Cyclodextrin Inclusion Complexes with Caffeoylquinic Acids as Bioactive Compounds

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Abstract

Neochlorogenic acid, a less-studied caffeoylquinic acid, isomer of chlorogenic acid, has been seen to possess antioxidant, antifungal, anti-inflammatory and anticarcinogenic effects, which makes it an interesting bioactive compound for incorporation in nutraceuticals, drugs or functional foods. However, its poor solubility in water and susceptibility to oxidation make such a task difficult. To overcome that, its encapsulation in cyclodextrins (CDs) is proposed. The fluorescence of neochlorogenic acid in different pH conditions was analyzed, and caffeic acid was proved to be the fluorescent moiety in the molecule. An encapsulation model whereby the ligand poses two potential complexation sites (caffeic and D-(-)-quinic moieties), showed that α-CD and HP-β-CD formed the best inclusion complexes with neochlorogenic acid, followed by M-β-CD, β-CD and γ-CD. Molecular docking with the two best CDs gave better scores for α-CD, despite HP-β-CD providing stabilization through H-bonds. The encapsulation of chlorogenic acid led to a similar CD order and scores, although constants were higher for α-CD, β-CD and M-β-CD, lower for HP-β-CD, and negligible for γ-CD. The solubility and the susceptibility to oxidation of neochlorogenic acid improved after complexation with α-CD and HP-β-CD, while the antioxidant activity of both isomers was maintained. These results could lead to obtaining more stable inclusion complexes with caffeoylquinic acids for applications in the pharmaceutical industry.

Introduction

Neochlorogenic acid (3-CQA) is a less known isomer of chlorogenic acid (5-CQA) formed by the ester binding between caffeic acid (CA) and D-(-)-quinic acid (QA) (Fig. 1A y 1B) and found in peaches, prunes, plums, coffee beans and rosemary leaves. Although 5-CQA is well-known by its biological activities, 3-CQA has also demonstrated to possess antioxidant, antifungal, anti-inflammatory and anticarcinogenic activities[1-4]. However, its low aqueous solubility and susceptibility to oxidation interfere with its potential applications in pharmaceutical industry.

Encapsulation in cyclodextrins (CDs) (Fig. 1C) have demonstrated to be a suitable way to overcome problems of this nature. CDs are torus-shaped oligosaccharides made up of α-(1,4) linked glucose units. They have a hydrophobic inner cavity, in which poorly water soluble compounds could interact non-covalently, unlike their mainly hydrophilic outer surface, that makes the whole molecule fairly polar and water soluble. These agents were effective in complexing the isomer 5-CQA in a competitive 1:1 model, in which CD could capture the guest molecule either by CA moiety or QA moiety. As 3-CQA have the same moieties, this model could be applied too. Indeed, this is the first work to analysed the encapsulation of 3-CQA.

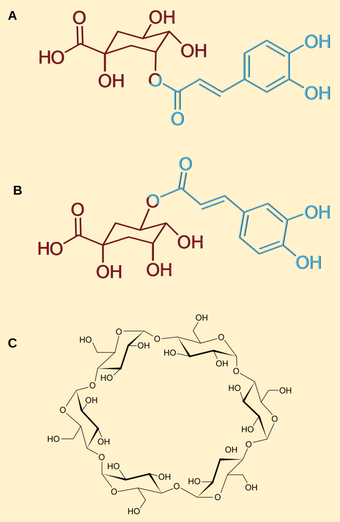

Fig. 1. Chemical structures of A) 3-CQA, B) 5-CQA and C) α-CD

Objectives

1. To evaluate the fluorescence of CQAs and the reason for the fluorescence
2. To analyse the encapsulation mechanism of 3-CQA by different types of natural and modified CDs in compare to 5-CQA (experimentally and computationally)
3. To analyse the solubility and susceptibility to oxidation of 3-CQA / CD complexes
4. To determine the antioxidant activity of CQAs in the presence and absence of CDs.

1 Fluorescence

By measuring the fluorescence of 3-CQA, 5-CQA, CA and QA, it was proved that CA was the fluorescent moiety of both CQAs, as QA was not fluorescent. Then, the fluorescence at maximum excitation wavelength (λexc) of the three fluorescent acids was analysed at different pH conditions (Fig. 2). It was found that there was almost no variation in the maximum emission wavelength (λem) of CQAs at pH 3 and pH 5, although CA decrease 10 nm at pH 5. Conversely, pH 9 induced an increase of more than 40 nm in the λem of all the molecules in compare to pH 5, and a high rise of fluorescence intensity of CA. This last fact was associated to the internal resonance of CA as dihydrocaffeic acid (which has the same structure as CA but does not present the internal double bond) was shown to be non-fluorescent.

