Inclusion of anthraquinone derivatives by the cucurbit[7]uril host†‡

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Two simple cationic anthraquinone derivatives were found to form stable complexes with the cucurbit[7]uril host, in which the anthraquinone residue is included by the host cavity. The corresponding equilibrium association constants were relatively low ($K \sim 10^3$ M^{-1})—compared to those of other complexes formed by the same host—suggesting that anthraquinone is not an ideal guest. However, the electrochemical behavior of the guests is strongly affected by inclusion inside the host cavity.

Introduction

Quinones are a class of molecules with a range of important properties and considerable biological relevance. In particular, anthraquinone and some of its derivatives have been proposed as artificial, recyclable DNA photonucleases, capable of cleaving duplex or single-stranded DNA upon exposure to light.[1,2] Some anthraquinone derivatives are efficient DNA intercalators,[3] and anthraquinone residues have also been used as covalently attached probes in oligonucleotides designed for fluorescent signaling of hybridization events.[4] The anticancer drug mitoxantrone,[5] a member of the anthracycline antibiotics, is also a cationic anthraquinone derivative, which intercalates between DNA base pairs at physiological pH. Fluorescent anthraquinone derivatives functionalized with platinum complexes have been utilized to investigate the distribution of platinum complexes in living cells.[6] Generally, cationic anthraquinone derivatives play an important role in all of these applications.

The molecular container properties of cucurbit[*n*]uril hosts are the subject of extensive attention.[7,8] Given our group's interest in these hosts, especially the cucurbit[*n*]urils with $n = 7$ (**CB7**)[9] and $n = 8$ (**CB8**),[10] we set out to investigate the binding interactions between the **CB7** host and two simple cationic anthraquinone derivatives. The guests **1** and **2** (shown in Fig. 1) differ only in the degree of substitution of the amine residue, which is attached to the anthraquinone through a methylene bridge. This leads to different steric influences on complex formation. Guest **1** bears a positive charge, while guest **2** can attain a positive charge in acidic media. The positive charges on these guests provide good aqueous solubility, which allows the investigation of the binding with **CB7** in aqueous media, thus overcoming the lack of aqueous solubility of many other anthraquinone derivatives. We demonstrate here that anthraquinone guests **1** and **2** form stable 1 : 1 complexes with **CB7**, in which the host engulfs the anthraquinone unit of the guest and the complex is stabilized by ion–dipole interactions between the ammonium cation and the carbonyl-laced portal of **CB7**. The stability of these complexes is influenced by the substituents attached to the positively charged nitrogen on the sidearm of the anthraquinone derivative.

Results and discussion

Guests **1** and **2** were prepared by reaction of 2-(bromomethyl)anthraquinone with an excess of the corresponding amine. The binding interactions between **CB7** and the anthraquinone derivatives were investigated by ^{1}H NMR spectroscopy. All the NMR measurements were recorded at 25 °C in 0.01 M DCl in order to guarantee the protonation of the amine group of guest **2**. Fig. 2 shows the ^{1}H NMR spectra of **1** in the presence of increasing concentrations of host. After addition of 1.5 equiv. of **CB7** to the solution of **1**, the aromatic proton resonances exhibit moderate upfield shifts (*ca.* 0.2 ppm). Their small magnitude suggests a less than ideal fit between the anthraquinone residue and the CB7 cavity, based on our own observations with other guests.[9] The proton signals corresponding to the aliphatic protons *a* and *b* shift 0.25 and 0.19 ppm downfield, respectively. The resonances corresponding to the methylene protons labeled *c* also experience a small

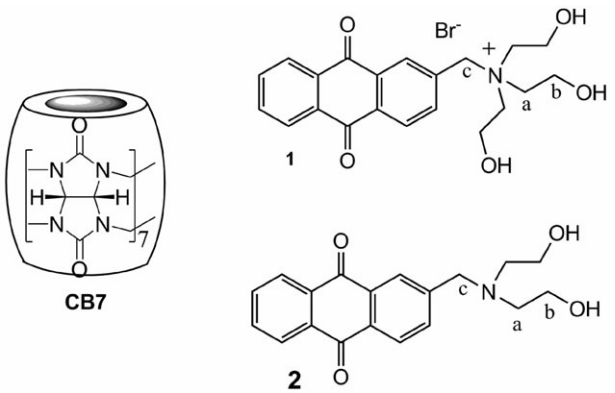

Fig. 1 Structures of the host and the guests used in this work.

