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Toward Reversible Control of Cucurbit[*n*]uril Complexes

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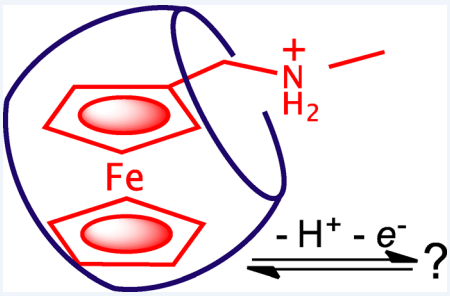
CONSPECTUS: The cucurbit[*n*]uril (CB*n*) host family consists of a group of rigid macrocyclic hosts with barrel-like shapes and limited solubility in aqueous media. These hosts are capable of reaching high binding affinities with positively charged hydrophobic guests. In optimum cases, equilibrium association constant (K) values as high as 10^{17} M^{-1} have been reported, exceeding the binding affinity of the avidin−biotin host−guest pair. The synthetic CB*n* receptors have shattered the notion that highly stable noncovalent complexes can form only when one of the partners is a molecule of biological origin.



The work described in this Account is concerned with the development of methods geared toward the reversible modulation of the binding affinity of CB*n* inclusion complexes under mild conditions. A good fraction of the research work has dealt with redox active guests, such as 4,4′-bipyridinium (viologen), ferrocene, and cobaltocenium derivatives. Our experimental results show that the thermodynamics and kinetics of the electron transfer reactions of these compounds can be substantially altered by complexation with CB*n* hosts, and therefore, electron transfer reactions can be used to exert a measure of control on the overall binding affinity of the CB*n* complexes. We have also developed systems in which proton transfer reactions have a strong effect on the binding affinity. With more structurally elaborate guests containing more than one adjacent binding sites, proton transfer reactions may affect the average location of the CB*n* host within the complexes.

A series of guest compounds containing paramagnetic 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) residues also exhibit interesting binding properties with CB7 and CB8. The latter host forms a very stable host−guest pair with TEMPO subunits, in which the nitroxide group resides inside the host cavity. Finally, with suitable ditopic guests, we have detected distinct microscopic complexes using experimental techniques with relatively slow time scales, such as NMR spectroscopy. These unusual findings are the result of the considerable thermodynamic and kinetic stability of CB*n* inclusion complexes.

INTRODUCTION

The cucurbit[*n*]urils (CB*n*) are a family of rigid macrocyclic hosts prepared by condensation of glycoluril with formaldehyde in acidic medium.[1−3] Two methylene groups act as bridges between adjacent glycoluril units and the resulting macrocyclic compound is shaped like a barrel without lids (Figure 1). The open ends serve as entrances ("portals") to the inner cavity and are lined by the carbonyl groups on the glycoluril units. CB*n* macrocycles have an equatorial plane of symmetry, which makes the two cavity portals identical. As in any barrel, the inner cavity is wider in the middle and narrows at both ends, which tends to slow the dissociation of any guests that may be included in the cavity.

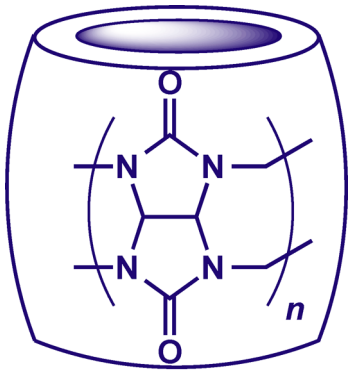


Figure 1. Structure and shape of the cucurbit[*n*]uril hosts.

While the solubility of most CB*n* hosts is limited, they are much more soluble in water than in organic solvents. In aqueous media, they tend to include hydrophobic compounds that fit in their cavities. Optimal binding affinities are reached when the included guest contains a hydrophobic residue whose shape fits well inside the cavity of the CB*n* host and has positively charged group(s) that are suitably located to develop ion−dipole interactions with the carbonyl oxygens lining the entrances of the cavity.[4] Extremely large binding affinities corresponding to equilibrium association constants (K) as large as 10^{17} M^{-1} have been recently reported by Isaacs and co-workers with cucurbit[7]uril (CB7) complexes,[5] clearly exceeding the binding affinity of the avidin−biotin biochemical host−guest pair.[6] The large binding affinities reached by some CB*n* hosts with suitable guests in aqueous solution are key to

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understand the growing interest in this family of hosts. It is fair to state that the CB*n* hosts have demonstrated that picomolar and even femtomolar binding regimes are possible with synthetic receptors and no longer the exclusive terrain of biomolecules.

Our work has focused on the investigation of CB*n* complexes containing redox active guests.[7] Our initial interest was to explore the possible use of electrochemical techniques to control the binding affinity or the structure of these complexes in a reversible way. We have also explored proton transfer reactions and spin exchange coupling processes in CB*n* complexes. This body of work, as well as recent results on the detection of microscopic complexes, constitutes the subject of this Account.

