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Binding Interactions between a Ferrocenylguanidinium Guest and Cucurbit[*n*]uril Hosts

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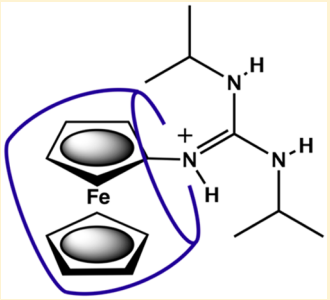
Supporting Information

ABSTRACT: The binding interactions between a novel ferrocenylguanidinium derivative (FcG^+) and the macrocyclic hosts cucurbit[7]uril (CB7) and cucurbit[8]uril (CB8) were investigated in aqueous solution. ^1H NMR spectroscopic experiments indicated that both hosts form stable 1:1 inclusion complexes with FcG^+, in which the ferrocenyl group is engulfed by the host cavity. The stoichiometry of the CB7·FcG^+ complex was also confirmed by electrospray mass spectrometric (ESI MS) experiments. The association equilibrium constants (K) were determined from NMR competition experiments. The measured K values were 3.5×10^9 and 2.5×10^8 M^{-1} for CB7 and CB8, respectively, in 50 mM sodium acetate-d_3 D_2O solution (pD 4.7). DFT computational studies confirmed the 1:1 stoichiometry and the inclusion character of both complexes. Voltammetric experiments were carried out to measure the complexation-induced shifts on the half-wave potentials for the one-electron oxidation of the ferrocenyl moiety. Complexation by CB7 led to a 12 mV anodic shift, while CB8 caused a larger 32 mV shift also in the positive direction. These potential shifts suggest that the delocalization of the positive charge on the side arm over the three nitrogens in the guanidinium unit results in electrochemical behavior similar to that observed with neutral ferrocene derivatives.



INTRODUCTION

Guanidine-based functional groups are common in biomolecules and may find applications in areas of chemistry as diverse as coordination compounds,[1] organocatalysis,[2] and anion recognition.[3,4] Bis(cyclopentadienyl)iron(II), better known as ferrocene, is an important organometallic compound that can be easily functionalized to yield a large number of derivatives. Very recently, some of us have reported the catalytic generation of ferrocene-containing guanidines and their use as precursors for the preparation of platinum(II) complexes with anticancer activity.[5] Since ferrocene derivatives are known to be excellent substrates for the formation of highly stable inclusion complexes with the cucurbit[7]uril (CB7) host,[6−10] we decided to investigate the binding interactions of ferrocenylguanidinium (FcG^+, see Figure 1 for structures) with the host CB7 and its larger analogue cucurbit[8]uril (CB8).[11] One of the most salient properties of ferrocene is its reversible one-electron oxidation to yield the positively charged ferrocenium. Ferrocene derivatives having a single substituent connected to one of the cyclopentadienyl rings form complexes with CB7 with binding affinities in the nanomolar to picomolar regime. (The corresponding equilibrium constants in aqueous media are in the 10^9−10^{12} M^{-1} range.) We have shown that, for ferrocene derivatives having a positively charged group on the side arm,[8] the half-wave potential ($E_{1/2}$) for ferrocene oxidation shifts to considerably more positive values upon complexation by CB7 ($\Delta E_{1/2} > 100$ mV, where $\Delta E_{1/2} = E_{1/2,\text{complex}} - E_{1/2,\text{guest}}$). Since the ferrocene moiety is included in the host cavity, as shown by NMR spectroscopic data, the observed potential shift is rationalized by the increased stability of neutral ferrocene and the decreased stability of the oxidized, positively charged ferrocenium, when both are held inside the hydrophobic cavity of CB7. In contrast, ferrocene derivatives with a neutral side arm show very small shifts in the $E_{1/2}$ value upon inclusion by CB7[8] ($\Delta E_{1/2} < 15$ mV). Since NMR spectroscopic data also indicate clearly that the ferrocene moiety of neutral guests is included in the cavity of the CB7 host, it is rather surprising that the CB7-induced potential shifts observed with neutral ferrocene guests are so much smaller than those measured with ferrocene guests having a positively charged side arm. Our key interest in guest FcG^+ relates to the fact that the positive charge on the side arm is delocalized through the three nitrogen atoms of the guanidinium group and, thus, can be seen as an "intermediate" case between guests with a positive

