Journal of Inclusion Phenomena and Macrocyclic Chemistry **44:** 395–397, 2002.

Inclusion of Polyphenol Oxidase Substrates in β-Cyclodextrin: A ^{1}H-NMR Study

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(Received: 7 May 2002; in final form: 1 October 2002)

Key words: apparent association constants, caffeic acid, chloragenic acid, β-cyclodextrin, enzymatic browning, polyphenol oxidase (PPO)

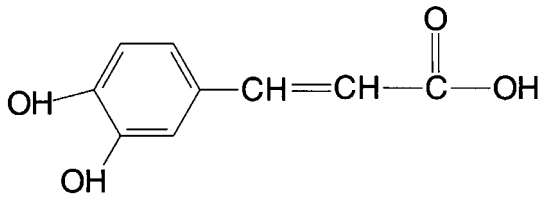
Abstract

A ^{1}H-NMR study of the interactions between β-cyclodextrin (β-CD) and included phenolic molecules (chloragenic acid and caffeic acid) in aqueous medium is reported. The results confirm that inclusion occurs. Data analysis by the continuous variation method shows that all the complexes have 1 : 1 stoichiometries. Values for the apparent association constants of the inclusion compounds are estimated and compared with previously reported values.

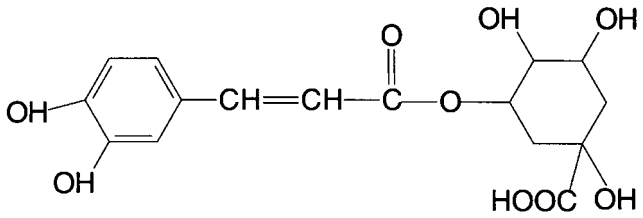
Introduction

In certain plant-derivated foods, such as juices, enzymatic browning – initiated by the enzyme polyphenol oxidase; *o*-diphenol: oxygen oxireductase EC 1.10.3.1 or PPO – occurs due to the oxidation and subsequent condensation of naturally occurring phenolic compounds, such as chloragenic acid (CGA) and caffeic acid (CA) (Scheme 1), and results in an undesirable pigmentation of the product. The control of enzymatic browning in fresh plant products is a problem for the food processing industry since the utilization of sulfites, the most effective inhibitor of browning, became restricted. Thus the minimally processed plant products offers a significant economic use for sulfite alternatives such as cyclodextrins. β-Cyclodextrin (cyclomalto-heptaose, β-CD) – a short, hollow, truncated cone shaped molecule – is a cyclic oligosaccharide composed by seven α(1–4) linked gluco-pyranose units in normal chair conformation. β-CD interacts with other molecules, which may get into the cavity thus originating inclusion compounds. β-CD is a chiral cyclic oligosaccharide whose natural enantiomer is R-(+). Both in the crystalline hydrate and in aqueous media, the β-CD molecule interacts with water molecules, some of which are removed when a guest of suitable size goes into the cavity.

In this work, a ^{1}H-NMR study of the interactions between β-CD and chloragenic acid (CGA) and caffeic acid (CA) in aqueous medium is reported.



Caffeic acid



Chloragenic acid

Scheme 1.

Materials and experimental methods

β-CD was obtained by Fluka, Switzerland., chloragenic acid, (1,3,4,5-tetrahydroxycyclohexanecarboxylic acid (CGA)), caffeic acid, (3,4-dihydroxycinnamic acid (CA)), D_2O and $CDCl_3$ (99.5% isotopic purity) were obtained from Aldrich, Madrid. Room temperature (T = 298 K) ^{1}H-NMR spectra were recorded with a Varian UNITY-500 NMR spectrometer, 499.824 MHz. D_2O was used as solvent