BINDING OF VIOLOGEN GUESTS

The derivatives of 4,4′-bipyridinum are commonly referred to as viologens[8] (V^{2+}, see Figure 2 for examples). These organic

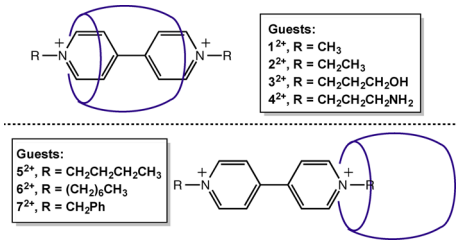

Figure 2. (top) Viologen encapsulation by the CB7 host with relatively hydrophilic guests. (bottom) Formation of external complexes between CB7 and more hydrophobic viologen guests.

dications usually undergo two consecutive one-electron reductions to produce, sequentially, a radical cation ($V^{+\bullet}$) and a neutral species (V). From an electrochemical standpoint both reduction processes are very fast (reversible in voltammetric jargon). The 4,4′-bipyridinium residue seems like a perfect, complementary fit for inclusion in the cavity of CB7, and in fact, our first publication on CB*n* hosts reported the formation of a symmetrical and stable inclusion complex between the simplest of viologens, methyl viologen ($\mathbf{1}^{2+}$), and CB7.[9] The binding affinity of this complex approaches the micromolar regime in aqueous medium ($K \approx 10^6$ M^{-1}). The electrochemical behavior of the complex reveals two interesting points: (1) a very small cathodic shift (~25 mV) upon CB7 complexation for the half-wave potential ($E_{1/2}$) corresponding to the first one-electron reduction of methyl viologen and (2) direct electron transfer between the inclusion complex and the electrode surface, that is, dissociation of the complex is not required for the electron transfer to occur. The small potential shift indicates a slight decrease in the stability of the complex, CB7·$\mathbf{1}^{2+}$, upon reduction to CB7·$\mathbf{1}^{+}$. In principle, this finding was not promising for further work geared to controlling the location of CB7 in more elaborate guests by changing the oxidation state of the viologen moiety.

We continued our work with viologen guests by investigating the binding interactions of the CB7 host with other viologens and quickly discovered that the encapsulation of the viologen moiety by the host only takes place when the *N*-substituents are either rather small or hydrophilic[10] (Figure 2, top). For instance, while methyl and ethyl viologens ($\mathbf{1}^{2+}$ and $\mathbf{2}^{2+}$) are encapsulated by CB7, the more hydrophobic butyl, heptyl, and benzyl viologens ($\mathbf{5}^{2+}$, $\mathbf{6}^{2+}$, and $\mathbf{7}^{2+}$) form external complexes with CB7 (Figure 2, bottom). The electrochemical behavior of the latter complexes has not been investigated in detail because these guests are very hydrophobic, especially after reduction, and they precipitate extensively on the electrode surface, leading to distortions in the voltammetric behavior, which make data interpretation almost impossible.

We went on to investigate the binding interactions between a series of more elaborate viologen derivatives and CB7 and found quickly that the viologen site is clearly not one of the best binding sites for CB7.[11] For instance, in guests $\mathbf{8}^{2+}$ and $\mathbf{9}^{2+}$, we can identify five possible binding sites for CB7 (two terminal benzyl/butyl groups), two viologen sites, and the central bis(pyridinium)-1,4-xylylene site. Our ^{1}H NMR experimental data demonstrate that CB7 selectively occupies the central site (Figure 3), happily bypassing the other four

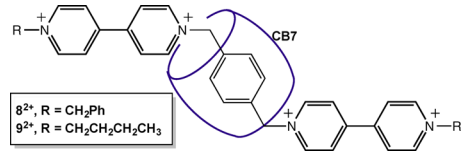

Figure 3. Selectivity of CB7 for the bis(pyridinium)-1,4-xylylene binding site in guests $\mathbf{8}^{2+}$ and $\mathbf{9}^{2+}$.

possible sites. This finding was used to prepare stable rotaxanes and strongly suggested to us that viologen sites are best used as secondary binding stations in our attempts to develop CB7-based rotaxanes in which we could control the relative location of the CB7 wheel.

