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Formation of Host-Guest Complex of Cucurbit[7]uril with Cytosine

YING HUANG[1], SAI-FENG XUE[2], QIAN-JIANG ZHU[2] and ZHU TAO[2,*]

[1]The Engineering and Research Center for Southwest Bio-Pharmaceutical Resources of National Education Ministry of China, Guizhou University, Guiyang 550025, P.R. China
[2]Key Laboratory of Macrocyclic and Supramolecular Chemistry of Guizhou Province, Guizhou University, Guiyang 550025, P.R. China

*Corresponding author: Tel./Fax: +86 851 3620906; E-mail: gzutao@263.net; yinghung128@163.com

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Interaction between cucurbit[7]uril (Q[7]) and cytosine in aqueous solution was investigated by ^{1}H NMR spectroscopy, UV absorption spectroscopy and HPLC analysis. The ^{1}H NMR analysis indicated that Q[7] selectively interacted with the cytosine moiety of the guest. UV absorption spectroscopy proved the formation of 1:1 inclusion complexes of Q[7]@guest. HPLC method was also introduced to explore the interaction between Q[7] and the cytosine.

Key Words: Cucurbit[7]uril, Cytosine, Inclusion complexes.

INTRODUCTION

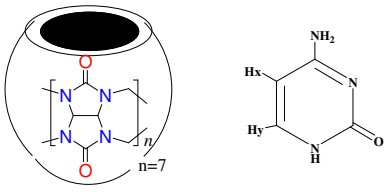
Cucurbituril and its homologues[1,2], as a new family of synthetic receptors, have been widely studied in recent years. The Q[n] family have common characteristic features, like a hydrophobic cavity and portal sizes lead to the formation of inclusion or exclusion complexes with different organic or inorganic species through a combination of dipole-ion, hydrogen bonding and hydrophobic interactions[3,4]. As other synthetic receptors, such as cyclodextrins (CDs) and calixarenes, cucurbit[n]urils and derivatives could be used as drug carriers with the aim to enhance the solubility, stability, bioavailability of drug molecules and to reduce the toxicity of drug molecules[5,6].

Pyrimidine base moieties in DNA play an important role in resistance of multiplication of human cancer cell and bio-synthesis of protein or RNA[7]. Both of pyrimidine and sugar rings of these compounds could bind with the cucurbit[n]urils and the formed inclusion or exclusion complexes could exhibited some novel properties. In this work, we studied the interactions between nucleoside analogue, cytosine with the water soluble Q[7] in aqueous solution (Fig. 1).

EXPERIMENTAL

Cucurbit[7]uril was prepared and purified according to the method developed in our laboratories. Cytosine (g) were obtained from Sigma and used without further purification.

To study the host-guest complexation of Q[7] and the guest, 2.0-2.5 × 10^{-6} mol sample of Q[7] in 5-7 × 10^{-4} L D_2O with an increasing concentrations of guest were prepared, the corresponding ^{1}H NMR spectra were recorded at 20 ºC on a Varian Inova-400 spectrometer.



Cucurbit[7]uril(Q[7] Cytosine(g)

Fig. 1. Structures of cucurbit[7]uril and cytosine

UV absorption spectra of the host-guest complexes were recorded on an Agilent 8453 Photospectrameter at room temperature. The aqueous solution of cytosine was prepared with a concentration of 1.00 × 10^{-3} M. An aqueous solution of Q[7] was prepared with a concentration of 2.00 × 10^{-4} M for absorption spectra determination. Samples of these solutions were combined to give solutions with a guest:Q[7] ratio of 0, 0.2:1, 0.4:1, 1:1, 1.5:1 and 2:1. The formation constants of the Q[7]-guest complexes (K) (1:1) were calculated according to curve fitting methods[8].

The high performance liquid chromatography (HPLC) system used for the study consisted of a pump, a UV-visible detector and an oven. A Nucleosil 250 mm × 4 mm ODS column (5 μm, particle size) supplied by Macherey-Nagel was used. The mobile phase (3 % formic acid) flow rate was at 0.8 mL min^{-1} and the column temperature was set at 50 ºC. The appropriate amounts of Q[7] (13.42-134.2 mg) were dissolved in

1 L of mobile phase and filtered through a 0.45 μm membrane filter. The wavelength at 276 nm for cytosine were used. The first peak caused by the change in UV absorbance of sample solvent of each injection was used as the dead time (t_0) of the system. The average t_0 of 2.10 min was used for all K' calculations.

