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ORIGINAL ARTICLE

Encapsulation of a β-carboline in cucurbit[7]uril

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Abstract Inclusion of a biological photosensitizer and prototype of β-carbolines, norharmane (NHM), into the cavity of cucurbit[7]uril (CB[7]) has been investigated for the first time, by using ^{1}H NMR and UV–visible spectroscopy, and ab initio calculations. Protonated NHM forms a very stable host–guest complex with CB[7] in aqueous solution, with a binding constant of $(9.0 \pm 0.5) \times 10^4$ M^{-1}. The encapsulation of NHM into CB[7] has driven the prototropic equilibrium of NHM to protonated NHM ($NHMH^+$) at neutral pH. A pH titration for the host–guest complex revealed a moderate shift of the acid–base equilibrium in the ground-state (from 7.2 to 7.9), which may be caused by the low polarity microenvironment of the CB[7] cavity. The CB[7] provides a binding pocket for the hydrophobic molecule, and the polar, carbonyl-lined portals offering an anchoring site for the positive charge of the cationic species $NHMH^+$.

Keywords Cucurbituril · Host–guest complexation · Norharmane · pK_a shift

Introduction

The assembly of biologically important molecules into organized microenvironments has been an important research topic during the past few decades. The encapsulation and organization of bioactive molecules into nano- or molecular containers where the polarity is different from that of bulk environment can often alter the guests' chemical and physical characteristics, including photophysical and photochemical properties, and mimic related biosystems, and therefore have high potential for biological and photochemical applications [1]. Several popular natural and synthetic molecular containers including cyclodextrins, calixarenes, and porphyrins, have been actively studied for their abilities in encapsulating bioactive molecules [2]. Conversely, similar investigations with cucurbituril host molecules are rarely available in the literature, compared with those for cyclodextrins.

The cucurbit[*n*]urils (CB[*n*], *n* is most commonly 5–8), a family of cyclic host molecules consisting of *n* glycoluril units bridged by 2*n* methylene groups, have a fairly rigid hydrophobic cavity of low polarity accessible through polar portals lined with carbonyl groups. Although the synthesis of cucurbit[6]uril (CB[6]) was reported more than 100 years ago [3], it was not until 1981 that its chemical nature and structure were fully characterized by Mock and co-workers [4]. For two decades thereafter CB[6] was extensively investigated with numerous reports of the formation of coordination complexes with a range of metal ions, and inclusion complexes with various guest molecules [5]. Unlike the cyclodextrin family of host molecules, the availability of only one relatively small cucurbituril cavity size had, until recently, limited the range of guest species that could be encapsulated. The discoveries of CB[5], CB[7], and CB[8] by Kim and co-workers expanded the CB[*n*] family, and provided hosts with a wide range of available cavity sizes [6]. The improved syntheses of CB[*n*], and in particular the superior solubility of CB[7] in aqueous solution, have prompted investigations into

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host–guest behaviour in water with a variety of aromatic and metal complex guest molecules [7–10], including bioactive species such as oxaliplatin and other anti-cancer platinum complexes [11, 12], and 5-aminosalicylic acid colon-specific drug molecules [13]. Recently, Wang and Macartney have reported the encapsulation of the histamine H_2-receptor antagonist ranitidine with CB[7], and observed that the pK_a values of the ranitidine have been increased upon its complexation with CB[7] [14]. Very recently, we have also discovered that CB[7] can modulate the base on/off process of vitamin B_{12} and coenzyme B_{12} due to the inclusion of the protonated dimethylbenzimidazole base of the B_{12} molecules in the cavity of CB[7], and increase the base's pK_a value, and therefore CB[7] is able to mimic the hydrophobic pocket of coenzyme binding protein [15].

