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The protonation thermodynamics of ferulic acid/γ-cyclodextrin inclusion compounds

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Abstract

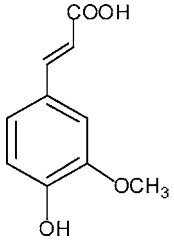
The physico-chemical properties of ferulic acid (FA) and its inclusion compounds with the γ-cyclodextrin (γ-CD) have been studied at 25 °C in aqueous solution (0.15 M NaCl) by potentiometry and solution calorimetry. The two basicity constants of the ionized FA, $\log K_1$ 8.94 and $\log K_2$ 4.48, in agreement with those of compounds of similar structure, were attributed to the protonation of hydroxyl and carboxyl ionized groups, respectively. Unlike $\log K_2$, the first basicity constant showed a slight decreasing pattern on increasing the amount of γ-CD. On the other hand, calorimetric data showed greater exothermicity during the protonation of the COO^- group in FA when in the presence of the γ-CD. Any increase of the latter led to greater enthalpy ($-\Delta H^\circ$) and lower entropy (ΔS°) changes. The increase of $-\Delta H^\circ$ and the decrease of ΔS° values reached a constancy only beyond γ-CD/FA molar ratios of 1. Thermodynamic data were consistent with the hypothesis of the inclusion complexation obtained by the penetration of the neutralized guest FA into the γ-CD host cavity.

Keywords: Ferulic acid; γ-Cyclodextrin; Inclusion compounds; Titration calorimetry; Enthalpy and entropy changes

1. Introduction

Phenolic compounds, as secondary plant metabolites found in fruits and vegetables, seem to be involved in the defense of plants against pathogens, like insects, viruses, etc. [1–3]. Among them, ferulic acid (FA) is a phenolic cinnamic acid derivative known to act as an in vivo substrate for peroxidase [4]. FA enhances the rigidity and the strength of plant cell walls by cross-linking with polysaccharides, thereby making the cell wall less susceptible to enzymatic hydrolysis during germination [5,6].

The chemical structure of FA, namely 3-methoxy-4-hydroxycinnamic acid,



shows an extended side chain conjugation, suitable to form a resonance stabilized phenoxy radical under UV absorption, which accounts for its strong antioxidant potential [7]. The ability to form stable radicals and to show a radical-scavenging activity [7,8], makes FA an interesting candidate for many industrial applications. Besides the various physiological protections against several diseases, FA may constitute the active ingredient in many skin lotions and sunscreens designed for photoprotection [7,9,10].

Recently, the Tsuno Rice Chemicals Co. Ltd., that improved a large-scale extraction and purification of natural FA from the byproduct of rice bran salad oil refining,

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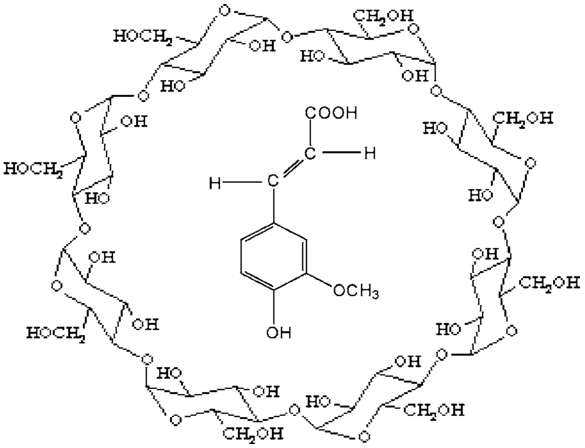
developed a novel product named ferudex™ [11]. Ferudex™ is a γ-cyclodextrin clathrate compound of FA that has been developed with the aim to improve the stability of FA, thus avoiding discoloration under light and at high temperatures. When it was applied to cosmetics, food, and drugs, it led to demothing commodity value of a feeling use. Moreover, the potential uses of cyclodextrins (CDs) in pharmaceutical, cosmetics, chemical products and technologies are widely reported [12,13]. CDs are cyclic oligosaccharides being formed by six (α-), seven (β-), or eight (γ-) glucose units [14]. In all cases, they display a torus-like or hollow truncated cone shape, with a cavity and two hydrophilic rims in which the primary and secondary hydroxyl groups are inserted. The main feature that makes CDs of interest is their ability to form inclusion compounds with a variety of guest molecules, in solution or in the solid phase. The first requirement for a molecule to form an inclusion compounds with CDs, is to fit in the cavity, either totally or partially. In addition to this, a favourable energetic balance is required, which depends on the nature of the guest and on the inner diameter of the CD (host). The main driving force of inclusion compound formation is the release of enthalpy-rich water molecules from the cavity. Water molecules are displaced by more hydrophobic guest molecules present in the solution to attain an apolar–apolar association and a decrease of the cyclodextrin ring strain, resulting in a more stable lower energy state [12]. The binding of guest molecules within the host cyclodextrin is not fixed or permanent but rather is a dynamic equilibrium. Binding strength depends on how well the host-guest complex fits together and on specific local interactions between surface atoms [15].