At that time, we combined these findings with the electrostatic repulsions that we detected between the CB*n* portals, laced with negative charge density owing to the presence of the carbonyl oxygen atoms on their rims, and negatively charged carboxylate groups to design an interesting class of switchable pseudorotaxanes.[12] Our axle compounds contain a central viologen unit and two terminal COOH-terminated aliphatic *N*-substituents. The dicationic compound $\mathbf{10}^{2+}$ (Figure 4) constitutes a prototypical example of these axle

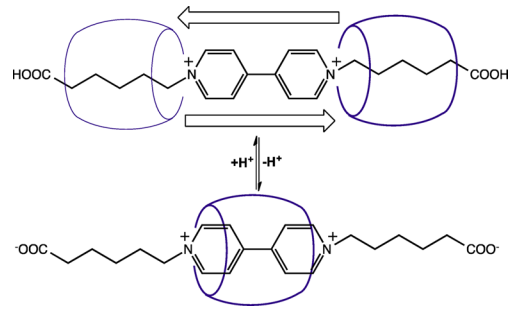

Figure 4. Switching and control on the location and movement of the CB7 wheel in the CB7·$\mathbf{10}^{2+}$ pseudorotaxane based on proton transfer reactions.

compounds. The CB7 wheel component forms very stable inclusion complexes with the axle, which can be characterized as pseudorotaxanes. Under mildly acidic conditions, when the two terminal COOH groups are protonated, our ^{1}H NMR data reveals that the CB7 wheel is shuttling between the two terminal aliphatic chains, quickly sliding over the central viologen residue. However, raising the pH enough so that the terminal carboxylate groups develop negative charges has a

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strong effect on the pseudorotaxane, because the CB7 portals suffer electrostatic repulsions from the terminal COO^- groups and the CB7 wheel is then forced to hover around the central viologen moiety, stopping the fast intramolecular exchange (shuttling) between the two terminal side chains. These switching properties are totally reversible and could also be monitored using UV–vis spectroscopic measurements.[12] The pH control on the shuttling and wheel location in CB7·$\mathbf{10}^{2+}$ demonstrates an effective mechanism to develop switchable molecules with this class of hosts.

The electrostatic barriers created by the terminal carboxylate negative charges may affect not only the shuttling and location of CB7 but also the kinetic rate of association and dissociation of these complexes. Recently, in collaboration with Vladimir Sindelar (Masaryk University) and Serena Silvi (University of Bologna), we have investigated this point,[13] using a cationic adamantyl derivative as the destination guest to force the dissociation of the CB7·**10** zwitterionic complex. Our results show that deprotonation of the COOH groups in $\mathbf{10}^{2+}$ (to form zwitterionic **10**) leads to an extraordinary decrease of ca. 6 orders of magnitude in both the association and dissociation rate constants with CB7, confirming that the negative charge on the terminal carboxylate creates a substantial activation barrier for the slipping of CB7 over the carboxylate group.

We have also taken advantage of the ability of the larger cavity host, CB8, to include two viologen groups when both are in the one-electron reduced, radical cation state ($V^{+\bullet}$), as first reported by Kim and co-workers.[14] Using unsymmetric dendronized viologens,[15,16] in which one *N*-substituent is rather small, typically an ethyl group, and the other *N*-substituent is a dendron of variable size, we can control the formation of dendrimers via dimerization of two dendronized viologens by selecting the oxidation state of the viologen group.[17] In the presence of CB8, the dicationic form (V^{2+}) of the dendronized viologen forms a 1:1 complex in which the host includes the viologen unit. However, upon one-electron reduction, two viologen radical cations are included inside the CB8 cavity. Therefore, the CB8 host serves as a connector to foster the dimerization of the two dendronized viologens. Since the one-electron reduction of the viologen residues is reversible, this dendrimer aggregation mechanism is also fully reversible. Charge transfer complexes between aromatic electron acceptors and donors are also highly stabilized by inclusion of the acceptor–donor moieties in the cavity of CB8.[18] We combined this CB8 property with its redox-switchable binding properties with viologen moieties to devise a rather unique mechanism for size selection (Figure 5), which favors dimerization between dendronized viologens, when these are reduced, and charge transfer complex formation between dendronized donors and dendronized viologens in the fully oxidized state.[19] This work affords reversible methods to control aggregate size based on the oxidation or reducing conditions of the medium.

BINDING OF FERROCENE AND COBALTOCENIUM DERIVATIVES

Bis(cyclopentadienyl)iron(II) (ferrocene) is a hydrophobic organometallic compound, which offers very good size complementarity for inclusion into the cavity of CB7. Our group reported in 2003 that both ferrocene and its one-electron oxidized form, ferrocenium, are excellent guests for inclusion complexation by CB7.[20] In fact, the positively charged analogue, bis(cyclopentadientyl)cobalt(III) (cobaltocenium) and its reduced form, cobaltocene, also bind strongly inside

