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Host-Guest Complexation of Apple Antioxidant Quercetin By Linked β-Cyclodextrin Dimer

HOA-DU NGUYEN[1,*], DUC-TRUC PHAM[1] and STEPHEN F. LINCOLN[2]

[1]Department of Chemistry, Vinh University, Nghe An, Vietnam
[2]School of Chemistry and Physics, The University of Adelaide, SA 5005, Australia

*Corresponding authors: E-mail: hoadu.nguyen@gmail.com (Hoa Du Nguyen); truc.dhv@gmail.com (Duc-Truc Pham)

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In aqueous phosphate buffer at pH 7.0 (3.3 % ethanol, I = 0.10 mol dm^{-3} at 298.2 K) quercetin forms host-guest complexes with β-cyclodextrin (βCD) and *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea (66βCD$_2$ur) of stoichiometry βCD.Qc and 66βCD$_2$ur.Qc, the analogous K_1 = 537 and 1901 dm^3 mol^{-1}, respectively, showing a good cooperative binding of two annuli of the dimer to the guest. The complexation of quercetin (Qc) by β-cyclodextrin (βCD) and *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrin)urea (66βCD$_2$ur) in D_2O was studied by 2D ROESY ^{1}H NMR provided evidence for complexation of quercetin into βCD annuli. The models of complexes were constructed and energy minimized.

Key Words: Host-guest complexation, Cyclodextrin, Quercetin, Fluorescence, ROESY.

INTRODUCTION

Fruits, such as apple, are the main natural antioxidant suppliers (polyphenols and flavonoids) in the human diet, which can protect against several disease[1]. Quercetin (Qc, 3,3',4',5,7-pentahydroxyflavone, Fig. 1) (pK_{a1} = 7.65[2]) is one of the flavonoids in apple which has been considered beneficial to health including antioxidative, free radical scavenging, anticancer and antiviral activities[3]. To improve solubility and stability of quercetin, recent studies utilizing β-cyclodextrin (βCD, Fig. 1) or mono substituted β-cyclodextrin to form host-guest complexes[4,5].

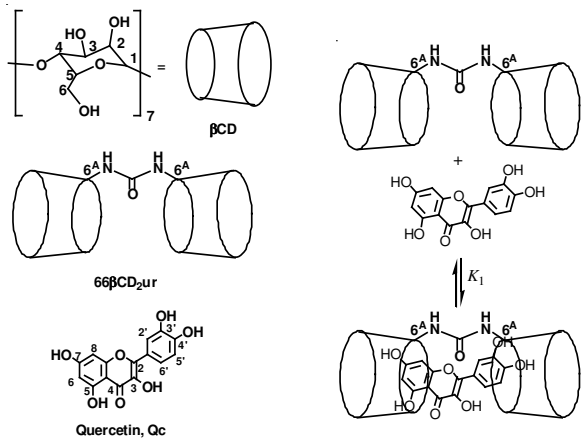

Fig. 1. Structures of β-cyclodextrin, *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)-urea, quercetin and complexation equilibrium for *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea and quercetin

To have a better host-guest complexation with quercetin, this study uses a linked β-cyclodextrin dimer, which previously reported having cooperative binding with several organic dyes and improved stability constants much higher than that of β-cyclodextrin[6,7]. The chosen dimer is *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea (66βCD$_2$ur) and host-guest measurements were studied for this dimer and native mono β-cyclodextrin under the same conditions for comparison and understanding.

EXPERIMENTAL

Fluorescence spectra were recorded at 1 nm intervals using a Cary Eclipse fluorimeter with excitation and emission slit widths of 10 and 20 nm, respectively. Samples were thermostated at 298.200 ± 0.02 K. Solutions were prepared from stock solution of quercetin 2 × 10^{-3} mol dm^{-3} in ethanol and of β-cyclodextrin 0.011 mol dm^{-3} or 66βCD$_2$ur 0.001 mol dm^{-3} in phosphate buffer at pH 7.0 and I = 0.10 mol dm^{-3}. The samples contained 3.3 % ethanol. The 2D ROESY ^{1}H NMR spectra for the two host-guest systems studied were run on a Varian Inova 600 NMR spectrometer operating at 599.957 MHz with a delay time of 300 ms. β-Cyclodextrin (βCD) was donated by Nihon Shokuhin Kako Co. Quercetin was obtained from Fluka (98 %) and recrystallized. *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea (66βCD$_2$ur)[6] and the freeze-dried complexes of β-cyclodextrin.quercetin[4] and *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea.quercetin were prepared similar as literature method.

