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Physicochemical, thermal and computational study of the encapsulation of rumenic acid by natural and modified cyclodextrins

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ABSTRACT

In this work the aggregation behavior of Rumenic acid (RA) is presented for the first time. The results point to a c.m.c. of 35 μM at pH 8 and 25 °C. This behavior can be modified by introducing CDs into the system to encapsulate the RA. The encapsulation process presented a 1:1 stoichiometry in all the cases studied but the complexation constants were strongly dependent on the type of CDs used, the pH and temperature. Firstly, the effect of the type of CD on the encapsulation process was studied. Among the natural and modified CDs analyzed HPβCD was the best for encapsulating RA. The pK_a determined for RA was 4.31. The K_F showed different behavior below and above 25 °C due to changes in the stoichiometry. Finally, molecular docking calculations provided further insights into how the different interactions influence the complexation constant.

1. Introduction

Dietary fatty acids (FA) are increasingly the subject of investigation due to their potential health-related properties. Rumenic acid (RA, Fig. 1 inset) is, a conjugated linoleic acid (CLA) found in the fat of ruminants and in dairy products. Along with vaccenic acid, it is formed by the biohydrogenation of dietary polyunsaturated fatty acids in the rumen. RA can be considered as the principal dietary form of CLA, accounting for as much as 85–90% of the total CLA content of dairy products, while the other isomer of CLA (t10, c12) only represents 1% of milk fat CLA (Yang et al., 2015).

A recent revision of the properties of RA in health and disease (Yang et al., 2015) emphasized the role of RA in cancer. *In vivo* the dietary supplementation of RA at 0.05–1% chemically inhibits induced tumors in many tissues (Białek, Jelińska, & Tokarz, 2015; Chen, 2003; Stawarska, Białek, Stanimirova, Stawarski, & Tokarz, 2015) or their metastasis (Chen et al., 2003; Sakai, Sasahira, Ohmori, Yoshida, & Kuniyasu, 2006). Furthermore, *in vitro* studies have demonstrated its anti-proliferative effect against a range of different cells lines (Beppu et al., 2006; Koronowicz, Dulińska-Litewka, Pisulewski, & Laidler, 2009; Ochoa et al., 2004). RA can decreases 5-lipoxygenase and cyclooxygenase expression, leading to decreased levels of prostaglandin E2 and thromboxane B2 (Ochoa et al., 2004), and can inhibit NF-kB activation (Rakib et al., 2013). In addition, RA might have therapeutic potential in the treatment of Th_1 mediated diseases (Loscher et al., 2005). A study in humans showed that the RA found in butter exerted immune-modulatory effects on healthy young adults, reducing the production of inflammatory biomarkers associated with overweight and obesity (Penedo et al., 2013).

For these reasons, RA might well be considered for use as a bioactive compound to fortify functional foods and nutraceutics. However, problems concerning the physico-chemical properties of RA have meant that few hydrophilic "*novel foods*" have been fortified with this antioxidant. Indeed the absence of information about some aspects of RA has meant that the European Food Security Agency (EFSA) has not lent its support to the health claims related with the use of FA in functional foods. Indeed, the EFSA has recently published (EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA), 2010, 2015) several negative reports about RA and has demanded new scientific data on the same.

To date, no study has been published on the possible aggregation behavior of RA in dissolution, as has been done in the case of other FA such as linoleic (LA), arachidonic (AA) and linolenic (LNA) acids, in both of which monomer/aggregate behavior has

Abbreviations: RA, rumenic acid; c.m.c., critical micellar concentration; CD, cyclodextrin; HPβCD, hydroxypropyl-beta-cyclodextrin; MβCD, methyl-beta-cyclodextrin.

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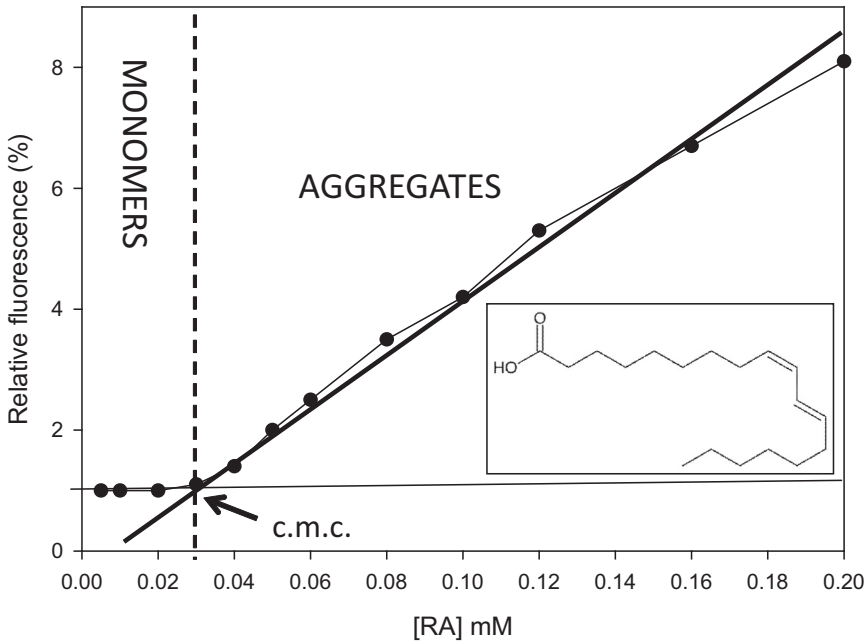