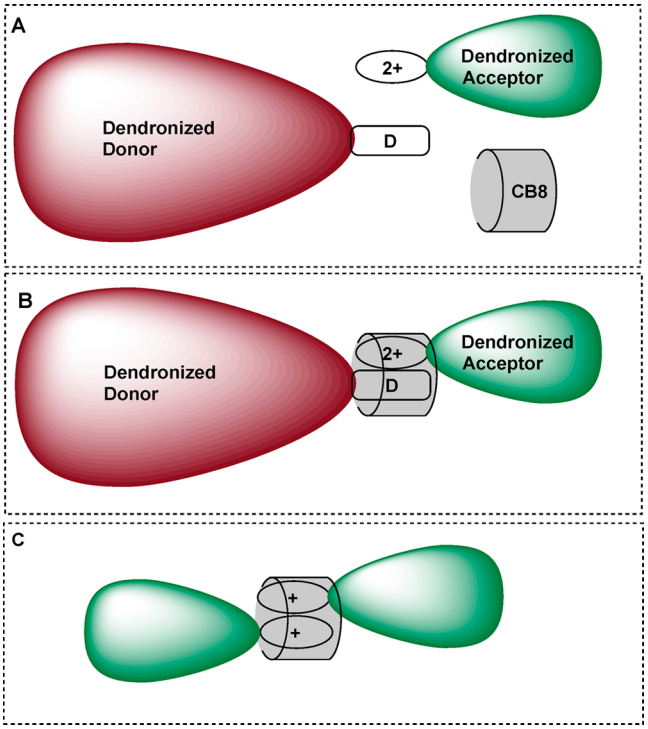

Figure 5. (A) Dendronized components for aggregate size selection. (B) Prevalent species when the dendronized viologen (acceptor) is oxidized. (C) Prevalent species when the dendronized viologen (acceptor) is reduced.

CB7.[20] The host shows a modest selectivity for the positively charged forms in both cases, but the respective half-wave potentials were little affected by the presence of CB7. Ferrocene is not very soluble in water, which makes the determination of *K* values difficult. We eventually determined the equilibrium association constant between CB7 and cobaltocenium as 5.7×10^9 M^{-1} at 25 °C in 50 mM sodium acetate solution[21] and have recently taken advantage of this association reaction to develop a simple method to establish the purity of CB7 and CB8.[22]

To continue our work with ferrocenyl guests, we started to investigate well-known ferrocene derivatives that are considerably more soluble in aqueous solution than their parent compound. Figure 6 shows the structures of the compounds

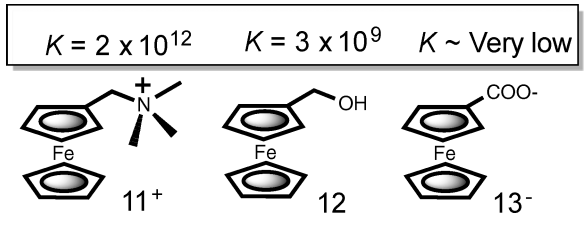

Figure 6. Water-soluble ferrocenyl guests and their equilibrium association constants (*K* in M^{-1}) with CB7 in pure water at 25 °C.

that we selected for the initial binding studies with CB7, which were done in collaboration with the groups of Kimoon Kim (Pohang University) and Yoshi Inoue (Osaka University). This work[23] led to rather surprising results, because the *K* values obtained using isothermal calorimetric (ITC) methods show an unexpectedly large binding affinity for CB7 and guest $\mathbf{11}^+$. Equally impressive is the fact that ferrocenecarboxylate binds

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very weakly to CB7 (no binding detected in our experiments), again highlighting the electrostatic repulsion between the carboxylate group and the host cavity portal.

Considering that CB7 and guest $\mathbf{11}^+$ are relatively simple synthetic compounds, the binding affinity, almost in the picomolar regime, is certainly remarkable. The voltammetric behavior of $\mathbf{11}^+$ changes considerably in the presence of CB7 (Figure 7). Upon complex formation, the half-wave potential

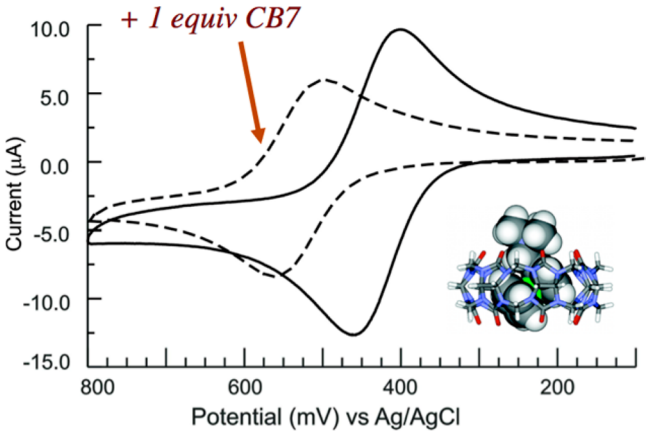

Figure 7. Cyclic voltammetric behavior on glassy carbon of guest $\mathbf{11}^+$ in the absence (continuous line) and in the presence (discontinuous line) of 1.1 equiv of CB7. Medium: 0.1 M NaCl. Scan rate: 0.1 V s^{-1}. The inset shows the energy-minimized (PM3) structure of the complex.

for the oxidation of the ferrocenyl residue shifts by 110 mV to more positive values and the voltammetric currents decrease markedly because the diffusion coefficient of the CB7·$\mathbf{11}^+$ complex is indeed lower than that of free $\mathbf{11}^+$. The observed anodic shift in the $E_{1/2}$ value can be rationalized by the hydrophobic nature of the CB7 cavity, which provides differential stabilization to the ferrocene relative to the ferrocenium form of the guest. While the crystal structure of this complex is not known, all of the experimental data as well as computational studies point to the confinement of the ferrocenyl residue in the cavity of CB7, while the ammonium nitrogen interacts with one of the host portals via ion−dipole interactions (Figure 7, insert).

