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Encapsulation of Vitamin B_1 and Its Phosphate Derivatives by Cucurbit[7]uril: Tunability of the Binding Site and Affinity by the Presence of Phosphate Groups

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Supporting Information

ABSTRACT: Vitamin B_1 (**1**) and its phosphate derivatives, thiamine monophosphate (**2**) and thiamine pyrophosphate (**3**), are shown to form stable 1:1 host−guest complexes with cucurbit[7]uril (CB[7]) in aqueous solution. The binding sites of CB[7] on these guests shift from the ethylthiazolium region of **1** to the pyrimidine moiety of **2** and **3** due to the presence of phosphate groups, leading to variations of binding affinities as well as C(2)−H/D exchange rate constants and C(2)−H pK_a values with these guest molecules.

Thiamine (vitamin B_1 (**1**), Figure 1) and thiamine diphosphate (**3**), its biologically active form, are important coenzymes in carbon−carbon bond formations and dissociations[1] such as decarboxylation of α-ketoacids, acyloin condensations, and transketolizations.[2] The most distinctive part of thiamine and its phosphates is the thiazolium ring, as the base-catalyzed abstraction of the C(2) proton forms a reactive thiazolium ylide (a stable equivalent of an acyl anion) which is a potent carbon nucleophile and a reasonably stable leaving group.[3] A significant portion of the catalytic rate acceleration is the stabilization of the unstable zwitterionic/dipolar intermediate (enamine/C(2) α-carbanion) by the protein environment. The role of the pyrimidine ring is thought to involve activation of the coenzyme through desolvation by preventing water from reaching the active site.[4] Simple thiazolium salts are used as catalysts in organic synthesis (e.g., benzoin condensation) and catalyze many of the enzymatic transformations, such as acetoin condensation, even in the absence of the enzymes.[2]

A number of thiamine diphosphate biomimics, using macrocyclic and other artificial receptors, have been reported.[4,5] Functionalized cyclodextrin monomers and dimers with thiazolium cations have demonstrated modest catalysis of benzoin condensation.[5a−c] The C(2)−H/D exchange reaction of thiamine and its phosphate esters has been studied for over 50 years, beginning with the work of Breslow.[6] Subsequent work[3a,b,7] has investigated the various factors governing the acidities and H/D exchange rates of thiamine, thiamine phosphates, and other thiazolium containing compounds. Haake et al. have determined, from the C(2)−H/D exchange rate constants of analogous oxazolium, thiazolium, and imidazolium cations, that the ratio of the exchange rate is approximately $10^{5.5}$:$10^{3.5}$:1.[7b] We have demonstrated that the inclusion of the α,α'-bis(3-(1-methylimidazolium))-*p*-xylene dication in the host molecule cucurbit[7]uril (CB[7], Figure

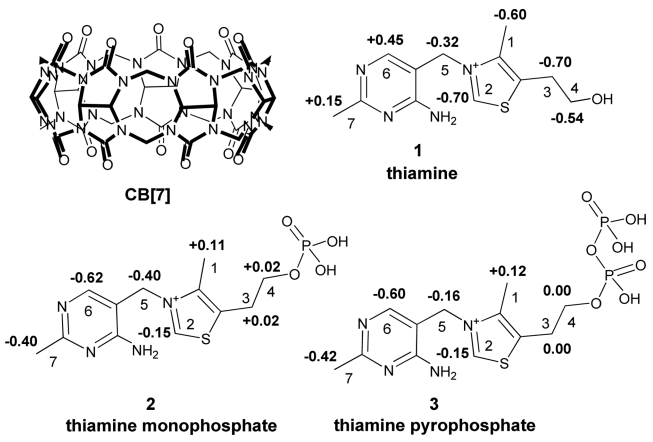

Figure 1. Molecular structures of cucurbit[7]uril and the three thiamine guests. The numbers in bold are the complexation-induced ^{1}H NMR chemical shift changes (ppm) for the resulting host−guest complexes with CB[7].