Fig. 1. Dependence of relative fluorescence intensity of diphenyl-hexatriene at 430 nm (excitatlon wavelength 358 nm) on RA concentration (pH 8 at 25 °C). Insert: Structure of RA.

been described (Bru, López-Nicolás, & García-Carmona, 1995; López-Nicolás, Bru, Sánchez-Ferrer, & García-Carmona, 1995).

RA shows very poor solubility in water (although it is more soluble in ethanol and other organic solvents), possesses low bioavailability and is easily oxidized by physico-chemical agents (Giua, Blasi, Simonetti, & Cossignani, 2013) all of which hinfer its use in functional foods or in nutraceutical products. The complexation of RA with molecules such as cyclodextrins (CDs), which might increase its bioavailability, solubility and stability in the face of prooxidant agents, is strongly desirable.

CDs are torus-shaped oligosaccharides made up of α-(1,4) linked glucose units. The most common CDs are α, β and γ-CD, which contain six, seven and eight glucose units, respectively (Del Valle, 2004; Szente & Szejtli, 2004). The cavity is carpeted by hydrogen atoms and so has a slightly hydrophobic nature, unlike the outer surface of the molecule, in which the primary and secondary hydroxyl groups are exposed to the solvent, thus making the whole molecule highly water-soluble (Del Valle, 2004; Szente & Szejtli, 2004). Poorly water-soluble compounds and hydrophobic moieties of amphiphilic molecules interact non-covalently with the CD cavity to form so-called inclusion complexes, which are also highly water-soluble (López-Nicolás et al., 1995; López-Nicolás & García-Carmona, 2008; López-Nicolás, Rodríguez-Bonilla, & García-Carmona, 2009). However, the solubility of these complexes depends on several factors such as the type of CD used. Because CDs are able to increase the bioavailability of different compounds and to protect different molecules against the action of external agents, their use in the pharmaceutical and food industries is increasing (Del Valle, 2004; López-Nicolás, Rodríguez-Bonilla, & García-Carmona, 2014; Szente & Szejtli, 2004).

To date, very few papers (Kim et al., 2000; Park et al., 2002) have studied the effect of CDs on RA and the complexation constants between RA and CDs have not been the subject of any study. Indeed, this is the first work where the complexation between CD and this potent antioxidant is reported. Knowledge of the stoichiometric coefficients and of the complexation constants (K_F) of the CD complexes is essential if this CLA isomer is to be used in the pharmaceutical and food industries.

Bearing the above in mind, the first objective of this work was to analyze the aggregation behavior of RA, studying the possible existence of monomers or aggregates that depend on the properties of the reaction medium. Secondly, the mechanism involved in RA complexation with different types of natural (α-CD and β-CD) and modified (hydroxypropyl-β-CD and methyl-β-CD) CDs under various experimental conditions (temperature and pH) are studied. The stoichiometry, K_F values and thermal behavior of the RA-CD complexes are also evaluated. For the study, a method which makes use of the changes in the fluorescence spectroscopic properties of RA in the presence of CDs, was used. Finally, the molecular interaction established in the complexation process was studied using *in silico* molecular docking.

2. Materials and methods

2.1. Materials

RA and β-CD, modified hydroxypropyl-beta-Cyclodextrin (HPβCD) and Methyl-beta-Cyclodextrin (MβCD) were purchased from Sigma Aldrich (Madrid, Spain). α-CD was purchased from Shanghai Soyoung Biotechnology (Shanghai, China). Diphenylhexatriene (DPHT) was a product of Fluka (Madrid, Spain) and tetrahydrofuran was from Merck (Darmstadt, Germany).

2.2. Fluorimetric determination of critical micellar concentration (c.m.c.)

The c.m.c. of RA was determined as a function of pH and temperature by means of the fluorescence spectroscopy method described by López-Nicolás et al. (1995). The required concentration of CD and RA was added to the desire buffer with 0.88 μM of DPHT (supplied in tetrahydrofuran). The samples were incubated for an hour at the desired temperature in the dark to reach equilibrium and, to prevent photoisomerization of the fluorescent probe.

Fluorescence intensity was measured at 430 nm (358 nm excitation wavelength) in a Kontron SFM-25 spectrofluorimeter