Complexation of quercetin with three kinds of cyclodextrins: An antioxidant study

Carolina Jullian [a,*], Lorena Moyano [b], Claudia Yañez [a], Claudio Olea-Azar [b]

[a] *Departamento de Química Orgánica y Fisicoquímica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla 233, Santiago 1, Chile*
[b] *Departamento de Química Inorgánica y Analítica, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, Casilla 233, Santiago 1, Chile*

Abstract

The slightly water-soluble flavonoid quercetin (QUE) and its inclusion with either β-cyclodextrin (βCD), hydroxypropyl-β-cyclodextrin (HP-βCD) or sulfobutyl ether-β-cyclodextrin (SBE-βCD) were investigated. The stoichiometric ratios and stability constants describing the extent of formation of the complexes have been determined by phase–solubility measurements; in all cases type-A_L diagrams have been obtained (soluble 1:1 complexes). The results showed that the inclusion ability of βCD and its derivatives was the order: SBE-βCD > HP-βCD > βCD.

Kinetic studies of DPPH$^\bullet$ with QUE and CDs complexes were done. The results obtained indicated that the QUE–SBE-βCD complex was the most reactive form.

The scavenging capability of QUE and CDs complexes with DPPH$^\bullet$ and galvinoxyl was studied using ESR spectroscopy. All complexes showed a higher scavenging capability with both radicals, compare quercetin in water. Beside, these results indicated that the complexes formed maintained the quercetin antioxidant activity.

Keywords: Quercetin; β-Cyclodextrin; Inclusion complex; Antioxidant; DPPH$^\bullet$

1. Introduction

Quercetin (Fig. 1) belongs to a large group of naturally occurring flavonoid compounds found in plants, foods and beverages. Flavonoids represent a sub-group of intensely colored polyphenolic phytochemicals. They contribute to plant color, providing a spectrum of colors from red to blue in flowers, fruit and leaves [1,2]. Due to some interesting health-benefiting properties, flavonoids are widely examined in terms of chemistry as well as biological activity. The antioxidant, antitumor and antibacterial activity of flavonoids is the focus of the attention of many researchers in pharmaceutical and medicine chemistry [3]. However, quercetin is sparingly soluble in water, which may be responsible for its limited absorption upon oral administration.

In pharmaceutical product development, β-cyclodextrins, a category of pharmaceutical excipients, have been widely used to improve solubility, chemical stability and bioavailability of a number of poorly soluble compounds.

Cyclodextrins (CDs) are cyclic oligosaccharides composed of glucopyranose units and can be represented as a truncated cone structure with a hydrophobic cavity [4]. The cavity of CDs is relatively hydrophobic compared to water, while the external faces are hydrophilic [5]. The most extraordinary characteristic of a cyclodextrin is its ability to form inclusion complexes with a variety of compounds, i.e., caging foreign molecules (guest) in its cavity (host). Generally, hydrophobic molecules or some hydrophobic residues have the highest affinity with the CD's cavity in aqueous solution. It has been well established that the ability of β-cyclodextrin to enhance the stability and solubility of drugs is mediated through the formation of inclusion complexes [6]. Unmodified or unsubstituted β-cyclodextrin, that is, with no substituent on the glucopyranose unit, has poor water solubility and is parenterally unsafe due to its nephrotoxicity. Therefore, several synthetically modified and relatively safe β-CD have been made and used in parenteral formulations, such as hydroxypropyl-β-cyclodextrin [7] (HP-βCD) and sulfobutyl ether-β-cyclodextrin (SBE-βCD) [8].

* Corresponding author. Tel.: +56 2 9782807/2865; fax: +56 2 9782809.
E-mail address: cjullian@uchile.cl (C. Jullian).

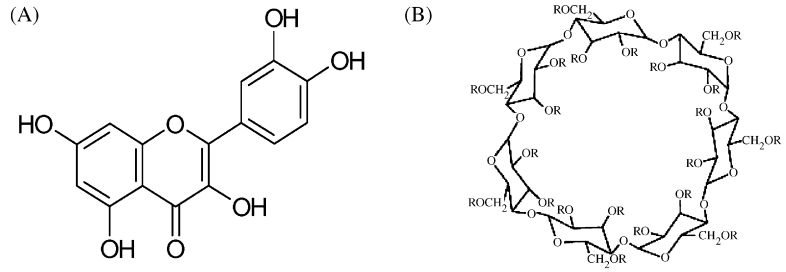

Fig. 1. (A) Molecular structures of quercetin. (B) Molecular structures of β-cyclodextrin, hydroxypropyl-β-cyclodextrin, and sulfobutyl ether β-cyclodextrin.

