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Guest Binding Dynamics with Cucurbit[7]uril in the Presence of Cations

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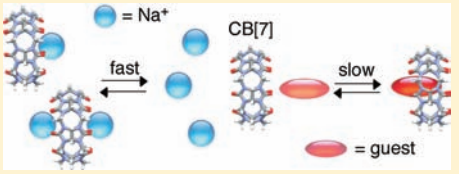
Supporting Information

ABSTRACT: The binding dynamics of *R*-(+)-2-naphthyl-1-ethylammonium cation (NpH^+) with cucurbit[7]uril (CB[7]) was investigated. Competitive binding with Na^+ or H_3O^+ cations enabled the reaction to be slowed down sufficiently for the kinetics to be studied by fluorescence stopped-flow experiments. The binding of two Na^+ cations to CB[7], i.e., CB[7]·Na^+ ($K_{01} = 130 \pm 10\ M^{-1}$) and Na^+·CB[7]·Na^+ ($K_{02} = 21 \pm 2\ M^{-1}$), was derived from the analysis of binding isotherms and the kinetic studies. NpH^+ binds only to free CB[7] ($(1.06 \pm 0.05) \times 10^7\ M^{-1}$), and the association rate constant of $(6.3 \pm 0.3) \times 10^8\ M^{-1}\ s^{-1}$ is 1 order of magnitude lower than that for a diffusion-controlled process and much higher than the association rate constant previously determined for other CB[*n*] systems. The high equilibrium constant for the NpH^+@CB[7] complex is a consequence of the slow dissociation rate constant of $55\ s^{-1}$. The kinetics results showed that formation of a complex between a positively charged guest with CB[*n*] can occur at a rate close to the diffusion-controlled limit with no detection of a stable exclusion complex.



INTRODUCTION

Cucurbit[*n*]urils (CB[*n*]s, Chart 1 for CB[7]) are unusual macrocylic hosts[1,2] because of the high equilibrium constants that can be achieved for their host−guest complexes.[3−8] Cucurbit[6]uril (CB[6]) was first synthesized over a hundred years ago[9] but was only characterized in the 1980s.[10−12] Development of the synthesis and purification of CB[*n*]s with different sizes[13,14] led to the widespread use of these supramolecular host systems in applications, such as self-sorting experiments,[4,15−17] catalysis,[11,18−25] porous materials,[26] supramolecular tandem enzyme assays,[27−29] protein capturing,[30] formation of supramolecular hydrogels,[31] electrochromism on TiO_2 films,[32] control of aggregate formation,[33,34] and drug stabilization or delivery systems.[35−46]

CB[*n*]s are torus-shaped host molecules with two equally sized portals, which are lined with carbonyl groups. One intriguing property of CB[*n*]s is their ability to form complexes with equilibrium constants[3−8,47,48] which are much higher than the host−guest equilibrium constants for other macrocyclic hosts,[47] such as cyclodextrins.[49,50] CB[*n*]s have two main molecular recognition elements: The carbonyl groups at the portals bind cations, while the guest's hydrophobic moieties are stabilized inside the cavity. The size of the portal and the volume of the interior cavities of CB[*n*]s vary depending on the number of glycoluril units, and the diameter of the interior of CB[*n*]s is larger than the diameter at the portals. In the case of CB[7] used in this work, the internal diameter is 7.3 Å, while the diameter for the portal is 5.4 Å (Chart 1).[13]

Molecular systems are characterized by structural determinations and thermodynamic studies. Kinetics play a role when there is interest on the reactivity of a molecule. In contrast, the dynamics of supramolecular systems are an inherent property that needs characterization because supramolecular systems are always reversible.[51−53] CB[*n*]s are no exception, and the importance of understanding the binding dynamics of guests with CB[6] was pointed out early on.[12] The complexation dynamics of guests with CB[*n*]s span a wide time range. Competition experiments using NMR showed that the dissociation of the guest is rate limiting, and the dissociation rate constant was influenced by the size of the guest.[11,12] The lifetimes of the complexes, defined as the inverse of the dissociation rate constants, for alkyl and aryl ammonium ions with CB[6] were between minutes to hours. The association rate constants between these guests and CB[6] were many orders of magnitude lower than for a process controlled by diffusion.[11,12] Kinetic studies for the binding of guests to CB[6] showed that cationic guests have slower dynamics than the neutral equivalents.[54] In the case of cationic guests, an exclusion complex, where only the positive charge interacts with the carbonyl groups at the portal of the CB[6], is formed in a fast reaction followed by the slow inclusion process of the hydrophobic moiety of the guest.[55,56] The presence and the nature of cations that bind to the portals of CB[6] affect the binding dynamics of guests.[54−57] Binding was

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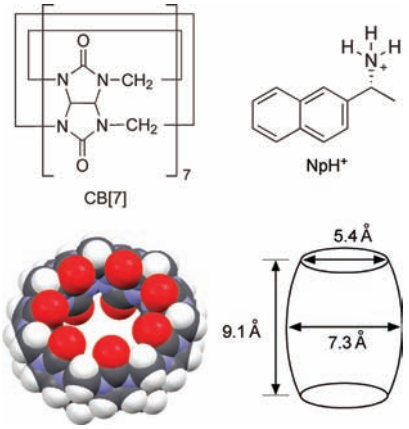
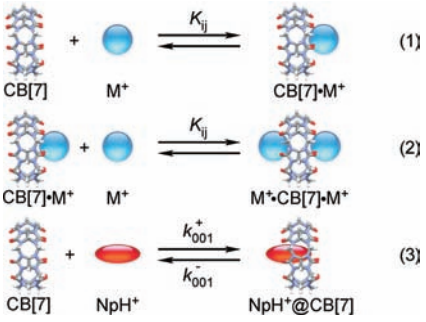
Chart 1. Structures for the Host Cucurbit[7]uril (CB[7], left) and the Guest *R*-(+)-2-Naphthyl-1-ethylammonium Cation (NpH^+, top right)[a]



[a]The dimensions for the portal diameter, inner cavity diameter, and height of CB[7] are shown in the bottom right corner.

Scheme 1. Mechanism for the Binding of NpH^+ with CB[7] in the Presence of H_3O^+ or Na^+ Cations (M^+)[a]



[a] The numbered subscripts correspond to the stoichiometries of the cations or guest bound to the CB[7] species shown on the right side of the equilibrium. From the left to the right the subscripts correspond to H_3O^+ ($i = 1$ or 2), Na^+ ($i = 0$ and $j = 1$ or 2), and NpH^+. Any "zeros" not followed by an integer are not shown. The symbol "•" indicates the binding of a cation to the portal of the CB[7], i.e., the formation of an exclusion complex. The symbol "@" indicates the formation of an inclusion complex.

proposed to occur to free CB[6] without any bound cation[55] or simultaneously to free CB[6] and CB[6] bound to one cation.[56]

All kinetic experiments for guest binding to CB[*n*]s were reported for CB[6] where slow dynamics were observed. NMR experiments provide examples where the host–guest system involving CB[*n*]s is under fast exchange,[4,12,39,43,58–61] indicating that the dynamics occur in milliseconds or faster. The objective of this work was to determine the kinetics of a CB[*n*] system with fast dynamics and compare its complexation mechanism with those proposed for complexes with slower dynamics. The dynamics of host–guest complexes are studied in real time, and the time-scale for the dynamics dictates the methodology employed. Dynamics in microseconds are studied by following the kinetics of a triplet state using laser flash photolysis, while dynamics in milliseconds are studied with stopped-flow experiments where changes in absorption or fluorescence intensities