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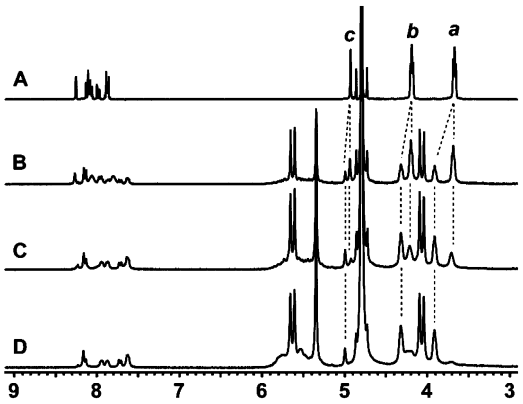

Fig. 2 ^{1}H NMR spectra (DCl–D_2O, pH = 2.0) of **1** (A) in the absence, and in the presence of (B) 0.5 equiv., (C) 1.0 equiv. and (D) 1.5 equiv. of **CB7**.

downfield shift of 0.06 ppm. The observed complexation-induced pattern for the proton resonances of **1** is consistent with the anthraquinone unit being located inside the host cavity and the methylene *a* and *b* protons positioned outside the host cavity. In addition to the hydrophobic forces that stabilize the inclusion of the anthraquinone residue by **CB7**, a second force stabilizing the inclusion complex is the ion–dipole interaction between the positively charged ammonium nitrogen on the guest and the carbonyl oxygens on the **CB7** portal. The formation of this complex was further confirmed by electronic absorption spectroscopy and MALDI-TOF mass spectrometry. The anthraquinone absorption band in the UV region is depressed in the presence of **CB7** and a major peak at 1532 *m*/*z*, which corresponds to the mass-to-charge ratio of the $[\mathbf{CB7 \cdot 1}]^+$ species, was clearly observed in the mass spectrum of **1**/**CB7** mixtures. (see ESI‡).

In the presence of 0.5 equiv. of host, signals corresponding to both the free and bound anthraquinone guest **1** are clearly evident. This observation is consistent with slow exchange between the free and bound guests on the NMR timescale. The distance between the two carbonyl oxygens in the anthraquinone residue is 5.34 Å, compared to the **CB7** portal, which has a 5.4 Å diameter, and the **CB7** internal cavity, which has a maximum inner diameter of 7.3 Å. Clearly, some stretching of the **CB7** macrocycle portals is required for inclusion of the guest and this structural factor may slow down the kinetics of the complex dissociation process. Furthermore, inclusion of **1** partially distorts the host cavity, as verified by molecular modelling computations (Fig. 3(A)). Integration of the resonances of the free and bound guest protons in the presence of variable amounts of **CB7** was used to determine the equilibrium association constant (*K*) of the **CB7 · 1** complex. We determined a *K* value of $(1.3 \pm 0.2) \times 10^3$ L mol^{-1}.

Similar experiments were also performed to investigate the supramolecular interaction between **CB7** and guest **2**. An analogous complexation-induced pattern of shifting NMR resonances was observed, as described above for the **CB7 · 1** complex. This finding is not surprising due to the similar structures of both guests. Key observations in the ^{1}H NMR spectra include upfield shifts of the aromatic proton resonances upon addition of 1.5 equivalents of **CB7** (Fig. 4), again indicating that the anthraquinone moiety is included within the host cavity. Simultaneous downfield shifts of the resonances for the aliphatic protons *a*, *b* and *c* are also consistent with and support the conclusion that the ethylenehydroxy chains remain outside of the host cavity, allowing the positively charged nitrogen to interact with the electron dense carbonyl oxygens on the host's portal. The formation of the 1 : 1 inclusion complex was also verified by MALDI-TOF mass spectrometry, through the detection of an intense peak at 1488 corresponding to the *m*/*z* ratio of $[\mathbf{CB7 \cdot 2}]^+$ species, and UV-Vis spectroscopy (see ESI‡).