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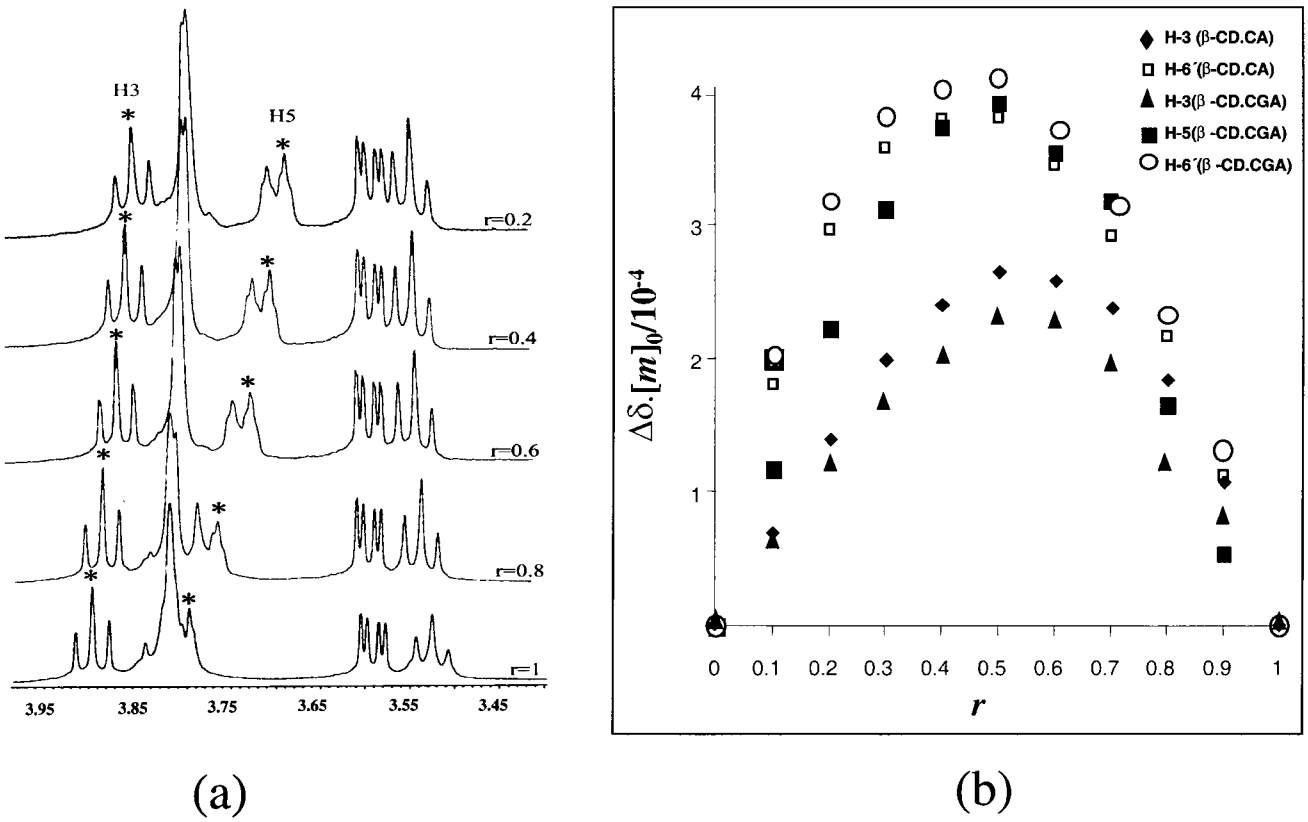

Figure 1. (a) Typical 500 MHz ^{1}H NMR spectra for mixtures of β-CD and Caffeic acid D_2O solutions with different values of r, in the region of the H(3) and H(5) signals. (b) Continuous variation plots of 500 MHz ^{1}H-NMR spectra, for mixtures of β-CD and Guest, (Chloragenic acid, CGA and Caffeic acid, CA)) in D_2O solutions with different values of r in the region of the H(5) and H(3) signals and H (6′) from Guest molecules.

and chemical shifts are given relative to external reference trichloromethane ($CDCl_3$, δ = 7.20 relative to TMS). The residual water signal was reduced by using Presat sequence.

The complexes stoichiometries were determined using the continuous variation method, Job plots [1]. 10 mM D_2O solutions of the guest (G) and of β-CD were mixed to constant volume (i.e., the sum of the initial concentrations of β-CD and G remains equal to 10 mM, $[\beta\text{-CD}]_0 + [G]_0$ = 10 mM), and to defined values of $r = [\beta\text{-CD}]_0/([\beta\text{-CD}]_0 + [G]_0)$ or $r = [G]_0/([\beta\text{-CD}]_0 + [G]_0)$ (r took values from 1/10 to 9/10, in steps of 1/10). The stoichiometries were finally determined by plotting $\Delta\delta.[\beta\text{-CD}]$ or $\Delta\delta.[G]$ against r and finding the r values corresponding to the maxima of these distributions.

The apparent association constant, K_{app}, measuring the extent of complex formation, was estimated by the Benesi-Hildebrand regression method [4]. The guest concentration was set at 0.2 mM and that of the β-CD varied from 5 to 10 mM, one of the species observed in the presence of a large excess of the other component.