The present work was designated to study the complexation of quercetin utilizing three different cyclodextrins (HP-βCD, SBE-βCD and βCD) to improve the solubility and to determine the effect of the complexation process on their antioxidant capacity.

2. Experimental

2.1. Apparatus

Spectrophotometric measurements were carried out with a UV_2 UNICAM spectrophotometer, using a 1 cm quartz cell.

ESR spectra were recorded in the X band (9.7 GHz) using a Bruker ECS 106 spectrometer with a rectangular cavity and 50 kHz field modulation.

2.2. Materials

Quercetin (3,3′,4′,5,7-pentahydroxyflavone), was purchased from Sigma (USA). βCD and HP-βCD [MS (average molar degree of substitution) = 1.0] was purchased from Sigma–Aldrich, Inc., St. Louis, MO. SBE-βCD [TDS (total degree of substitution) = 6–7; Captisol®] was purchased from CyDex, Inc. DPPH• (2,2-di(4-*tert*-octylphenyl)-1-picrylhydrazyl), free radical and galvinoxyl (2,6-di-*tert*-butyl-α-(3,5-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-*p*-tolyloxy) free radical, was purchased from Sigma–Aldrich, Inc., St. Louis, MO. All solvents employed in the spectrophotometric analyses were of spectroscopic reagent grade, from Merck. Other reagents were all analytical grade and double distilled water was used throughout.

2.3. Method

2.3.1. Phase–solubility measurements

Phase–solubility measurements were carried out according to the method of Higuchi and Connors [9]. Excess amount of quercetin (5 mg) was added to 5 mL of deionized water containing increasing amounts of βCD, HP-βCD and SBE-βCD (ranging from 0 to 0.010 M). The resulting mixture was equilibrated in a Julabo thermostatic shaking water bath for 24 h at 30 °C after which the equilibrium was reached. To minimize photochemical degradation flask were covered with aluminium foil. Then, suspensions were filtered through 0.45 μm cellulose acetate membrane filter to remove undissolved solid. An aliquot from each vial was adequately diluted and spectrophometrically analyzed at 375 nm.

The apparent stability constant (K_s) of the complexes were calculated from the phase–solubility diagrams according to the following equation:

$$K_s = \frac{\text{slope}}{S_0(1 - \text{slope})} \quad (1)$$

where S_0 is the solubility of quercetin at 30 °C in absence of cyclodextrin and slope means the corresponding slope of the phase–solubility diagrams, i.e., the slope of the drug molar concentration versus CDs molar concentration graph.

2.3.2. Determination of antioxidant activity by the scavenging of the stable radical DPPH•

The antioxidant activity was measured, wherein the bleaching rate of a stable free radical, DPPH• is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH• absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases.

A volume of 2000 μL of 4.94×10^{-5} M DPPH• was used. Furthermore, DPPH• is insoluble in aqueous solution the scavenging study was performed in mixture of methanol–water (20:80).

The reaction was started by addition of 20 μL of QUE, QUE–βCD, QUE–HP-βCD and QUE–SBE-βCD samples, which correspond to the 3 mM cyclodextrin concentration from the phase–solubility studies. The bleaching of DPPH• was followed at 517 nm.

The decrease in absorbance at 517 nm was measured against a blank of pure methanol to estimate the radical scavenging capacity of each antioxidant sample.

2.3.3. Data analysis

The curve-fittings of the absorbance versus time plots were carried out on a Pentium PC using the Scientist program Origin 7.0. Curvefittings were achieved through least-squares regression and yielded optimized values for the parameters. We used

