Contrasting pK\textsubscript{a} Shifts in Cucurbit[7]uril Host–Guest Complexes Governed by an Interplay of Hydrophobic Effects and Electrostatic Interactions

Nuno Basílio,* Sandra Gago, A. Jorge Parola,* and Fernando Pina

Laboratório Associado para a Química Verde (LAQV), Rede de Química e Tecnologia (REQUIMTE), Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal

**Supporting Information**

ABSTRACT: Cucurbit[7]uril inclusion complexes with guests bearing dimethylamino groups show the expected upward pK\textsubscript{a} shifts, whereas their diethylamino counterparts display a decrease in pK\textsubscript{a} due to the preferential stabilization of the unprotonated form. These results identify the diethylamino group as the substituent of choice to avoid receptor-assisted protonation of guest molecules and present new evidence for the role of the hydrophobic effect as a driving force in cucurbituril complexation.

INTRODUCTION

Cucurbit[n]urils (CB\textsubscript{n}) are water-soluble macrocyclic receptors holding a rigid barrel-shaped hydrophobic cavity and highly electronegative portals lined by carbonyl groups.\textsuperscript{1,2} Owing to these structural and electronic characteristics, CB\textsubscript{n} display high affinity and selectivity for guest molecules with complementary size, shape, and charge/polarity. Their remarkable binding properties enabled the development of potential applications based on the reversible formation of host–guest complexes within the fields of sensing, catalysis, self-assembled materials, drug delivery, etc.\textsuperscript{3–17} Likewise, fundamental investigations on CB\textsubscript{n} molecular recognition also contributed to progress the knowledge on crucial aspects of supramolecular chemistry and noncovalent interactions in aqueous media. Some examples include recent reports on the nonclassical hydrophobic effect associated with the release of high-energy water molecules from hydrophobic cavities, which was invoked to rationalize the exceptionally high binding affinities of CB\textsubscript{n} receptors toward some neutral organic guests.\textsuperscript{18–25}

Although CB\textsubscript{n} can display high affinity for some neutral guests, these molecules are traditionally known for their ability to selectively complex organic cations. In fact, for most basic guests, such as amines, CB\textsubscript{n} display stronger affinity for the positively charged protonated species (BH\textsuperscript{+}) with association constants from 1 to more than 4 orders of magnitude higher in comparison with those determined for the respective neutral conjugated bases (B). The higher stability of the complexes formed with the protonated guest leads to upward complexation-induced pK\textsubscript{a} shifts that are proportional to the relative stabilization of this species with respect to this conjugated base.

Mathematically, this is elegantly expressed by eq 1, where $K_a$ and $K_a'$ are the acid dissociation constants of the guest in bulk solution and in the encapsulated form, respectively, whereas $K_{BH^+}$ and $K_B$ are the association constants for the formation of the complex with BH\textsuperscript{+} and B, respectively.

$$K_a' = K_a \frac{K_B}{K_{BH^+}}$$  \hspace{1cm} (1)

According to eq 1, for guests with $K_{BH^+}$ values more than 4 orders of magnitude higher than $K_B$, complexation-induced pK\textsubscript{a} shifts higher than 4 units are predicted.\textsuperscript{26–28} This special feature has been explored in the framework of supramolecular catalysis, drug delivery, indicator displacement assays, and dye stabilization, which in the case of the flavylum family compounds is of major importance for their applications, for example, as food colorants and dye-sensitized solar cells.\textsuperscript{29–35} The trend in the recognition properties of CB\textsubscript{n}, showing selectivity factors ($K_{BH^+}/K_B$) for positively charged species covering various orders of magnitude, depending on the guest, is not completely understood and, consequently, in most cases, the magnitude of the pK\textsubscript{a} shifts cannot be predicted. Herein, by using flavylum cations and water-soluble trans-chalcones (Scheme 1) to form inclusion complexes with cucurbit[7]uril (CB7), we show that the magnitude of this shift is very sensitive to small structural variations. Our study puts in evidence that whereas guests with dimethylamino groups display the...
traditional upward $pK_a$ shifts, their counterparts with diethylamino substituents revealed downward $pK_a$ shifts. These results establish the diethylamino group as the substituent of choice for applications where the complexation-assisted protonation should be avoided and provide new hints into the recognition properties of CB$_n$ receptors.