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1) significantly slows (about 1000-fold) the C(2)−H/D exchange reaction in D_2O and shifts the pK_a value up by about 3 pK_a units[7g] and have recently reported similar behavior with the CB[7] complex of the corresponding α,α'-bis(thiazolium)-*p*-xylene dication (ΔpK_a = 0.7), a model antimalarial drug.[7h] Schmitzer and co-workers have observed that the C(2)−H/D exchange is inhibited for dibenzylimidazolium cations included in the cavity of β-cyclodextrin.[8]

The cucurbit[*n*]urils (CB[*n*], where *n* = 5−8, 10, 14) are a family of macrocyclic host molecules comprised of methylene-bridged glycoluril units whose remarkable binding behaviors toward cationic guests have been of considerable recent interest.[9] The CB[*n*] hosts possess a hydrophobic interior cavity, accessible through restrictive portals rimmed with ureido carbonyl groups, and CB[7], with its superior solubility in aqueous solution[10] and the capacity to include guests bearing aromatic rings, is particularly attractive to host a variety of organic cations of biological and medicinal interest, both in vitro[11] and in vivo.[12] One of the more interesting effects of guest inclusion in the cavity of cucurbiturils is the increase in the ground- and excited-state pK_a values of protonated forms of basic guests through preferential binding of the (cationic) protonated species.[13]

In this paper, we describe the encapsulation of three thiamine compounds, **1**, **2** (an intermediate in the biosynthesis of thiamine), and **3** (Figure 1), by CB[7] in aqueous solution, employing UV and ^{1}H NMR spectroscopy and ESI-MS spectrometry. The anticipated 1:1 host−guest complexation stoichiometry is supported by positive-ion mode ESI-MS measurements that contain strong signals for singly charged species at m/z = 1428 ($[M]^+$), 1508 ($[M + H]^+$), and 1588 ($[M + 2H]^+$), for the CB[7] complexes ([M]) of **1**, **2**, and **3**, respectively (Figures S1−S3).

In the ^{1}H NMR spectra of the CB[7] host−guest complexes with the thiamine (Figure 2) and the thiamine phosphates (Figure 2 and Figure S4), the complexation-induced shift changes (CIS, $\Delta\delta = \delta_{bound} - \delta_{free}$) exhibited by guest protons can provide valuable information regarding the average position of the guest protons with respect to the CB[*n*] cavity.[9] Downfield shifts ($\Delta\delta > 0$ ppm) are observed for deshielded guest protons located adjacent to carbonyl oxygens of the portals, while shielded guest protons located within the CB[*n*] cavity exhibit upfield CIS values ($\Delta\delta < 0$ ppm). The CIS values (Figure 1) and the NMR spectra (Figure 2) show that for the host−guest complex **1**−CB[7] the resonances corresponding to the ethyl group protons (H3 and H4), the methyl protons on the thiazolium ring (H1), the linking methylene protons (H5), and the C(2)-proton in the ^{1}H NMR spectrum of the inclusion complex have shifted upfield from those of the free guest, thus indicating that they are positioned within the cavity of CB[7]. Meanwhile, the other methyl proton resonance (H7) and the aromatic proton on the pyrimidine ring (H6) exhibited downfield shifts, indicating that the methylated pyrimidine ring is located outside of the cavity but near the portal. Conversely, the substituted pyrimidine ring of **2** and **3** is preferentially bound by CB[7], rather than the ethylthiazolium portion, because of the presence of polar (negatively charged) phosphate groups on the ethyl groups of **2** and **3**, thus shifting the CB[7] over to the pyrimidine side of the guest.

UV spectroscopy was employed to further confirm the CB[7] binding stoichiometry and host locations and to determine the host−guest stability constants ($K_{CB[7]}$). The Job's plots of the thiamine guests with CB[7] are consistent with 1:1 host−guest