RESULTS AND DISCUSSION

Fluorimetric studies: The relative fluorescence increases shown by quercetin on complexation by β-cyclodextrin is best-fitted by an algorithm for the formation of β-cyclodextrin. quercetin characterized by $K_1 = 537 \pm 5$ ($dm^3\ mol^{-1}$) (Fig. 2). The greater fluorescence changes induced by the linked β-cyclodextrin dimers forming *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea.quercetin with $K_1 = 1901 \pm 11$ ($dm^3\ mol^{-1}$) (Fig. 3). It is seen that the stability constant of 66βCD_2ur.Qc is 3.5 times higher than that of βCD.Qc showing the cooperative binding of two β-cyclodextrin annuli of the dimer with the guest.

In the free state in 3.3 % ethanol and phosphate buffer pH = 7.0 (I = 0.1 mol dm^{-3}, 298.2 K), the quercetin fluorescence maxima, λ_{max}, occur at 534 nm with relative fluorescence of 93 au (arbitrary units). The fluorescence of β-cyclodextrin. quercetin (547 nm, 192 au) is consistent with complexation changing the quercetin environment and enhancing fluorescence as a consequence of partial complexation in the β-cyclodextrin annulus. Similar in λ_{max} and increases in relative fluorescence occurs for *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea.quercetin (547 nm, 322 au) as a result of quercetin experiencing an increased environmental change when complexed in two β-cyclodextrin annuli.

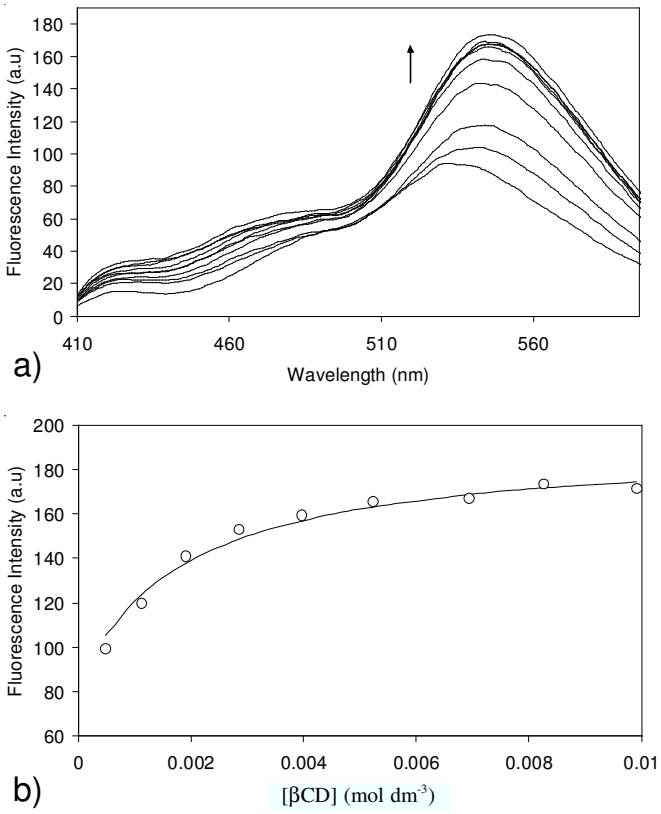

Fig. 2. (a) Increase in the relative fluorescence of quercetin (6.67×10^{-5} mol dm^{-3}) with [β-cyclodextrin]total in the range 0-9.9×10^{-3} mol dm^{-3} at pH 7.0, I = 0.1 mol dm^{-3}, 298.2 K. The excitation wavelength is 370 nm (b) Fluorescence intensities of quercetin at 550 nm *versus* differences β-cyclodextrin concentrations, data fitted by the Specfit/32[8]

2D ROESY ^{1}H NMR Study: The 2D ROESY ^{1}H NMR spectrum of a D_2O solution (pD = 7.0) of 5.0×10^{-3} mol dm^{-3} βCD.Qc (Fig. 4a) and 66βCD_2ur.Qc (Fig. 4b) shows cross-peaks (enclosed in the rectangle) arising from NOE interactions between the annular protons of the β-cyclodextrin with the aromatic protons of quercetin. This indicates the formation of host-guest complexes of βCD.Qc and 66βCD_2ur.Qc. (In ROESY experiments, a NOE cross-peak between a proton of the β-cyclodextrin annulus and a proton of the guest will be observed if the protons are closer than 4 Å through space, which infers that the guest was inside the annulus)[9].

