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The inclusion complex of oxyresveratrol in modified cyclodextrins: A thermodynamic, structural, physicochemical, fluorescent and computational study

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ABSTRACT

The interaction between oxyresveratrol (a type of stilbene with high biological activity) and modified cyclodextrins (CDs) was studied. Using HPLC-RP, was seen to form a 1:1 complex with all the CDs tested. The best CD in this respect was MβCD ($K_F = 606.65 \pm 30.18\ M^{-1}$), the complexation showing a strong dependence on pH and temperature: The complexation constant (K_F) decreased as the pH and temperature increased. The thermodynamic parameters studied ($\Delta H°$, $\Delta S°$ and $\Delta G°$) showed negative entropy, enthalpy and Gibbs free energy change at 25 °C. In addition, fluorescence signal of oxyresveratrol increased when MβCD was added. The oxyresveratrol emission and excitation spectra were obtained for first time. A ^1H NMR was carried out to study the structure of the complex and, DSC studied demonstrated the complexation. A computational study by molecular docking was made to complement the structural study.

1. Introduction

Oxyresveratrol (*trans*-3,5,2′,4′-tetrahydroxystilbene, OXY, Fig. 1**A**) a stilbenoid presents in mulberry fruits (*Morus alba* L.) and twigs is a naturally occurring resveratrol (3,5,4′-trihydroxy-*trans*-stilbene) analogue with an additional hydroxyl group (situated in "meta" position respect to a –OH group of resveratrol) in the aromatic ring (Fig 1**A**). A recent review (Lim, Kim, & Kim, 2015) is about its pharmacological properties: including a wide range of biological activities, such as antioxidant, antiviral, anti-inflammation, anti-obesity, cholesterol lowering, hepato- and neuroprotection and photo-protective effects. Furthermore, OXY presents cyclooxygenase and tyrosinase-inhibitory activities. Indeed, OXY has demonstrated great potential in several pre-clinical studies (Bertram et al., 2010; Chen, Yeo, Elhennawy, & Lin, 2016).

Despite all these health-related properties, several problems related with the chemical properties of OXY prevent its use as a fortifier of nutraceutical or functional foods. This bioactive molecule presents low solubility in aqueous solutions, poor bioavailability and is easily oxidized, making it necessary to seek new strategies to improve its solubility, bioavailability and resistance to oxidation. In this paper we analysed the encapsulation of OXY by a type of molecule with a known high complexation capacity: cyclodextrins (CDs).

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 (Szente & Szejtli, 2004). These types of natural CDs have two GRAS statuses and are appear in the lists of additives approved for alimentary use with the corresponding E-numbers α-, β- and γ-CD: E-457, E-459 and E-458, respectively. The cavity of CDs is carpeted by hydrogen atoms and is therefore of a rather hydrophobic nature, unlike the outer surface of the molecule, in which the primary and secondary hydroxyl groups are exposed to the solvent, 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. However, the solubility of these complexes depends on several factors such as the type of CD used (López-Nicolás, Bru, Sánchez-Ferrer, & García-Carmona, 1995; López-Nicolás & García-Carmona, 2008a, 2008b; López-Nicolás, Rodríguez-Bonilla, & García-Carmona, 2009). 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 both the pharmaceutical and food industries is increasing (Del Valle, 2004; López-Nicolás, Rodríguez-Bonilla, & García-Carmona, 2014; Szente & Szejtli, 2004). Among the guest mole-

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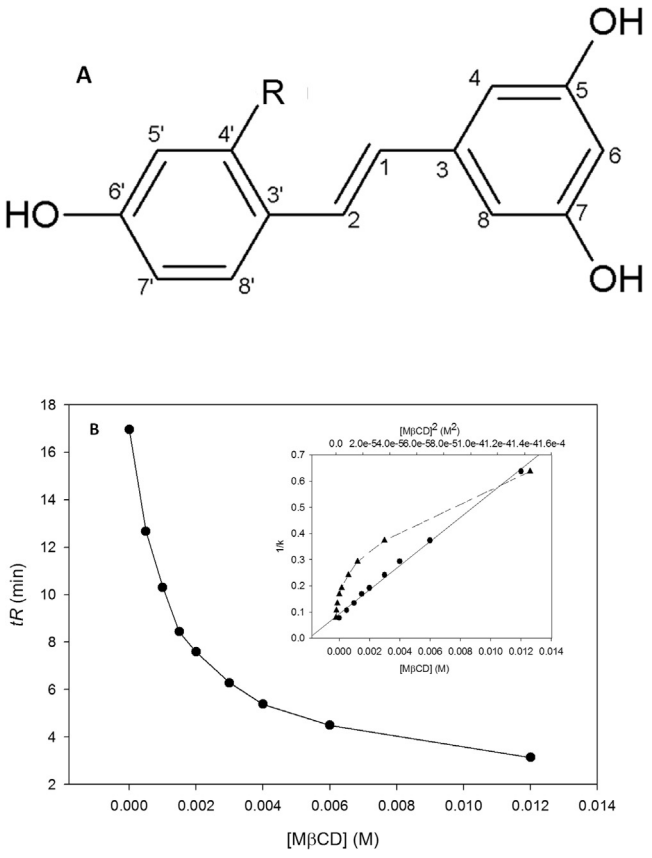

Fig. 1. (A) Structure of Oxyresveratrol (R = –OH) or Resveratrol (R = –H). (B) Effect of increasing concentration of MβCD on retention time of OXY (C18 column, 30:70 MeOH/water 1.0 mL/min at 25 °C). Inset: linear fit of OXY complexed with MβCD to determine the stoichiometry of OXY/MβCD complexes: 1/k vs [MβCD] (circles, 1:1 complex and triangles, 2:1 complex).

cules encapsulated by CDs are fatty acids (Matencio, Hernández-Gil, García-Carmona, & López-Nicolás, 2017), vitamins (Vilanova & Solans, 2015), and phenols (López-Nicolás & García-Carmona, 2008a, 2008b).

