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Research Article

Supramolecular Encapsulation of Vitamin B_6 by Macrocyclic Nanocontainer Cucurbit[7]uril

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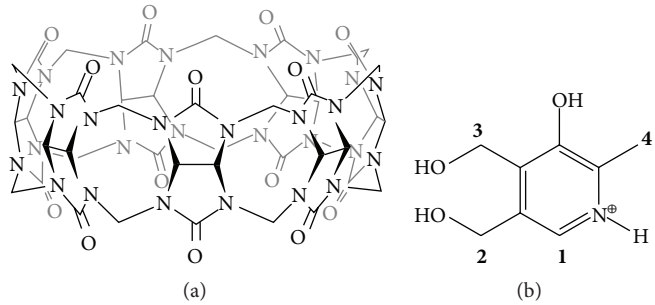
A pharmaceutically and biologically relevant molecule, pyridoxine hydrochloride (vitamin B_6), was encapsulated inside the cavity of a molecular container, cucurbit[7]uril (CB[7]), in aqueous solution. The CB[7] based "nanocapsule" of vitamin B_6 has been investigated for the first time, via ^{1}H NMR and UV-visible spectroscopic titrations (including Job's plot) and *ab initio* molecular modeling. The results have demonstrated that vitamin B_6 forms stable host-guest complexes within CB[7] in 1 : 1 stoichiometry, with a binding affinity of $(4.0 \pm 0.5) \times 10^3$ M^{-1}. Such a nanocapsule could potentially find application in vitamin B_6 formulation for the purpose of enhancing the stability, absorption, and delivery of this important vitamin.

1. Introduction

The self-assembly of pharmaceutically and biologically relevant molecules to form organized microenvironments or nanocarriers has been an important research topic during recent decades. Very often, nontoxic polymers act as the shells or encapsulation matrix of nanocapsules and supramolecular containers. For instance, there have been reports of vesicles or micelles that are composed of functional polymers that can encapsulate molecules into their nanoscale cores [1]. In addition, various natural and synthetic molecular containers including cyclodextrins and cucurbiturils have been investigated for their abilities to direct the encapsulation of bioactive molecules and formation of nanocapsules [2–4]. Nanocapsules have a myriad of applications, especially in the biomedical, food, and health sciences fields, such as drug delivery, food enhancement, and nutraceutical formulation [5, 6]. The benefits of such encapsulation can range from the protection of included substances from adverse environments to controlled release and to precision targeting [6, 7].

While cyclodextrins have been well known for decades for their encapsulation of bioactive molecules [8, 9], during the past fifteen years the cucurbit[*n*]urils (CB[*n*], n = 5–8, 10, and 14) have attracted increasing attention and have been highlighted in numerous reviews, due to their potential applications in drug encapsulation, formulation, and delivery [3, 4]. CB[*n*]s consist of n glycoluril units that are linked by $2n$ methylene groups. These molecules possess a hydrophobic cavity as well as two identical carbonyl-lined portals with negative dipole potentials. The reaction of glycoluril and excess formaldehyde in the presence of concentrated acid such as sulfuric acid or hydrochloric acid at a temperature of ~110°C produces the macrocyclic CB[*n*] compounds through an acid-catalyzed condensation reaction. Initially only CB[6] was isolated and characterized [10]. In the years of 2000 and 2001, respectively, Kim and Day independently reported the isolation of other species including CB[5], CB[7], and CB[8], when they synthesized these macrocycles using a lower temperature (75–90°C) [11, 12]. This exciting discovery has consequently attracted more scientists to join the research field and look into the CB[*n*] chemistry and its various potential applications. In particular, during recent years, CB[*n*]s have exhibited outstanding molecular recognition behavior and strong interactions with a wide variety of



(a) (b)

Figure 1: Structural diagrams of CB[7] (a) and pyridoxine hydrochloride (PH) (b).

neutral and cationic molecules, particularly those bearing imine or amine groups, many of which are biologically and medically relevant compounds [3, 4, 13].