The equilibrium association constant for the **CB7 · 2** complex was also determined by ^{1}H NMR spectroscopy and found to be $(2.8 \pm 0.3) \times 10^3$ L mol^{-1}. This value is more than two times higher than the equilibrium constant measured for the **CB7 · 1** complex. The guests differ only on the number of ethylenehydroxy units attached to the nitrogen connected to the anthraquinone residue *via* a single methylene. The

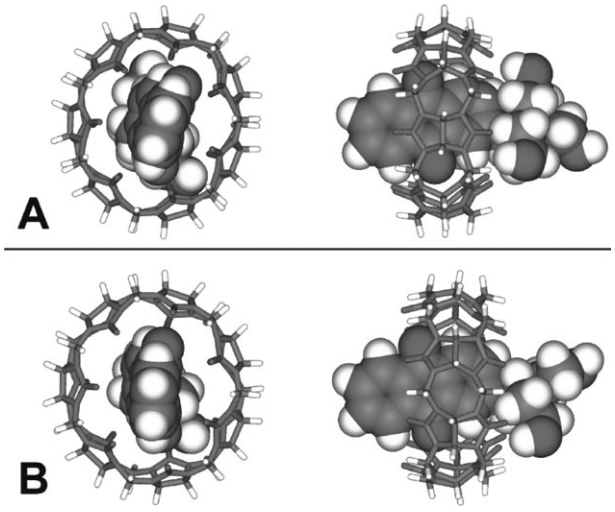

Fig. 3 Energy-minimized (AM1) structures of (A) **CB7 · 1** and (B) **CB7 · 2** complexes.

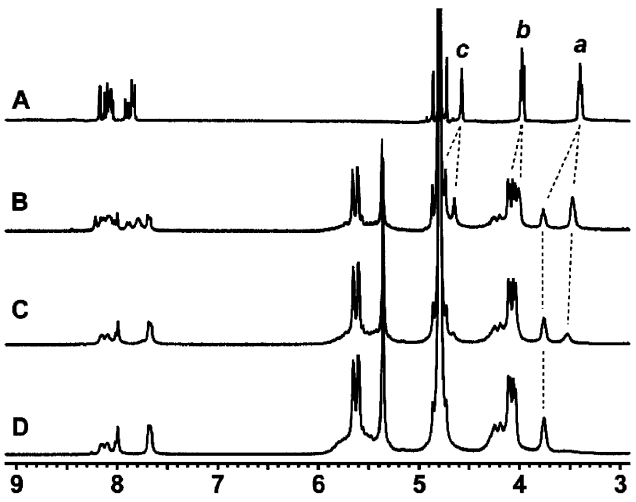

Fig. 4 ^{1}H NMR spectra (DCl/D_2O, pH = 2.0) of **2** (A) in the absence, and in the presence of (B) 0.5 equiv., (C) 1.0 equiv. and (D) 1.5 equiv. of **CB7**.

hydrophobic interactions between the anthraquinone unit and the inner **CB7** cavity are significantly weakened by the presence of two carbonyl oxygens on the guests. Therefore, the predominant driving force for inclusion complexation is the ion–dipole interaction between the positively charged nitrogen on the guest and the rim of carbonyl oxygens on the **CB7** portal. Molecular modelling (see Fig. 3) provides some clues to understand the minor binding differences of guests **1** and **2** with **CB7**. The larger volume of the substituents attached to the quaternized nitrogen in guest **1** appears to sterically hinder the cationic nitrogen from reaching the most thermodynamically advantageous position close to the host portal. On the other hand, the replacement of an ethylenehydroxy substituent by a proton in guest **2** allows the optimal positioning of the positively charged nitrogen against the electron rich carbonyls of the **CB7** portal. As a result of these steric factors, the complex **CB7**·**2** is more stable than the complex **CB7**·**1**. The structure of both guests suggests that upon complexation they would disrupt the equatorial symmetry of **CB7**, giving rise to environmental differences between the two portals of the host, as observed in other **CB7** inclusion complexes. The anticipated splitting of the methylene proton resonances of **CB7** is observed in the ^{1}H NMR spectra of the **CB7**·**2** complex. This phenomenon, however, was not so well observed in the case of **CB7**·**1**, possibly due to weaker host–guest interactions.