Further studies on the electrochemistry of this complex and other CB7 complexes of ferrocenyl guests[24] indicate that the standard rate constant ($k°$) for the heterogeneous electron transfer processes decreases substantially upon complexation. As mentioned before, most ferrocene derivatives exhibit reversible voltammetric behavior ($k°$ > 1.0 cm s^{-1}), and the exact value of $k°$ is not measurable with conventional electrodes. However, their CB7 inclusion complexes show quasi-reversible behavior and $k°$ values that are easily measurable, in the range 0.1−0.4 cm s^{-1}. The effect of CB7 encapsulation on the $k°$ value can be understood because the presence of the host hinders the mixing between the molecular orbitals in the guest and the electronic levels in the electrode. It is interesting to point out that no decrease in the $k°$ value was detected with CB7-encapsulated viologen guests.[25] This contrasting behavior may be due to the effective shielding achieved by the engulfing host on the guest's molecular orbitals that actually participate in the electron transfer process. In the case of ferrocenyl guests, the relevant process is an oxidation and the guest's HOMO is highly localized on the ferrocene residue, which is well within the host cavity in the complex. The situation is very different with viologen complexes, because the relevant electrochemical process is a reduction and the LUMO of the guest protrudes out of the cavity at the two ends of the guest, leading to molecular regions of the complex where the mixing of electronic energy levels is not substantially impeded by the presence of the CB7 host.

In this regard, we must also note here that Yuan and Macartney[26] have investigated the self-exchange electron transfer between the CB7·$\mathbf{11}^+$ complex and its oxidized form, CB7·$\mathbf{11}^{2+}$. They found a slight acceleration in the corresponding rate constant compared with that between the unbound guest $\mathbf{11}^+$ and its oxidized form $\mathbf{11}^{2+}$. These results are at variance with our electrochemical data, and we are still actively looking for ways to rationalize the homogeneous and heterogeneous rate constant data.

The outstanding binding affinity between $\mathbf{11}^+$ and CB7 recently led us to evaluate how well the stability of this complex would tolerate small structural variations in the guest. In joint work with Isabel Cuadrado (Universidad Autónoma, Madrid), we investigated two other guests, structurally similar to $\mathbf{11}^+$ but having either zero or two methylenes as the linker between the ferrocenyl group and the trimethylammonium group.[27] Our experimental results show that all three complexes are highly stable, but CB7·$\mathbf{11}^+$ has a K value about an order of magnitude higher that the other two complexes. A single methylene as linker allows the simultaneous optimization of the interactions between the ferrocenyl residue and the host cavity and those between the positively charged ammonium group and one of the cavity portals.

After the discovery of the unusually stable CB7·$\mathbf{11}^+$ complex, the obvious next step was to search for related guests that would exhibit even higher binding affinities with CB*n* hosts. A dicationic ferrocene derivative, 1,1′-bis-(trimethylammoniomethyl)ferrocene ($\mathbf{14}^{2+}$), was selected as the target guest, and its binding affinity with CB7 was studied in aqueous media.[6] Not surprisingly, the measured K value was 3 × 10^{15} M^{-1}, which positions this complex in a rarefied atmosphere of high stability, similar to that measured with the biochemical host−guest pair avidin−biotin. The electrochemical behavior of the complex was similar to that observed with CB7·$\mathbf{11}^+$, although the CB7-induced half-wave potential shift approaches 200 mV, and the crystal structure of the CB7·$\mathbf{14}^{2+}$ complex was solved, showing that the ferrocenyl group was fully engulfed by the CB7 cavity and that each of the trimethylammonium groups interacts with one of the host portals. The determination using ITC of the thermodynamic parameters for the complexation reactions between the CB7 host and the $\mathbf{11}^+$, **12**, and $\mathbf{14}^{2+}$ guests reveals that the increasing K values in going from **12** to $\mathbf{11}^+$ to $\mathbf{14}^{2+}$ are driven by decreasing entropic penalties associated with complex formation. In fact, the enthalpies for all three complexation processes are roughly the same. More recent studies strongly suggest that the small entropic penalties and high binding affinities can be rationalized by the release of high-energy water molecules included in the cavity of the free host prior to complexation.[28] Release of water molecules solvating the trimethylammonium groups on the guest may also contribute to the observed entropic values.[33]

The biochemical host−guest pair avidin−biotin is essentially the "gold standard" in chemistry when noncovalent interactions are used to bring molecular structures together. An extremely high K value guarantees strong association, to the point that