**RESULTS AND DISCUSSION**

Flavylium derivatives 1a and 1b (Scheme 1) were previously investigated and were found to form inclusion complexes of equivalent stability with CB7 at pH = 2.36 The amino groups in flavylium compounds are weakly basic with $pK_a$ values of $-0.35 \pm 0.05$ and $0.60 \pm 0.02$ for 1a and 1b, respectively (see Figure S1). Figure 1 shows the spectral variations observed for 1a and 1b upon addition of CB7 in the presence of a concentration of HCl required to adjust the H$^+$ activity near the $pK_a$ value.

In the case of 1a, upon addition of CB7, the absorption band centered at 530 nm (flavylium) decreases and the band centered at 428 nm increases (protonated flavylium). This result is in line with the displacement of the acid–base equilibrium toward the dicationic species and therefore with an expected complexation-induced upward $pK_a$ shift. On the other hand, in the case of 1b, the equilibrium is shifted toward the monocationic species upon addition of CB7, suggesting that the complexes formed with this species are more stable than those formed with the dicaticionic species. This behavior is compatible with an unexpected downward $pK_a$ shift and is in contrast to that observed for 1a. Fitting the absorbance data reported in Figure 1 to a 1:1 binding model allows the recovery of the apparent binding constants for 1a ($K = (7.9 \pm 0.8) \times 10^5$ M$^{-1}$) and 1b ($K = (2.6 \pm 0.3) \times 10^5$ M$^{-1}$). However, these association constants cannot be compared, as the experiments were performed for different concentrations of H$^+$, which is a known competitor for CB7 complexation.37 Additionally, under
these conditions, the apparent binding constants also depend on the $pK_a$ value of the guest and on the association constants for complexation of monocationic and dicationic species.\(^{38}\)

Owing to the very acidic and, thus, unfavorable conditions required for the determination of $pK_a$ of the inclusion complexes and the association constants of the dicationic species, it was decided to investigate other possible guests with higher $pK_a$ values (which also would confirm the generality of the observed behavior). With this purpose in mind, water-soluble trans-chalcones 2a and 2b (Scheme 1) were readily synthesized through a Claisen−Schmidt condensation.

The N-protonation of the trans-chalcones, 2a and 2b, can be readily followed by ultraviolet−visible (UV−vis) absorption spectroscopy according $pK_a$ values of 3.50 ± 0.02 and 4.90 ± 0.03, respectively (see Figure S2), with the diethylamino substituted 2b being more basic, as expected. The formation of inclusion complexes between both trans-chalcones and CB7 was also investigated by UV−vis absorption spectroscopy at pH = 9. Upon addition of increasing concentrations of CB7, the characteristic absorption of 2a (centered at 425 nm) and 2b (centered at 440 nm) gradually decreases and a new red-shifted band (ca. 50 nm) concomitantly appears (Figure 2). The spectral variations were fitted to a 1:1 binding model with $K = (3.9 \pm 0.4) \times 10^4$ M\(^{-1}\) and $K = (2.3 \pm 0.2) \times 10^5$ M\(^{-1}\) for 2a and 2b, respectively.

To rationalize the observed selectivity for 2b, complementary $^1$H NMR (Figure 3) and isothermal titration calorimetry (ITC) (see Figure S3) experiments were carried out. The complexation-induced chemical shifts ($\Delta \delta$) observed upon addition of CB7 to 2a and 2b suggest that the amino groups and the respective phenyl ring are included within the cavity of the receptor (complementary 2D NMR experiments were carried out for complete assignment of the $^1$H NMR signals, see the Supporting Information). Nevertheless, the phenyl ring of 2a ($\Delta \delta = -0.586, -1.092, and -0.336$ ppm for protons g, f, and e, respectively) seems to be completely included in the hydrophobic cavity, whereas in the case of 2b ($\Delta \delta = -0.628, -0.638, -0.702, and 0.021$ ppm for protons h, g, f, and e), the diethylamino group is deeply enclosed in the CB7 cavity with the phenyl ring partially exposed to the solvent, as shown in Scheme 2.\(^{36}\)

On the other hand, ITC experiments afforded binding constants compatible with those obtained by UV−vis and revealed that the association process is enthalpy driven with a small unfavorable entropic component (Table 1). The higher enthalpic change observed for 2b is in line with the

