Host-Guest Binding Interactions

Molecular Encapsulation by Cucurbit[7]uril of the Apical 4,4′-Bipyridinium Residue in Newkome-Type Dendrimers**

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Molecular recognition phenomena in biological systems often involve host or guest residues which are attached to the surface of organized assemblies (membranes) or incorporated into large macromolecular structures (proteins or nucleic acids). The high degree of structural complexity and molecular size that can be attained with dendritic structures can be utilized to model the complexity of biological systems. Therefore, the investigation of dendrimers as partners in host–guest binding interactions may have considerable biological relevance. Dendrimers have been used extensively as hosts, either by taking advantage of their inner cavities[1] or by designing and creating binding sites in their interior,[2] but their utilization as guests has been more limited.[3] We have previously reported the binding interactions of dendrimers containing organometallic guest units with cyclodextrin hosts,[4] as well as those of dendrimers containing a single dansyl residue with cyclodextrin and polyclonal anti-dansyl antibodies.[5] Here, we focus our attention on a new host–guest interaction recently reported both by Kim and coworkers[6] and our own research group,[7] which focuses on the strong complexation of 4,4′-bipyridinium (viologen) derivatives by cucurbit[7]uril[8] (**CB7**) in aqueous solution. The dendrimers selected as guests (**1-V^{2+}**, **2-V^{2+}**, and **3-V^{2+}**) contain a single viologen residue covalently attached to the focal points of carboxylate-terminated, Newkome-type dendrons (Scheme 1).

Dendrimers **1-V^{2+}**, **2-V^{2+}**, and **3-V^{2+}** were prepared by hydrolysis of their *tert*-butyl ester precursors with formic acid,[9] followed by ion exchange to their bromide salts. The resulting dendrimers were fully characterized by ^{1}H and ^{13}C, COSY, and HMQC NMR spectroscopies, FAB or MALDI-TOF mass spectrometry, UV/Vis spectroscopy, and electrochemical techniques. Host **CB7** was synthesized according to literature reports.[8] The water-soluble, viologen dendrimers **1-V^{2+}**, **2-V^{2+}**, and **3-V^{2+}** have 3, 9, and 27 surface carboxylic acid groups, respectively. As a consequence of their polyprotic acid character, the pH value of the solution is expected to influence the overall charge and the conformation of these dendrimers. Therefore, we decided to investigate their binding interactions with host **CB7** at two solution pH values, namely, pH 7 and 3, which were selected to bracket the average pK_a value of the carboxylic acid groups. Methylviologen (**MV^{2+}**) was used as a model compound to provide baseline values for the complexation of the viologen residues of **CB7** unhindered by dendritic components.

^{1}H NMR spectroscopic data readily confirm that the viologen dendrimers are complexed by **CB7**. For example, Figure 1 shows the spectra (in 0.1 M Na_2SO_4/D_2O) of **3-V^{2+}** in the absence and in the presence of one equivalent of **CB7**. Clearly, the host has a pronounced effect, primarily on the resonances of the bipyridinium protons. In fact, the bipyridinium β/β′ protons shift to higher fields and undergo considerable broadening, which is in agreement with the results that we had previously reported for the complexation of simple viologens by **CB7**.[7] Similar results were obtained with the other two viologen dendrimers, thus indicating that inclusion complexation of the viologen residue by the **CB7** host takes place regardless of the growth of the dendrimer (1st to 3rd generation).

MALDI-TOF mass spectrometry also supplied strong evidence in support of complex formation between the viologen dendrimers and **CB7**. Figure 2 shows the spectra obtained from 1:1 mixtures of **CB7** and each of the viologen dendrimers using 2,5-dihydroxybenzoic acid matrices. In the case of the first generation dendrimer, the spectrum is dominated by a single signal at m/z 1691.3 (Figure 2A), which corresponds to the monocationic complex **CB7·1-V$^{+\cdot}$**. In the case of the second generation dendrimer, the spectrum contains two major signals (Figure 2B). The most intense peak again corresponds to the one-electron-reduced complex **CB7·2-V$^{+\cdot}$**, but the free dendrimer **2-V$^{+\cdot}$** is also clearly observed at m/z 1216.6. The spectrum for the third generation viologen dendrimer shows increased complexity, but all the major signals can be ascribed (Figure 2C). The most prominent signal observed still corresponds to the one-electron-reduced complex **CB7·3-V$^{+\cdot}$**. The clear spectroscopic detection of the radical cation complexes (**CB7·*n*-V$^{+\cdot}$**, $n = 1$, 2, and 3) reveals two things: 1) One-electron reduction of the viologen residues takes place under the mass spectrometric conditions, and 2) the resulting viologen cation radicals are