During our investigation it became clear that the presence of metal cations significantly influences the extent of complexation of guests **1** and **2** with **CB7**. Therefore, we titrated the **CB7**·**1** complex with increasing concentrations of NaCl (see ESI‡). Not surprisingly, an increasing amount of salt leads to the reappearance of the signals corresponding to unbound guest. Concurrently, signals of the complexed host are replaced by those of the free host at higher chemical shifts. After addition of ~60 equiv. NaCl (corresponding to a 0.3 M concentration of NaCl) only resonances of the free guest **1** and uncomplexed **CB7** are present. This experiment shows that sodium cation successfully competes with anthraquinone guest **1** for the **CB7** cavity. Similar behavior was observed for complex **CB7**·**2** (see ESI‡). However, 230 equivalents of NaCl were needed to complete the dissociation of the guest **2** from **CB7**. The larger concentration of NaCl needed to complete the dissociation of this complex is indeed in agreement with the higher equilibrium constant of complex **CB7**·**2** compared to complex **CB7**·**1**. The observed decrease of the binding constant in the presence of the increasing sodium cation concentrations has been observed in other cucurbit[*n*]uril complexes.[11]

To further quantify the influence of sodium cation on the formation of these complexes, we used ^{1}H NMR spectroscopy to determine the equilibrium constants at variable concentrations of NaCl (Fig. 5). As expected, the values of the equilibrium constants decrease with increasing concentration of NaCl and this effect is more pronounced for the weaker complex.

The electrochemical behavior of both guests is also strongly affected by the presence of host **CB7**. In acidic solution (0.1 M HCl) both guests exhibit a cathodic wave at −0.25 V *vs.* Ag/AgCl and its associated anodic wave at *ca.* 0.02 V, corresponding to the rather slow two-electron reduction process coupled

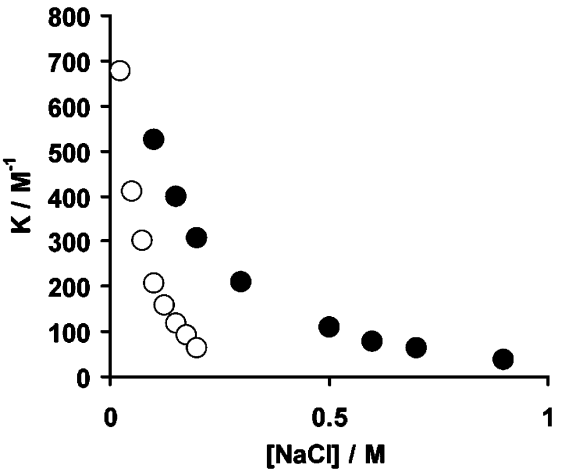

Fig. 5 Dependence of the equilibrium constants for **CB7**·**1** (○) and **CB7**·**2** (●) complexes as a function of the concentration of NaCl.

to the uptake of two protons [**1** (or **2**) + 2 H + 2 e = **1**·H_2 (or **2**·H_2)]. This electrochemical process is expected to be hindered by inclusion of the anthraquinone residue inside the **CB7** cavity. Indeed, Fig. 6 shows the effects of **CB7** additions on the electrochemical behavior of guest **2**. As the host is added to the solution, the electrochemical response of the anthraquinone residue disappears, revealing its gradual inclusion complexation. In the presence of 2.0 equiv. **CB7**, only residual current waves were observed for both the cathodic and the anodic processes. A similar voltammetric response was recorded for guest **1**.

We also performed additional cyclic voltammetric experiments in different media, but the results were similar. For instance, in 0.1 M pH 2 phosphate buffer also containing 0.1 M NaCl, we also observed attenuation of the voltammetric response of **2** upon increasing concentrations of **CB7**. However, the attenuation at a given concentration of **CB7** was less pronounced in this medium than in 0.01 M HCl, reflecting the larger concentration of sodium ion, which competes with the anthraquinone guest and decreases the extent of complexation.