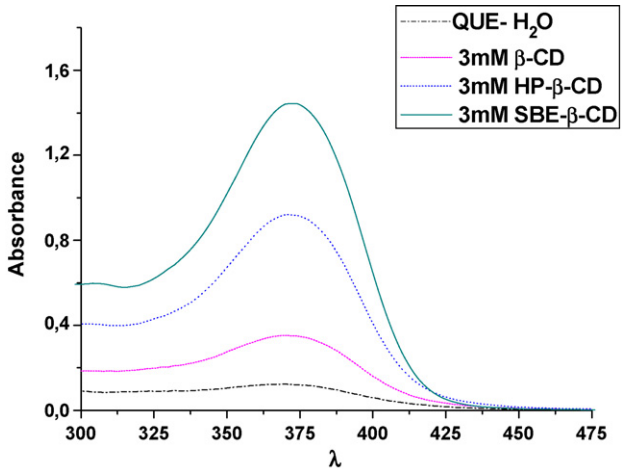

Fig. 2. Absorption spectra of quercetin in aqueous solutions with the different CD.

the slope initial rate methodology to obtain two parameters to be analyzed: consumption of DPPH• and initial rate. The curve-fitting procedures typically gave good (>0.99) to excellent (>0.999) correlation coefficients.

2.3.4. ESR

Three millimolars cyclodextrin concentration from the phase–solubility study in mixture methanol/water (20:80) and DPPH• or galvinoxyl (2 mM) were deoxygenated under a stream of nitrogen gas. Aliquots (2 mL) were transferred to Hamilton gastight syringes coupled to a pneumatic ram and connected to a two-stream ESR quartz flow cell. In situ reactions at room temperature were initiated by rapidly evacuating the syringes. Spectra and decay curves were obtained on a Bruker ECS 106 spectrometer. The measurement of the decreasing ESR signal was measurement at 10 min after the start reaction.

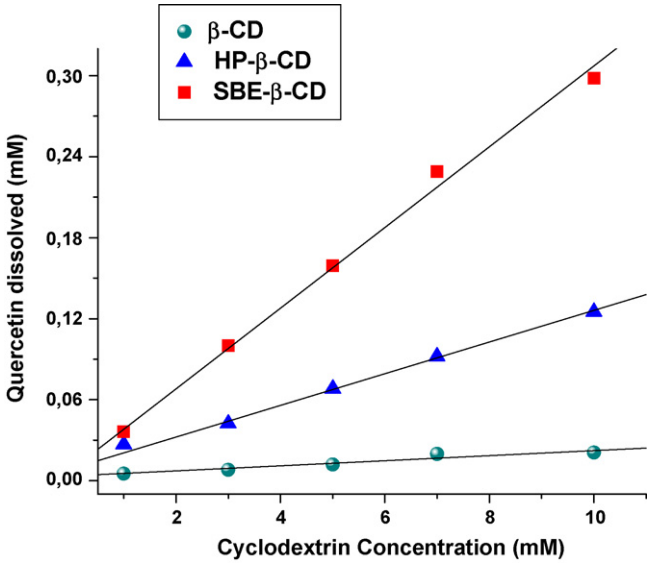

Fig. 3. Phase–solubility diagrams of QUE–βCD, QUE–HP-βCD and QUE–SBE-βCD system in water at 30 °C.

Table 1
Apparent stability constant (K_S) of quercetin inclusion complexes

QUE complex	K_s (M^{-1})
QUE–βCD	602
QUE–HP-βCD	1419
QUE–SBE-βCD	4032

3. Results and discussion

3.1. Phase–solubility measurements

All three CDs enhanced the poor aqueous solubility of quercetin, thus proving a certain degree of its inclusion complexation in aqueous solutions (Fig. 2).

All phase–solubility diagrams of quercetin with β-, HP-, and SBE-βCD within the concentration range studied displayed a typical A_L type diagram (i.e., linear increase of quercetin solubility with increasing β-CD concentration), consistent with a 1:1 molecular complex formation for all three β-CDs (Fig. 3). The result observed showed a linear behavior which is unequivocal for all CDs studied ($r^2 = 0.996$ or better). The binding constant K_s of the complexes was calculated from the slopes of the linear phase–solubility plots according to the methodology described before. Results are summarized in Table 1. As shown in Table 1 and Fig. 3, the binding constant and solubility of quercetin determined with the three CDs followed the rank order SBE-βCD > HP-βCD > βCD, reflecting and enhancement of binding and solubility with an increasing in substitution and hydrophilicity of the CDs. The same order was reported by Zheng et al. [10]. In the case of QUE–SBE-βCD the strongly bound is expected, because the substituents groups are probably assisting in the binding.