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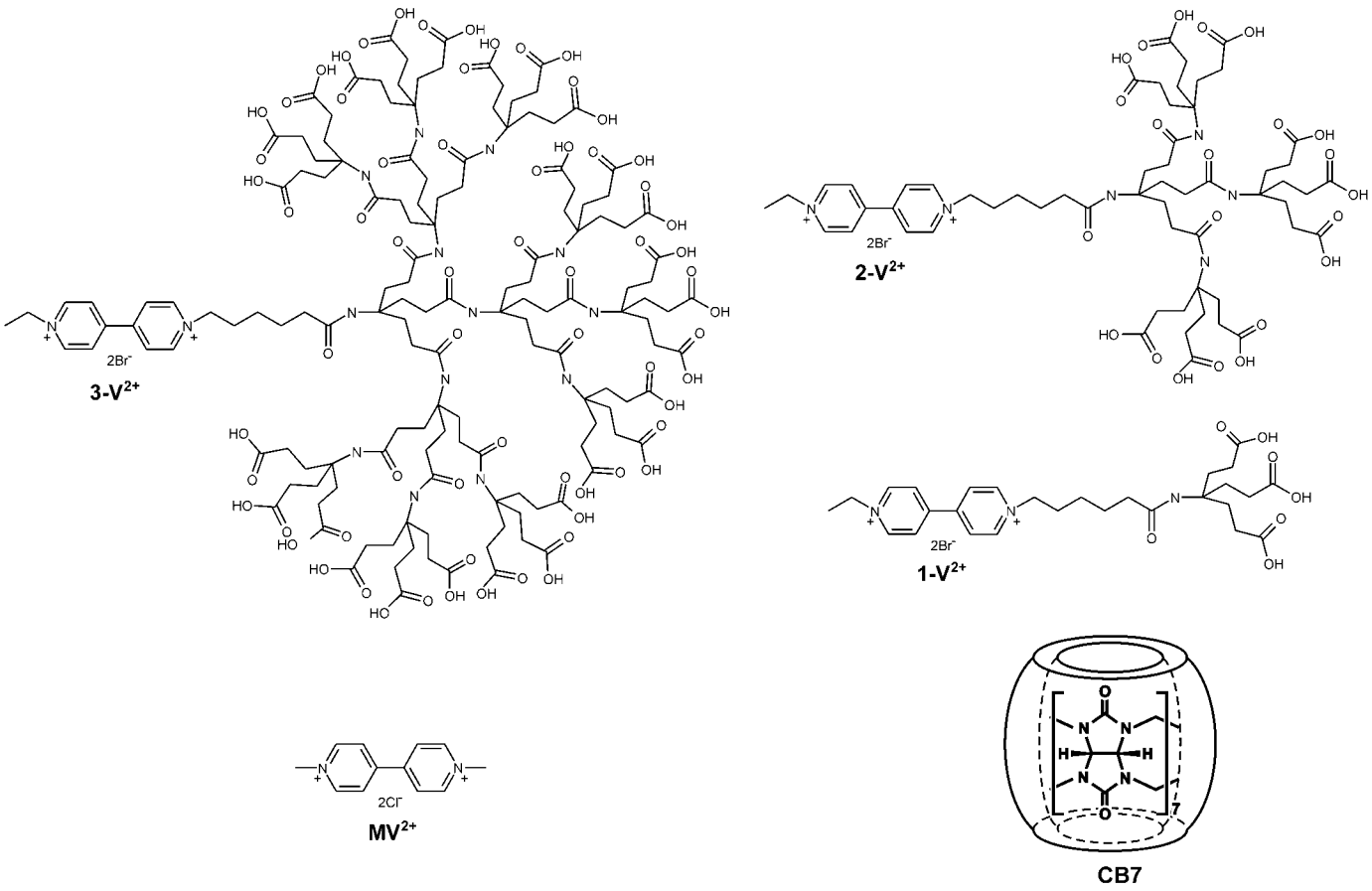
[**] This work was supported by the NSF (to A.E.K.; CHE-9982014 and CHE-0240295).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

  DOI: 10.1002/ange.200250214



Scheme 1. Structures of the host and guest compounds.