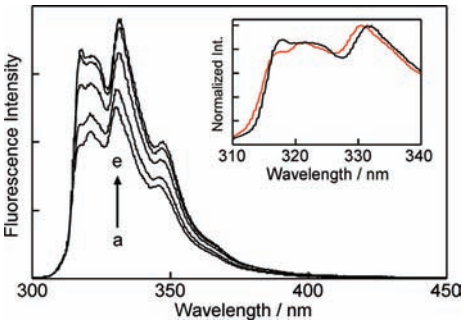


Figure 1. Fluorescence emission spectra for NpH^+ (50 μM) in a 0.20 M HCl aqueous solution in the absence (a) and presence of CB[7] (b) 9.1, (c) 27, (d) 46, and (e) 100 μM. The inset shows an expanded region for the normalized spectra of NpH^+ in the absence of CB[7] (red) and in the presence of 100 μM CB[7] (black).

are followed.[51,52,62] At the onset of this project, the time domain for the host–guest dynamics was unknown, and a guest with the naphthalene moiety was chosen because its photophysics was suitable for both types of experiments. The guest, *R*-(+)-2-naphthyl-1-ethylammonium cation (NpH^+, Chart 1) contains the naphthyl moiety, which is expected to be included inside the cavity, and a positively charged moiety, which interacts with the carbonyl groups at the portals of CB[7].

RESULTS

Adopted Mechanism. The presence of cations needs to be considered when studying the binding dynamics of guests with CB[*n*]s. The studies described below are consistent with a mechanism in which the sequential binding of two cations (Na^+ or H_3O^+) occurs (eqs 1 and 2, see Scheme 1) and the guest binds to free CB[7] (eq 3, see Scheme 1). The mechanism shown in Scheme 1 will be used in the description of the results, and the elimination of competitive mechanisms will be discussed in a later section.

Characterization of the NpH^+@CB[7] Complex. All experiments were performed at a pH where NpH^+ is protonated (pK_a = 7.6).[63] Complexation of NpH^+ to CB[7] led to a fluorescence increase of NpH^+ and to a change in the shape of the emission spectrum (Figure 1). Singlet excited state lifetimes of guests are frequently diagnostic for the formation of supramolecular complexes, leading to a lengthening of this lifetime when the guest is bound in supermolecules.[53,64] The lifetime for NpH^+ in water was measured as 36.2 ± 0.4 ns, while in the presence of CB[7] ($[NpH^+]_T = 10\ \mu M$; $[CB[7]_T] = 18\ \mu M$; and $[H_3O^+] = 2.0$ mM), the lifetime was determined to be 62.6 ± 0.6 ns. These decays followed a monoexponential function in both cases, suggesting that all NpH^+ was bound to CB[7] for the CB[7] concentration employed. The lifetimes for the excited singlet states are too short for the relocation of the excited guests to occur during their lifetimes.[53] At lower concentrations of CB[7] ($[NpH^+]_T$ = 10 μM; $[CB[7]_T] = 5\ \mu M$; $[H_3O^+] = 0.2$ mM), where not all NpH^+ is bound, the fluorescence decay did not follow a monoexponential function, suggesting that more than one NpH^+ species was detected. The fit to the sum of two exponentials led to lifetimes of 36 and 63 ns with pre-exponential factors of 0.32 and 0.68, respectively, showing that a portion of NpH^+ was free in water while another portion was bound to CB[7]. The significant lengthening of the fluorescence lifetime is similar to that observed

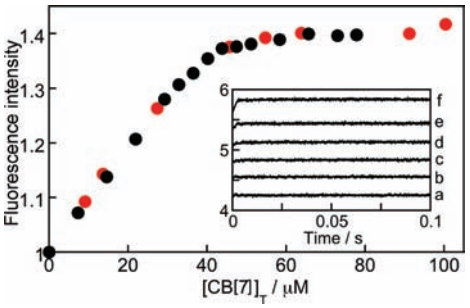


Figure 2. Dependence for the changes in the emission intensity measured by steady-state fluorescence (red circle) or stopped-flow measurements (black circle) with the addition of CB[7] to 50 μM NpH^+ in the presence of 0.2 M H_3O^+. The intensities in the absence of CB[7] were normalized to 1. The inset shows the stopped-flow traces for NpH^+ (50 μM) mixing with CB[7] in the presence of 0.2 M H_3O^+. [CB[7]] = 0 (a), 7.3 (b), 14.6 (c), 21.9 (d), 29.2 (e) and 43.8 μM (f).

for naphthalene derivatives bound to other macrocycles, such as cyclodextrins,[65−68] and is consistent with the inclusion of the naphthalene moiety of NpH^+ inside the CB[7] cavity.

The absorption spectra for NpH^+ and Np are the same (Figure S1, Supporting Information), while a change in the shape of the fluorescence spectra is observed for these two species (Figure S2, Supporting Information). In addition, the lifetime for Np was determined to be 2.7 ± 0.1 ns consistent with an intramolecular quenching mechanism when the nitrogen on Np is not protonated. Complexation of protonated guests to CB[*n*]s was shown to lead to an increase for the guest's pK_a.[69−71] The shape of the fluorescence spectrum of NpH^+ bound to CB[7] changes when compared to the spectrum free in water, suggesting that the environment around the ammonium cation is changed in the host−guest complex (inset Figure 1), probably due to the ion−dipole interaction between the carbonyl groups on CB[7] and the ammonium moiety on NpH^+.

At high photon flux and in the presence of oxygen, the slow formation of a fluorescent product was observed (Figure S3, Supporting Information). The formation of this product is completely inhibited when the photon flux is kept low, making it possible to use aerated solutions for the kinetic experiments. In addition, the use of deaerated solutions is not possible with the stopped-flow system, since consistent deaeration cannot be achieved. The different concentrations of oxygen between experiments lead to large changes in the fluorescence intensities, precluding intensity comparisons, such as those shown in Figure 2.

Values for the overall (β) or individual (K) equilibrium constants were recovered from binding isotherms where the change in the NpH^+ emission intensity with the concentration of CB[7] was measured either by steady-state fluorescence experiments (Figure 1) or from the intensity increase observed in the stopped-flow experiments after completion of the kinetics (inset Figure 2). The intensity changes observed for these two types of experiments were the same (Figure 2), indicating that the kinetics of complex formation were finished within 0.1 s. This is an important result in view of kinetic studies with CB[6] that showed that the kinetics for complex formation can occur in minutes to hours.[12,54] The results for NpH^+ binding with CB[7] suggest that the dynamics of guest complexation with CB[*n*]s occur over very different time domains depending on the structure of the guest and the size of the CB[*n*].