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dissociation becomes a rare event and complex lifetimes are quite long. Substantial research efforts have been directed to temper the avidin–biotin binding affinity via site-directed mutagenesis on avidin-type proteins. Often, if dissociation of avidin–biotin complexes is desired, the best resource is to submit the system to boiling temperatures, which causes dissociation of the complexes through the hardly elegant outcome of avidin denaturation. Given this state of affairs, would it not be possible to temper the binding affinity of the very stable supramolecular complexes formed between ferrocenyl guests and CB7 hosts? To address this question, we focused on a ferrocenyl guest that we thought could be easily changed via proton and electron transfer reactions. The protonated form of guest **15** should bind to CB7 with *K* values similar to those exhibited by guest $\mathbf{11}^{+}$.[29] However, one-electron oxidation should decrease the stability of the complex, while proton removal (to give the amine form on the side arm) should further decrease the *K* value (Figure 8). These

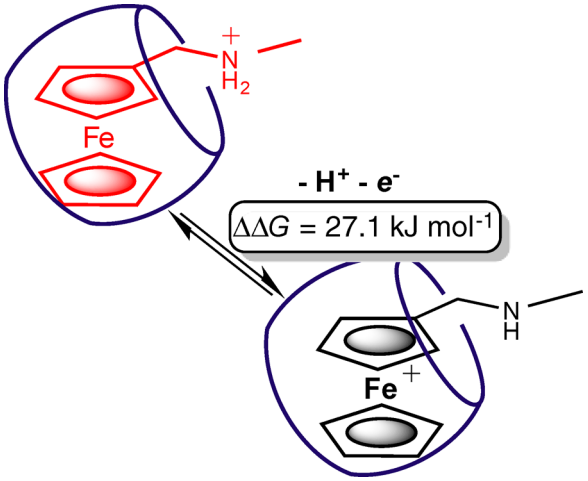

Figure 8. Proton and electron transfer control of the binding affinity of guest $\mathbf{15H}^{+}$ with the CB7 host.

expectations were confirmed by our experimental results. The *K* value for association between the protonated form of the guest ($\mathbf{15H}^{+}$) and CB7 was measured as 1.3×10^{12} M^{-1}, while deprotonation leads to a value of 3.4×10^{9} M^{-1} for association with **15**. This represents a drop in $\Delta G°$ of 14.8 kJ mol^{-1} for complexation by CB7. Furthermore, one-electron oxidation of **15** to $\mathbf{15}^{+}$ leads to a further decrease in the *K* value, which now drops to 2.4×10^{7} M^{-1}, corresponding to an additional drop in $\Delta G°$ of 12.3 kJ mol^{-1}. Clearly, these are mild reactions that have a large effect on the stability of the inclusion complexes. Should further decreases in the *K* values be necessary, other methods can be envisioned, such as addition of electrolytes or organic solvents, in order to lower the complex stability to levels in which dissociation would prevail. Perhaps, the most important lesson here is that these experiments show the way to modulate with mild, reversible reactions the binding affinities of CB7–ferrocenyl complexes in ways that are inaccessible with the avidin–biotin pair.

With guest $\mathbf{15H}^{+}$, we confirmed that electrochemical oxidation of a ferrocenyl site leads to a decrease in its binding affinity with CB7. This fact was used before by our group to design, prepare, and characterize an electrochemically switchable pseudorotaxane.[30] The axle compound in this case ($\mathbf{16}^{2+}$, see Figure 9) is composed of three adjacent binding sites: A

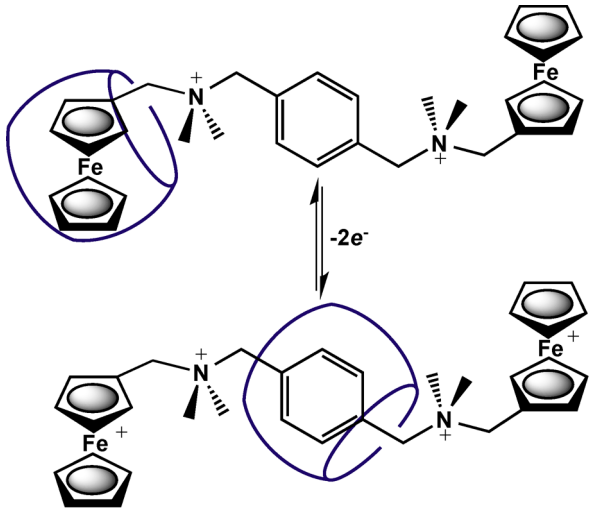

Figure 9. Electrochemical switching of the CB7 position in the CB7·$\mathbf{16}^{2+}$ pseudorotaxane.

central diammonium-1,4-xylylene and two terminal ferrocenylmethylammonium sites. In the presence of 1.0 equiv of CB7, one of the ferrocenyl sites is included. As expected, exchange of CB7 between the two identical terminal sites is slow in the ^{1}H NMR time scale. Electrochemical data reveal that oxidation of the ferrocenyl sites leads to the CB7 sliding over to occupy the central binding site. The process is reversible and reduction of the axle returns the CB7 to one of the terminal binding sites. The known binding thermodynamics of CB7 with the individual sites[4] composing the axle suggests that, upon oxidation, the two positively charged ferrocenium sites contribute to stabilizing the CB7 host on the central binding site.