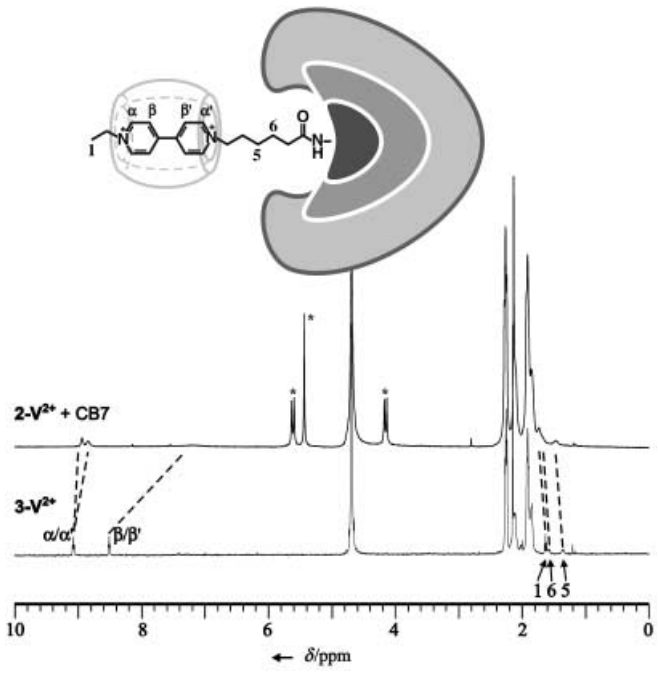


Figure 1. ^{1}H NMR spectra (500 MHz, 0.1 M Na_2SO_4/D_2O) of dendrimer **3-V^{2+}** in the absence (bottom) and in the presence (top) of one equivalemt of host **CB7**. Host resonances are labeled with an asterisk.

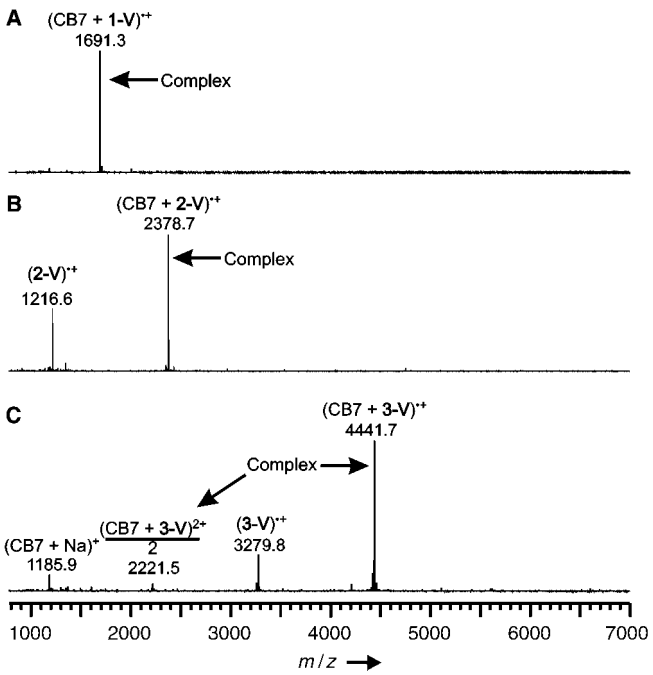


Figure 2. MALDI-TOF mass spectra obtained with 1:1 mixtures of each of the viologen dendrimers and host **CB7** in 2,5-dihydroxybenzoic acid matrices.

also strongly bound by the **CB7** host. These findings have precedents in our previously reported data with simple viologen derivatives.[7]

The complexation equilibrium between any viologen derivative and the **CB7** host can be conveniently monitored by electronic absorption spectroscopy, since the molar absorptivity coefficient (ε) of the characteristic UV band of viologens (λ_{max} ca. 260 nm) is depressed upon formation of the **CB7** inclusion complex.[7] Figure 3 shows, for example, the