The aim of this paper is to evaluate the solution behaviour during the protonation process of the ionized FA, when it is by alone and when it is in the presence of the γ-CD. This allows a deep knowledge of the host–guest mechanism that will be of scientific interest in view of technological applications. To this purpose we have undertaken a systematic thermodynamic study to clarify the role played by the γ-CD and the stoichiometry of the FA/γ-CD inclusion compound in aqueous media (0.15 M NaCl and 25 °C) by using mainly potentiometric and solution calorimetric techniques.

2. Experimental

2.1. Materials

Ferulic acid (FA) and γ-cyclodextrin (γ-CD) were obtained from Tsuno Rice Chemicals Co. Ltd. (Japan). Ferudex™, a clathrate compound of FA with γ-CD, was synthesized by the Tsuno company [11]. Solutions were prepared with double distilled water at a constant ionic strength (0.15 M NaCl). Standardized NaOH and HCl solutions (0.1 M) were used throughout.

2.2. Potentiometry

Potentiometric titrations were carried out in aqueous solution at 25 °C by using a TitraLab 90 titration system from Radiometer Analytical. TitraLab 90 consists of three components: the TIM900, a powerful Titration Manager, the ABU901, a high-precision autoburette and the SAM7, a convenient sample stand. A Windows based software (TimTalk 9) was used in connection with the TIM900 Titration Manager for remote control. Titrations of the compounds were performed in a thermostated glass cell filled with 100 mL of 0.15 M NaCl, in which a weighed amount of solid materials (0.16 mmol of FA; 0.040 mmol of γ-CD) and a measured volume of standard hydrochloric acid solution were dispersed by magnetic stirring, under a presaturated nitrogen stream. Ferudex™ was dissolved in the alkaline solution and titrated with 0.1 M HCl. Forward and backward titrations with respectively 0.1 M NaOH and 0.1 M HCl standards showed rielable results. Basicity constants were evaluated with the Superquad program [16], running on PC. The results of three replicates were averaged.

2.3. Calorimetry

Calorimetric titrations were carried out in aqueous solution at 25 °C with a Tronac calorimeter (mod. 1250) operating in the isothermal mode. The aqueous solution (25 mL of 0.15 M NaCl), containing a weighed amount of compounds (0.13 mmol of FA; 0.013–0.26 mmol of γ-CD) and a measured volume of standard sodium hydroxide, was titrated with standard 0.1 M HCl solution at a constant BDR (Buret Delivery Rate) of 0.0837 mL/min through a Gilmont buret. Calorimetric titrations of FA with γ-CD solutions were performed in acetate and phosphate buffered solutions (0.01M) at pHs 4.21 and 7.31, respectively. All the experiments were automatically controlled by the Thermal program, from Tronac Inc., that was renewed to operate through a NI-DAQ driver software in Windows, from National Instruments. The graphical programming language LabVIEW was used to create the application. Calibration of the apparatus and corrections for the heats of dilution of the titrant were made before each titration run. The enthalpy change values were computed with the Fifth program [17], whereas the entropy change values were calculated by the relation $\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T$, with ΔG°

Table 1
Basicity constants of ferulic acid (FA) protonation in the absence and in the presence of γ-CD (0.15M NaCl and 25 °C)

Compound	γ-CD/FA molar ratio	Reaction step[a]	log K[b]
FA	0.00	First	8.94 (1)
		Second	4.48 (1)
FA + γ-CD	0.25	First	8.88 (2)
		Second	4.51 (3)
Ferudex™	0.76	First	8.69 (1)
		Second	4.47 (1)

[a] First: $L^{2-} + H^+ = LH^-$; second: $LH^- + H^+ = LH_2$ (L^{2-} is the complete ionized form of FA).

[b] Values in parentheses are standard deviations.

= $-RT \ln K$. The results of at least three replicates were averaged.