Scheme 2. Overall Equilibrium for the Binding of NpH^+ with CB[7]

$$CB[7]_{GF} + NpH^+ \underset{}{\overset{\beta_{11}}{\rightleftharpoons}} NpH^+@CB[7] \quad (4)$$

$$[CB[7]_{GF}] = [CB[7]] + [CB[7]{\bullet}Na^+] + [Na^+{\bullet}CB[7]{\bullet}Na^+] \quad (5)$$

The rate constants measured for the host−guest complex formation are always related to the sum of the association and dissociation processes.[62,72] The association process can be slowed by decreasing the concentration of the reagents since this reaction is a bimolecular process, while the dissociation process is unimolecular and cannot be influenced by changes in the concentration of reagents. Addition of cations that bind to the CB[7] portals decreased the concentration of free CB[7] available to bind NpH^+ and therefore decreased the rate for the association process (see below). The binding of Na^+ to CB[7] is more efficient than the binding of H_3O^+. For this reason, a lower Na^+ concentration is required to slow down the reaction sufficiently for the complexation kinetics to be studied by stopped flow. The binding isotherms saturated at higher CB[7] concentrations as the concentration of Na^+ was raised (Figure S4, Supporting Information), as is expected for the competitive binding of CB[7] with Na^+. The addition of 0.4 mM H_3O^+ in the presence of 0.2 M Na^+ did not alter the binding isotherm (Figure S4, Supporting Information), suggesting that at the low H_3O^+ concentrations required to protonate NpH^+, the binding of H_3O^+ to CB[7] can be ignored.

The binding isotherm for the change in the NpH^+ fluorescence intensity with the addition of CB[7] at a given Na^+ concentration corresponds to the overall binding where the concentration of guest free CB[7] ($[CB[7]_{GF}]$) is equal to the sum of free CB[7] and bound CB[7] to one or two Na^+ cations (Scheme 2).

The overall binding constant β_{11} is defined by eq 6 and is related (eq 7) to the equilibrium constants between CB[7] and one Na^+ cation (K_{01}), two Na^+ cations (K_{02}), and NpH^+ (K_{001}, see derivation in the Supporting Information):

$$\beta_{11} = \frac{[NpH^+@CB[7]]_{eq}}{[CB[7]_{GF}]_{eq}[NpH^+]_{eq}} \quad (6)$$

$$\beta_{11} = \frac{K_{001}}{1 + K_{01}[Na^+] + K_{01}K_{02}[Na^+]^2} \quad (7)$$

At low Na^+ concentrations, the last term in the denominator is small, and a linear relationship is obtained for the double reciprocal plot (eq 8). Binding isotherms were measured for Na^+ concentrations between 2 and 8 mM and were fit using a numerical analysis method (see the Supporting Information for the model used). The binding isotherms fit well assuming a 1:1 binding stoichiometry (Figures S5 and S6, Supporting Information), and the β_{11} values decreased from (8.3 ± 0.7) × 10^6 M^{-1} in the presence of 2 mM Na^+ to (5.2 ± 0.2) × 10^6 M^{-1} in the presence of 8 mM Na^+, while the relative quantum efficiency for bound NpH^+ with respect to aqueous NpH^+ remained unchanged (Table S1, Supporting Information). The double reciprocal plot is linear (Figure 3), and the values recovered from the fit to eq 8 were 130 ± 10 M^{-1} for K_{01} and

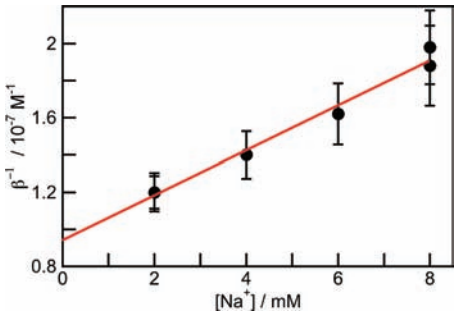


Figure 3. Dependence with the Na^+ concentration of the inverse of the overall binding constant for the formation of the Np@CB[7] complex.

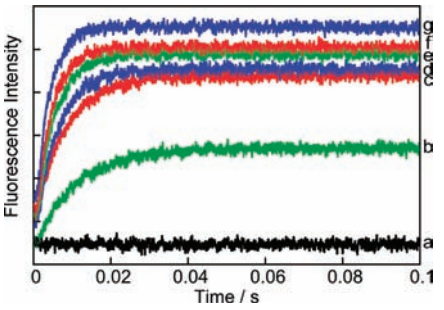


Figure 4. Kinetics for the formation of NpH^+@CB[7] complex by mixing a NpH^+ (0.5 μM)/Na^+ solution with a CB[7]/Na^+ solution at various CB[7] concentrations ([Na^+] = 100 mM and [H_3O^+] = 0.1 mM). $[CB[7]]_T$ = 0 (a, black), 2.5 (b, green), 5.0 (c, red), 7.5 (d, blue), 10.0 (e, green), 12.5 (f, red), and 15 μM (g, blue). All concentrations indicated are the final ones after mixing.

$(1.06 \pm 0.05) \times 10^7\ M^{-1}$ for K_{001}.

$$\frac{1}{\beta_{11}} = \frac{1 + K_{01}[Na^+]}{K_{001}} \qquad (8)$$

Kinetics for the Formation of the NpH^+@CB[7] Complex. The kinetics for the formation of the complex between NpH^+ and CB[7] measured by fluorescence stopped-flow experiments follow a monoexponential growth (Figure S7, Supporting Information), indicating that only one relaxation process was observed when the guest and host were mixed. This result shows that the binding of both Na^+ cations to CB[7] is a fast process and can be treated as pre-equilibrium steps. The observed rate constants (k_{obs}) are recovered by fitting the growth kinetics to eq 9, where I_0 and I_∞ are, respectively, the intensities at time zero and the total amplitude for the kinetics. Increasing concentrations of CB[7] led to an increase in the amplitude of the signal and to faster kinetics, i.e., higher k_{obs} values (Figure 4). The kinetics started at the intensity value for NpH^+ in water. The lack of an offset indicated that no fast kinetic process involving NpH^+ occurred within the 1 ms dead time of the stopped-flow experiment, and only one relaxation process needs to be considered.

$$I = I_0 + I_\infty(1 - e^{-k_{obs}t}) \qquad (9)$$

The expression for k_{obs} was derived for the mechanism shown in Scheme 1 by assuming that the total NpH^+ concentration is much lower than the total concentration for CB[7] (eq 10, see

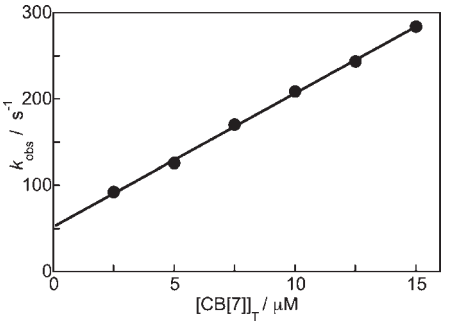


Figure 5. Dependence of the observed rate constant for the formation of the NpH^+@CB[7] complex with the concentration of CB[7] for the mixing of NpH^+ (0.5 μM) with CB[7] at constant Na^+ (100 mM) and H_3O^+ (0.1 mM) concentrations.