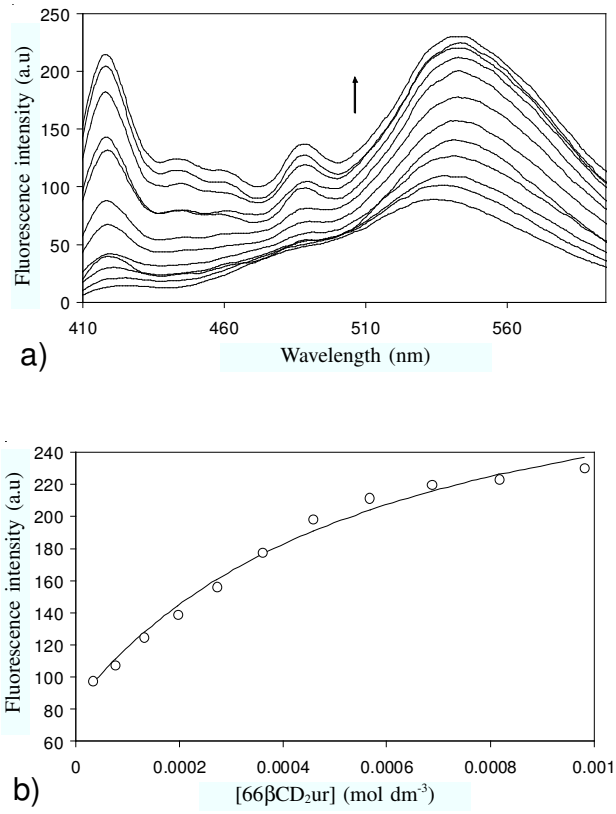

Fig. 3. Increase in the relative fluorescence of quercetin (6.67×10^{-5} mol dm^{-3}) with [66βCD_2ur]total in the range 0-9.8×10^{-4} mol dm^{-3} at pH 7.0, I = 0.1 mol dm^{-3}, 298.2 K. The excitation wavelength is 370 nm (b) fluorescence intensities of quercetin at 550 nm *versus* differences β-cyclodextrin concentrations, data fitted by the Specfit/32[8]

Molecular modelling: In MM2 Chem3D[10] energy minimized gas phase models of the linked β-cyclodextrin-dimers (Fig. 5), in which the β-cyclodextrin annuli are constrained to an approximately common axis by the quercetin guest, the distance from $O3^B$-$O6^B$ in the glucopyranose unit of β-cyclodextrin is 780 pm. The distances measured from the mid-point of this distance projected into the centre of the linked β-cyclodextrin annuli of *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea is 980 pm with the angles between the planes of the two β-cyclodextrin macrocycles is *ca.* 40º. The distances measured from H6 to H4' of quercetin is 930 pm. While hydrogen bonding between the β-cyclodextrin hydroxy groups and water occurs, the structural constraints discussed above probably maintain the relative sizes of the interannular distances. Therefore, the length of guest is longer than that of β-cyclodextrin but fit well inside the linked β-cyclodextrin dimer.