3. Results and Discussion

3.1. Basicity Constants

The analysis of potentiometric titration curves allowed to check the purity of FA (98.5%), and evaluate the two basicity constants (log K). These were easily measured, because the two different buffer regions were separated by a sharp end-point of ca. 4 pH units. This occurred for either simple FA and for FA in the presence of γ-CD. The basicity constants for the different investigated systems are reported in Table 1. The first log K value was attributed to the protonation of the ionized hydroxyl group in FA, this being the more basic, while the second log K was associated with the carboxylate anion. Their values were close to those showed by many phenolic compounds [18] and very similar to those reported by Merdy, that studied lignin model compounds, including FA [19]. In the latter case, log K_1 was slightly lower (8.71, ref. [19]) because of the higher ionic strength (1 M KNO_3), whereas log K_2 was the same (4.46, ref. [19]). Unlike the log K_2, the magnitude of the log K_1 values was also affected by the presence of γ-CD, showing a slight decreasing pattern as the γ-CD/FA molar ratios increased. This trend may be attributed to a shielding effect exerted on the negative charges of FA by the γ-CD molecules, similarly to what showed by simple salts. Potentiometric analysis revealed that the ferudex™ forms an inclusion compound containing more than one FA molecule per γ-CD cavity. In fact, the γ-CD/FA molar ratio was 0.76.

3.2. Enthalpy and entropy changes

Unlike the basicity constants, the enthalpy ($-\Delta H^\circ$) and entropy (ΔS°) changes for the protonation of basic groups in FA were more sensitively dependent on the presence of the γ-CD. Any increase of the latter led to an increased exothermicity, expecially when the protonation of the COO^- group was concerned. Fig. 1 shows a typical plot of the heat evolved during the protonation reaction of ionized hydroxyl and carboxylate groups in FA alone and when complexed in ferudex™.

As shown in Fig. 1, the slope of the –Q/α plot is greater for ferudex™ than for FA. Table 2 reports the values of $-\Delta H^\circ$ and ΔS° along with the $-\Delta G^\circ$ for the simple and the complexed FA in ferudex™. The $-\Delta H^\circ$ values were obtained by taking into account the dependence of the log K_1 on the γ-CD/FA molar ratios, together with the analytical data, and all the calorimetric titration points (heat evolved/mL of titrant). The $-\Delta G^\circ$ and the ΔS° were calculated using the formulas $RT \ln K$ and $(\Delta H^\circ - \Delta G^\circ)/T$, respectively.

The protonation of both phenolic and carboxyl ionized groups in FA showed both positive $-\Delta H^\circ$ and ΔS° values. Due to the lack of literature data for molecules strictly related to FA, we found suitable correlations by taking into account enthalpy and entropy changes of a phenolic compounds series (Table 3) [20].

In this series we can observe that the increased substitution of a weakly electron withdrawing group in 2-,4-,6- positions led to a regular decrease of $-\Delta H^\circ$ and to an increase of ΔS° (compounds **1**,**2** and **3**). In line with this, the presence of the vinyl substituent showed greater electron withdrawing properties to allow the lower $-\Delta H^\circ$ of 17.6 kJ mol^{-1} found in FA (Table 2). On the other hand, the ΔS°, that was expected to increase in FA, showed a decrease to 112 J mol^{-1} K^{-1}. This trend may be likely attributed to intramolecular hydrogen bonds between the protonated hydroxyl and the neighboring methoxy oxygen in FA. The five-membered ring structure resulted in a more stable and ordered geometry at lower ΔS°. This hypothesis may be corroborated from the results

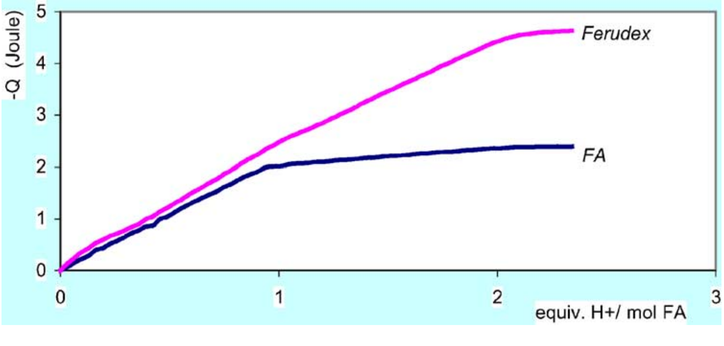


Fig. 1. Calorimetric titrations of ionized FA and ferudex™: heat evolved (Q, Joule) in relation to the degree of protonation α (equivalent H^+/mol FA).