BINDING OF TEMPO-CONTAINING DERIVATIVES

Because of related supramolecular work, we became interested in the binding interactions of compounds containing the 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) residue with the CB7 and CB8 hosts. TEMPO moieties contain a stable nitroxide radical, which shows a simple electron paramagnetic resonance (EPR) spectrum composed of three lines. The separation (in gauss) between any two adjacent spectral lines affords the hyperfine splitting constant, $\langle a_N \rangle$, which is a measure of the relative polarity of the microenvironment surrounding the nitroxide radical. Initially, we investigated guest $\mathbf{17}^{+}$ (see Figure 10), which contains both cobaltocenium and TEMPO subunits.[31] Our experimental findings, using several spectroscopic techniques, indicate that $\mathbf{17}^{+}$ has clearly bimodal binding interactions with CB7 and CB8, that is, CB7 includes the cobaltocenium residue, while CB8 prefers to engulf the TEMPO group. This finding suggests that CB8 and TEMPO form a highly stable host–guest pair and, in fact, the *K* value for the formation of the CB8·$\mathbf{17}^{+}$ complex was determined as 2.1×10^{8} M^{-1} at 25 °C in water.[31] The hyperfine splitting constant decreases substantially upon complexation by CB8, which reveals that the nitroxide radical is included inside the hydrophobic cavity of CB8. In contrast to this, complexation of $\mathbf{17}^{+}$ by CB7 leads to a small increase of the $\langle a_N \rangle$ value.

Guest $\mathbf{18}^{+}$ contains two nitroxide radicals, located at the end of its side arms. The central cobaltocenium residue acts as a ball bearing allowing scissoring motions of the two side arms. Through-space interactions between the two nitroxide radicals

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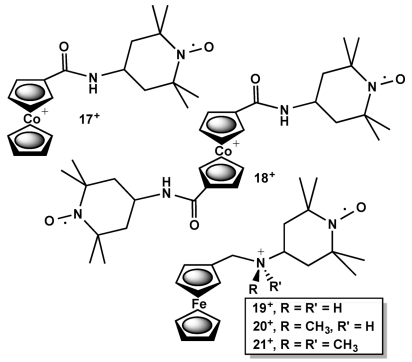

Figure 10. Structures of TEMPO-containing guests for binding with CB7 and CB8.

fluctuate with time, and the EPR spectrum shows an average situation in which the spectrum contains five lines due to the spin exchange coupling interactions between the two radicals. Similarly to guest $\mathbf{17}^+$, CB8 interacts with the biradical guest $\mathbf{18}^+$ by forming inclusion complexes with the terminal TEMPO groups. Since these complexes are highly stable, they form quantitatively at micromolar concentrations of guest and host. Therefore, addition of 2.0 equiv of CB8 to an aqueous solution of $\mathbf{18}^+$ leads to the formation of a ternary complex (2 CB8 hosts and 1 guest) in which the nitroxide radicals are as far away as possible, showing a simple three-line spectrum that reflects the total absence of spin exchange coupling. This process is completely reversible and the addition of a competing guest, trimethylammoniumadamantane, results in the dissociation of the $(CB8)_2{\cdot}\mathbf{18}^+$ complex and the regeneration of the five-line EPR spectrum.[31] We were able to isolate single crystals of the ternary complex and, thus, confirm its proposed structure (Figure 11).

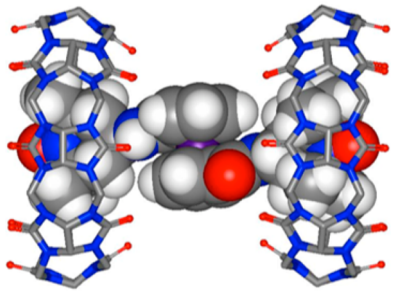

Figure 11. Crystal structure of the $(CB8)_2{\cdot}\mathbf{18}^+$ ternary complex.