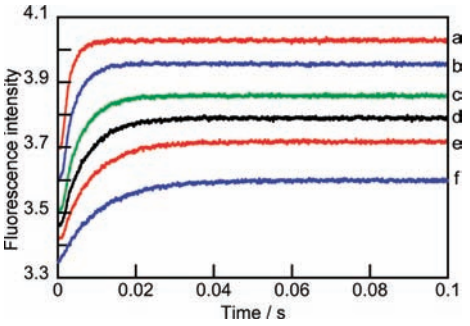


Figure 6. Kinetics for the formation of the NpH^+@CB[7] complex ($[NpH^+]_T$ = 1.0 and $[CB[7]]_T$ = 10 μM; and [H_3O^+] = 0.1 mM). [Na^+] = 0.050 (a, red), 0.075 (b, blue), 0.100 (c, green), 0.125 (d, black), 0.150 (e, red), and 0.200 M (f, blue).

derivation in the Supporting Information). The expression for the slope in eq 10 is dependent on the mechanism assumed, but the intercept is defined as k_{001}^-. The dependence of k_{obs} with the concentration of CB[7] was linear (Figure 5), and the average value recovered for k_{001}^- from 7 independent experiments was 55 ± 7 s^{-1}. These experiments were performed at Na^+ concentrations between 75 and 200 mM and at H_3O^+ concentrations of 0.1 or 1 mM (Table S2, Supporting Information). No dependence of the extrapolated k_{001}^- value was observed when the concentrations of Na^+ or H_3O^+ were changed.

$$k_{obs} = k_{001}^+ \frac{1}{1 + K_{01}[Na^+] + K_{01}K_{02}[Na^+]^2}[CB[7]]_T + k_{001}^- \qquad (10)$$

The kinetics for the formation of the NpH^+@CB[7] complex was studied by varying the Na^+ concentration (Figure 6). Conceptually, based on the mechanism in Scheme 1, an increase in the Na^+ concentration traps CB[7] as CB[7]·Na^+ and Na^+·CB[7]·Na^+ complexes, which are unavailable for binding with NpH^+. As a consequence, the association process was slowed down, and the final concentration of NpH^+@CB[7] was lower when the concentration of Na^+ was raised (Figure 6).

At all Na^+ concentrations the kinetics were first order (Figure S8, Supporting Information), and the k_{obs} values were recovered from the fit of the data to eq 9. Information on the association rate constant between NpH^+ and CB[7] (k_{001}^+) is contained in

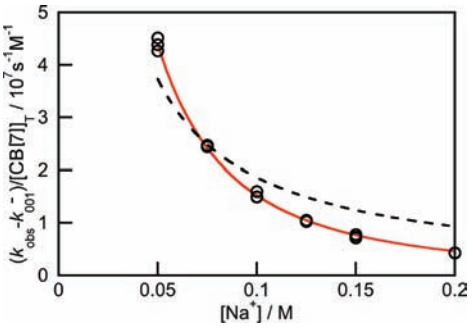


Figure 7. Dependence of the overall association rate constant for the formation of the NpH^+@CB[7] complex with the concentration of Na^+. Three independent experiments are shown for each Na^+ concentration. The solid red line corresponds to the fit of the data to eq 11. The dashed line corresponds to the fit of the data when the binding of only one Na^+ to CB[7] is considered.

the first term of eq 10. The value of k^-_{001} was determined independently, and the $[CB[7]]_T$ value is known. Equation 10 can be rearranged into eq 11, where the expression on the right side corresponds to the overall association rate constant. Three independent experiments at various Na^+ concentrations were performed at two different NpH^+ and CB[7] concentrations ($[NpH^+]_T$ = 1 and $[CB[7]]_T$ = 10 μM or $[NpH^+]_T$ = 0.5 and $[CB[7]]_T$ = 5 μM). The data fit well to eq 11 but did not fit if the binding of only one Na^+ was considered (Figure 7). This result shows that two Na^+ cations bind to CB[7]. The same conclusion is reached for a double reciprocal plot of eq 11 (Figure S9, Supporting Information), where a linear relationship was expected if only one Na^+ binds to CB[7], but a clear upward curvature was observed. Relatively large errors were recovered when k^+_{001}, K_{01}, and K_{02} were left as free parameters in the fit of the data to eq 11, but the value for K_{01} was always larger than for K_{02}. The value of K_{01} was determined independently from the determination of binding isotherms in steady-state fluorescence studies (see above), and the value of 130 M^{-1} was fixed for the fit of the data to eq 11. The recovered value for K_{02} was 21 ± 2 M^{-1}, and the value for k^+_{001} was (6.3 ± 0.3) × 10^8 M^{-1} s^{-1}. The equilibrium constant for the NpH^+@CB[7] complex (K_{001}) calculated from the ratio of k^+_{001} and k^-_{001} is (1.2 ± 0.2) × 10^7 M^{-1}, which agrees well with the value determined from the binding isotherm studies (1.06 ± 0.05) × 10^7 M^{-1}).

$$\frac{k_{obs}-k^-_{001}}{[CB[7]]_T} = \frac{k^+_{001}}{1 + K_{01}[Na^+] + K_{01}K_{02}[Na^+]^2} \qquad (11)$$

An alternate analysis to differentiate between the mechanism where one or two Na^+ cations bind to CB[7] is to simultaneously fit the kinetic data at the different Na^+ concentrations using a global analysis method. The values for k^-_{001} and K_{01} were fixed. A systematic deviation was observed for the residuals for the model where only one Na^+ binds to CB[7] (Figure S10, Supporting Information), while random residuals were observed for the model where two Na^+ cations bind (Figure S11, Supporting Information). Despite the larger errors recovered for k^+_{001} and K_{02}, the global analysis method is very diagnostic for the differentiation between the two mechanisms and supports the assignment that two Na^+ cations bind to CB[7].

The kinetics for the formation of the NpH^+@CB[7] complex were also investigated in the presence of H_3O^+. High concentrations of H_3O^+ (0.5 M − 2.0 M) were required to sufficiently slow down the reaction for the formation of NpH^+@CB[7] complex in the stopped-flow experiments. The results in the presence of H_3O^+ should be taken with caution because at the high concentrations of H_3O^+ used, the activity coefficients of the solutes are likely to be different from those in the experiments with Na^+, and the analysis used in both cases was based on concentrations of solutes. Qualitatively the same kinetic behavior as for Na^+ was observed when the concentration of CB[7] (Figure S12, Supporting Information) or H_3O^+ (Figure S13, Supporting Information) was varied. In all cases the kinetics followed a monoexponential function. The dependence of k_{obs} with the concentration of CB[7] is linear (Figure S14, Supporting Information), and a value of 36 ± 4 s^{-1} was recovered for k^-_{001} from a fit using an equation analogous to eq 10. The dependence of $(k_{obs} - k^-_{001})/[CB[7]]_T$ with the H_3O^+ concentration was only adequately fit if the binding of two H_3O^+ was considered (Figure S15, Supporting Information). Unfortunately the changes for β_{11} for the concentration range where only one H_3O^+ cation binds to CB[7] were too small for an independent determination of K_1 to be feasible. For this reason, the data were fit by assuming that the ratio between the equilibrium constants for the first and second cation binding was the same for H_3O^+ as observed for Na^+, i.e., K_1/K_2 is equal to K_{01}/K_{02}. Based on this assumption, the estimated values were 8 × 10^8 M^{-1} s^{-1} for k^+_{001}, 11 M^{-1} for K_1, and 2 M^{-1} for K_2. The calculated value from the ratio of k^+_{001} and k^-_{001} for K_{001} was 2 × 10^7 M^{-1}.