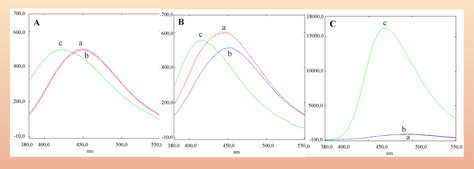

Fig. 2. Fluorescence emission signal of (a) 3-CQA, (b) 5-CQA and (c) CA at A) pH3, B) pH 5 and C) pH 9

2 Encapsulation in Cyclodextrins

EXPERIMENTAL STUDY

The relative fluorescence at pH 5 of both CQAs at increasing concentration of α-CD, β-CD, γ-CD, HP-β-CD and M-β-CD was measured and the encapsulation constants for the competitive model 1:1 determined with R^2>0.97 in every case. It was found that α-CD (K_{F1} = 458 ± 23 M^{-1}) and HP-β-CD (K_{F1} = 504 ± 25 M^{-1}) formed the most stable inclusion complexes (Fig. 3) with 3-CQA followed by M-β-CD (K_{F1} = 315 ± 16 M^{-1}), β-CD (K_{F1} = 160 ± 8 M^{-1}) and γ-CD (K_{F1} = 22 ± 1 M^{-1}). The results were similar for 5-CQA, although constants were higher for α-CD (K_{F1} = 530 ± 27 M^{-1}), β-CD (K_{F1} = 312 ± 16 M^{-1}) and M-β-CD (K_{F1} = 382 ± 19 M^{-1}), lower for HP-β-CD (K_{F1} = 440 ± 22 M^{-1}) and negligible for γ-CD.

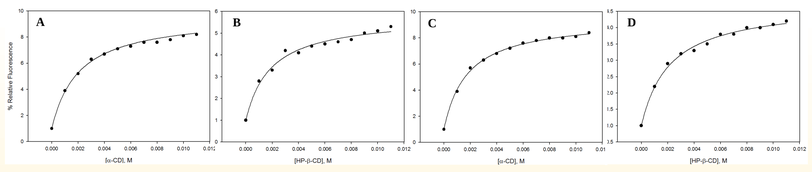

Fig. 3. Complexation curves of α-CD and HP-β-CD with 3-CQA (A and B) and 5-CQA (C and D) (25 ºC pH 5)

COMPUTATIONAL STUDY

Molecular modelling of the most likely inclusion complexes with CQAs showed a better binding force with α-CD than with HP-β-CD, although HP-β-CD complexes possess H-bonds (Fig. 4). Comparing both acids, 3-CQA gave a slightly better score than 5-CQA.

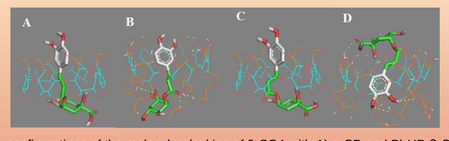

Fig. 4. Most likely configurations of the molecular docking of 3-CQA with A) α-CD and B) HP-β-CD, and 5-CQA with C) α-CD and D) HP-β-CD. Flexible atoms of CDs are orange and non-flexible atoms blue. H-bonds are yellow dotted lines.

3 Solubility and Oxidation

After 3 days of incubation in water, the two best CDs selected previously (α-CD and HP-β-CD) increased in 17 % and 26 % the aqueous solubility of 3-CQA, respectively, at the highest concentration analysed (Fig. 5A). Moreover, enzymatic oxidation by polyphenol oxidase (PPO) showed that 50 % of free 3-CQA was lost after 10 minutes reaction. Meanwhile, the complexation with HP-β-CD reduced this percentage to 24 %, and less than 6 % with α-CD (Fig. 5B).

In general, HP-β-CD gave the best solubility results, despite α-CD being more effective in avoiding the oxidation of 3-CQA by PPO. In both experiments, the differences between the treatments at intermediate and high concentration of CD and the controls without CD were significant.

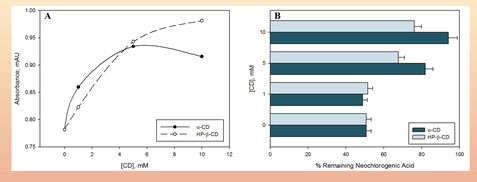

Fig. 5. A) Aqueous solubility and B) enzymatic oxidation with PPO of 3-CQA with α-CD and HP-β-CD (λ_{max} = 324 nm) T-test significance of p < 0.05

4 Antioxidant Activity

The measurement of the percentage of DPPH scavenging activity of 3-CQA and 5-CQA revealed that the antioxidant capacity of both bioactive compounds was maintain after encapsulation process, without significant differences among the CD concentration tested (Fig. 6). These outcomes are in agreement with previous studies[5] of antioxidant activity of 5-CQA/β-CD inclusion complexes by the same method. However, there are no previous studies of 3-CQA/CD inclusion complexes to compare.

When comparing the isomers, it highlights that 3-CQA, free or complexed, had slightly higher antioxidant activity than 5-CQA. Despite not being significant at the concentration used, these results are of great interest for future investigations on this less-studied isomer of 5-CQA.

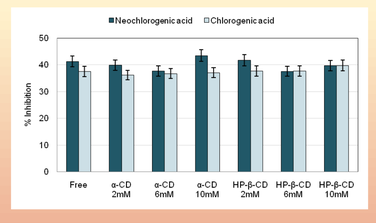

Fig. 6. Antioxidant activity of CQAs in the presence and absence of α-CD and HP-β-CD. Ethanol was used as a negative control. T-test significance of p < 0.05

Conclusions

The fluorescence of caffeoylquinic acids in different pH conditions was characterized, and caffeic acid was demonstrated to be the fluorescent moiety of neochlorogenic and chlorogenic acids. At pH 5, HP-β-CD and α-CD formed the best inclusion complexes with both caffeoylquinic acids. Molecular docking provided better scores for α-CD, although HP-β-CD showed stabilization through H-bonds. Complexation with cyclodextrins demonstrated to improved the solubility and protection against oxidation of neochlorogenic acid. Antioxidant activity of caffeoylquinic acids was maintain after the encapsulation, independently of the cyclodextrin concentration used. Overall, these findings could lead to obtaining more stable inclusion complexes with caffeoylquinic acids for application in pharmaceutical industry.

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