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Pronounced pH effects on the kinetics of cucurbit[7]uril-based pseudorotaxane formation and dissociation†

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Deprotonation of the two terminal COOH groups on a 4,4′-bipyridinium linear derivative leads to a pronounced slow down on the kinetics of threading and unthreading by the cucurbit[7]uril host.

Among the many applications of the cucurbit[*n*]uril (**CBn**) hosts,[1] their use as 'wheels' in rotaxanes and pseudorotaxanes is particularly attractive.[2] The rigid structures of **CBn** hosts and their high association equilibrium constants with cationic compounds lead to the favourable threading of suitable elongated guests ('axles'), resulting in highly stable pseudorotaxane inclusion complexes. If required, rotaxanes can also be prepared by further synthetic elaboration. Recently, we investigated a series of pseudorotaxanes[3] formed by cucurbit[7]uril (**CB7**), as the wheel component, and various threading guests containing a 4,4′-bipyridinium (viologen) central unit connected to two identical carboxylic acid-terminated, aliphatic *N*-substituents (see Fig. 1 for an example). We found that, under acidic conditions, when the two terminal –COOH units are protonated, the **CB7** host shuttles back and forth between the two aliphatic ends of the guest. However, if the pH of the solution is raised enough to cause the deprotonation of the terminal carboxylates, the **CB7** host locates itself around the aromatic viologen unit, residing on this central binding site and discontinuing the shuttling motions between the terminal aliphatic sites. In other words, the **CB7**-based pseudorotaxane behaves like a molecular shuttle[4] at low pH, while **CB7** simply engulfs the central viologen unit at high pH. Excess **CB7** at low pH gives rise to the formation of a 2 : 1 complex. A crucial factor underlying these findings is the existence of substantial electrostatic repulsions between the carboxylate negative charges on the ends of the axle component and the carbonyl oxygens on the two identical portals of the **CB7** host. Here, we report more detailed results on the kinetic and thermodynamic parameters describing the binding interactions between **CB7** and a representative COOH-terminated viologen guest (compound $\mathbf{1}^{2+}$). Conceptually related work on the pH dependence of **CB6** slippage over amine/ammonium groups has been reported by the groups of Nau[5] and Masson.[6]

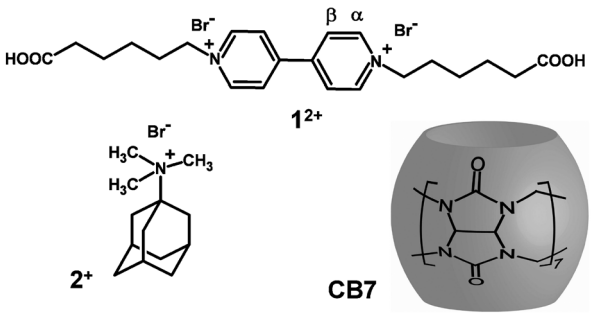

Fig. 1 Structures of the guests (viologen $\mathbf{1}^{2+}$ and adamantane $\mathbf{2}^{+}$) and the **CB7** host used in this work.

The formation of the different **CB7**·$\mathbf{1}^{2+}$ adducts can be followed by means of UV-Vis spectroscopic titrations.[3] The experiments were performed in aqueous solution also containing 50 mM NaCl, in order to control the concentration of Na^+ in solution.[7] The absorption spectrum of $\mathbf{1}^{2+}$ shows the typical absorption band of viologen derivatives, with a maximum at 261 nm, whose shape and intensity are independent of the pH of the solution. Titration experiments of $\mathbf{1}^{2+}$ with **CB7** have been performed at several pH values, ranging from 2 to 7. At pH values between 2 and 4 the titration curves show two distinct segments, consistent with the formation of two complexes between **CB7** and $\mathbf{1}^{2+}$, namely the 1 : 1 and 2 : 1 adducts (Fig. 2). At pH values comprised between 5 and 7 the titrations suggest the formation of a single adduct, with 1 : 1 stoichiometry (Fig. 2). At higher pH values, the spectroscopic changes take place so slowly that titration experiments become impractical.