DISCUSSION

CB[n]s are sparingly soluble in water, and the enhancement of its solubility with the addition of acid and cations was established early on.[9] This enhancement is due to the formation of cation$_m$·CB[n] complexes, and it is the reason why many studies were performed in 1:1 formic acid:water mixtures,[10,11,55,56,73−75] especially for the less soluble CB[6] and CB[8]. The binding of cations to CB[n] can be competitive with the binding of guests, and in the presence of these competitive reactions, the overall equilibrium constants for guest@CB[n] formation can be changed with the addition of salts.[56,59,76−80]

Analysis of supramolecular systems containing CB[n]s needs to explicitly take into account the formation of the cation$_m$·CB[n] complexes. Nonlinear relationships between the overall binding constants with the Na^+ concentration were observed for CB[7] binding to berberine[79] or to the cationic form of neutral red.[80] These nonlinear relationships suggested that more than one cation was bound to CB[7]. The equilibrium constants determined for the binding of the first Na^+ with CB[7] were 120 M^{-1} in the study with berberine[79] and 80 M^{-1} for neutral red.[80] The binding constant for the second Na^+ cation was determined to be 20 M^{-1} in both studies. Considering two noninteracting binding sites, one would expect the binding constant for the second Na^+ to be four times lower than the binding constant of the first Na^+ cation.[81]

The equilibrium constant for the binding of Na^+ cations to CB[7] was determined from a combination of binding isotherm and kinetic studies. The value of K_{01} of 130 ± 10 M^{-1} was determined from the β_{11} values measured at low Na^+ concentrations. The linearity of the plot defined by eq 8 (Figure 3) suggests that the binding of the second Na^+ does not interfere with the determination of K_{01}. Indeed based on the K_{01} and K_{02} values determined, the fraction of Na^+·CB[7]·Na^+ for the total sodium

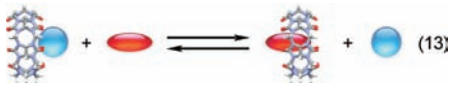
bound CB[7] is less than 8% for 8 mM Na^+. The K_{01} value determined is within the range of the values previously reported (80−120 M^{-1}).[79,80] The value of K_{02} of 21 ± 2 M^{-1} was determined from the fit of the kinetic data. This value is somewhat lower than the equilibrium constant expected based on statistical considerations (33 M^{-1}), which could have been interpreted as a small repulsive effect between the Na^+ cation bound to both portals of CB[7]. However, when the data in Figure 7 were fit by assuming a ratio of four between K_{01} and K_{02} very similar statistical parameters, i.e., χ^2 values and correlation coefficients, were obtained suggesting that we cannot differentiate between the K_{01}/K_{02} ratio obtained in our experiments (6.2 ± 0.8) and the theoretical ratio of 4. Therefore, any repulsion between the two Na^+, if it occurs, is minor. The K_{01} and K_{02} values recovered when the ratio between these two constants was 4 were, respectively, (92 ± 5) and (23 ± 1) M^{-1}.

The binding of H_3O^+ with CB[7] is much weaker than the binding of Na^+, and a higher concentration of H_3O^+ is required to achieve the same slow down for the formation of the NpH^+@CB[7] complex as observed in the presence of Na^+. Analysis of the kinetic data indicated that the binding of two H_3O^+ had to be considered. The estimates recovered from the fit of the kinetic data, 11 and 2 M^{-1}, are in line with the qualitative result that the binding affinity toward CB[7] is much lower for H_3O^+ than Na^+.

The equilibrium constants between guests and CB[7] determined in the presence of Na^+ are overall, sometimes called conditional, equilibrium constants (β). The comparison of data for different experimental conditions, i.e., different concentrations of Na^+, or the use of different metal cations needs to be taken into account when comparing binding efficiencies. In addition, knowledge of the role of the cation in the supramolecular system needs to be considered. In the case of CB[n]s with no included guest, the metal cations bind to the portals. However, the type of guest used defines the role of the metal cation.[56] For example, for neutral guests the cations were proposed to act as "caps" for the included guest,[56,75,78] while for neutral guests with metal binding abilities, such as carbonyl groups, the metal cations can lead to enhanced stability of the complex.[59,79,82] In the case of positively charged guests, the metal cation acts as a competitor[56,76−78,80] to the binding site at the portal, and one has to consider for monocationic guests if a metal cation is bound to the opposite portal of CB[7]. The different roles for the metal cations in the guest@CB[n] complexes make comparison at the same concentrations of metal cations difficult unless one can assume that the role of the cation is the same for the guests that are being compared. The kinetic studies in this work are consistent with a guest@CB[7] complex with no sodium bound (see below). The relationship (eq 12, which is derived from eq 7) between the guest@CB[7] equilibrium constant (K_{001}) and overall equilibrium constants (β_{11}) can be used to calculate K_{001} values without the independent determination of the equilibrium constants in the presence of different concentrations of Na^+. This calculation assumes that only the guest@CB[7] complex is formed, and an independent validation of such an assumption is required. A good test for this assumption would be to measure β_{11} at two different Na^+ concentrations and check if the same K_{001} values are recovered by using eq 12.

$$K_{001} = \beta_{11}[1 + (130 \pm 10)[Na^+] + (2.7 \pm 0.3) \times 10^3[Na^+]^2] \quad (12)$$

Scheme 3. Displacement Reaction between NpH^+ and CB[7]·Na^+



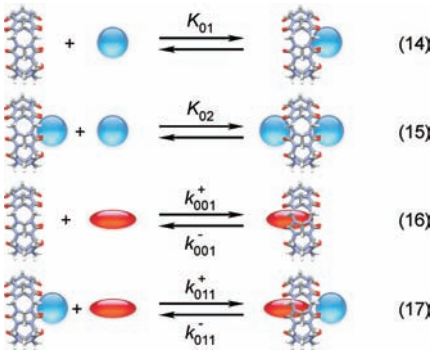
Comparisons of overall binding constants for aqueous solutions and formic acid/water solutions are difficult because of the different nature of the solvent and an uncertainty on the role that formic acid plays in the presence of guest@CB[n] complexes. However, β_{11} values have been determined frequently at given Na^+ concentrations. The β_{11} value for NpH^+ with CB[7] in the presence of 50 mM Na^+ is (7.4 ± 0.6) × 10^5 M^{-1}. This value is 1 order of magnitude lower than for 4-aminotoluene (8 × 10^6 M^{-1}) and slightly higher than for 4-aminomethyl-pyridine (3.6 × 10^5 M^{-1}) determined from NMR competition experiments in the presence of 50 mM Na^+.[4] The values for these aromatic guests are much lower than for bulkier and spherical monocation guests containing adamantane or ferrocene moieties determined for the same experimental conditions (>10^{11} M^{-1}).[4]

The measured relaxation process (k_{obs}) corresponds to the sum of the rate constants for the association and dissociation processes. In the case of a bimolecular reaction, the relaxation process is related to the product of the bimolecular rate constant and the equilibrium concentration of the reactants. Therefore, the dependence of a process with the concentration of reactants provides information on the molecularity of the reaction.

The dissociation rate constant of NpH^+ from the CB[7] complex (k_{001}^-) did not change when the concentrations of Na^+ was raised. This result shows that the dissociation reaction is unimolecular and eliminates the possibility that binding of Na^+ to the second portal of the NpH^+@CB[7] complex leads to the displacement of NpH^+ from the NpH^+@CB[7] complex (see below the discussion on the evidence why the NpH^+@CB[7]·Na^+ complex is not formed). In the case of such a displacement reaction, the dissociation term in k_{obs} was expected to increase when the concentration of Na^+ was raised. This result also eliminates, based on the microreversibility argument, the possibility that the NpH^+@CB[7] complex is formed from the reaction of NpH^+ with the CB[7]·Na^+ complex (eq 13, Scheme 3).