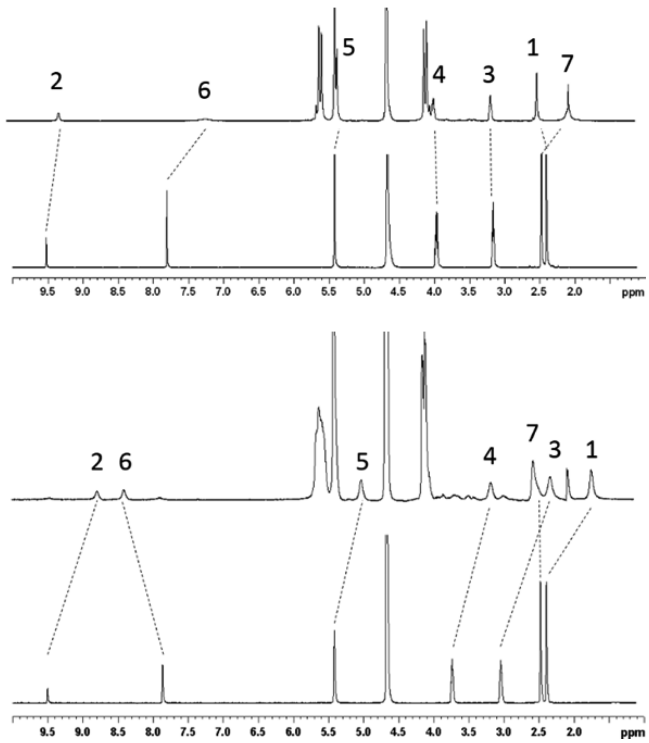

Figure 2. ^{1}H NMR spectra of **1** (2 mM) and **1**−CB[7] (1.1 equiv) (lower), and **2** (2 mM) and **2**−CB[7] (1.1 equiv) (upper) in D_2O. Proton labeling as found in Figure 1.

stoichiometries (Figures S5−S7). The ultraviolet spectrum of thiamine and the thiamine phosphates in aqueous solution are very similar to one another, with peaks at 232 ($\varepsilon \approx 1 \times 10^4$ M^{-1} cm^{-1}) and 262 nm ($\varepsilon \approx 8 \times 10^3$ M^{-1} cm^{-1}). The 232 and 262 nm bands are associated with the $\pi-\pi^*$ transitions of the pyrimidine ring and thiazolium ring, respectively.[14] When CB[7] is added to the solution of **1**, the thiamine spectrum changes in a different fashion compared to those of the thiamine phosphates (Figure 3

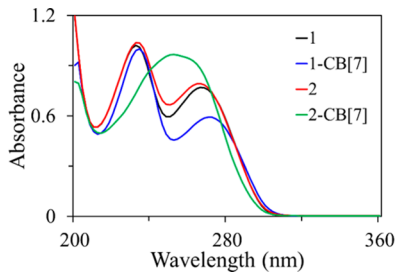

Figure 3. UV spectra of the thiamine **1** (0.1 mM) and thiamine monophosphate **2** (0.1 mM) guests and their CB[7] (2.0 equiv) host−guest complexes in aqueous solution.

and Figure S8). In the formation of **1**−CB[7], there is only a small shift in the 232 nm peak to 234 nm, with a very slight decrease in intensity. The peak at 262 nm, however, exhibits a bathochromic shift to 270 nm with a larger reduction in the intensity (also observed in the weak binding of thiamine to β-cyclodextrin[15]). The spectra of **2** and **3**, upon CB[7] inclusion, exhibit a significant bathochromic shift in the 232 nm peak to 252 nm with increases in the intensities. The peak at 266 nm becomes a shoulder on the 252 nm peak, with little change in the wavelength. The different changes observed upon inclusion of the thiamine guests in the cavity of CB[7] are consistent with the

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^{1}H NMR CIS changes and are indicative of the respective portion of the guest which resides in the cavity.

The host–guest stability constants ($K_{CB[7]}$) were determined from UV titrations of the thiamine guests with CB[7] at pH 7 (Figure 4) and are found to be on the order of **1**–CB[7] > **2**–CB[7] > **3**–CB[7] (Table 1). The trend is most simply explained in terms of the increasing negative charge on the guest as the number of phosphate groups, which would engage in ion–dipole repulsions with the CB[7] portal, increases, as we have observed with CB[7] complexes with cholines of different charges.[16] The shift in the binding from the thiazolium group in **1** to the pyrimidine group in **2** and **3** may also play a role in defining binding affinities. Similarly, Schrader and co-workers[5d] reported the binding of thiamine and thiamine diphosphate to a molecular clip with naphthalene side walls, with binding constants of 1.9×10^4 and 1.4×10^4 M^{-1}, respectively, in D_2O at 20 °C.

