Available online at www.sciencedirect.com
ScienceDirect
Spectrochimica Acta Part A 71 (2008) 269–275
SPECTROCHIMICA ACTA PART A
www.elsevier.com/locate/saa

Complexation of morin with three kinds of cyclodextrin A thermodynamic and reactivity study

Carolina Jullian [a,*], Teresita Orosteguis [b], Fernanda Pérez-Cruz [b], Paulina Sánchez [b], Fernando Mendizabal [c], 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*
[c] *Departamento de Química, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile*

Received 23 August 2007; received in revised form 12 December 2007; accepted 15 December 2007

Abstract

Properties of inclusion complexes between morin (M) and β-cyclodextrin (βCD), 2-hydroxypropyl-β-cyclodextrin (HPβCD) and Heptakis (2,6-*O*-di methyl) β-cyclodextrin (DMβCD) such as aqueous solubility and the association constants of this complex have been determined. The water solubility of morin was increased by inclusion with cyclodextrins. The phase-solubility diagrams drawn from UV spectral measurements are of the A_L-type. Also $ORAC_{FL}$ studies were done. An increase in the antioxidant reactivity is observed when morin form inclusion complex with the three cyclodextrin studied. Finally, thermodynamics studies of cyclodextrin complexes indicated that for DMβCD the inclusion is primarily enthalpy-driven process meanwhile βCD and HPβCD are entropy-driven processes. This is corroborated by the different inclusion geometries obtained by 2D-NMR.

Keywords: Morin; Cyclodextrin; Reactivity; ROESY

1. Introduction

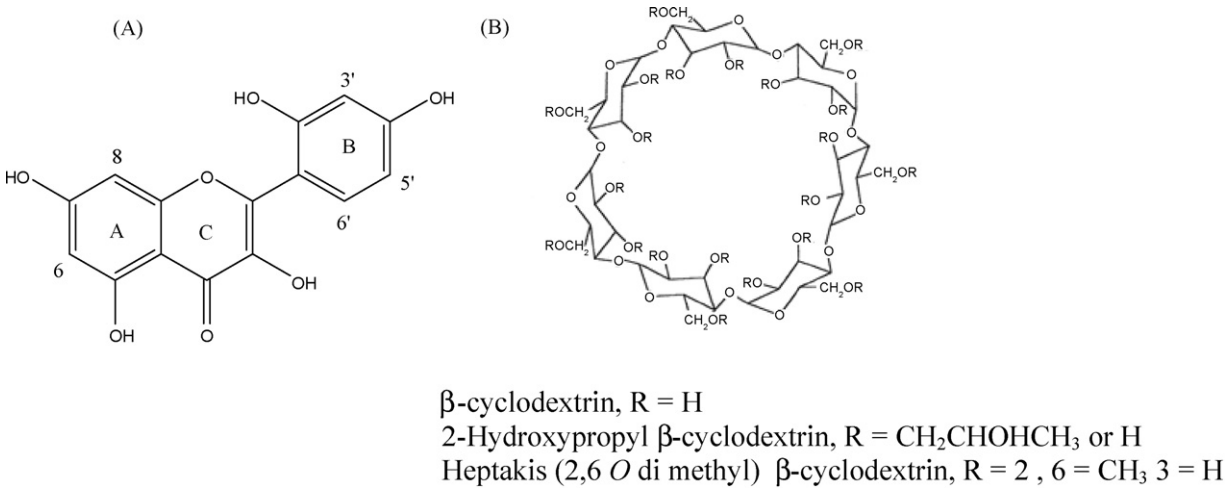
Flavonoids have recently attracted great interest as potential therapeutic agents against a variety of diseases, most by involving radical damage. These polyphenolic compounds, ubiquitous in higher plants, are commonly major dietary constituents. The biological and medicinal properties of flavonoids have been reviewed extensively, with wealth of data on their activity as reducing agents, hydrogen-donating antioxidants and singlet oxygen quenchers; in some cases metal chelating has been proposed [1–5]. Morin (2′,3,4′,5,7-pentahydroxyflavone) is a flavonoid widely distributed in tea, coffee, cereal grains and a variety of fruits and vegetables [6] (Scheme 1), and has two aromatic rings (A and B) linked by an oxygen-containing heterocyclic (ring C). Abundant in the human diet, morin, with potent antioxidant and metal ion chelating capacities, possesses various biological and biochemical effects including anti-inflammatory, anti-neoplastic, and cardioprotective activities [7,8]. They have aroused considerable interest due to their broad pharmacological activity, but morin is sparingly soluble in water, which limits its absorption in oral administration.