The ferrocenyl-TEMPO derivatives, compounds $\mathbf{19}^+$–$\mathbf{21}^+$ (Figure 10), also exhibit interesting binding behavior. While CB7 includes the ferrocenylmethylammonium binding site in all cases,[32] the behavior of CB8 was found to vary depending on the level of methylation of the ammonium nitrogen in the middle of the guest. In this way, the complex between the fully protonated guest $\mathbf{19}^+$ and CB8 is centered on the ferrocenylmethylammonium site. On the other extreme, complexation of the fully methylated $\mathbf{21}^+$ by CB8 takes place by inclusion of the TEMPO residue. Not unexpectedly, complex CB8·$\mathbf{20}^+$ is composed of a roughly equimolar mixture of the two microscopic complexes, in which the CB8 encapsulates either the ferrocenyl or the TEMPO residue. The effect of methylation on the preferred binding site for CB8 was unexpected and, although poorly understood at this time, is probably related to the solvation around the ammonium group.[33]

DETECTION OF MICROSCOPIC COMPLEXES

As illustrated by guest $\mathbf{20}^+$, a ditopic guest containing two different binding sites for a given host may form two distinct microscopic complexes.[34] With many hosts, detection of the individual microscopic complexes is hampered by fast exchange kinetics of the host among the binding sites. In this regard, the kinetic and thermodynamic stability of many CB*n* inclusion complexes should facilitate the detection of these species. Guest $\mathbf{22}^+$ (see Figure 12), which contains both ferrocenylmethylammonium and adamantylammonium residues, offers a remarkable example.[35] When mixed in aqueous solution with 1.0 equiv of CB7, both microscopic complexes are clearly observed using either ^{1}H NMR spectroscopic or voltammetric techniques. Initially, both complexes are observed at relatively similar concentrations, but the mixture gets progressively richer in the adamantyl-included microscopic complex. The mechanism and kinetics of this conversion were investigated in detail.[35]

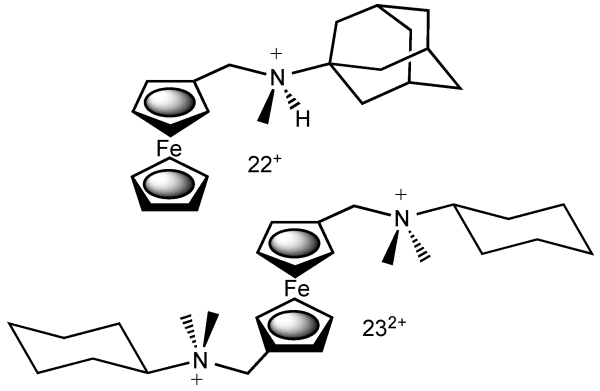

Figure 12. Ferrocenyl-containing guests whose CB7 complexation gives rise to the detection of distinct microscopic complexes.

The fully methylated ammonium guest compound $\mathbf{23}^{2+}$ provides more interesting examples of detectable microscopic complexes.[36] Upon mixing with 1.0 equiv of CB7, the host clearly engulfs one of the terminal cyclohexyl units. However, this complex evolves with time to the more stable form in which the host is centered, encapsulating the ferrocenyl subunit. We investigated the intramolecular kinetics of this sliding process, which is characterized by a half-life of 46 min at 25 °C. Equally interesting are the binding interactions with CB8.[36] In this case, different complexes are observed depending on the prevalent [host]/[guest] ratio in the solution. When [host] < 1.0 equiv, the predominant form of the complex is that in which the host is centered in the middle binding site. However, when [host] > 1.0 equiv, the prevalent form is the ternary complex in which each of the cyclohexyl units is encircled by a CB8 host.

CONCLUDING REMARKS

The body of work described here provides only a starting point on the variety of structures and properties available with unmodified CB*n* complexes. Clearly, complexation of redox active moieties by CB*n* receptors may have substantial effects on the thermodynamics and kinetics of the associated electron transfer reactions. However, the binding affinities and

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selectivities of CB*n* hosts can reach extremely high values, and very careful design is required to prepare molecular systems that may be controllable via electron transfer processes. Some of the systems described here can also be switched effectively with proton transfer reactions.

The high binding affinity reached between CB7 and suitable ferrocenyl guest suggests the possible use of this host–guest pair as a synthetic replacement of the biochemical avidin–biotin pair. In fact, we have shown that it is possible to modulate the binding affinity substantially, using the combined effect of proton and electron transfer processes under mild conditions. This modulation of the binding affinity is fully reversible.

Recent improvements in the synthetic methods available to functionalize CB*n* receptors are already starting to open research avenues to a wealth of new chemistry with this family of hosts.[37,38] Novel applications have been proposed,[39] and more are certain to follow as an increasing number of groups explore the fascinating chemistry of these macrocycles.

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Notes

The authors declare no competing financial interest.

Biography

Angel E. Kaifer was born in Madrid, Spain, where he completed his undergraduate studies in chemistry at Universidad Autónoma. He earned his Ph.D. (with Luis Echegoyen) in 1984 at the University of Puerto Rico, Rio Piedras, and did postdoctoral work (with Allen Bard) at the University of Texas, Austin. In 1985, he joined the faculty of the University of Miami, where he has been Professor of Chemistry since 1994. His research interests focus on the electrochemistry of supramolecular systems, with current emphasis on molecular capsules and the binding properties of cucurbit[*n*]uril hosts.

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