the enthalpic and entropic contributions have not been explored [8–12]. Isothermal titration calorimetry (ITC) is a powerful technique that can be used to determine these two thermodynamic contributions from a single experiment, which is not possible to accomplish by other techniques. In this work, we obtained the thermodynamic parameters of FER complexation with three CDs using ITC at 298.1 K and pH 9.0. Due to the moderate solubility of FER in water, the pH of the solution was fixed to 9.0 in order to achieve concentrations of FER and CDs high enough to perform reliable calorimetric titrations for these low affinity systems.

2. Experimental

2.1. Materials

The specifications of chemicals used are presented in Table 1. Scheme 1 shows the molecular structure of *trans*-ferulic acid.

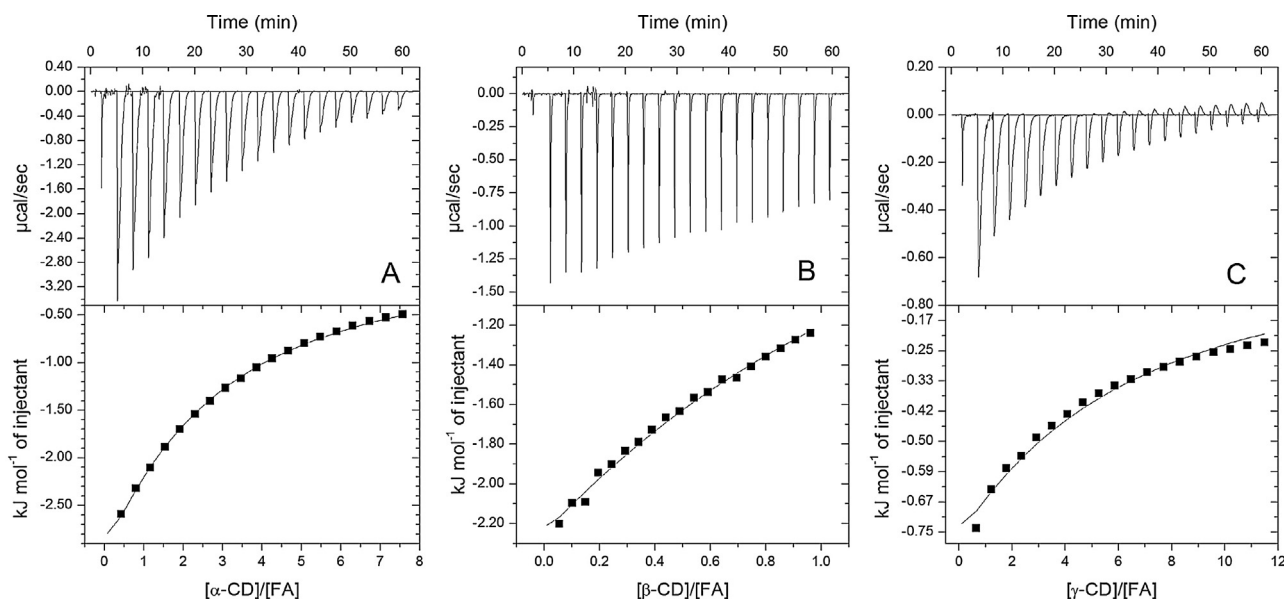### 2.2. Isothermal titration calorimetry

Calorimetric titrations at constant temperature were performed with a high-precision iTC$_{200}$ microcalorimeter (Microcal,

Table 1
Chemical samples.

Compound	CAS No.	Molecular weight(g mol^{-1})	Purification method[a]	Mass fraction purity[b]	Water content	Source
α–CD	10016-20-3	972.84	None	0.98	11%	Wacker
β–CD	7585-39-9	1135.0	None	0.98	11%	Wacker
γ–CD	17465-86-0	1297.0	None	0.98	11%	Wacker
trans-Ferulic acid	537-98-4	194.2	None	0.99	–	Sigma-Aldrich
Sodium Borate	1303-96-4	381.37	None	0.995	10 H_2O	J.T. Baker
Water	–	18.02	MilliQ	Deionized ultrapure	–	Our laboratory

[a] No further purification.
[b] As stated by the supplier.

Fig. 1. Isothermal titration calorimetry of ferulate with α-CD (A), β-CD (B) and γ-CD (C) in 0.050 mol dm^{-3} sodium borate buffer at pH 9.0 and 298.1 K. The concentration of ferulate was 0.0035 mol dm^{-3} in the sample cell, and the syringe contained 0.130, 0.0165 or 0.197 mol dm^{-3} of α-CD, β-CD or γ-CD, respectively.
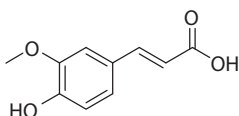
Scheme 1. Molecular structure of *trans*-ferulic acid.

Inc.). Ferulic acid and the cyclodextrins were all dissolved in a 0.050 mol dm^{-3} sodium borate solution at pH 9.0. The sample cell was filled with a 0.0035 mol dm^{-3} FER solution, and the pipette contained the working CD solution. The CD concentrations were 0.197, 0.130, and 0.0165 mol dm^{-3} for γ-CD, α-CD and β-CD, respectively. Concentrations were selected according to the solubilities of the respective compounds, in order to achieve the highest possible concentration of the complex in the calorimeter cell, which in turn results in more reliable heat measurements. All of the solutions were freshly prepared and previously degassed under a pressure of 61.3 kPa, with moderate magnetic stirring for 5 min at 298.1 K. The titrations consisted of a preliminary 0.4 μL (dummy) injection followed by 19 additions of 2.0 μL, with a 3 min interval between injections. The experiments were performed at 298.1 K, with a stirring speed of 1000 rpm. To determine the heat of the dilution of the CDs, titrations were performed with buffer alone under the same conditions in the sample cell. These values were subtracted from the measured heats in the presence of FER. Nonlinear fitting of the normalized titration data, based on an identical and independent binding sites model [13], generates the complexation constant (K_c), the enthalpy change (ΔH_c) and the stoichiometry (n).

2.3. Molecular modelling and docking simulations

Molecular modeling was performed with the MOE 2013.08 software package (www.chemcomp.com). The cyclodextrin structures (α-, β- and γ-cyclodextrin) were obtained from the Protein Data Bank (www.pdb.org) from files with IDs 4D5B, 2ZYN and 1D3C, respectively. The ferulic acid structure was constructed using MOE–Build, and then, the carboxyl and phenolic groups were both deprotonated, fixing their charges to −1. Prior to docking, all of the structures were minimized using the MMFF94x force field up to a gradient of 0.004 kJ mol^{-1} Å^{-2}.

MOE–Dock_Induced_Fit was the docking protocol used to model the interaction between FER and each CD. *Triangle Matcher* was the method employed for placing the ligand conformers onto the CD cavity, and *London DG* was the scoring function for the rigid poses that were generated. The structure of the generated complexes was further refined, allowing for position readjustments through molecular mechanics calculations, using the MMFF94x force field and the *GBVI/WSA dG* rescoring function. Finally, the best scoring complexes for each CD were re-submitted to an energy minimization up to a gradient of 0.004 kJ mol^{-1} Å^{-2}.

2.4. Accessible surface area calculations

The calculations of accessible surface area A_i were done with the program NACCESS [14], based on the Lee and Richards algorithm [15], using a probe radius of 1.4 Å and the atomic coordinates of the modeled complexes and FER molecule. The atomic coordinates