The binding dynamics of Na^+ with CB[7] was fast occurring on a time scale shorter than microseconds. This result is supported by electron paramagnetic resonance line broadening experiments, where K^+ was chelated to the carbonyl group of a guest inside CB[7]. The association rate constant for K^+ with CB[7] was determined to be ca. 10^{10} M^{-1} s^{-1}, while the dissociation rate constant was 10^7 s^{-1}.[82] Binding of cations to the portals of CB[7] is likely faster when there is no stabilization by a guest, leading to higher dissociation rate constants. Therefore the binding of Na^+ occurs on the nanosecond time scale. The formation of the complex between NpH^+ and CB[7] is a bimolecular process, and in principle, the complex could be formed with free CB[7] and with the various CB[7]·$(Na^+)_m$ species. The presence of Na^+ was shown to slow down the relaxation reaction (Figure 6), suggesting that the presence of this cation affected the association process. The smaller amplitude for the kinetics in the presence of Na^+ cations indicates that the concentration of complexed NpH^+ is lower in the presence of added Na^+ cations. Both these observations are consistent with

Scheme 4. Mechanism for the Simultaneous Binding of NpH^+ with CB[7] and CB[7]·Na^+



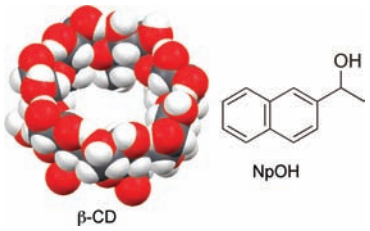
the "trapping" of CB[7] in an unreactive form toward NpH^+. However, this qualitative analysis does not indicate if the unreactive host is CB[7]·Na^+, Na^+·CB[7]·Na^+, or both. Binding of NpH^+ to Na^+·CB[7]·Na^+ would lead to the displacement of one Na^+ and such a mechanism can be eliminated since the value for the dissociation rate constant did not depend on the concentration of Na^+ (see above). In addition, if NpH^+ was bound to Na^+·CB[7]·Na^+, the final concentration of complexed NpH^+ should not diminish in the presence of Na^+. Binding of NpH^+ to CB[7] leads to an increase in the fluorescence intensity and lengthening of the excited-state lifetime for NpH^+. Only one lifetime was observed when all NpH^+ was bound, indicating either that only one complex was formed or that, in the case of simultaneous formation of NpH^+@CB[7] and NpH^+@CB[7]·Na^+, the lifetime for NpH^+ in both complexes is the same. In the latter case the two complexes cannot be differentiated in the kinetic experiment. One relaxation process would be observed for the simultaneous binding of NpH^+ with CB[7] and CB[7]·Na^+ because CB[7] and CB[7]·Na^+ are in fast equilibrium, and the binding of the second Na^+ would lead to the unreactive CB[7] species (Scheme 4). It is important to note that the binding of two Na^+ cations in fast pre-equilibrium is the same for the different mechanisms shown in Schemes 1 and 4. The difference between these two mechanisms is that NpH^+ binds to CB[7] only in one of them (Scheme 1), while it binds simultaneously to CB[7] and CB[7]·Na^+ (Scheme 4) in the second mechanism. The dissociation rate constants for NpH^+ from NpH^+@CB[7] and NpH^+@CB[7]·Na^+ for the mechanism shown in Scheme 4 (eqs 14−17) were assumed to be the same since no dependence with the Na^+ concentration was observed for the dissociation process (see eq. S56, Supporting Information for the case where the dissociation rate constants are different). The association process for the mechanism in Scheme 4 is given by eq 18 (see Supporting Information for derivation), where the parameter n is a multiplier that relates k^+_{001} with k^+_{011}. On statistical grounds the maximum value for n is 0.5 since only one portal is available for the binding of NpH^+ with CB[7]·Na^+ when compared to the binding with CB[7]. The data were fit to eq 18 by fixing the values for K_{01}, K_{02} and k^-_{001}, and by incrementally increasing the value of n. The fits with an n value of 0.5 were very similar to the fit assuming that only one Na^+ binds to CB[7]. This result is reasonable since if the values of k^+_{001} and k^+_{011} were similar, then the binding to CB[7]·Na^+ would predominate because of its much higher concentration when compared to CB[7] and the reaction leading to the slow down of the kinetics would be the binding to CB[7]·Na^+ of the second Na^+ cation. This mechanism is equivalent to the binding of only one cation to CB[7]. Adequate fits of the data to eq 18 were obtained for n values of 0.025, 0.020, and 0.01 when K_{02} was respectively 50, 33, and 20 M^{-1} (see Figure S16, Supporting Information). The value of 33 M^{-1} corresponds to the maximum value expected considering the K_{01} value determined, and the other two values of K_{02} were used to determine the sensitivity of the fit to the variation in this equilibrium constant. This analysis shows that the value for the association rate constant of NpH^+ with CB[7]·Na^+ is at least 40 times lower than with CB[7] and can be considered negligible. The reasons for the lower association rate constants of NpH^+ when a Na^+ cation is bound with CB[7] are speculative at this point. One contribution could be charge repulsion between NpH^+ and Na^+ because the CB[7] cavity is filled with an aromatic moiety and not with water, which shields charges to a greater extent. A second possibility is that the naphthyl moiety is sufficiently long to sterically impede the binding of the Na^+ cation to the second portal. The latter suggestion is supported by the observation that increasing the length of alkyl ammonium cations leads to the displacement of the second Na^+ cation on CB[6] when the alkyl group is sufficiently long to span the cavity of CB[6].[78]

$$\frac{k_{obs} - k^-_{001}}{CB[7]_T} = \frac{k^+_{001}(1 + nK_{01}[Na^+])}{1 + K_{01}[Na^+] + K_{01}K_{02}[Na^+]^2} \qquad (18)$$

Several kinetic studies of guests with CB[6] were previously reported.[11,12,54−57] Competition experiments where the rate limiting step is the dissociation of a guest showed that the dissociation dynamics of alkyl and arylammonium cations included in CB[6] is slow ($10^{-5} - 10^{-2}$ s^{-1}) and depends on the size of the guest.[11,12] The association process is also slow, with rate constants (1−6000 M^{-1} s^{-1})[11,12] many orders of magnitude smaller than the diffusion-controlled rate constant for a bimolecular reaction in water (6.5×10^9 M^{-1} s^{-1}, 20 °C).[83] The complexation dynamics of 4-methylbenzylammonium cation with CB[6] was studied in 1:1 water/formic acid[11,55] and in 1 M aqueous HCl solutions.[57] The dynamics were faster for the reaction in aqueous HCl solutions[55,57] showing that experimental conditions, such as the presence and concentrations of H_3O^+ cations and the total concentration of guest and CB[6], affect the binding kinetics. In formic acid/water the dependence of the observed rate constant with the concentration of reagents showed a downward curvature,[55] which is consistent with the formation of an exclusion complex (guest·CB[6]), where the positive charge of the guest interacts with the carbonyl groups of the CB[6] portal followed by the inclusion of the guest into the cavity to form the guest@CB[6] complex.