3.2. Scavenging study of DPPH• by free or complexed-quercetin

DPPH• is a stable free radical generating a deep violet solution in organic solvents. Its progressive discoloration when in

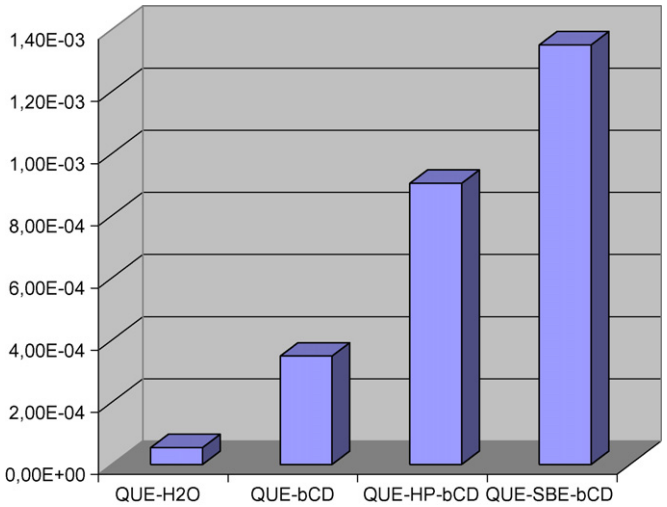

Fig. 4. Consume of DPPH• (mM^{-1}) in presence of QUE free and complexes forms.

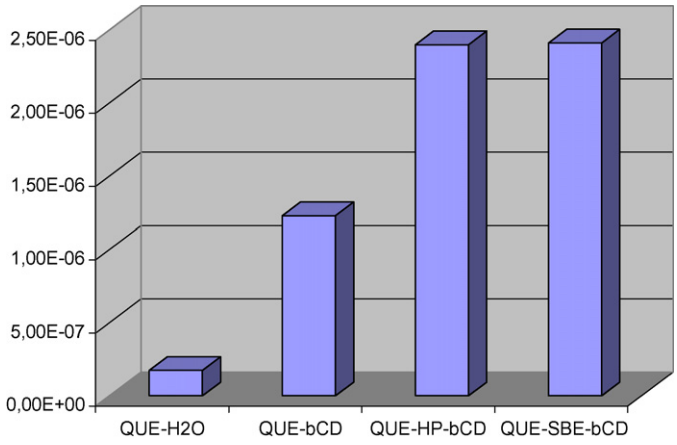

Fig. 5. Initial rate of QUE free and complexes forms.

the presence of quercetin indicated that it is acting as an antioxidant. Furthermore, since the mechanism of DPPH• reduction is known, the amount remaining of both reagents may be determined.

The rate of the DPPH•-scavenging reaction was measured by monitoring the decrease in absorbance at 517 nm due to DPPH•. The decay of the absorbance at 517 nm due to DPPH• corresponded to a second order decay. When cyclodextrin was mixture with DPPH• no decay was observed. Fig. 4 shows the consumption of DPPH• which indicates that the complexed quercetin–CDs were more effective than free quercetin, with the QUE–SBE-βCD complex being the best. The same results were found for the initial rate studies (Fig. 5).

3.3. ESR study of the scavenging ability of free and complexed quercetin toward DPPH• and galvinoxyl

The scavenging ability of quercetin toward DPPH• and galvinoxyl radicals in the absence and presence of CDs was also investigated. The scavenging ability was measured as a relative scavenging between DPPH• and galvinoxyl alone versus DPPH• and

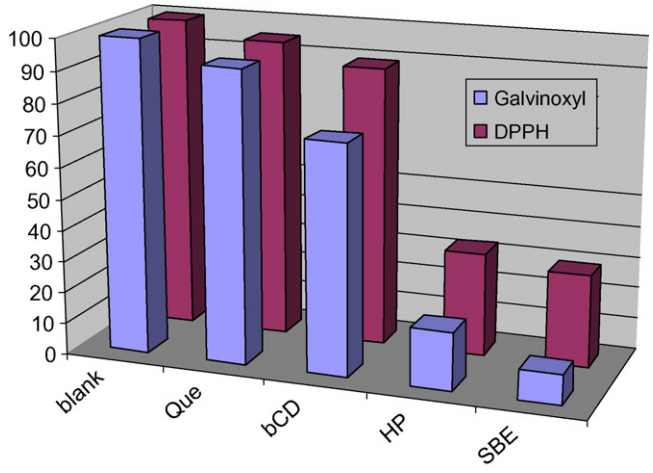

Fig. 6. Radical DPPH• and galvinoxyl scavenging activity. The initial concentration was 2 mM for DPPH/galvinoxyl in all reaction mixtures. The data were recorded at 10 min of reaction and expressed as % DPPH/galvinoxyl remaining.