Within the CB[*n*] family, CB[7] (shown in Figure 1(a)) has attracted perhaps the greatest interest as a candidate for drug encapsulation and delivery applications due to its excellent water-solubility and matching size with numerous organic and organometallic drugs [3, 4]. It has been demonstrated by numerous groups that CB[7] can effectively encapsulate whole or part of molecules including a variety of drugs and bioactive molecules including the beta blocker atenolol [14], the tuberculosis drug pyrazinamide [15], platinum-containing anticancer drugs such as cisplatin [16–18], and prilocaine (a local anaesthetic) [19]. We have previously encapsulated ranitidine and bis(thiazolium) model drug [20, 21] and coumarins (coumarin and coumarin-6) [22, 23] with CB[7], and these encapsulations helped to protect the guest drugs against thermal degradation and enhanced cellular uptake. Consequently, this encapsulation has the potential to extend the shelf-lives of these drugs. Similarly, we investigated the complexation of vitamin B_{12} and coenzyme B_{12} with CB[7], through interactions with the 5,6-dimethylbenzimidazole α-nucleotide base, and found that this complexation stabilized the base-off forms of the vitamin B_{12} compounds [24]. As far as we are aware, this vitamin B_{12} investigation has been the very first and so far the only study of CB[7]'s complexation of a vitamin compound.

Pyridoxine hydrochloride (PH), better known as vitamin B_6 (Figure 1(b)), is soluble in water, and, just like vitamin B_{12}, it is a member of the vitamin B complex family. The active species of this vitamin is pyridoxal phosphate. Vitamin B_6 serves as a coenzyme to many other enzymes in the body that are mainly responsible for the metabolic reactions of amino acids, glucose, and lipids, as well as the synthesis of neurotransmitters, histamine, and hemoglobin and function as well as gene expression [25]. PH is the chemical form of vitamin B_6 often found in vitamin dietary supplements. Both the free and the bound species of PH are widely distributed in foods. In the medical world, it has been used for decades as a treatment for treating nausea and vomiting in early pregnancy, often in combination with other drugs such as metoclopramide or doxylamine [26]. In addition, according to a prospective clinical study conducted out in Netherlands, the consumption of vitamin B_6 from either food or supplements could cut by half the risk of Parkinson's disease among smokers [27]. Interestingly, it has been previously reported that PH can be encapsulated by cyclodextrins [28] and ApoC-opC protein [29], for potential vitamin formulation related applications. These studies together with our previous experience with CB[7]'s encapsulation with vitamin B_{12} and other drug molecules have led us to extend our efforts to study the supramolecular encapsulation of vitamin B_6 by CB[7]. We report herein the formation of a stable 1 : 1 host-guest nanocapsule complex of PH@CB[7] in aqueous media, and such molecular encapsulation was examined by different spectroscopic methods including ^{1}H NMR as well as UV-visible spectroscopy, in addition to as *ab initio* calculation based molecular modeling.

2. Materials and Methods

2.1. Materials. CB[7] was synthesized according to a procedure described in the literature, with minor modifications [12]. Briefly, 10 g of glycoluril (70 mmol) and 4.22 g of paraformaldehyde (140 mmol) were initially mixed together thoroughly. Ice-cold concentrated HCl (14.2 mL) was subsequently added to this powder mixture, and this mixture was stirred vigorously to yield a gel-like mixture. This gel-like mixture was then gradually heated to 100°C and this temperature was maintained for ~20 h, and the mixture was subsequently allowed to cool to room temperature. The remaining solid in the mixture was removed via filtration. The filtrate was concentrated to one fourth of its original volume and water (5 mL) was added to again yield a cloudy solution, which was filtered again to remove any solid. Approximately 35 mL of methanol was added to the remaining filtrate, which was then stirred overnight to give a precipitate that was collected by vacuum filtration. The off-white crude product was dissolved in 100 mL of hot 20% aqueous glycerol, heated, and stirred for 30 min to give a relatively clear solution. A white precipitate was produced by the addition of cold methanol and was collected by vacuum filtration before it was washed several times with methanol to remove the glycerol. The product was dried overnight under vacuum to give a white powder (yield: 2.70 g, 25%; ^{1}H NMR (400 MHz, D_2O): δ 5.73 (d, J = 15.4 Hz, 14H), 5.48 (s, 14H), and 4.19 (d, J = 15.4 Hz, 14H) ppm; ESI-MS: m/z = 1186 $(M + Na)^+$, calculated m/z = 1186).

Pyridoxine hydrochloride (vitamin B_6, analytical standard) was purchased from Sigma-Aldrich and was not purified further.