The kinetics for the binding of cyclohexylmethylamine with CB[6] was studied for the protonated and neutral guests.[54] The mechanism for the binding dynamics is affected by the presence of the positive charge. The neutral guest enters the cavity directly, while the ammonium cation first forms an exclusion complex followed by a unimolecular inclusion, "flip−flop", reaction. Detailed mechanistic studies of this system showed that Na^+ cations led to a slow down of the kinetics because of the competitive binding between the guest and Na^+ to the CB[6] portal.[56] The kinetic data are consistent with the binding of the guest to CB[6] and CB[6]·Na^+, which contrasts to the proposed exclusive binding of 4-methylbenzylammonium cations to free CB[6].[55] The extrapolated values in pure aqueous solutions for

Chart 2. Structure of β-Cyclodextrin (β-CD) and 2-Naphthyl-1-ethanol (NpOH)



the association and dissociation rate constants of cyclohexylmethylammonium cation with CB[6] were 0.44 M^{-1} s^{-1} and 4×10^{-6} s^{-1}, respectively.[56]

The binding studies for NpH^+ with CB[7] showed that cationic guests can form complexes without the detection of the intermediate exclusion complex, as a linear dependence was observed for the relaxation rate constant with the CB[7] concentration. Therefore, formation of a complex between a cationic guest and CB[*n*]s does not necessarily proceed through a two-step mechanism. An encounter complex is always formally formed in a bimolecular reaction, but the detection of this encounter complex as an intermediate depends on the relative magnitude of the rate constant for the reaction in the encounter complex, i.e., inclusion into the CB[7] cavity, and the dissociation of the encounter complex. The lack of the detection of the $NpH^+ \cdot$CB[7] exclusion complex when compared to cyclohexylmethylammonium$\cdot$CB[6] (or guest$\cdot$CB[6]$\cdot Na^+$) is due to the inherently faster inclusion of NpH^+ into CB[7]. The association rate constant of NpH^+ with CB[7] of 6.3×10^8 M^{-1} s^{-1} is much higher than the association rate constants measured for CB[6] as described above, suggesting that inclusion of NpH^+ into the CB[7] is not significantly impeded. The fact that the association rate constants of guests with CB[*n*]s span by more than 10^9 orders of magnitude shows that any generalization of the complexation mechanism of guests with CB[*n*]s is premature and further structure−dynamics relationship studies will be required before a comprehensive mechanistic understanding will emerge.

CB[*n*]s form host−guest complexes with much higher equilibrium constants[3−8,48] than observed for other macrocycles.[47] The binding constants of 10^{12} to 10^{15} M^{-1} observed for some CB[*n*] complexes are similar to the binding constants observed for biological systems with high affinities, such as biotin−avidin.[84] In contrast, the high equilibrium constants for other macrocycles, such as cyclodextrins (CDs), are of the order of 10^5 to 10^7 M^{-1}.[47,49,50] A comparison of the binding of guests to CDs and CB[*n*]s is instructive since these macrocycles have similar cavity volumes, i.e., 279 Å^3 for CB[7][13] and 263 Å^3 for β-CD (Chart 2).[85] CB[*n*]s are longer (9.1 Å)[13] than CDs (7.8 Å).[85] Other structural features that are different between these two macrocycles are that CDs have wide portals lined with hydroxyl groups, while the portals of CB[*n*]s are narrower than their internal cavities, and the portals are lined with carbonyl groups. In addition, the two portals for CB[7] are the same, while in the case of CDs the two portals are of different sizes, where the larger one is lined with secondary hydroxyl groups, while the smaller portal is lined with primary hydroxyl groups. The driving force for guest binding with CDs is the hydrophobic effect, while in the case of CB[*n*]s, in addition to the hydrophobic effect, there is also the possibility of charge−dipole interactions between the positive charges on the guest and the carbonyl groups. It is important to note that with both hosts solvation of the host and guest also needs to be considered. Comparative studies for binding of the same guests to β-CD and CB[7] showed that the neutral form of a guest is preferentially bound to β-CD, while the protonated guests do not bind to β-CD and bind strongly to CB[7].[70,86]

The association rate constants for the formation of 1:1 complexes between CDs and guests that fit within its cavity were determined to be of the order of 10^8 to 10^9 M^{-1} s^{-1},[51,62,67,72,87−89] while lower rate constant are observed when the fit of the guest with the cavity is not optimal.[51,62,72,90] The association rate constant for NpOH (Chart 2) with β-CD[67] is 3×10^8 M^{-1} s^{-1}, which is very similar to the association rate constant measured between CB[7] and NpH^+ (6.3×10^8 M^{-1} s^{-1}). These rate constants are 1 order of magnitude lower than the rate constant for a diffusional process in water (6.5×10^9 M^{-1} s^{-1}).[83] In the case of β-CD, similar association rate constants were obtained for different guests,[51,67,87−89] suggesting that the same mechanism operates for the association of host with guest. The entropy of activation for the association process of xanthone with β-CD is positive suggesting that desolvation of the host and/or guest plays a role in the association process.[91] Since the association rate constants are similar for guests with different solvation, the desolvation of the CD cavity is likely the predominant factor. In addition, the rate could also decrease due to the requirement of a geometrical alignment between the host and guest for the formation of the host−guest complex, i.e., if the guest hits the exterior sidewall of the CD, the encounter complex is likely to dissociate instead of leading to the formation of the complex. Therefore, the rate constants of 5×10^8 to 1×10^9 M^{-1} s^{-1} are likely to be the upper limit for the association of guests with CDs. Desolvation of the CB[7] cavity and geometrical considerations are also relevant for the complexation of NpH^+ with CB[7]. Although at this point only one example is at hand for such a fast association process, the rate constant of 6×10^8 M^{-1} s^{-1} for NpH^+ with CB[7] is likely to be close to the upper limit for the association rate constants of guests with CB[*n*]s. The high association rate constant for NpH^+ with CB[7] shows that guest association with CB[*n*]s does not have to follow a constricted binding mechanism where a significant barrier has to be overcome and that fast binding is possible with CB[*n*]s. Fast binding is a desirable feature for applications where competitive bimolecular reactions occur, such as sensing in environments where the analyte can be bound to multiple components of the system as frequently encountered in biological systems.

Structural changes to the guest[67,87,88,92] or the CD[93] have a much larger effect on the dissociation rate constant of the guest−CD complex than on the association process. Development of a positive charge increases the dissociation rate constant to above 10^8 s^{-1},[92] while increased hydrophobicity and depth of penetration into the CD cavity slow down the dissociation.[67,87] The dissociation rate constant for NpOH with β-CD is 1.8×10^5 s^{-1}.[67] This rate constant is 3000 times higher than the dissociation rate constant for NpH^+ from the complex with CB[7] (55 s^{-1}). Therefore, the slow down of the dissociation of NpH^+ is responsible for the high equilibrium constant for the NpH^+@CB[7] (1.06×10^7 M^{-1}) system when compared to NpOH@β-CD (1.7×10^3 M^{-1}). The low value for k_{001}^- cannot be due to constrictive binding because the constriction by the portal would have influenced both the association and dissociation processes. The slow dissociation could be related to the fact

that CB[7] is not very soluble in water, and release of the guest would cost the penalty of stabilizing CB[7] with bound water and metal cations. In this respect the amphiphilic nature of the guest with a hydrophobic surface and a positive charge makes NpH^+ a better solubilization agent of CB[7] than metal cations. The situation is different in the case of β-CD, which is more soluble in water than the guest.