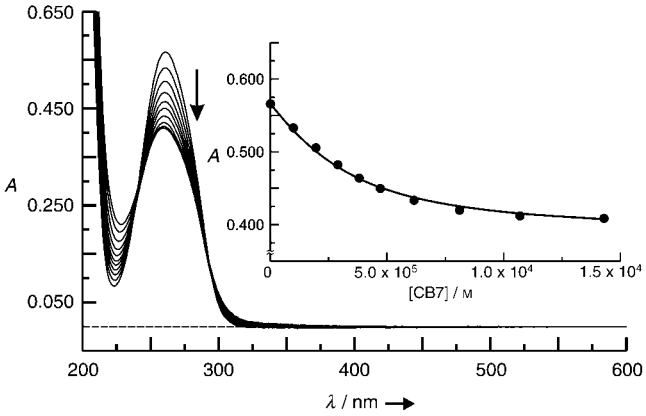


Figure 3. Electronic absorption spectra of aqueous solutions (0.03 M Tris buffer, pH 7.3) containing a fixed concentration (27 μM) of dendrimer **2-V²⁺** and variable concentrations of **CB7**. The inset shows the experimental absorbances at 260 nm (filled circles) and the best fit line obtained with the parameters listed in Table 1.

UV spectra obtained with aqueous solutions (0.03 M tris(hydroxymethyl)aminomethane (Tris) buffer, pH 7.3) containing a fixed concentration of **2-V²⁺** (27 μM) and variable concentrations of **CB7**. The absorption band of the viologen exhibits progressively lower absorbance as the concentration of host is increased. This observation reflects the increasing concentration of the **CB7·2-V²⁺** complex and the decreasing concentration of free **2-V²⁺** in the solution. The fitting of these data, using regression analysis, to a 1:1 binding isotherm model affords the corresponding binding constant (K) and the molar absorptivity coefficient of the complex (ε_c). The values obtained at the two surveyed solution pH values with methylviologen and the three viologen dendrimers are given in Table 1.

Table 1: Equilibrium association constants (K), free energies of complexation (ΔG^0), and molar absorptivity coefficients (ε_c) for the inclusion complexes formed between the viologen guests and host **CB7** in aqueous solutions at 25 °C.

	Medium					
	0.2 M formic acid buffer, pH 3.2			0.03 M Tris buffer, pH 7.3		
Guest	K [L mol^{-1}][a]	ΔG^0 [kcal mol^{-1}]	ε_c [M^{-1} cm^{-1}]	K [L mol^{-1}][a]	ΔG^0 [kcal mol^{-1}]	ε_c [M^{-1} cm^{-1}]
MV²⁺	2.9×10^5	−7.45	11 500	2.2×10^5	−7.28	11 500
1-V²⁺	5.9×10^5	−7.87	12 900	5.5×10^4	−6.46	12 900
2-V²⁺	6.2×10^5	−7.90	14 100	5.7×10^4	−6.49	14 200
3-V²⁺	3.4×10^5	−7.54	13 600	1.3×10^4	−5.61	19 200

[a] Estimated error margin: ±12%.

The first important fact that emerges from the data in Table 1 is that the binding affinity between the underivatized **MV²⁺** guest and **CB7** depends on the composition of the medium. The 0.2 M formic acid buffer solution (pH 3.2) used in our experiments contains approximately 0.04 M Na^+ ions (introduced as NaOH in the preparation of the buffer). The binding constant measured in this medium ($K = 2.9\times10^5$ L mol^{-1}) is clearly larger than the value previously determined by us in unbuffered 0.2 M NaCl (1.0×10^5 L mol^{-1}).[7] Since neither the host or the guest are pH-sensitive and sodium ions are known to interact strongly with cucurbituril hosts,[10] we conclude that increasing concentrations of sodium ions depress the apparent K value for the binding of viologens by **CB7**. The binding constant measured in Tris buffer solution at pH 7.3 (2.2×10^5 L mol^{-1}) is very close to the value reported by Kim and co-workers in a similar medium (2.0×10^5 L mol^{-1} in 0.05 M Tris buffer, pH 7).[6] Since this medium does not contain any sodium ions, the measured K value indicates that protonated Tris also interacts with **CB7**.