In pharmaceutical product development, β-cyclodextrins (Scheme 1), a category of pharmaceutical excipients, have been widely used to improve solubilities, chemical stabilities and bioavailabilities 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 [9]. The cavity is relatively hydrophobic, while the external faces are hydrophilic [10]. The most extraordinary characteristic of a cyclodextrin is its ability to form inclusion complexes with a variety of compounds, i.e., by trapping foreign molecules (guest) in its cavity (host). Generally, hydrophobic molecules or those with hydrophobic residues have the highest affinity with the CD cavity in aqueous solution, and it is well established that the ability of β-cyclodextrin to enhance drug stability and sol-

* Corresponding author. Tel.: +56 2 9782865.
E-mail address: cjullian@uchile.cl (C. Jullian).

1386-1425/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.saa.2007.12.020



Scheme 1. (A) Structures of morin. (B) Structures of β-cyclodextrin, 2-hydroxypropyl-β-cyclodextrin, and Heptakis-2,6-*O*-di methyl-β-cyclodextrin.

ubility depends on formation of inclusion complexes [11]. Unmodified or unsubstituted β-cyclodextrins, i.e., those with no substituent on the glucopyranose unit, have poor water solubility and are parenterally unsafe due to nephrotoxicity. Therefore, several synthetically modified and relatively safe βCD have been made and used in parenteral formulations, such as hydroxypropyl-β-cyclodextrin [12] (HPβCD) and Heptakis-2,6-*O*-di methyl-β-cyclodextrin (DMβCD).

We recently reported a study of quercetin with a number of cyclodextrins and with antioxidant measurements. The results indicated that the complexes formed maintained the quercetin antioxidant activity [13].

Here, we report the preparation of inclusion complexes of morin with three different cyclodextrins, (HPβCD, DMβCD and βCD) in order to improve the aqueous solubility of the drug. Thermodynamic parameters, from van't Hoff plots, were analyzed in order to gain information about the association mechanism. In relating the thermodynamic parameters with the inclusion geometries, we have also examined 2D-ROESY a NMR spectra of the inclusion complexes. We also report the effect of complexation on antioxidant capacity.

2. Experimental

2.1. Apparatus

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

A luminescence spectrometer LS 50B (PerkinElmer, Boston, MA, USA), a heating circulator bath DC1–B3 (Haake Fisons, Karlsruhe, Germany) and quartz cuvettes were used for the $ORAC_{FL}$ assay.

NMR spectra were recorded at 300 K on a Bruker Avance DRX spectrometer 300 MHz for ^1H, in unbuffered D_2O.

2.2. Materials

Morin (3,2′,4′,5,7-pentahydroxyflavone), was purchased from Sigma (USA).

βCD (β-cyclodextrin), DMβCD (Heptakis-2,6-*O*-di methyl-β-cyclodextrin), HPβCD (2 Hydroxypropyl-β-cyclodextrin) [M.S. (average molar degree of substitution) = 1.0] AAPH (2,2′-azobis(2-methylpropionamidine) dihydrochloride), FL (Fluorescein disodium salt) and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), were from Sigma-Aldrich, Inc., St. Louis, MO. All solvents used in spectrophotometric analyses were of spectroscopic reagent grade, Merck.

2.3. Method

2.3.1. Phase-solubility measurements

Phase-solubility measurements were carried out by the method of Higuchi and Connors [14]. Excess amount of morin (5 mg) was added to 5 mL of deionized water containing increasing amounts of βCD, HPβCD and DMβCD (from 0 to 0.010 M). The resulting mixture was equilibrated in a Julabo thermostatic shaking water bath for 24 h at variable temperature (293, 298, and 303 K) until equilibrium was reached. To minimize photochemical degradation, the flasks were covered with aluminium foil. Suspensions were filtered through 0.45 μm cellulose acetate membrane filters to remove undissolved solid. An aliquot from each vial was diluted and analyzed spectrophometrically at 366 nm. Cyclodextrin did not interfere in the spectrophotometric assay of morin.

The apparent stability constants (K_a) of the complexes were calculated from the phase-solubility diagrams according to the following equation:

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

where S_0 is the solubility of morin at 303 K in the 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. The experiment was carried out in triplicate at each temperature.