RESULTS AND DISCUSSION

When a guest interacts with a Q[n], it could experience a cavity interaction or a portal interaction or a combined cavity interaction and portal interaction with the host Q[n]. The environment of protons of the guest can be changed by the shielding effect of the cavity of the Q[n] or deshielding effect of the portals of the Q[n]. Therefore, ^{1}H NMR technique is a powerful method to investigate the interaction and structure characteristics of the guest and the host.

Fig. 2 shows ^{1}H NMR spectra of cytosine in the absence (a) and in the presence of 0.4 equiv (b) and 0.8 equiv (c) of Q[7]. Two undeuterated protons Hx and Hy of the guest g underwent a gradually upfield shift with increasing equiv of Q[7] (from bottom to top), suggesting that Q[7] can include cytosine base into its cavity with a fast ingress and egress exchange rate. Chemical shift changes of certain proton resonances of the guest or host with increasing or decreasing equiv of the guest or host can be used to study host-guest interaction. However, it was difficult to read the accurate chemical shift and the integrity of the guest for the Q[7]-g interaction system due to the broad proton resonances of the guest.

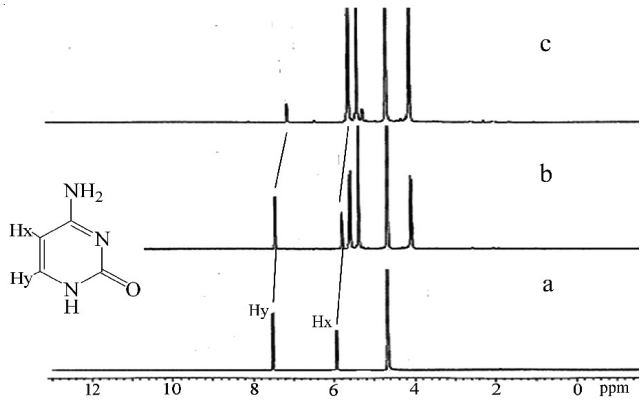


Fig. 2. ^{1}H NMR spectra (400 MHz, D_2O) of g in the absence (a) and in the presence of 0.4 equiv (b) and 0.8 equiv (c) of Q[7]

The ^{1}H NMR spectroscopy revealed that Q[7] bound the guest and formed the host-guest inclusion complexes. But it was difficult to measure the ratio of the host and the guest in these complexes due to the fast ingress/egress of the guest and the broad resonance signals. To determine quantitatively the stability of the host-guest inclusion complexes formed from Q[7] and guest, UV spectra of the aqueous solutions containing a fixed concentration of the guest (40 μM) and variable concentrations of Q[7] were recorded at pH = 3.0 for the interaction system (Fig. 3).

The absorption spectra of guest exhibited a progressively lower absorbance with a slight red shift as the ratio of $N_{Q[7]}/N_g$ was increased and the host showed no absorbance in the range of > 210 nm. The absorbance (A) *vs*. ratios of mole of the host

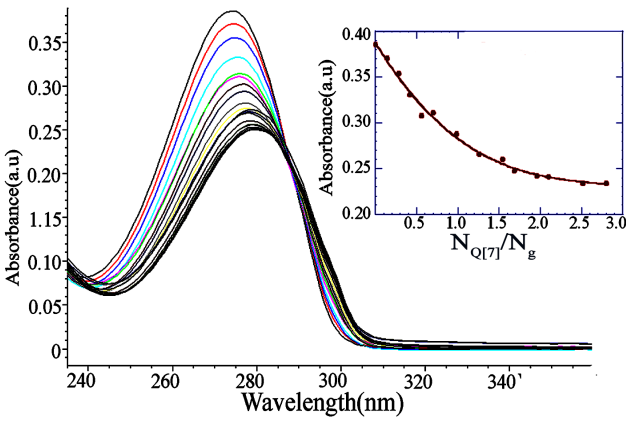


Fig. 3. UV absorption spectra of guest in the presence of increasing concentrations of Q[7] and corresponding absorbance *versus* $N_{Q[7]}/N_g$ curves (insert) at 274 nm

Q[7] and the guest ($N_{Q[7]}/N_g$) data can be fitted into a 1:1 binding model for the system of Q[7]-g at λ = 276 nm. The corresponding formation constant (K) is (6.41 ± 1.12) × 10^4 L mol^{-1}.