The data in Table 1 reveal that dendrimer growth has a relatively small effect on the stability of the complexes formed between **CB7** and guests **1-V²⁺**, **2-V²⁺**, and **3-V²⁺**. In buffered formic acid solution, the K values are higher for the first and second generation dendrimers, while the binding constant for the third generation is closer to that measured with **MV²⁺**. A reasonable explanation for these results takes two factors into account. First, one side of the viologen unit in the viologen dendrimers is connected to a large organic structure, which may partially hinder solvation by water molecules. This arrangement will enhance the ion–dipole interactions between the carbonyl portals of the host and the quaternary ammonium groups of the guest and will tend to increase the stability of the complex. Second, dendrimer growth may create some steric problems for the approach of the host to the viologen residue. This factor does not appear to be extremely important in these dendrimers, but it is probably responsible for the decrease in the K value from **2-V²⁺** to **3-V²⁺**. Finally, another trend clearly visible in Table 1 is that the binding constants between the viologen dendrimers and **CB7** are at least one order of magnitude lower in neutral solutions (pH 7.3) than in acidic solutions (pH 3.2). This pH effect is of a larger magnitude that can be attributed to medium effects, as evidenced by the much closer K values observed for the **CB7·MV²⁺** complex. We rationalize the stronger pH effect detected with the **CB7·*n*-V²⁺** ($n = 1$, 2, and 3) complexes as a result of the ionization of the peripheral carboxylic acid groups. The presence of the negatively charged carboxylates probably leads to some degree of intramolecular ion pairing with the positively charged viologen residue, which hinders the **CB7**–viologen interaction. This effect becomes stronger as the dendrimer generation (and the number of carboxylates) increases, which explains the lower K values observed and their relative variation from **1-V²⁺** to **3-V²⁺**.

We can define the thermodynamic free energy parameter $\Delta\Delta G^0$ as the difference

$\Delta G^0_{3rd} - \Delta G^0_{st}$ to describe quantitatively the loss in complex stabilization from the first to the third generation of the dendrimer. From the values in Table 1, we obtain $\Delta\Delta G^0$ values of 0.33 and 0.85 kcal mol^{-1} at solution pH values of 3.2 and 7.3, respectively, for the host–guest system investigated in this work. These $\Delta\Delta G^0$ values are comparable to the value (0.61 kcal mol^{-1}) that can be obtained from our reported binding data on the host–guest system composed by dansyl-containing dendrimers and a polyclonal anti-dansyl antibody.[5] On the other hand, the values reported here are significantly smaller than the $\Delta\Delta G^0$ values calculated from our reported binding data on ferrocene-labeled dendrimers and β-cyclodextrin[4c] (1.74 kcal mol^{-1}), as well as from dansyl-labeled dendrimers and β-cyclodextrin[5] (2.91 kcal mol^{-1}). An important difference between the viologen dendrimer guests described in this work and the related ferrocenyl[4c] and dansyl[5] dendrimer guests reported before is the length of the tether connecting the guest residue to the Newkome-type dendron. While the ferrocenyl and dansyl residues were directly connected to the corresponding dendrons through a simple amide linker, the viologen residues in dendrimers **1-V^{2+}** to **3-V^{2+}** are separated by a 5-methylene chain from the amide group at the focal point of the dendrimer. However, in regard to the formation of inclusion complexes by guests at the focal point of Newkome-type dendrons, our $\Delta\Delta G^0$ data suggest that stable complexes ($K > 10^4$ L mol^{-1}) are less affected by dendrimer growth than weaker complexes ($K < 10^3$ L mol^{-1}). It is important to note that the literature contains reports on other dendrimer systems that exhibit different trends, such as large $\Delta\Delta G^0$ values for $K > 10^4$ L mol^{-1}[11] and small $\Delta\Delta G^0$ values for $K < 10^4$ L mol^{-1}.[12] Clearly, more thermodynamic binding parameters on host–guest systems involving dendrimers are necessary to fully rationalize the results presented here. Overall, this work underscores the complexity of host–guest binding interactions involving dendrimers and may suggest that the nature of the intermolecular forces between the host and the guest may be an important factor to determine the magnitude of dendrimer growth effects on the binding affinity.

Experimental Section

Dendrimers **1-V^{2+}, 2-V^{2+}**, and **3-V^{2+}** were prepared by hydrolysis of their *tert*-butyl ester precursors[9] (see Supporting Information for synthetic details and full spectroscopic characterization data). The host **CB7** was synthesized as reported by the research groups of Kim and Day.[8] ^{1}H NMR spectra were obtained on Bruker Avance 400 and 500 MHz NMR spectrometers. MALDI-TOF mass spectra were recorded in a Bruker Biflex IV system using either 2,5-dihydroxybenzoic acid or α-cyano-4-hydroxy

cinnamic acid as the matrix compounds. Electronic absorption spectra were recorded in a Shimadzu UV-2101PC spectrophotometer. The determination of the binding constants with the **CB7** host was done using the method previously reported for **MV^{2+}**.[7]

Received: September 23, 2002
Revised: November 19, 2002 [Z50214]

Keywords: cucurbituril · dendrimers · host–guest systems · self-assembly · thermodynamics

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