Table 2
Thermodynamic functions of ferulic acid (FA) and ferudex^{TM} protonation in 0.15 M NaCl and 25 °C

Compound	Reaction step	$-\Delta G^{\circ a}$ (kJ mol^{-1})	$-\Delta H^{\circ\ b}$(kJ mol^{-1})	$\Delta S^{\circ b}$ (J mol^{-1} K^{-1})
FA	First	51.0	17.6 (4)	112.2 (1.3)
	Second	25.6	3.4 (2)	74.5 (7)
Ferudex^{TM}	First	49.6	21.1 (2)	95.4 (7)
	Second	25.5	18.5 (7)	23.4 (2.3)

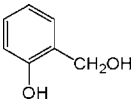
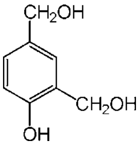
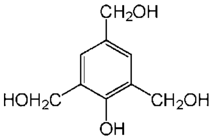
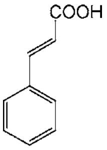
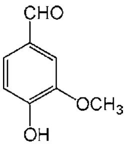
[a] $-\Delta G^\circ = 5.708 \log K$.

[b] Values in parentheses are standard deviations.

showed by the 4-hydroxy-3-methoxy-benzaldehyde (compound **5**, Table 3), that revealed lower ΔS°, as lower was also $-\Delta H^\circ$; this is again in line with our data for FA. Moreover, the protonation of the COO$^-$ group in FA showed thermodynamic functions very close to those reported by the cinnamic acid (compound **4**, Table 3). Even if in the latter case the heats of reactions were evaluated by the van't Hoff method [21].

Regarding the simple steps, the protonation mechanism of FA in the presence of the γ-CD seemed more complicated. In ferudex^{TM}, $-\Delta H^\circ{}_1$ was greater of ca. 3 kJ mol^{-1} and ΔS_1° lower of ca. 17 J mol^{-1} K^{-1} (Table 2). These results may be likely attributed to H-bonding interactions of FA being protonated and surrounding γ-CD molecules. Instead, when the COO$^-$ group in FA is considered, $-\Delta H^\circ{}_2$ showed a very high value that could not be attributed only to simple H-bonds, but also to other overcoming mechanisms. This reflects a sharp decrease of ΔS_2°. All this means that another strong interaction between the FA and the γ-CD molecules should overcome the simple H-bonding process. The likely hypothesis is that the complete neutralization of the FA made this molecule more hydrophobic, thus making easier its interaction with the hydrophobic cavity of γ-CD. The main contribution to the inclusion process of FA into the γ-CD was the enthalpic one, because the hydrophobic cavity of the γ-CD was filled more effectively. So, a systematic calorimetric study of the interaction between the FA and the γ-CD was undertaken by varying the γ-CD/FA molar ratios from 0 to 2. Fig. 2 shows the $-\Delta H^\circ$ and ΔS° values in relation to the γ-CD/FA molar ratio.

Table 3
Thermodynamic values of related phenolic compounds protonation [20]

Number	Compound	log *K*	$-\Delta H^\circ$ (kJ mol^{-1})	ΔS° (J mol^{-1}·K^{-1})
1		9.95	22.6	114.7
2		9.79	20.5	118.9
3		9.56	18.8	119.7
4		4.40	2.5	75.8
5		7.40	15.7	89.2

1: 1-hydroxy-2-hydroxymethyl-benzene; 2: 1-hydroxy-2,4-dihydroxymethyl-benzene; 3: 1-hydroxy-2,4,6-trihydroxymethyl-benzene; 4: cinnamic acid; 5: 4-hydroxy-3-methoxy-benzaldehyde.