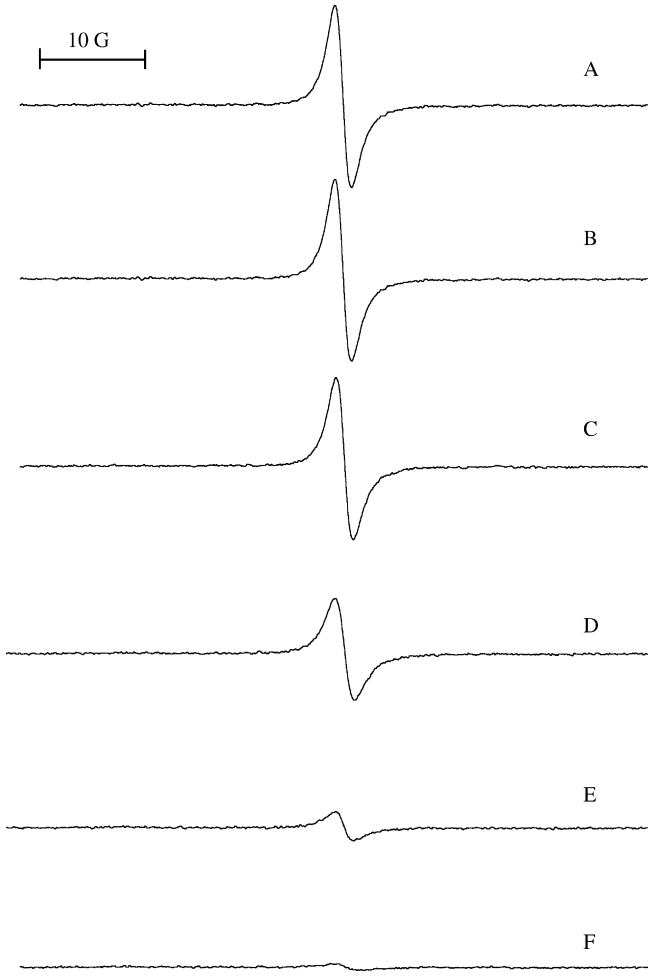

Fig. 7. ESR spectrum of (A) galvinoxyl; (B) galvinoxyl in presence of βCD (similar behavior is founded by the other cyclodextrins) (C) QUE; (D) QUE–βCD; (E) QUE–HP-βCD; (F) QUE–SBE-βCD.

galvinoxyl in presence of free or complex quercetin. We choice the 3 mM cyclodextrin concentration from the phase–solubility study to compare the scavenging ability. Not decay was observed when cyclodextrin alone was mixture with DPPH• and galvinoxyl. Fig. 6 depicts a decrease in DPPH• and galvinoxyl signal with increasing solubility obtained for the different CDs. Fig. 7 shows the ESR spectrum of galvinoxyl with QUE free and its complexes forms. Figs. 6 and 7 are in according with scavenging ability is related with enhanced solubility of quercetin. Also theses results indicated that the complexes formed maintained the quercetin antioxidant activity.

4. Conclusions

The results from this study indicated an interaction between quercetin and CDs in water. This interaction increases the solubility of the flavonoid forming 1:1 inclusion complex with β-cyclodextrin and its derivative. The solubility of quercetin increased with increasing CD concentration in the following order βCD < HP-βCD < SBE-βCD.

Kinetic studies of DPPH• indicated that the QUE–SBE-βCD complex was the most reactive form.

ESR is a useful methodology for determining reactions involving quercetin free or complexed with radicals like DPPH$^{\bullet}$ or galvinoxyl. The solution containing CDs and QUE showed a scavenging capability in both radical. This property is related with enhanced solubility of QUE. Also theses results indicated that the complexes formed maintained the quercetin antioxidant activity.

Acknowledgments

C. Jullian thanks financial support of Departamento de Posgrado y Postitulo PG/57/2005 of University of Chile and CEPEDEQ from Chemical and Pharmaceutical Science Faculty of University of Chile.

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