In recent years our research group has published several works concerning the ability of CDs to encapsulate different molecules of the stilbene family, such as resveratrol (López-Nicolás & García-Carmona, 2008a, 2008b), piceatannol (Matencio, García-Carmona, & López-Nicolás, 2016), α-methylstilbene (Matencio, Hernandez-Garcia, Garcia-Carmona, & Nicolás, 2016), pterostilbene (López-Nicolás, Rodríguez-Bonilla, Méndez-Cazorla, & García-Carmona, 2009) or pinosilvyn (López-Nicolás et al., 2009) by CDs. Although our research group has encapsulated OXY in natural CDs (Rodríguez-Bonilla, López-Nicolás, & García-Carmona, 2010). However, the effect of pH on the encapsulation constant (K_F) was not evaluated, despite of the importance of this in the stability of the resulting complex in nutraceutical products. Furthermore, a more exhaustive study of OXY using modified CDs is necessary, because modified CDs are the most used in nutraceutical solutions (Challa, Ahuja, Ali, & Khar, 2005).

Bearing the above in mind, the objective of this study is to analyze the encapsulation mechanism of OXY by different types of modified (HPβCD and MβCD) CD. To carry it out, some points will be evaluated:

1) To determine the effect of temperature and pH on the encapsulation mechanism of OXY.
2) To study the effect of the addition of CDs on OXY fluorescence behavior.
3) To determine the stoichiometry, K_F values and thermodynamic parameters for the OXY-CD complexes.
4) To study the physical interactions between OXY and CD using NMR and molecular docking.

To characterize the resulting inclusion complexes HPLC, was used as a technique increasingly used for observing and characterizing CD-guest inclusion (López-Nicolás, Escorial Camps, Pérez-Sánchez, & García-Carmona, 2013; Matencio, Bermejo-Gimeno, García-Carmona, & López-Nicolás, 2016; Rodríguez-Bonilla et al., 2010). Modifications of the retention properties of molecules with different CD concentrations in the mobile phase were found to be related to the stoichiometry and stability of the inclusion complexes thus formed (Fujimura, Ueda, Kitagawa, Takayanagi, & Ando, 1986).

2. Materials and methods

2.1. Materials

Hydroxypropyl-beta- and methyl-beta-cyclodextrin (HPβCD and MβCD, CIDs 44134771 and 10171019) were purchased from Carbosynth (Berkshire, UK). Oxyresveratrol (CID 5281717) was purchased from Sigma-Aldrich (Madrid, Spain) and used as received. The samples were stored in darkness. Methanol (HPLC grade, Pubchem CIF 887) was purchased from Fisher (Madrid, Spain). MQ water was obtained using a Milli-Q Advantage A10 system by Merck Millipore (Madrid, Spain). Binary mixtures of water/methanol were used without further purification.

2.2. Equipment and experimental procedure

2.2.1. Inclusion complex characterization

The methodology was used as Rodríguez-Bonilla et al. (2010) said, with slight modifications as follows: 0.5 μL of OXY (0.01 g/mL in Methanol) was analysed in an Agilent 1100 series HPLC system (CA, USA) and a 1200 series module UV–VIS detector with a Kromasil 150 C18 column (Análisis Vínicos S.L.Tomelloso, Spain) (150 mm × 4.6 mm, 5 μm particle size). The mobile phase flow rate was set and automatically controlled at 1.00 ± 0.01 mL/min with Methanol/Water (30/70 v/v – pH 7 20 mM Tris-HCl) with different concentrations of CDs at 25 °C. The UV detector was operated at 328 nm (Rodríguez-Bonilla et al., 2010).

To determine the K_F value for the OXY/CD complexes, Eq. (1), which relates the capacity factor, k, and the CDs mobile-phase concentration, [CD], was used (López-Nicolás, Núñez-Delicado, Pérez-López, Barrachina, & Cuadra-Crespo, 2006). In this equation two conditions are assumed: 1) the complex has a 1:1 stoichiometry and 2) any interaction of the OXY/CD complexes with the stationary phase is negligible (Fujimura et al., 1986).

$$\frac{1}{k} = \frac{1}{k_o} + \frac{K_F}{k_o}[\mathrm{CD}] \quad (1)$$

where k is the capacity factor of the solute, k_0 is the solute capacity factor in the absence of CD, K_F is the apparent formation constant of the inclusion complex and [CD] is the CD mobile-phase concentration. Values of R^2 close to 1 indicate a 1:1 model.

Eq. (2) is an extension of Eq. (1) and includes a second-order term that accounts for the possibility of a 1:2 OXY/CDs complex formation (Moeder, O'Brien, Thompson, & Bicker, 1996):

$$\frac{1}{k} = \frac{1}{k_o} + \frac{K_{F_{12}}}{k_o}[\mathrm{CD}]^2 \quad (2)$$