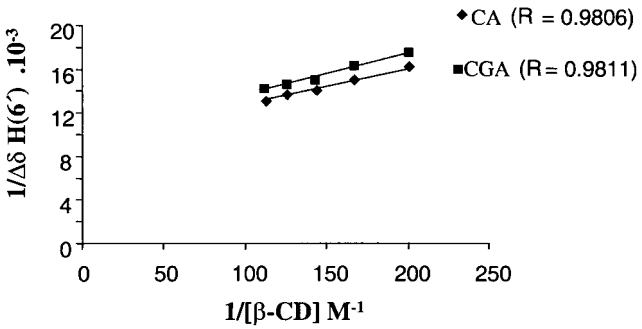

Figure 2. Benesi-Hildebrand plots obtained using H(6′) protons of Guest (Chloragenic acid, CGA and Caffeic acid, CA). The total concentration of the Guest was kept constant (0.2 Mm).

Results and discussion

The H(3) and H(5) protons of β-CD form two inner 'crowns' of hydrogen atoms, in the wider and narrower rims of β-CD, respectively. These 'crowns' of protons have strategic positions for reporting host-guest interactions in the cavity. Both H(3) and H(5) are appreciably shifted to lower frequencies and point to the inside of the cavity, it can be inferred that the formed species are inclusion complexes (Figure 2a). In other hand, the $\Delta\delta$s of H(6′) resonances of guest molecule shows significant chemical shifts perturbations. So, we used the H(5), H(3) and H(6′) NMR signals for probing the *β-CD.guest* interaction (Figure 1a and b).

Stoichiometry of the inclusion compounds

For a 500 MHz spectrometer, and a typical value of the largest observed chemical shift difference ($\Delta\delta_{max}$ = 0.2), the fast exchange condition (i.e., the exchange rate larger than the reciprocal of the largest observed frequency shift in Hz)

Table 1. Chemical shift differences for the H(6′) protons of the Guest ([chlorogenic acid] = [caffeic acid] = 0.2 mM in mixtures of a D_2O solution of β-CD

	$\Delta\delta$ H(6′)/Hz	
$[\beta\text{-CD}]_0$/mM	*CGA*	*CA*
0	3525	3533
5	3468	3475
6	3464	3470
7	3458.5	3468
8	3456.5	3466
9	3455	3462.5

implies that inclusion and release of the guest should occur at least 100 times/s. Under these conditions, the frequency of a proton signal is obtained by averaging the frequencies of the free and complexed species, weighted by their mole fractions. From this relationship, one easily arrives at $[C]/[\beta\text{-CD}]_0 = \Delta\delta/\Delta\delta_{max}$, that is, $\Delta\delta$ provides a means for measuring the concentration of the inclusion complex, [C] [2]. By plotting $\Delta\delta.[\beta\text{-CD}]_0$ against r ($\Delta\delta$ is the chemical shift difference for H(5), $\Delta\delta = \delta_{(\text{free }\beta\text{-CD})}\text{-}\delta_{(\beta\text{-CD.G})}$), one obtains maxima at $r = 0.5$ in all cases (Figure 2b), pointing to the formation of 1 : 1 complexes. These 'Job plots' are roughly symmetrical, suggesting that one type of inclusion compounds should be dominant, as competitive formation of complexes would give rise to asymmetric curves [2].

Apparent association constants

The equilibrium for the inclusion process in aqueous solution involves hydrated forms of β-CD and G, and represents a substitution of water molecules in the β-CD cavity by the incoming guest molecule. The apparent association constant, K_{app}, measuring the extent of complex formation, was estimated by the Benesi-Hildebrand method [4] (Table 1, Figure 2). The apparent association constant K_{app} obtained from this procedure was 504 M^{-1} for the *β-CD.Chloragenic acid* inclusion compound and 936 M^{-1} for *β-CD.caffeic acid* inclusion compound.

Conclusion

The results herein reported for K_{app} indicate that these systems satisfy the basic requirements (K_{app} in the range 10^2–10^4 M^{-1}) for use in the pharmaceutical and food industries. In addition, the K_{app} value herein obtained for β-CD.CGA is of the same order of magnitude of a previously determined value obtained by ultra violet-visible spectrophotometry [5].

Acknowledgements

The authors acknowledge support from Fundação da Ciência e Tecnologia (F.C.T.), Lisboa, to the research units Molecular Physical Chemistry and Ressonance Magnetic Nuclear Spectroscopy, Coimbra University.

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