Norharmane (9H-pyrido[3,4-b]-indole, NHM) is a bioactive β-carboline alkaloid molecule and widespread in plants and animals frequently acting as monoamine oxidase inhibitors [16]. In addition, it has been reported to work as a very efficient biological photosensitizer in the presence of oxygen [17]. Moreover, it has been widely accepted that four different species including cationic, neutral, zwitterionic and anionic species are present in solution, depending on the pH of the solution, with intensive literature reports of the pK_a values at various stages [18]. Numerous studies have dealt with the modulation of the prototropic transformation of NHM, mainly between the neutral and protonated form (Scheme 1) through supramolecular complexation and/or encapsulation by cyclodextrin [19, 20], micelles [21], serum albumins [22], and solvents [23] at physiological pH. These studies have led us to investigate the CB[7] host–guest chemistry with NHM in aqueous solution. The preferential complexation of the protonated $NHMH^+$ form modulates the acid–base equilibrium of $NHMH^+$ upon its inclusion into the cavity of CB[7].

Materials and methods

Materials

Cucurbit[7]uril was synthesized according to a literature method [24] and characterized by both ^{1}H NMR spectroscopy and electrospray ionization (ESI) mass spectrometry. All other materials, including norharmane (Sigma) were used as received. Acetate (0.10 M NaAc/HAc) and phosphate (0.10 M NaH_2PO_4/Na_2HPO_4) buffer solutions were prepared to carry out the pH titration between pH 4.0 and 12.0.

Apparatus

The ^{1}H NMR spectra were measured on a Bruker AV-400 M NMR spectrometer. The UV–visible spectra were acquired on a Hewlett Packard 8452A diode array UV–visible spectrometer using quartz cells with a 1.00 cm path length. The modelled structure of the host–guest complex was computed by energy-minimizations using Gaussian 03 (Revision C.02) programs run on the computing facilities of the High Performance Virtual Computing Laboratory (HPVCL) at Queen's University. The calculation method was HF (Hartree-Fock) and the basis set used for the calculations was 3-21G**.

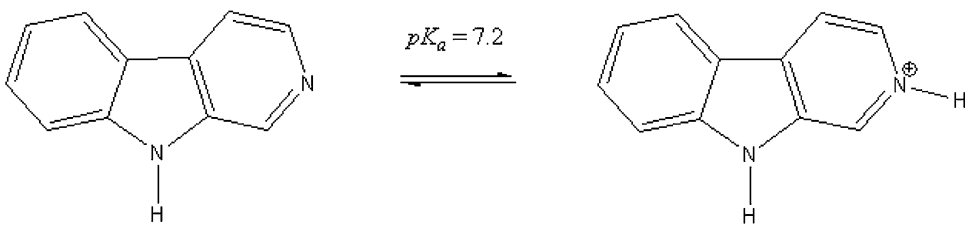
Preparation of complex solutions

For the ^{1}H NMR spectra, 1.0 mM solutions of NHM in D_2O were mixed with 0.70 and 1.5 equivalents of CB[7] and sonicated for 3 min. The 1:1 guest–host complex {$NHMH^+$•CB[7]} was prepared in the same way in water with excess of CB[7] for the pH titration monitored by UV–visible spectroscopy.

Results and discussion

Examination of $NHMH^+$•CB[7] by ^{1}H NMR and ab initio calculation

The formation of a 1:1 guest–host complex between cationic NHM ($NHMH^+$) and CB[7] has been demonstrated by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of the 1:1 guest–host complex of the $NHMH^+$ with CB[7] (Fig. 1a) reveals complexation-induced upfield chemical shifts (0.1–1.1 ppm) for the majority of the aromatic guest resonances from H3 to H7, Fig. 1, consistent with their inclusion in the shielding hydrophobic cavity. The negligible shift for the H1 proton and the slight downfield shift



Scheme 1 Equilibrium between neutral and protonated NHM species, with pK_a value indicated

of the H2 proton suggest that they are located in line with the carbonyl groups of the portals, which have been observed to deshield guest protons [7–10, 14, 25]. With a limiting amount of CB[7] (the middle spectrum of Fig. 1a), the proton resonances of NHM guest have no splitting into free and bound species but rather only one set of migrating chemical shifts, indicative of a fast rate of complexation–decomplexation processes between NHM and CB[7] on the ^{1}H NMR timescale.