![Figure 3. $^1$H NMR spectra of 2a (0.5 mM, pH = 9) and 2b (0.5 mM, pH = 7) in the absence and presence of CB7 (1.2 and 0.5 mM for 2a and 2b, respectively). pH = pH* + 0.4, where pH* is the direct reading taken from the pH meter; see ref 39.](image1.png)

**Scheme 2. Proposed Structures for the Inclusion Complexes Formed between 2a and 2b with CB7**

![Scheme 2](image2.png)
The values estimated from the observed complexation-induced chemical shifts observed in the 1H NMR (see Figure S7 and S8) for the protonated forms of 2a and 2b are similar for both compounds. Particularly, the signals of the aromatic protons of the aniline group are shifted upfield in both cases, whereas the magnitude of the shift is lower for diethylamino protons of 2b. Conversely, this suggests that the binding mode is similar for 2a in the basic and protonated forms, whereas in the case of 2b, the diethylamino group is displaced from the interior of the cavity to the proximity of the portals upon protonation. The co-conformational movement is due to a change in the complexation driving force from hydrophobic to electrostatic (ion–dipole), which is associated with a relevant energetic penalty. The net result is a lower binding constant for the protonated form of 2b and hence a downward pKa shift.

**CONCLUSIONS**

In conclusion, the present work shows that the general assumption regarding the selectivity of CB7 receptor for positively charged species has exceptions and reveals a simple structural motif to avoid or reverse this selectivity. This can be achieved by substitution of diethylamino by diethylamino groups in selected guests, leading to an inversion of the pH-dependent selectivity and consequently of the complexation-induced pKa shift. These results support the increasing evidence for the higher contribution of enthalpic hydrophobic effects over ion–dipole interactions in the complexation of specific guests with cucurbiturils.\(^{16,18}\) It is also remarkable that this effect arises from a small structural variation within similar guest molecules. In addition to an obvious fundamental interest, we envisage that such contrasting pKa shifts observed for a family of structurally similar guest molecules can find applications in several fields such as molecular machines, pH-driven self-sorting systems, or in selective supramolecular catalysis, where reactants might be not selectively recognized but selectively activated by complexation-assisted protonation.\(^{29,41}\)

**MATERIALS AND METHODS**

**Materials.** All solvents and chemicals employed for synthesis and for preparation of samples were of reagent grade and were used as received. Ultrapure Millipore grade...
water was used. Cucurbit[7]uril and flavlylium cations were available from previous studies.36

**Synthesis of 2a and 2b.** 2a and 2b were synthesized using a similar procedure (Scheme 3). 4′-(1-Sulfo-4-butyloxy)-acetophenone sodium salt (0.1 g, 0.34 mmol) and 4-(dimethylamino)benzaldehyde (0.051 g, 0.34 mmol) or 4-(diethylamino)benzaldehyde (0.060 g, 0.34 mmol) were dissolved in 0.4 mL methanol and the solution was cooled in an ice bath. After addition of 0.044 mL of 40% NaOH, the solution was allowed to warm to room temperature and stirred overnight. The reaction mixture was diluted in 5 mL of distilled water, neutralized with 1 M HCl, and extracted with diethyl ether. The aqueous phase was concentrated by evaporation, and the crude product was purified by reverse-phase (C18) column flash chromatography with gradient elution from 100% H2O to 70% H2O/30% CH3CN. After evaporation of the solvent and drying in high vacuum, 2a (0.094 g, 69% yield) and 2b (0.110 g, 75% yield) were obtained as orange solids.

**Methods.** The pH of the solutions was adjusted with HCl and NaOH and measured with a Crison basic 20+ pH meter. UV/vis absorption spectra were recorded using a Varian Cary 100 Bio or a Varian Cary 5000 spectrophotometer. NMR experiments were run on a Bruker AMX 400 instrument, operating at 400 MHz (1H) and 101 MHz (13C). The solutions for NMR were prepared in D2O and the pD adjusted with DCl water, neutralized with 1 M HCl, and extracted with diethyl ether. The aqueous phase was concentrated by evaporation, and the crude product was purified by reverse-phase (C18) column flash chromatography with gradient elution from 100% H2O to 70% H2O/30% CH3CN. After evaporation of the solvent and drying in high vacuum, 2a (0.094 g, 69% yield) and 2b (0.110 g, 75% yield) were obtained as orange solids.

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