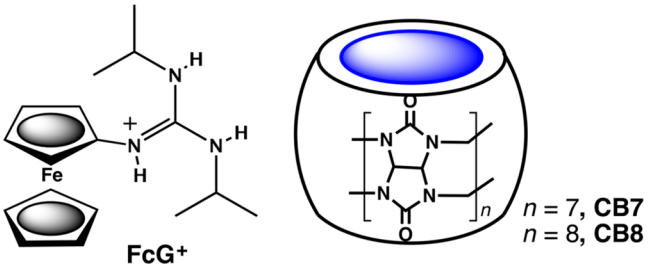


Figure 1. Structure of the guest and the hosts used in this work.

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charge localized on an ammonium nitrogen and those with no charge on the side arm.

RESULTS

The initial investigation of the binding interactions between FcG^+ and CB7 was carried out using ^1H NMR spectroscopy. Figure 2 shows the ^1H NMR spectrum of free FcG^+ in D_2O. Addition of 0.5 equiv of CB7 leads to the observation of two sets of resonances, corresponding to the free guest and a new species, CB7·FcG^+, the host–guest complex of FcG^+ with CB7. The simultaneous observation of resonances for these two species indicates that the host-exchange process is slow on the time scale of the NMR experiments. Once the amount of added CB7 reaches a full equivalent, the peaks for free FcG^+ are no longer observed and the peaks for CB7·FcG^+ become fully developed. The presence of CB7 leads to substantial upfield shifts for all of the ferrocene proton resonances (labeled c–e in the figure), which is a well-established indication that the ferrocene moiety is included inside the CB7 cavity.[11] At the same time, the terminal methyl protons on the side arm shift downfield, revealing that they are held in the proximity of the carbonyl-laced host portal but outside the cavity. We also noted that each of the two doublets corresponding to the methylene protons on the CB7 host is split into two overlapping doublets, reflecting the environmental differences between the two CB7 portals upon inclusion of FcG^+. In other words, one of the CB7 portals is exposed to the guanidinium side arm while the other one is not.

Identical NMR experiments with the larger cavity host CB8 yield similar results (see Figure S1). The ferrocene protons undergo CB7-induced shifts to higher fields, and the terminal methyl protons on the guest's side arm shift slightly downfield. Again, these findings indicate clearly that the ferrocene group is bound in the host cavity, as was the case with CB7. On the other hand, the guest-induced splitting of the methylene host protons is only clearly observed for the proton signal at 5.7 ppm, which corresponds to the "inside" protons on the methylene bridges. This observation is consistent with the wider cavity of CB8, which leads to a looser fit between the host and the guest in the inclusion complex, reducing the guest-induced environmental differences between the two portals and probably decreasing the lifetime of the complex.

The NMR experiments suggest a 1:1 stoichiometry for both complexes because host additions beyond 1.0 equiv do not result in further spectral changes. We also performed electrospray (ESI) mass spectrometric experiments to confirm this point. In the case of CB7, we detected signals associated with the 1:1 complex with FcG^+ without much difficulty. Figure 3 shows the observed ESI MS spectrum, in which we identified intense peaks corresponding to the species CB7·FcG^{2+} and Na^+·CB7·FcG^+. The first species has two positive charges due the one-electron oxidation of the ferrocene