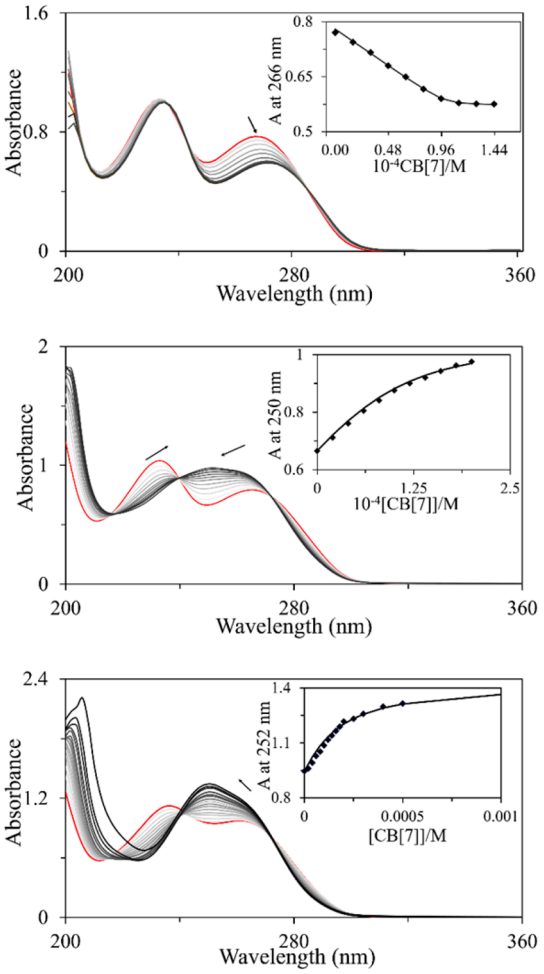

Figure 4. Titrations of the thiamine **1** (top), thiamine monophosphate **2** (middle), and thiamine diphosphate **3** (bottom), 0.1 mM each, with CB[7] in water at pH 7.0. The solid curves are calculated using the values of $K_{CB[7]}$ given in Table 1.

Table 1. Host–Guest Stability Constants in Aqueous Solution under Room Temperature and the C(2)–H/D Exchange Rate Constants and C(2)–H pK_a Values for the Thiamine Guests in the Absence and in the Presence of CB[7], Determined at Room Temperature in DAc/Ac$^-$ Buffer solution in D_2O with NaCl (0.2M) for Ion Strength Adjustment

	$K_{CB[7]}$ (M^{-1})	k_{DO} ($M^{-1}s^{-1}$)[a]	pK_a[b]	ΔpK_a
1	$(6.5 \pm 1.0) \times 10^5$	8.7×10^6	18.4	
1-CB[7]		1.2×10^6	19.3	0.9
2	$(2.5 \pm 0.5) \times 10^4$	6.4×10^6	18.6	
2-CB[7]		3.2×10^6	18.9	0.3
3	$(8.0 \pm 1.0) \times 10^3$	4.3×10^6	18.7	
3-CB[7]		1.9×10^6	19.1	0.4

[a]Error limits of 10%. [b]Error limits of ±0.1 pK unit.

We also employed ab initio gas-phase calculations (HF method with 3-21G** basis set,[17] details in Figures S15–S17 and Tables S1–S3, Supporting Information) to further support the CB[7] binding site selectivity for these guest molecules. As illustrated in Figure 5, the binding sites of the guests inside the CB[7] cavity are consistent with the ^{1}H NMR results and the CIS values (Figures 1 and 2) and the changes in the UV spectra (Figure 3). The molecular modeling structure of **3**–CB[7] is similar to that of **2**–CB[7].

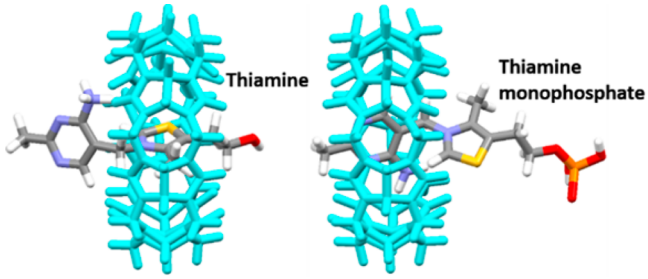

Figure 5. Energy-minimized gas-phase structures (HF/3-21G** basis set) of **1**–CB[7] (left) and **2**–CB[7] (right).