2.2. Apparatus. The ^{1}H NMR spectra were acquired using a Bruker AV-400M NMR spectrometer. The UV-visible spectra were recorded using a Hewlett Packard 8452A diode array UV-visible spectrometer using quartz cells having a 1.00 cm path length. The predicted structure of the host-guest complex was calculated via energy-minimizations using the Gaussian 03 (Revision C.02) program, which was run at the High Performance Virtual Computing Laboratory (HPVCL) at Queen's University. The HF/3-21G** basis set was used for these calculations.

2.3. Preparation of the Complex Solutions. In order to prepare solutions in order to perform ^{1}H NMR characterization, a 1 mM solution of pyridoxine hydrochloride (PH) in D_2O was simply mixed with various amounts of CB[7] to reach 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 mM of CB[7], respectively, without changing the PH concentration, and sonicated for 3 min and was characterized via ^{1}H NMR spectroscopy.

In order to perform the UV-visible spectroscopic titrations, 0.1 mM aqueous solutions of PH were initially prepared. These PH solutions were subsequently titrated with various volumes of solutions containing 0.1 mM PH and 0.5 mM CB[7] to achieve different ratios of PH : CB[7] while allowing the PH concentration to remain constant during the entire titration.

In order to prepare solutions for continuous variation titrations, solutions with total concentrations of PH and CB[7] of 0.1 mM were prepared. Among these solutions, the ratio of CB[7]/([PH] + CB[7]) was varied from 0, 0.1, 0.2, . . ., up to 1.0 (i.e., at intervals of 0.1).

3. Results and Discussion

3.1. ^{1}H NMR Investigation. In the ^{1}H NMR spectra of the CB[n] host-guest complexes, the guest protons exhibited complexation-induced shift (CIS, $\Delta\delta = \delta_{\text{bound}} - \delta_{\text{free}}$) that can provide valuable information regarding the average position of the guest protons in comparison with the CB[n] cavity. Downfield shifts ($\Delta\delta > 0$) are observed when guest protons are positioned adjacent to the carbonyl oxygen at the portals. In contrast, guest protons located deep within the CB[n] cavity become shielded by the hydrophobic cavity and exhibit upfield CIS values ($\Delta\delta < 0$). With this knowledge in hand, the complexation behavior between PH and CB[7] can be readily monitored via ^{1}H NMR spectroscopy. As shown by Figure 2, in the presence of increasing amounts of CB[7], the resonances corresponding to the aromatic proton (H1) and methylene protons (H2 and H3) exhibit upfield CIS (−0.1 to −0.5 ppm), implying their inclusion within the shielding hydrophobic cavity of CB[7]. Conversely, the subtle downfield CIS (<0.1 ppm) exhibited by the methyl protons (H4) suggests that the methyl group of PH is likely located slightly outside of the carbonyl groups of the CB[7] portal and thus is only affected minimally. In addition, when the amounts of CB[7] are increased (e.g., from 0.5 to 2.5 equivalents of CB[7]), the encapsulated protons exhibit broadening behavior in the NMR spectrum. These broadened proton signals thus do not exhibit splitting into free and bound peaks but instead only appear as one set of signals with migrating chemical shifts, which is indicative of a fast exchange rate of complexation-decomplexation processes between the free and bound PH species on the ^{1}H NMR timescale [22, 23, 30]. When these observations are taken together with the moderate CIS values of the guest protons upon complexation, it can be concluded that the guest PH mainly occupies the shallow regions of the CB[7] cavity, rather than regions deep within the cavity.

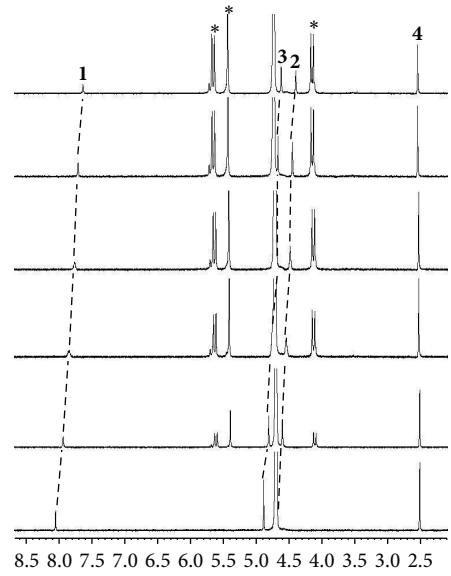


Figure 2: ^{1}H NMR spectra of PH (1 mM) alone (the bottom spectrum) and in the presence of increasing amounts (0.5, 1.0, 1.5, 2.0, and 2.5 mM) of CB[7].