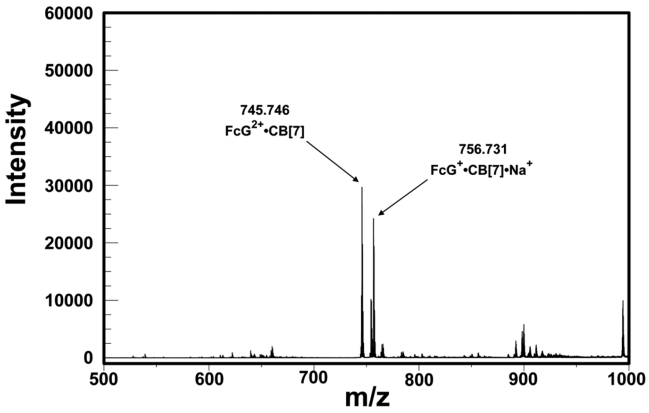

Figure 3. ESI mass spectrum of a solution containing 2.0 mM FcG^+ and 2.0 mM CB7.

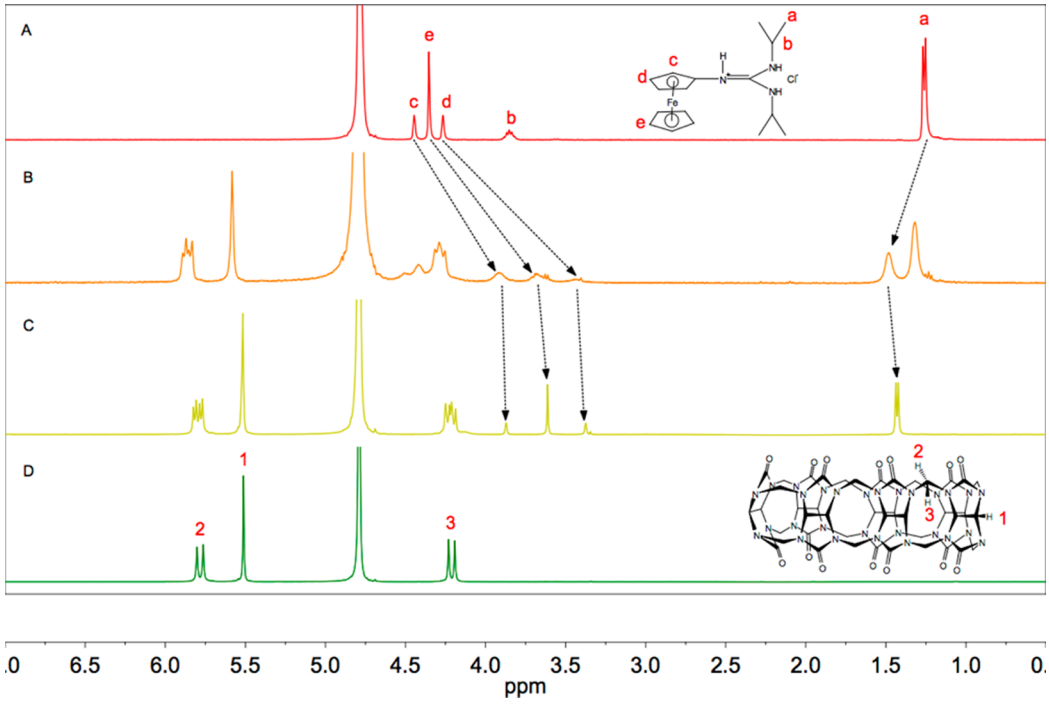

Figure 2. ^1H NMR spectra (D_2O, 400 MHz) of (A) 2.0 mM FcG^+, (B) 2.0 mM FcG^+ + 1.0 mM CB7, (C) 2.0 mM FcG^+ + 2.0 mM CB7, and (D) CB7.

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group under ESI conditions. In the second species the ferrocene is not oxidized, but a sodium ion is attached to the CB7 portal opposite the one occupied by the guanidinium side arm. Similar species have been obtained in MS experiments and reported in the recent past by our group,[9] as oxidation of ferrocene groups and attachment of Na^+ ions often take place under ESI conditions. Therefore, the unequivocal detection of these species confirms the formation of a stable CB7·FcG^+ complex with 1:1 stoichiometry. Unfortunately, in the case of CB8, we could not detect any species under ESI conditions that may serve to confirm the 1:1 stoichiometry suggested by the NMR data.