An energy-minimized structure (Fig. 1b) of the NHMH$^+$•CB[7] complex from ab initio (HF method with 3-21G** basis set) calculations seems in general agreement with the NMR results, in terms of the complexation geometry, with the protonated pyridium headgroup situated at the portal and the rest located mainly within the CB[7] cavity. Please note that, however, the H4–H7 (especially H6 and H7) are sitting slightly out of the cavity, which is in fact inconsistent with their upfield proton chemical shifts from the ^{1}H NMR spectra (Fig. 1a) upon guest complexation; This inconsistency is primarily caused by the inherent difference between the two methods: ^{1}H NMR is a solution-based characterization whilst the ab initio calculation is a gas-phase simulation. Still, the interaction of the positively charged nitrogen group with the portal carbonyl groups on CB[7] evidently stabilizes the inclusion complex in the computed structure.

NHM titrated with CB[7] and NHMH$^+$•CB[7] binding constant determination

The UV–visible absorbance spectrum of NHM at neutral pH exhibits typical absorption peaks at both 345 and 372 nm representing neutral and protonated species respectively, consistent with literature reports [17–21]. The inclusion of the NHM in cucurbit[7]uril can be conveniently monitored using UV–visible spectroscopy, especially if the encapsulation of NHM into CB[7] can affect the guest acid–base equilibrium. The gradual addition of CB[7] (0.5 and 1.1 equivalent of NHM) into NHM solution at neutral pH has resulted in a absorption reduction at 345 nm and a simultaneous increase at 372 nm (Fig. 2), suggesting a transition from the neutral NHM species into the protonated species in the presence of CB[7] without changing pH (buffered solution). Because at neutral pH both protonated