The association constants determined from the titration experiments are gathered in Table 1. At pH $\leq$ 4 the two carboxylic groups at the extremities of $\mathbf{1}^{2+}$ are protonated, therefore, both aliphatic chains are available for the interaction with **CB7**. The association constant for the formation of the 1 : 1 adduct is on the order of 3×10^6 M^{-1}. The binding constant for the 2 : 1 adduct is around 2×10^4 M^{-1}, thus denoting that the system is anti-cooperative, that is, after complexation of the first aliphatic site, the second one is less accessible to **CB7**.

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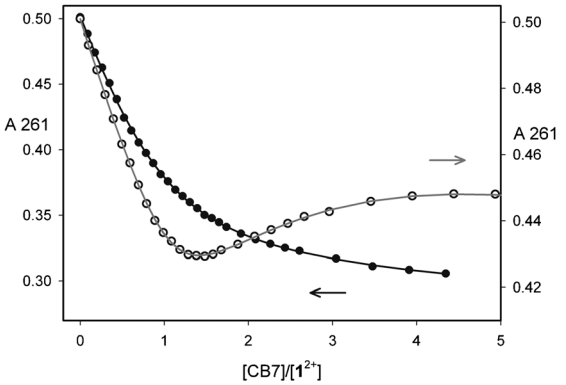

Fig. 2 Spectrophotometric titration curves of a 2.1 × 10^{-5} M solution of $\mathbf{1}^{2+}$ with **CB7** at pH 4 (circles) and pH 7 (dots). The solid lines represent the fittings of the experimental data.

Table 1 Solution pH dependence of thermodynamic and kinetic parameters for the complexation of $\mathbf{1}^{2+}$ with **CB7** in aqueous solution at 25 °C

pH	K_1[a]/M^{-1}	K_2[b]/M^{-1}	k_{on}[c]/$M^{-1}s^{-1}$	k_{off}[d]/s^{-1}	k_{on}/k_{off}
2	3 × 10^6	3 × 10^4	1.4 × 10^6	0.45	3 × 10^6
3	3 × 10^6	1 × 10^4	—	—	—
4	3 × 10^6	3 × 10^4	—	—	—
5	5 × 10^5	—	—	—	—
6	2 × 10^5	—	7.5 × 10^3	0.02	4 × 10^5
7	2 × 10^5	—	—	—	—
11	—	—	0.6	3.9 × 10^{-6}	2 × 10^5

[a] Equilibrium constant for the formation of the 1 : 1 complex between **CB7** and $\mathbf{1}^{2+}$. [b] Equilibrium constant for the formation of the 2 : 1 complex between **CB7** and $\mathbf{1}^{2+}$. [c] Rate constant for the formation of the 1 : 1 complex between **CB7** and $\mathbf{1}^{2+}$. [d] Rate constant for the dissociation of the 1 : 1 complex between **CB7** and $\mathbf{1}^{2+}$ upon addition of $\mathbf{2}^{+}$.

On increasing the pH value, the –COOH units are deprotonated and the aliphatic chains no longer constitute a good binding station for the **CB7** host. Under these conditions, **CB7** interacts with the viologen unit. Between pH 5 and 7 the titration experiments are consistent with the formation of a 1 : 1 adduct, in which the **CB7** resides on the bipyridinium unit of the axle. The binding constant is obviously lower relative to that for the 1 : 1 adduct in which the macrocycle resides on the extremities of the axle, even taking into account the statistical factor, and was found to be between 1.5 and 5 × 10^5 M^{-1} (in line with literature data).[8]

In order to study the unthreading of the **CB7**·$\mathbf{1}^{2+}$ pseudorotaxane, we used the cationic adamantane derivative $\mathbf{2}^{+}$ as a competitive guest (Fig. 1).[9] This is necessary to create a driving force for the unthreading process (**CB7** dissociation). We performed titration experiments of a solution containing $\mathbf{1}^{2+}$ and **CB7** in 1 : 1.2 ratio, in the pH range between 2 and 7. As expected on the basis of the high affinity of $\mathbf{2}^{+}$ for **CB7** (binding constant up to 10^{12} M^{-1}),[7] the dissociation of the **CB7**·$\mathbf{1}^{2+}$ adduct is quantitative.