To assess the thermodynamic stability of the complexes formed between guest FcG^+ and the two hosts, CB7 and CB8, we measured the equilibrium association constants (K) of these complexes. Our ^{1}H NMR spectroscopic experiments indicate that complex formation is quantitative at the millimolar concentrations typical of these experiments. Previous reports[6−10] on the binding of ferrocenyl guests with either CB7 or CB8 suggests high K values, above the range easily measurable in NMR experiments. Therefore, we decided to measure the K values using competition experiments in which 1.0 equiv of host is added to a solution containing 1.0 equiv of FcG^+ and 1.0 equiv of a second (reference) guest, whose binding constant with the host is already known. The relative level of complexation of both guests once equilibrium is reached allows the calculation of the K value between FcG^+ and the host. These experiments were done in 50 mM sodium acetate-d_3 D_2O solution (pD 4.7) because this medium has become the practical standard for host−guest binding studies involving CB7 and CB8 hosts after the seminal work of Isaacs and co-workers.[12] The relative concentrations at equilibrium were measured by integrating suitable ^{1}H NMR peaks of bound and free guests. To determine the K value between FcG^+ and CB7 we used p-xylylenediamine ($K = 1.84 \times 10^9$ M^{-1} for association with CB7[12]) as the reference competing guest, and 1,3-bis(4-aminomethylphenyl)triazene ($K = 5.78 \times 10^{10}$ M^{-1} for association with CB8[12]) was used to measure the binding constant with CB8.[12] More details of these experiments can be found in the Supporting Information. Our measurements yielded K values of 3.5×10^9 and 2.5×10^8 M^{-1} for CB7 and CB8, respectively, at room temperature in the aforementioned aqueous medium. These values are consistent with previous observations, as the stability of the CB7 complex of FcG^+ is larger than that of the CB8 complex, probably as a result of the more snuggly fit between the ferrocenyl unit and the inner cavity of CB7.

We also measured the diffusion coefficients (D_0's) of FcG^+ and its complexes with CB7 and CB8. Using two-dimensional DOSY NMR experiments, we obtained a value of 4.6×10^{-6} cm^2 s^{-1} for free FcG^+ in D_2O solution. The D_0 values for the CB7·FcG^+ and the CB8·FcG^+ complexes were determined to be 2.5×10^{-6} cm^2 s^{-1} and 2.4×10^{-6} cm^2 s^{-1}, respectively, in the same medium. We also measured the D_0 values for free CB7 and CB8 as 2.8×10^{-6} cm^2 s^{-1} and 2.5×10^{-6} cm^2 s^{-1}, respectively. As expected the inclusion complexation of FcG^+ by either host leads to a pronounced decrease in the D_0 values, due to the increase in molecular size. The difference between the values for the CB7 and CB8 complexes is however very small.

We must note that FcG^+ is not stable in aqueous solution for long periods of time. Experimentation can be conducted for a few hours without any measurable effects from decomposition. However, millimolar aqueous solutions of FcG^+ are a pale yellow and we noticed discoloration and some precipitation around 24 h after preparation. ^{1}H NMR spectroscopic experiments do not reveal any new resonances, but the original peaks from FcG^+ decrease in intensity as time elapses and eventually disappear, which is consistent with insoluble decomposition products. While we did not investigate this slow decomposition reaction in more detail, we notice that, in the presence of CB7 or CB8, no decomposition takes place and the aqueous solutions are perfectly stable for weeks. Clearly, the stabilization afforded to FcG^+ by inclusion complex formation slows the kinetics of the decomposition reaction to a point in which it becomes unobservable on a time scale of days.