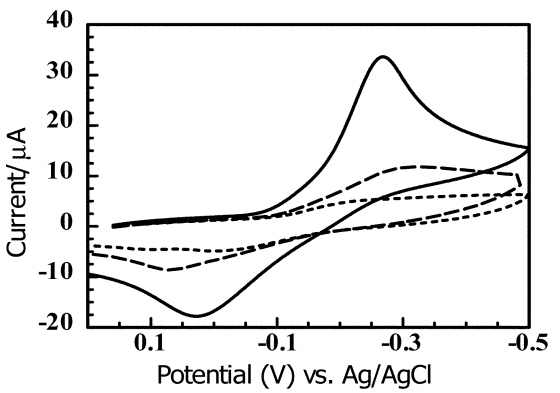

Fig. 6 Cyclic voltammetric response on glassy carbon (0.071 cm^2) of guest **2** in 0.1 M HCl solution in the absence (continuous line) and in the presence of 1.0 equiv. (discontinuous line) and 2.0 equiv. (dotted lined) of CB7. Scan rate: 0.1 V s^{-1}.

Experimental

General procedures

^{1}H and ^{13}C NMR spectra were obtained at fields of 300 and 400 MHz. FAB mass spectra were recorded using 3-nitrobenzyl alcohol as the matrix. MALDI-TOF mass spectra were recorded from α-cyano-4-hydroxycinnamic acid solid matrices.

Synthesis

Cucurbit[7]uril (**CB7**) was prepared according to a reported procedure.[12]

Guest 1. 2-(Bromomethyl)anthraquinone (0.5 g, 1.66 mmol) was stirred in excess triethanolamine (3 mL) at 80 °C for 10 h. Then, $CHCl_3$ was added to obtain a precipitate, which was filtered off and dried under vacuum. The dry solid was dissolved in water and precipitated by adding a concentrated aqueous solution of NH_4PF_6. The resulting sticky solid was washed with water and dried under vacuum. The dry solid was dissolved in acetone from which the pure compound was precipitated (by addition of a few drops of conc. HBr) as its bromide salt (299 mg, 40%).

^{1}H NMR (300 MHz, D_2O–DCl) δ 8.17 (s, 1H), 8.13–8.06 (m, 3H), 8.00 (d, 1H), 7.85–7.82 (m, 2H), 4.57 (s, 4H), 3.97 (t, 4H), 3.39 (t, 4H); ^{13}C NMR (300 MHz, D_2O–DCl) 183.3, 183.2, 137.5, 136.0, 135.6, 133.8, 133.4, 132.2, 129.6, 128.3, 127.3, 57.2, 55.6, 55.3; MS (FAB): m/z 370 $[M]^+$.

Guest 2. 2-(Bromomethyl)anthraquinone (0.5 g, 1.66 mmol) was stirred in excess diethanolamine (3 mL) at 90 °C for 6 h. Then, water (25 mL) was added and the resulting precipitate was collected by filtration. The solid compound was dissolved in dilute HCl, and impurities were extracted with diethyl ether. NH_4OH was added to the acidic solution until it became basic. The resulting yellow precipitate was washed with water and recrystallized from ethanol–water to yield the pure product (281 mg, 52%).

^{1}H NMR (300 MHz, D_2O–DCl) δ 8.25 (s, 1H), 8.13–8.06 (m, 3H), 8.00 (d, 1H), 7.88–7.85 (m, 2H), 4.92 (s, 2H), 4.19 (t, 6H), 3.67 (t, 6H); ^{13}C NMR (300 MHz, D_2O–DCl) 183.5, 183.4, 139.6, 135.7, 134.0, 133.8, 132.6, 131.9, 128.2, 127.4, 64.5, 61.3, 55.5; MS (FAB): m/z 326 $[M]^+$.

Conclusions

We have prepared two new water-soluble anthraquinone derivatives **1** and **2** and investigated their 1 : 1 inclusion complexes with **CB7** using ^{1}H NMR and MALDI TOF mass spectroscopy, as well as cyclic voltammetry. The apparent equilibrium association constants of the **CB7**·**1** and **CB7**·**2** complexes decrease in the presence of increasing NaCl concentrations. Inclusion complexation was found to slow down the electrochemical processes associated with reduction of the anthraquinone residues. The evidence presented here suggests that anthraquinone residues can serve as reasonable substrates for inclusion by cucurbit[7]uril, but the resulting binding affinities are much lower than those observed with other guests.

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