3.2. UV-Visible Study. The formation of 1 : 1 host-guest complexes between PH and CB[7] in aqueous media was also supported by UV-visible absorbance measurements of PH (0.1 mM) titrated with different amounts of CB[7]. The incremental addition of CB[7] to a solution of PH resulted in a decrease in the absorbance peak at 290, along with a subtle bathochromic shift (Figure 3), which was consistent with the inclusion of the guest into the hydrophobic microenvironment. The nonlinear least squares fit (Figure 3, inset) is consistent with a 1 : 1 binding stoichiometry model and provides a binding constant K_a of $(4.0 \pm 0.5) \times 10^3\ \text{M}^{-1}$ [31]. The strength of this complexation is moderate in comparison between CB[7] and many other cationic species [32]. However, since this complexation is with a fairly water-soluble guest molecule, the binding strength in this order of magnitude is fairly strong, especially considering that the complexation driving force is mainly cation-dipole forces and hydrogen bonding, and this binding process involves minimal hydrophobic effect, which often plays a major role in the complexation of other organic guests with CB[n] hosts. This binding affinity is even comparable with the binding constants reported for the inclusion of other aromatic molecules, such as coumarins [22, 23].

The 1 : 1 binding stoichiometry was also confirmed by Job's plot titration method. Indeed, a Job's plot for the

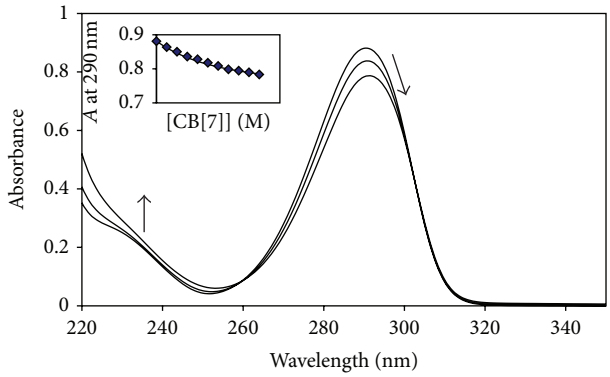


Figure 3: The UV spectrum of PH (0.1 mM) at different concentrations of CB[7] (0, 0.2, and 0.5 mM, following the direction as indicated by arrows). Inset image: a binding curve obtained based on the UV absorbance measurement of PH (0.1 mM) titrated with various amounts of CB[7]. This plot is based on the absorbance at 290 nm versus the concentration of CB[7] (M) and indicated a binding constant of $(4.0 \pm 0.5) \times 10^3$ M^{-1} for PH@CB[7] complex at pH = 5 [31].

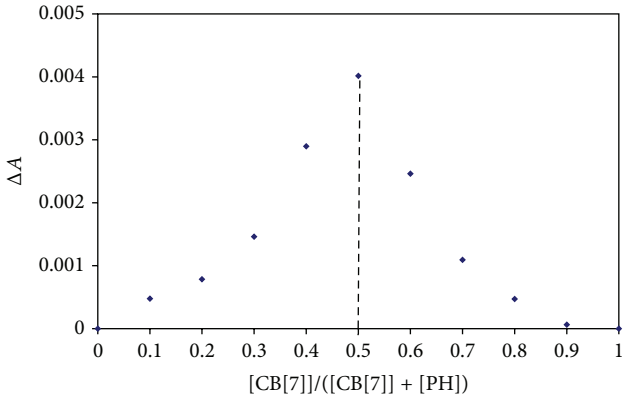


Figure 4: Job's plot of the CB[7]-PH host-guest complexes: this plot was prepared via monitoring the changes of UV-visible absorbance during the continuous variation titration.

PH@CB[7] system (with [CB[7]] + [PH] fixed to 0.1 mM), as monitored using UV-visible spectroscopy (Figure 4), reached a maximum at a ratio of 0.50 for [CB[7]]/[CB[7]] + [PH]]. This behavior indicates that the 1 : 1 complex between CB[7] and PH represented the dominant species in this concentration range.