Our best efforts to crystallize these complexes failed to produce single crystals of sufficient quality for X-ray diffraction analysis. Therefore, we carried out DFT calculations in order to collect further information on the structures of the CB7·FcG^+ and CB8·FcG^+ complexes. Using the M062X functional with the 6-31g(d,p) basis set, we obtained the energy-minimized structures shown in Figure 4. We also obtained similar structures with

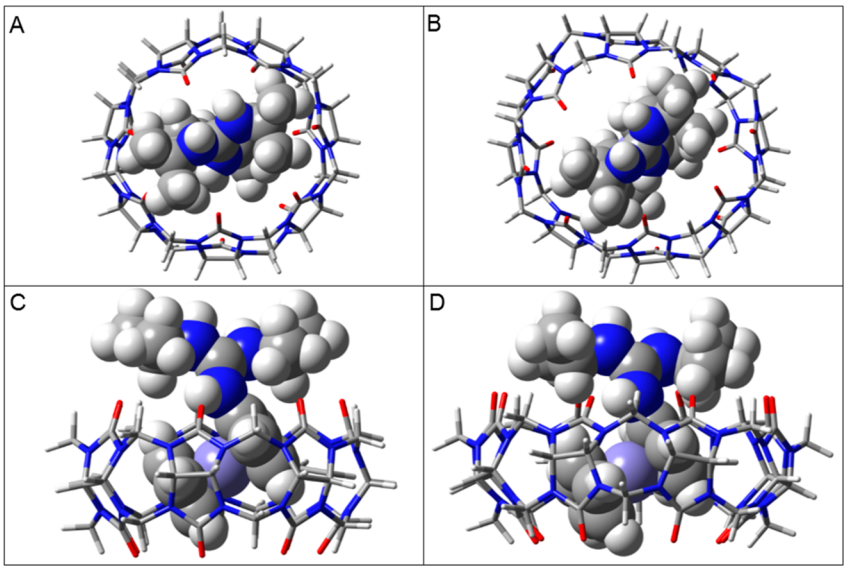

Figure 4. Energy-minimized structures obtained by the DFT method at the M062X level for the complexes formed between FcG^+ and the hosts CB7 (left panels) and CB8 (right panels). The top panels show top views and the bottom panels show side views of the complexes.

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the B3LYP functional (see the SI). However, the complex formation energies determined with the M062X functional parallel better the measured thermodynamic stabilities (K values) measured in the NMR competition experiments. Clearly, the ferrocene moiety is included in the cavity of both hosts, in full agreement with the NMR spectroscopic results, while the guanidinium side arm hovers next to one of the carbonyl-laced portals of the host. Both complex structures are similar, although the ferrocene seems to penetrate more deeply into the host cavity in the CB8 complex. This small difference might be the result of stronger interactions between the positive charge delocalized among the three nitrogens on the guanidinium group and the carbonyl oxygens lacing the cavity entrance in CB8, which is clearly distorted from the expected D_{8h} symmetry.

Our NMR, MS, and DFT computational data clearly demonstrate the formation of highly stable inclusion complexes between FcG^+ and the two hosts. The binding is effective in the nanomolar regime ($K \sim 10^9$ M^{-1}) and is, thus, essentially quantitative at the millimolar concentrations typically used in voltammetric experiments. We set out to determine the effects of inclusion complexation by CB7 and CB8 on the electrochemical behavior of guest FcG^+. The anodic behavior of FcG^+ in cyclic voltammetry (CV) and square wave voltammetry (SWV) experiments is shown in Figure 5. The reversible