We carried out threading and unthreading kinetic experiments at three paradigmatic pH values, that is, pH 2, 6, and 11. At pH 2 and 6 the experiments have been performed by means of the stopped flow apparatus, whereas at pH 11 the processes are much slower, and were followed by means of a spectrophotometer. The rate constants determined from the kinetic experiments are gathered in Table 1. The kinetic traces (Fig. 3, and ESI†) show the absorption change at 261 nm; threading

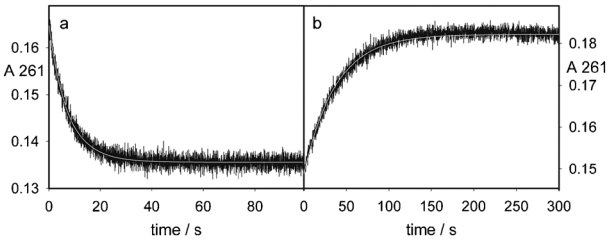

Fig. 3 Stopped-flow kinetic traces, recorded at 293 K and pH 6, for the absorbance change at 261 nm obtained upon mixing (a) $\mathbf{1}^{2+}$ and **CB7** (concentration after mixing 9 × 10^{-6} M) and (b) **CB7**·$\mathbf{1}^{2+}$ (9 × 10^{-6} M) and 4.5 equivalents of $\mathbf{2}^{+}$ (H_2O, 50 mM NaCl, path length = 1.0 cm). Black lines = experimental data, white lines = fitting.

experiments were performed by mixing equimolar amounts of $\mathbf{1}^{2+}$ and **CB7**, while unthreading experiments were carried out by mixing a solution of **CB7**·$\mathbf{1}^{2+}$ with an excess of $\mathbf{2}^{+}$. From the kinetic rate constants we can calculate the binding constant at pH 11, as k_{on}/k_{off} ~2 × 10^5 M^{-1}, which is consistent with **CB7** encircling the bipyridinium unit.[8,9]

At low pH values, when both –COOH units are protonated, the threading and unthreading processes are fast ($t_{1/2}$ for unthreading is 1.5 seconds). Under basic conditions, when both –COOH units are deprotonated, the threading process is *six orders of magnitude slower*, and $t_{1/2}$ for unthreading is 49 hours. Clearly, the charged $-COO^-$ units slow down the slipping of **CB7** over the ends of the axle. Nevertheless, the association constant is on the order of 10^5 M^{-1}, thus suggesting that the thermodynamics of the association is not influenced by the negative charges at the terminal points of the axle.

At intermediate pH values, NMR experiments and spectroscopic titrations indicate that **CB7** resides on the viologen unit. Spectroscopic pH titrations on $\mathbf{1}^{2+}$ suggest that the two –COOH units are independent, *i.e.*, they have the same pK_a. Therefore, at pH 6 a complex equilibrium exists involving completely deprotonated axles, partly deprotonated axles, and fully protonated axles. **CB7** can slip quickly through the protonated –COOH termini, but, with a pK_a of *ca.* 4.5,[10] (see also data in the ESI†) at pH 6 the ratio between COOH/COO^- units is around 1 : 20. Therefore both the threading and unthreading rate constants measured under these experimental conditions are apparent values, because they are influenced by the protonation–deprotonation equilibrium. Simple simulations of the kinetic experiments confirm this hypothesis (see ESI†).

^{1}H NMR spectroscopic data helped us to support the conclusions derived from the UV-Vis spectroscopic measurements. Unthreading of the **CB7** macrocycle from the **CB7**·$\mathbf{1}^{2+}$ pseudorotaxane in the presence of the competitive guest $\mathbf{2}^{+}$ was studied only at high pH as the process in acidic media was too fast to be monitored with this technique. At basic pH, the **CB7** host encircles the viologen unit of the guest, as evidenced by the significant downfield shift of the β* protons of the bound viologen guest, as compared to the free guest β protons (see Fig. 4). After addition of the competitive guest $\mathbf{2}^{+}$ to the pseudorotaxane-containing solution, the signals of the free viologen guest $\mathbf{1}^{2+}$ started to reappear while those corresponding to its bound form became weaker (Fig. 4). Simultaneously, the peak corresponding to the methyl protons (CH_3) on the free guest $\mathbf{2}^{+}$ is gradually replaced by a new upfield peak (CH_3^*) for the **CB7**-bound guest.