The kinetics of the exchange by deuterium of the weakly acidic thiazolium C(2) proton of the guests in buffered D_2O (pD = 3.0–5.5) at 25 °C and I = 0.20 M (NaCl), in the absence or in the presence of CB[7], were followed by ^{1}H NMR spectroscopy[18] (Figures S9–S14). The second-order rate constants and pK_a values of the three guests in the absence and presence of CB[7] are listed in Table 1. The rate constant of 8.7×10^6 M^{-1} s^{-1} for thiamine is in good agreement with the value of 8.4×10^6 M^{-1} reported by Washabaugh and Jencks,[7e] as is the pK_a value of 18.4 compared their value of 18.0.[7e] The effects of CB[7] complexation on the k_{DO} and ΔpK_a values is more pronounced in the case of **1** compared with **2** and **3** and very similar to those of the α,α'-bis(thiazolium)-*p*-xylene dication, suggesting that the inclusion of the thiazolium ring stabilizes the C(2) proton, with respect to dissociation, more so than if the pyrimidinium ring is bound.

In conclusion, the rate of C(2)–H/D exchange on the thiazolium ring of three thiamine guests was observed to decrease upon 1:1 CB[7] complexation, with a concomitant increase in the C(2)–H pK_a value, while the strength and location of the CB[7] binding of these guests can be tuned by the presence of an anionic phosphate/diphosphate group on the molecular structures. The tunability of binding location of CB[7] as well as associated influence on the activity of C(2)–H may find application in thiamine related enzyme biomimics.

EXPERIMENTAL SECTION

General Information. Commercially available thiamine hydrochloride, thiamine monophosphate chloride dihydrate, and thiamine pyrophosphate were used as received. The CB[7] host molecule was prepared according to a method from Day's group.[10b] The acetate buffer solutions (total buffer concentration of acetate for each kinetic

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experiment was 0.05 M) were prepared by the requisite addition of DCl (35 wt % in D_2O) to D_2O solutions of sodium acetate-d_3. NaCl was utilized to adjust the ionic strength to 0.20 M. A 400 M NMR spectrometer was used to record the ^1H NMR spectra. All of the UV spectra were acquired on a diode array UV−vis spectrometer using 1.00 cm path length quartz cells. A single quadrupole MS spectrometer equipped with an ESI/APcI multiprobe was used to acquire the ESI-MS spectra. The stability constants of host−guest complexes were determined by utilizing a UV spectroscopic titration of the thiamine with increasing amounts of CB[7] in aqueous solution, fitting the absorbance changes to a nonlinear least-squares 1:1 binding model.[19] All of the modeled host−guest structures involved in this investigation were calculated by energy minimizations using Gaussian 03 (Revision C.02) programs. The calculations were performed at the High Performance Virtual Computing Laboratory (HPVCL) at Queen's University. The structures of the host−guest complexes were originally constructed using ChemDraw and Chem3D (ChemOffice 7.0t) programs and subsequently imported into Gaussian 03. The basis set of HF/3-21G** was used for the calculations. ^1H NMR spectra of the guest molecules in the absence and in the presence of CB[7] were recorded during the deuterium exchange of the C(2)-proton at different pD (pD = pH + 0.41) conditions (DAc/Ac^- in D_2O as buffer, NaCl to adjusted I = 0.20 M, 400 MHz NMR). The proton integrations of the methylene protons (1H and 7H) resonance in guests, were employed as an internal reference resonance for determining the integration of the C(2)-proton. A relaxation delay (between the pulses) of d_1 = 75 s (>5T_1) was used to acquire accurate integrals for the C(2)-proton.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b02666.

Calculations of C(2)−H/D kinetics, ^1H NMR and ESI-MS spectra of the CB[7]−thiamine host−guest complexes, Job's plots, calculations of the C(2)−H/D exchange kinetics, details of energy-minimized structure calculations, and the full citation to ref 17 (PDF)

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Notes

The authors declare no competing financial interest.

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