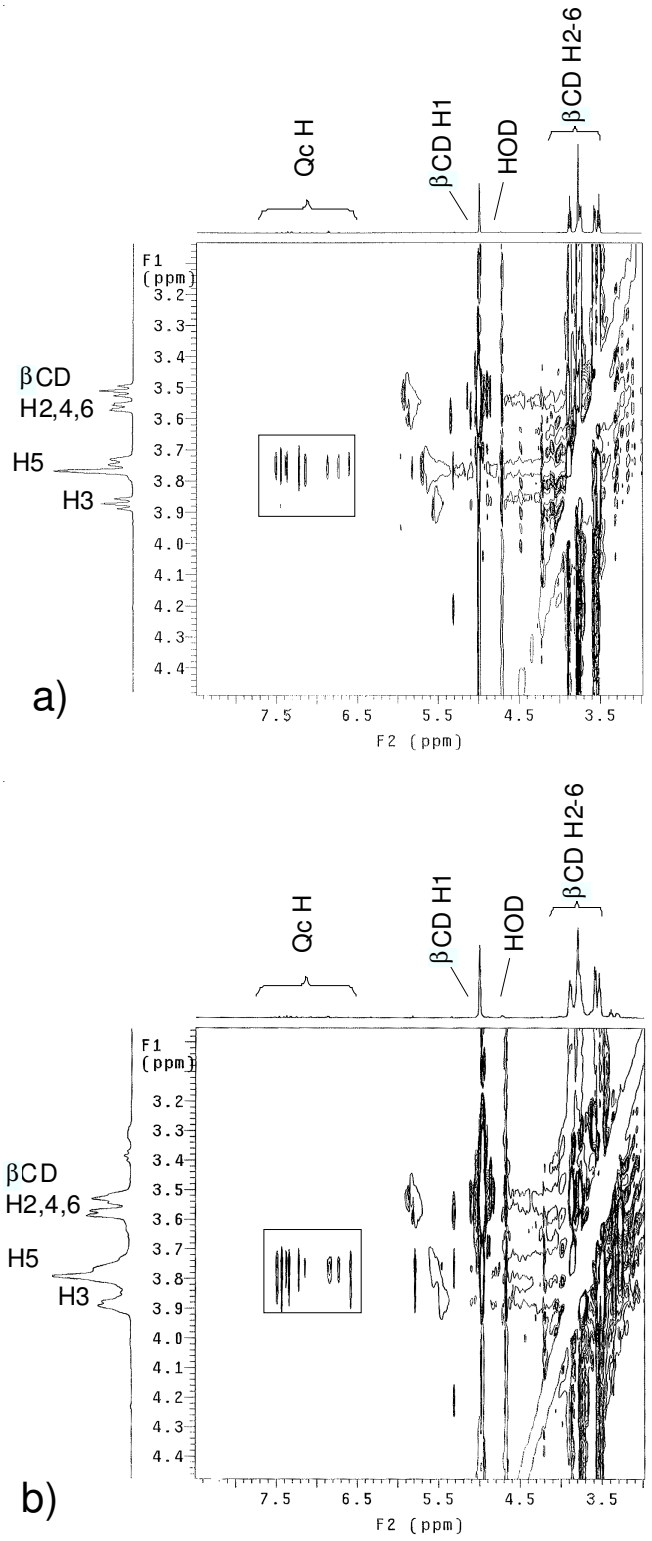

Fig. 4. 2D ROESY ^{1}H NMR (600 MHz) spectrum of a D_2O solution (pD 7.0) equimolar at 5.0×10^{-3} mol dm^{-3} in (a) β-cyclodextrin.quercetin and (b) *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea.quercetin. The rectangle contains the cross-peaks arising from the NOE interactions between the annular protons of β-cyclodextrin and the aromatic protons of quercetin

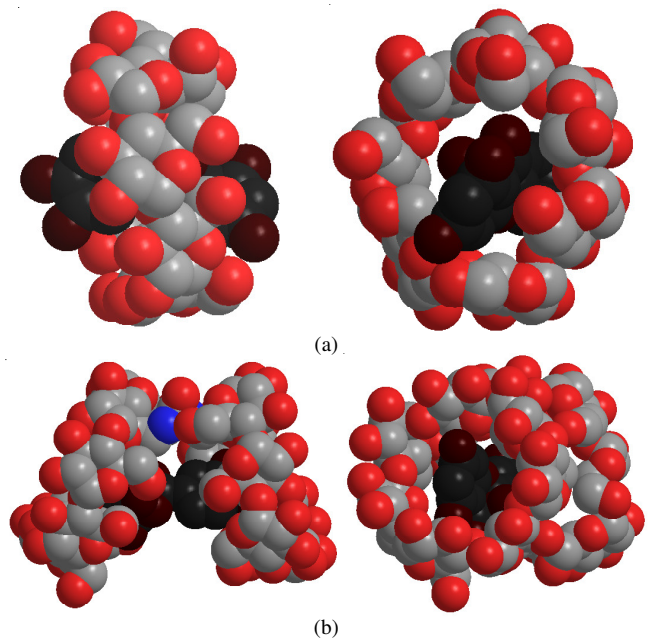

Fig. 5. Energy minimized models of (a) β-cyclodextrin.quercetin (E = 28.17 KJ/mol) and (b) *N,N'-bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea. quercetin (E = 67.95 KJ/mol). The quercetin's atoms are in darken colours. Hydrogen atoms are omitted

Conclusion

The stability constants for the linked β-cyclodextrin dimer N,N'-*bis*(6^A-deoxy-6^A-β-cyclodextrinyl)urea with quercetin complexes is 3.5 times greater than that for βCD.Qc showing a good cooperative binding of two annuli of the dimer. The host-guest complexation of βCD and the linked βCD dimer 66β-CD_2ur with quercetin have been studied by 2D ROESY ^{1}H NMR and molecular modelling. Further study for solid state of these complexes will be carried on by DSC, DTA and anticancer testing.

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