High performance liquid chromatography with β-cyclodextrin modified mobile phase has been extensively used for the study of the interactions of β-cyclodextrin with various guests and for the determination of the formation constants of the related inclusion complexes[9]. In this work, we tried to use this method to explore the interaction and stability between the host Q[7] and the guest g. The following expression is true when the cucurbituril has no retention in the LC system[10].

$$K' = \frac{(K'_G - K')}{KQ[7]m} + K'_{G\cdot Q} \quad (1)$$

where K' is the retention factor of the guest at a particular concentration of Q[7], $[Q[7]_m]$ is the concentration of Q[7] in the mobile phase and K'_G is the retention factor in the absence of Q[7]. For the inclusion compound with a 1:1 stoichiometry ratio, a plot of K' *vs*. $(K'_G - K')/[Q[7]_m]$ yields a straight line which could give K' and K'_G. Thus, the formation constants (K) of the Q[7]-guest systems can be calculated based on the eqn. 1.

The chromatograms of guest with different concentrations of Q[7] added to the mobile phase as a modifier were shown in Fig. 4a. The column temperature was kept at 50 ºC. The retention time of the g (t_R) increased slightly as the concentration of Q[7] increased, suggesting that the free guest was retained on the reversed stationary phase shorter when no Q[7] was added to the mobile phase. The correlation of K' upon $(K'_g - K')/[Q[7]_m]$ is graphically illustrated in Fig. 4b. According to eqn. 1, the calculated formation constant for the host-guest inclusion complexe is (4.55 ± 1.25) × 10^4 L mol^{-1}. They are consistent with the corresponding formation constant obtained by UV spectroscopy analysis.

Conclusion

The interaction between cucurbit[7]uril and cytosine in aqueous solution has been studied by ^{1}H NMR spectroscopy, UV absorption spectroscopy and high performance liquid chromatography. The ^{1}H NMR spectra analysis indicated

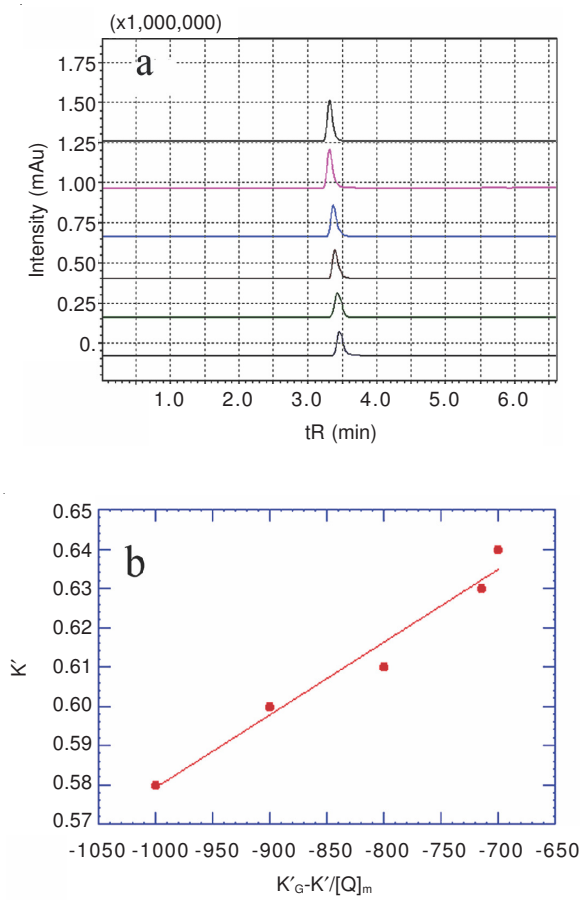


Fig. 4. Chromatogram of guest with various concentrations of Q[7] in the mobile phase for g (a). The concentration of Q[7] were 0, 1×10^{-5}, 3×10^{-5}, 5×10^{-5}, 7×10^{-5} and 1×10^{-4} M from top to bottom. The correlation plot of eqn. 1 for guest (b)

that Q[7] interacted with the cytosine moiety of the guest. Ultraviolet absorption spectroscopy and HPLC analysis have been used to determine quantitatively the stability of the host-guest inclusion complexe. The formation constant were $(6.41 \pm 1.12) \times 10^4$ L mol^{-1}(UV), $(4.55 \pm 1.25) \times 10^4$ L mol^{-1} for Q[7]-g, respectively (HPLC).

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