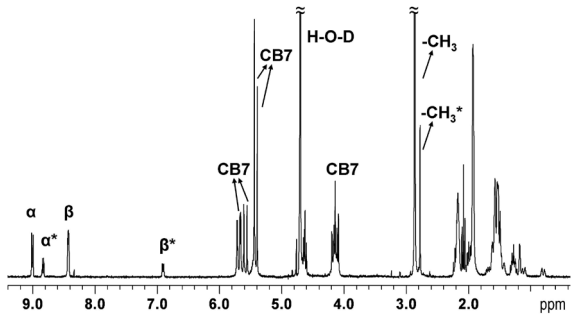

Fig. 4 ^{1}H NMR spectrum (300 MHz, D_2O–NaOD, pD 10.8) of the **CB7**·$\mathbf{1}^{2+}$ pseudorotaxane (3.54 mM) in the presence of guest $\mathbf{2}^+$ (7.24 mM) recorded 8 h after mixing.

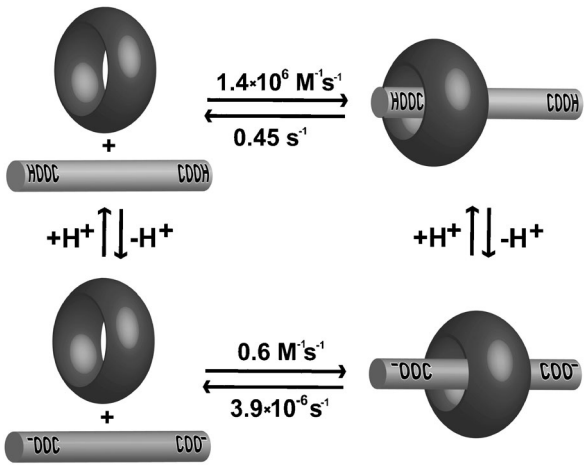

Fig. 5 Schematic representation of the pH effects on the kinetics of pseudorotaxane **CB7**·$\mathbf{1}^{2+}$ formation and dissociation.

The observed spectral changes correspond to the replacement of the viologen guest inside **CB7** by the cationic adamantane guest. The presence of two complexes, **CB7**·$\mathbf{1}^{2+}$ and **CB7**·$\mathbf{2}^+$, in the solution is also illustrated by the appearance of two separate sets of signals for each of the **CB7** protons. The complexation between **CB7** and both guests is slow on the NMR timescale. Thus, integration of a particular guest signal (β^* and CH_3^*) allowed us to follow the decrease of the pseudorotaxane concentration with time and to calculate the rate constant for the dissociation of the 1 : 1 complex between **CB7** and $\mathbf{1}^{2+}$. We performed four independent measurements in the pD range from 10.0 to 11.7. In this pD region the carboxylic groups are fully deprotonated, therefore, similar values for the pseudorotaxane dissociation constant were expected. Indeed, the obtained values are identical within experimental error and the average dissociation rate constant value is $(1.8 \pm 0.7) \times 10^{-5}$ s^{-1}. Although the dissociation rate constant from NMR experiments is about 4.5 times higher than the value calculated from UV-vis experiments (Table 1), we believe that these values are in relative good agreement considering the experimental differences between both techniques.

In conclusion, our experimental data show that deprotonation of the terminal carboxylic acid groups in the viologen guest $\mathbf{1}^{2+}$ leads to an extraordinary decrease in the kinetic rates of **CB7** based pseudorotaxane association and dissociation (Fig. 5). This considerable kinetic slow down is due to the electrostatic repulsions between the carbonyl oxygens lining the portals of the **CB7** cavity and the carboxylate negative charges, which create a substantial activation barrier to the slipping of **CB7** over any of the terminal carboxylate groups. From the experimental results presented here, we suggest that two very different kinetic regimes for **CB7**-based pseudorotaxane formation and dissociation are likely to exist with many other linear guests as long as they contain ionizable termini.

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