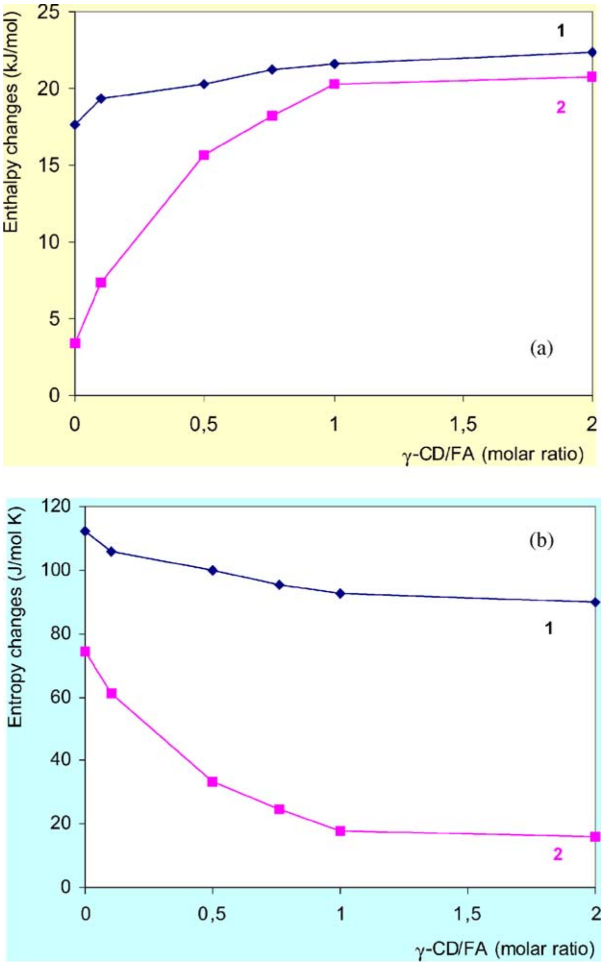


Fig. 2. (a) Enthalpy, $-\Delta H^\circ$, and (b) entropy, ΔS°, changes of FA protonation in relation to the γ-CD/FA molar ratio (0.15 M NaCl and 25 °C).

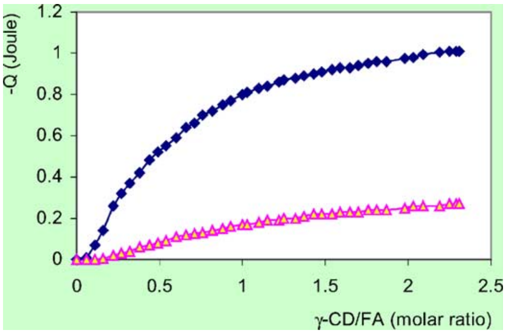


Fig. 3. Calorimetric titrations of the γ-CD titrant solution (concentration 0.10 mol L^{-1}) in the FA titrate solution (0.10 mmol in 25 mL) buffered to pHs 4.21 (upper curve) and 7.31 (lower curve).

Unlike the slight differences in $-\Delta H^\circ$ and ΔS° for the first protonation step of FA, the protonation of the COO^- group showed a regular $-\Delta H^\circ$ increase and a concomitant ΔS° decrease until the γ-CD/FA molar ratio of 1:1 was reached. Beyond the latter value, the thermodynamic functions remained constants. These results are also in agreement with the 1:1 stoichiometry of the γ-CD/FA inclusion compound.

To better ascertain the above mentioned process, that derived from the inclusion of the neutral FA molecule into the γ-CD cavity, direct calorimetric interactions were performed; this allowed to measure the heat evolved only for the formation of the inclusion compound. Fig. 3 shows typical plots of the heat evolved by adding the γ-CD to the FA aqueous solutions at two different pH values. These were chosen according to the different amount of carboxyl groups in the more hydrophobic FA molecule.

The two buffer solutions had pHs 4.21 and 7.31 corresponding respectively to 65 and 0.15% of COOH form in FA as evaluated by the log Ks. Any attempt to decrease the solution pH to less than 4.2 failed because of the low solubility of FA at high concentrations. The results of Fig. 3 are in agreement with the inclusion complexation mechanism that occurs only when FA is completely protonated. A greater amount of FA in the COOH form, improves a greater exothermicity and the FA/γ-CD inclusion complex forms to a stoichiometric ratio of 1:1, as can be seen by extrapolating the two curves. The slope of the flatter part of the curve, even lower, indicates that the forming inclusion compound is not fixed or permanent but rather is a dynamic equilibrium of low stability.

4. Conclusions

The study of ferulic acid protonation showed enthalpy and entropy changes closer to phenolic related compounds. This permitted to give an evaluation of intramolecular hydrogen bonds between the phenolate group being protonated and the neighboring methoxy oxygen. The same interaction was recently predicted on the basis of different pK_a values, obtained by studying four monomeric molecular models of lignin [19].

Moreover, the protonation of FA in the presence of γ-CD showed greater enthalpy and lower entropy changes. The enthalpy-driven process was accomplished with charge neutralization of the FA, because the hydrophobic cavity of the γ-CD was filled more effectively. The inclusion complexation showed that the FA/γ-CD stoichiometry was unitary, even though a greater amount of FA was retained in the ferudex^{TM}. This should be probably accounted with FA molecules entrapped outside the γ-CD during the phase separation process to ferudex^{TM}.

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