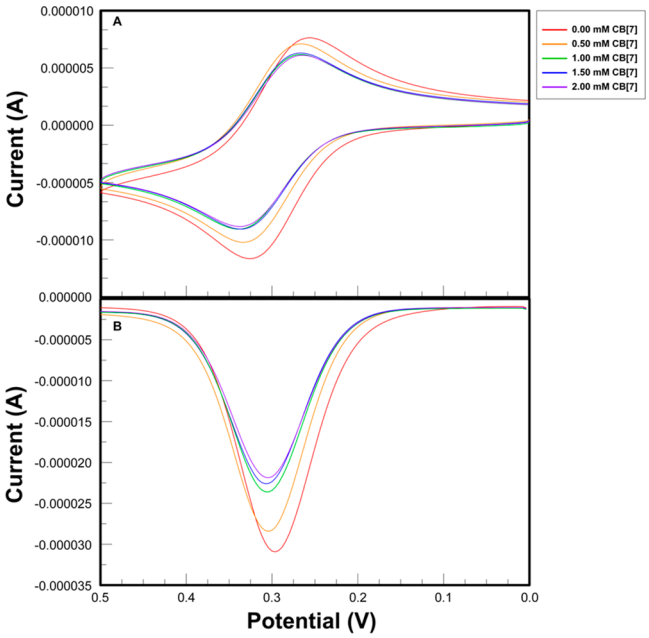

Figure 5. (A) Cyclic voltammetric behavior of 1.0 mM FcG^+ on glassy carbon (0.071 cm^2) in the presence of variable amounts of CB7 and 0.1 M NaCl. Scan rate: 0.1 V s^{-1}. (B) Square wave voltammetric behavior of the same solutions.

one-electron oxidation of the ferrocene moiety takes place at a $E_{1/2}$ of 0.296 (±0.005) V vs Ag/AgCl. In the CV experiments, the potential difference observed between the anodic and the cathodic peaks is ca. 70 mV, which is close to the theoretical value for a reversible redox couple, exhibiting fast electrochemical kinetics. Addition of CB7 decreases the current levels observed in the voltammetric wave and has only a small effect on the half-wave potential, which shifts to more positive values by ca. 12 mV upon addition of 1.0 equiv of CB7. Further host additions have no effect on the voltammetric response.

The voltammetric results with CB8 are similar (see Figure S4). The presence of this host causes a reduction in the current levels associated with the voltammetric wave for the one-electron oxidation of the ferrocene group. The host-induced current decrease is more pronounced in this case than it was with CB7, which is consistent with the larger size of CB8 and the larger molecular weight of its FcG^+ complex, leading to a larger decrease in the diffusion coefficient of the complex as compared to the free guest. Upon addition of 1.0 equiv of CB8 the $E_{1/2}$ value shifts about 32 mV to more positive values. Therefore, the CB8-induced shift of the half-wave potential for ferrocene oxidation is more pronounced than that observed with CB7.

DISCUSSION

Beck and Winter have shown that CB7 forms a stable complex with a guest related to FcG^+, bis(acetyl-guanidinium)ferrocene, leading to a disruption of the binding interactions of this dicationic ferrocene derivative with dicarboxylates in aqueous media.[3] Our experimental results clearly show that the monocationic ferrocenyl guanidinium (FcG^+) guest forms stable complexes with both hosts, CB7 and CB8. The CB7·FcG^+ complex exhibits higher thermodynamic stability than its CB8 counterpart. The formation free energies for the CB7 and CB8 complexes are calculated from the K values as −54.5 and −47.9 kJ mol^{-1}, respectively, resulting in a $\Delta\Delta G^\circ$ = 6.6 kJ mol^{-1} when CB7 is replaced by CB8 in the complex. However, both hosts are effective binders in the nanomolar concentration regime, as suggested by the measured K values in D_2O solution also containing 50 mM sodium acetate-d_3 (pD = 4.7). In the case of the CB7 complex, the 1:1 stoichiometry of the complex was verified in ESI MS experiments, but our experimental and computational body of evidence strongly suggests that the CB8 complex also has the same stoichiometry. The stabilization afforded by inclusion complex formation with both hosts is also evident in the considerable slowdown experienced by the decomposition of FcG^+ in aqueous media.