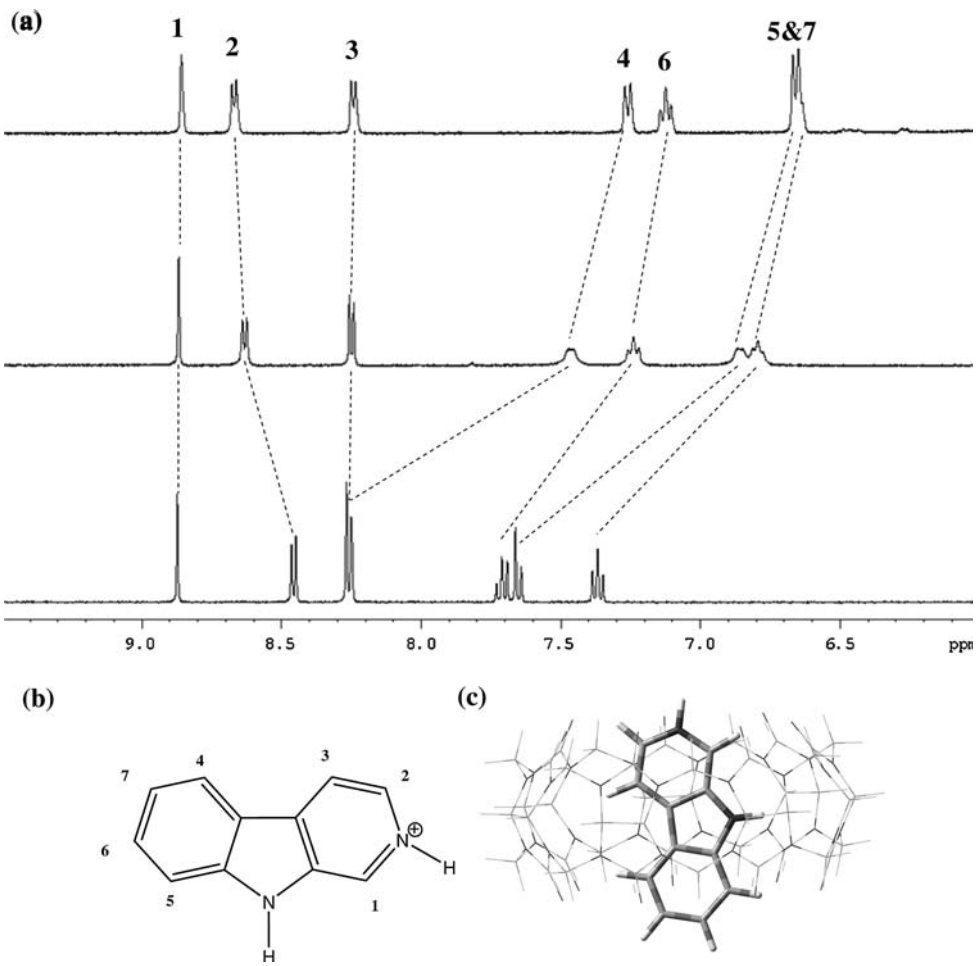


Fig. 1 ^{1}H NMR (400 MHz, D_2O) spectra of NHMH$^+$ in the absence (*bottom*) and in the presence of 0.7 (*middle*) and 1.5 (*top*) equivalent CB[7] (**a**), proton labelled structure of cationic NHM (**b**), and energy-minimized complex structure (**c**)

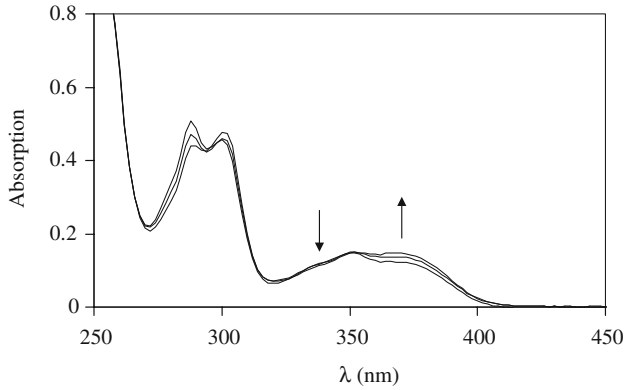


Fig. 2 The UV spectrum of NHM (0.5 mM) at various concentrations of CB[7] (0, 0.25 and 0.55 mM, following the direction as indicated by *arrows*)

and neutral species of NHM are present in the solution, in order to eliminate the complication due to species transition that exists at neutral pH, a spectrophotometric titration at pH = 5 was conducted (at this pH, cationic species is virtually the only species), which provided a stability constant for $NHMH^+$•CB[7] of $(9.0 \pm 0.5) \times 10^4\ M^{-1}$ (Fig. 3), which is similar to the values reported for the inclusion of other cationic aromatic molecules, such as methylviologen ($K = 2 \times 10^5\ M^{-1}$) in CB[7] [26, 27].

Measurements of pK_a value

It has been demonstrated before that the pK_a values of protonated guest molecules included in the cavity of cucurbiturils may be shifted through non-covalent interactions with the polar carbonyl-lined portals [14, 15, 25, 28, 29]. The effect of the inclusion of $NHMH^+$ in CB[7] on the cationic to neutral species equilibrium constant was investigated with a UV pH titration (Fig. 4), monitoring the