3.3. Ab Initio Molecular Modeling. In addition to ^{1}H NMR and optical spectroscopy measurements, we also employed *ab initio* molecular modeling to further evaluate the presence of the 1 : 1 host-guest complexes and verify the inclusion complexes formation and the host binding sites selectivity on the guest molecules. The gas-phase structure of the CB[7] supramolecular complexes with PH was determined using *ab initio* calculations (HF method with 3-21G** basis set). As demonstrated by Figure 5, the host-guest complex formations and the binding sites of the guest within the CB[7] cavity are consistent with the deductions from the experimental shifts of the guest proton resonances ($\Delta\delta$ values) in the presence of CB[7] on ^{1}H NMR spectra (Figure 2). The entire aromatic ring of the guest is preferentially encapsulated by CB[7], leaving the substituted methyl group mostly outside of the cavity. The positively charged nitrogen atom sits right at the portal, in line with the carbonyl groups for optimal cation-dipole interactions, evidently stabilizing the complex. In addition, the energy-minimized structure of the complex also reveals multipoint N–H···O=C and O–H···O=C hydrogen bonding contacts. As summarized from Table 1, the distances between the nitrogen-based hydrogen and the carbonyl oxygen are 2.78 and 2.98 Å at one of the portals. Meanwhile, the distances between hydroxyl hydrogen and the carbonyl oxygen are 1.91 and 1.89 Å on one portal and 2.06 Å on the other portal, while the hydrogen bond angles range between 109° and 143°. The CB[7] host molecule acts not only as a steric barrier (to the whole aromatic ring) but also a hydrogen bond acceptor for the nitrogen-based proton and hydroxyl protons, by positioning the guest within its cavity to facilitate optimal hydrogen bonding interactions.

Table 1: A summary of the hydrogen bonding angles and distances derived from Figure 5.

Active PH hydrogen	H-bond angle (°)	H···O distance (Å)
N-**H**	109.9, 110.5	2.78, 2.98
C(2)-O-**H**	131.5	1.91
C(3)-O-**H**	142.5	1.89
Ar-O-**H**	128.1	2.06

4. Conclusion

As reported herein, the supramolecular encapsulation of PH by CB[7] was characterized by ^{1}H NMR, UV-visible spectroscopy (including Job's plots), and *ab initio* calculations. ^{1}H NMR characterization has clearly demonstrated that PH and CB[7] undergo 1 : 1 complexation with a fast exchange rate between the free and bound species on the NMR timescale. The binding constant of the host-guest complexes was determined from UV-visible spectroscopic titration of PH with various quantities of CB[7]. The complexation of a water-soluble guest, PH, can still reach a binding constant with an order of magnitude 10^3 M^{-1}. The present investigation provides the first example of CB[7]'s complexation with vitamin B_6 and may find potential application in stabilization and sensing for vitamin B6 and even other biologically and medically relevant applications for other vitamins formulation and delivery by CB[7], especially with improved understanding of its biocompatibility profile [33].

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Wanying Li and Shengke Li contributed to the work equally.

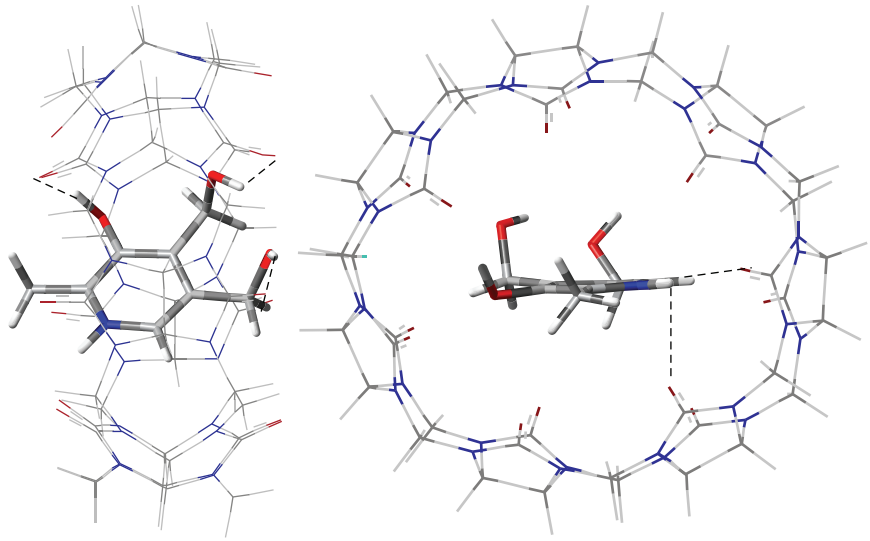


Figure 5: Molecular modeling of 1 : 1 PH@CB[7] complexes based on *ab initio* calculations using the HF/3-21G** basis set.

Acknowledgments

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