As stated in the Introduction, we were interested in measuring the change in the half-wave potential suffered upon CB7 complexation by the one-electron oxidation of the ferrocene group in FcG^+. Our CV and SWV data clearly show that the presence of CB7 has a very minor effect on the $E_{1/2}$ value. From the free guest to the CB7 complex, the $E_{1/2}$ value shifts by ∼12 mV to more positive values. This seems to align this guest with ferrocene guests having a neutral side arm.[8] Clearly, the fact that FcG^+ has a positive charge fully delocalized over the three nitrogens on the guanidinium unit brings it closer to the voltammetric behavior observed with neutral ferrocenyl guests. It seems that a more localized positive charge, such as that in a side arm containing a trialkylammonium group connected to ferrocene by a single methylene, is required to observe a larger CB7-induced $E_{1/2}$ shift.[6,8] This can be rationalized by the effect of the localized positive charge on the side arm, which may help lock the ferrocene unit inside the CB7 cavity because of the attractive ion−dipole forces between the ammonium group and the rim of carbonyl oxygens lacing the host portal. In the absence of the localized positive charge, this anchoring effect does not take place, or it does to a lesser extent, and upon oxidation, the ferrocene group probably shifts closer to one of the host portals, becoming more exposed to solvating water molecules and, thus, minimizing the measured potential shift.

It is indeed surprising that CB8, with a larger cavity than CB7, actually induces a larger $E_{1/2}$ shift (∼32 mV) from free

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FcG^+ to its complex, CB8·FcG^+. Our computational results suggest that the ferrocene nucleus is more deeply included in the host cavity in the case of the CB8 complex. In fact, we measured for both complexes the distance between the iron atom and the plane formed by the carbonyl oxygens of the portal that interacts with the guanidinium side arm. Using the energy-minimized structures obtained with the M062X density functional, we measured 2.558 and 2.988 Å as the distances in the CB7 and CB8 complex, respectively. Similar data were obtained with the B3LYP functional. The distortion of the CB8 host also suggests that it can adapt its oxygen-laced portal to interact with the guanidinium group in a more effective way, leading to a deeper inclusion of the ferrocene nucleus inside the host cavity and, thus, a slightly larger complexation-induced shift in the $E_{1/2}$ value. However, the CB7 complex is more stable overall because hydrophobic effects are more important than ion−dipole interactions at driving the formation of these complexes[13] and the ferrocene group fits tightly inside the inner cavity of the CB7 host, while the fit is looser in the case of the larger cavity host, CB8. While these arguments provide a reasonable explanation for the overall thermodynamic stability and voltammetric properties of the CB7·FcG^+ and CB8·FcG^+ complexes, we recognize that we have limited data related to the effects of CB8 on the voltammetric behavior of ferrocene derivatives. We are currently trying to collect additional electrochemical data with CB8 and also carrying out additional computational work to improve our understanding of these phenomena.

In conclusion, the considerable stability of these CB7 and CB8 complexes, which prevents the decomposition of the FcG^+ guest in aqueous media, can be utilized in future work to develop water-soluble, redox-active probes for interaction and detection of specific anionic (carboxylate) motifs in biomolecules.

EXPERIMENTAL SECTION

Ferrocenylguanidinium, FcG^+, was synthesized by direct reaction of the previously reported neutral guanidine[5] [Fc−NH=C(NH^iPr)$_2$] with 1.0 M HCl in diethyl ether. CB[7] and CB[8] were prepared according to a literature procedure.[14] *p*-Xylylenediamine is commercially available, and 1,3-bis(4-aminomethylphenyl)triazene was synthesized following a literature procedure.[12] A glassy carbon working electrode, an Ag/AgCl reference electrode, and Pt wire counter electrode were used in the voltammmetric experiments. All ^{1}H NMR experiments were performed on a 400 MHz NMR spectrometer. Details on the computational work are given in the Supporting Information.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b02508.

Experimental and computational details; additional NMR spectroscopic, computational and voltammetric data (PDF)

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Notes

The authors declare no competing financial interest.

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