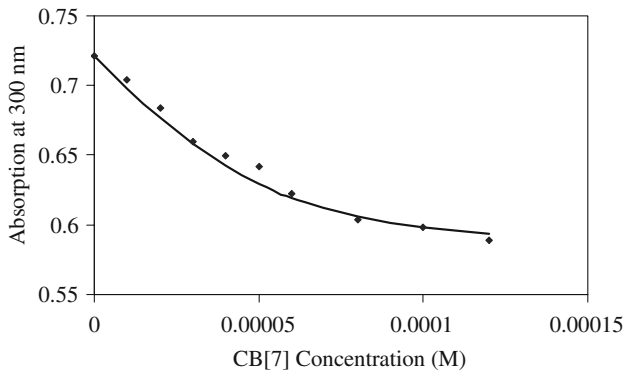


Fig. 3 The binding curve from the UV absorbance measurement of $NHMH^+$ (50 μM) titrated with different amounts of CB[7]. The plot is based on absorbance at 300 nm versus concentration of CB[7] (M), gives a binding constant of $9 \times 10^4\ M^{-1}$ for $\{NHMH•CB[7]\}^+$ complex at pH = 5

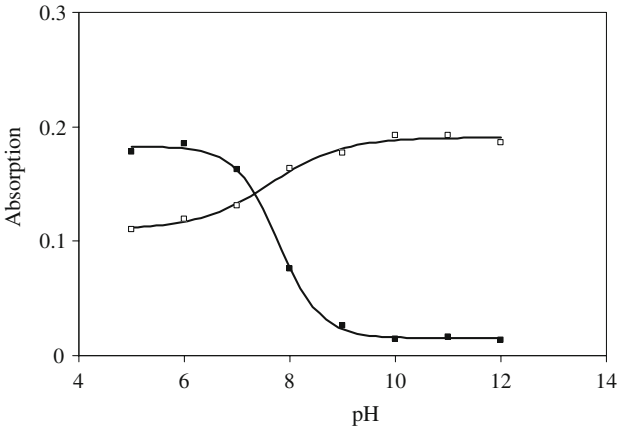


Fig. 4 pH titrations of the $\{NHM•CB[7]\}^+$ host–guest complex monitored at 348 nm (*unfilled square*) and 372 nm (*filled square*), with the *solid curves* both corresponding to a pK_a of 7.9)

changes in the absorbances at 348 and 372 nm with pH in the range of 5–12. The titration of the $NHMH^+$•CB[7] from acid into base pH results in an increase in the peak at 348 nm and a simultaneous decrease at 372 nm, corresponding to protonated to neutral transition. From the pH dependent UV spectral changes, the titration gives a value of p$K_a = 7.9 \pm 0.1$ (according to Eq. 1) upon guest inclusion into CB[7], which is increased moderately from the reported value of 7.2 of $NHMH^+$ without any host molecules [19–23].

$$pK_a = pH + \log\left(\left[\{NHMH \bullet CB[7]\}^+\right]/[\{NHM \bullet CB[7]\}]\right). \quad (1)$$

The increased pK_a value of the included guest $NHMH^+$ is presumably caused by the low polarity cavity of the host CB[7] and the cation–dipole interactions (seen on Fig. 1b) between the positively-charged pyridium of the guest and polar carbonyl portals of the host molecule upon the guest ($NHMH^+$) complexation with CB[7], as such interactions apparently stabilize the protonated species.

Conclusion

In summary, the complexation of NHM with CB[7] was investigated by ^{1}H NMR, ab initio calculation, and UV–visible spectroscopy in aqueous solution. ^{1}H NMR has demonstrated clearly the 1:1 complexation with fast exchange rate between free and included guest in NMR time scale. The stability constant of inclusion complexes was calculated to be $(9.0 \pm 0.5) \times 10^4\ M^{-1}$ based on the CB[7] titration of NHM with UV–vis measurements. Interestingly, the complexation has moderately modified the pK_a of the guest NHM. The present investigation of

NHM complexation by CB[7] provides the first example of cucurbiturils' capacity to strongly complex with β-carboline, and may have potential biological and medical applications for drug formulation and delivery, as β-carboline molecules are biologically and pharmaceutically important. We are currently expanding the study to other β-carboline molecules.

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