CONCLUSIONS

Kinetic studies for the binding of NpH^+ with CB[7] led to mechanistic information of the association and dissociation rate constants, the CB[7] species to which NpH^+ is bound, and the role of Na^+ or H_3O^+. The association rate constant was 1 order of magnitude lower than for a diffusion-controlled process and was much higher than measured previously for guest binding to CB[6]. The high equilibrium constant for the formation of the NpH^+@CB[7] complex is due to a slow dissociation process when compared to the dissociation processes observed for similar guests in cyclodextrins. However, the dissociation rate constant was shown to be higher than for guest·CB[n] complexes where constricted binding occurs. Two Na^+ cations bind to CB[7], and NpH^+ does not form complexes with either CB[7]·Na^+ or Na^+·CB[7]·Na^+. The stopped-flow experiments employed to investigate the fast dynamics of the NpH^+@CB[7] formation can be applied to study the dynamics of other guests with CB[n]s for which a fast exchange is observed by NMR, precluding kinetic experiments with the latter technique. In addition, we are developing a competitive methodology to study the binding dynamics for guests that do not show absorption or fluorescence changes when complexed to CB[n]s.

CB[n]s are being developed for many applications, such as drug delivery,[35−46] tandem enzyme assays,[27−29] and switching of supramolecular hydrogels.[31] The rates for the association and dissociation of guests with CB[n]s play a role in these applications, and knowledge on how the dynamics of the system can be altered will be important to optimize these applications. The current study shows that fast association of guests with CB[n]s is possible, and for this reason, applications where competitive binding with CB[n]s are required can be achieved at low guest concentrations (nM to μM). The wide range of association and dissociation rate constants reported in this work and by others and the variability in the mechanisms for formation of the guest complexes with CB[n]s make this host more versatile than other macrocycles to develop applications.

EXPERIMENTAL SECTION

Materials. CB[7] was synthesized as previously described.[5,13] The actual mass of CB[7] in each stock solution was assayed (Figure S17, Supporting Information) using ferrocene (Fluka ≥98%) as a competitive guest with NpH^+ because the ferrocene equilibrium constant with CB[7] is fairly high.[94] The CB[7] concentrations were corrected for the fraction of CB[7] in each sample. Water and residual acid or salts from the purification steps are the other components in the sample. Initial experiments were performed with a sample containing 73% CB[7], while a 99% pure sample was used at a later stage. After correction for the actual CB[7] concentration, the data were the same for both samples. Possible interference from smaller CB[n]s was tested in an independent experiment with CB[6], which showed that NpH^+ does not bind to this smaller CB[n]. *R*-(+)-2-naphthyl-ethylamine (Np, Fluka, ≥ 99%) was used as received, and no fluorescent impurities were present, since a single exponential decay was observed for the singlet excited state of NpH^+. Standardized volumetric solutions of hydrochloric acid (Anachemia, ACS reagent grade), standardized volumetric solutions of sodium hydroxide (Anachemia, ACS reagent grade), sodium chloride (BDH), and methanol (Caledon Laboratories, spectrograde, > 99.8%) were used as received.

Solution Preparation. HCl (1.000 M) and NaOH (1.000 M) stock solutions were prepared by diluting the respective standardized solutions. NaCl (2.000 M), NpH^+ (625 μM), and CB[7] (800 μM) stock solutions were prepared by dissolving the appropriate amount of solid in deionized water (Barnstead NANOpure deionizing systems ≥17.8 MΩ cm^{-1}). Solutions with lower concentrations of NaCl, NpH^+, or CB[7] were prepared by dilution for the stopped-flow experiments. Aliquots of a CB[7] stock solution (380 μM) were injected into 3.00 mL of a NpH^+ solution for the determination of the fluorescence binding isotherms. The stock solution for ferrocene (1 mM) was prepared in methanol. Aerated samples were used for all experiments because deaeration cannot be achieved for stopped-flow experiments.

Equipment. UV−vis absorption spectra were recorded by using a Varian Cary 1 spectrophotometer at room temperature. A PTI QM-2 fluorimeter was employed to measure steady-state fluorescence spectra (λ_{ex} = 280 nm, λ_{em} = 300−550 nm, and excitation/emission monochromator bandwidth = 2 nm). The spectra were corrected by subtracting a baseline spectrum, which corresponds to the emission for a solution containing all chemicals with the exception of NpH^+. The baseline spectrum contains the Raman peak for water and a small emission from CB[7] (≤15% of the Raman peak for $[CB[7]]_T$ = 25 μM). The fluorescence intensities were obtained by integrating the corrected spectra between 315 and 350 nm and were normalized by dividing each measurements by the intensity measured for NpH^+ in the absence of CB[7]. Time-resolved fluorescence decays were measured with an Edinburgh OB920 single photon counting (SPC) system. Samples were excited with an EPLED280 (Edinburgh) light emitting diode (λ_{ex} = 277 nm). The emission was detected at 330 nm (monochromator bandwidth = 16 nm) until the number of counts in the channel with highest intensity was 10 000. The scattering excitation light from a Ludox solution was used to determine the instrument response function (IRF). Quartz cells with dimensions of 10 × 10 mm or 10 ×2 mm were employed for steady-state and time-resolved fluorescence experiments. The samples for both experiments were kept at 20.0 ± 0.1 °C using a circulating water bath for at least 15 min before an experiment was conducted.

The fluorescence decays were fit to a monoexponential function (i = 1) or to the sum of two exponentials (i = 2, eq 19) using the Edinburgh software. The calculated fit was reconvoluted with the IRF and compared to the data. The fits were deemed adequate if the χ^2 values were between 0.9 and 1.2 and the residuals were random.[95]

$$I(t) = I_0 \times \sum_1^i (A_i \times e^{-t/\tau_i}) \quad (19)$$

A SX20 stopped-flow system (Applied Photophysics) was employed for the kinetic measurements. The solutions were excited at 280 nm (monochromator bandwidth = 4.7 nm), and the fluorescence was detected at 90° to the excitation beam by either using a monochromator set to 330 nm (bandwidth = 37 nm) or by using a 310 nm cutoff filter (Schott WG 310). A 1:1 mixing ratio was used, and the two syringes were kept at 20 °C for at least 15 min before the start of the experiment. The voltage for the photomultiplier of the detector was kept constant throughout each experiment. The voltage was set to achieve an intensity of ca. 6 V, which is below the detector's saturation level, for the solution with the highest fluorescence intensity in a particular experiment. For each experiment, at least 20 kinetic traces were averaged. When the data were analyzed using the global analysis method, an average of 75 individual kinetic traces was obtained to improve the signal-to-noise ratio. The intensity values for control experiments where water or

aqueous CB[7] were contained in both syringes were recorded for each experiment. This intensity corresponds to less than 0.5% of the intensity measured for the fluorescence of NpH^+ in water. For each experiment, the intensity of aqueous NpH^+ was determined by mixing NpH^+ in one syringe with water and in the second syringe where both syringes contained the same H_3O^+ or Na^+ concentrations. For the determination of the final amplitude of a kinetic trace, the fluorescence intensity was corrected by subtracting the baseline for the mixing of the same solutions that contained all chemicals with the exception of NpH^+. This corrected intensity was normalized by dividing the intensity values by the corrected intensity of NpH^+ in aqueous solution in the absence of CB[7] but in the presence of the same concentrations of H_3O^+ or Na^+.

The relaxation kinetics in the stopped-flow experiment were analyzed by fitting individual experiments to a monoexponential function (eq 9) with the Pro-Data Viewer software (Applied Photophysics) or by using a global analysis method in the Pro-Kinetics II software (Applied Photophysics). In the global analysis method, kinetic traces obtained for different experimental conditions were fit simultaneously. The quality of the fit was judged by the randomness of the residuals.

■ ASSOCIATED CONTENT

Supporting Information. Absorption and fluorescence spectra, formation of byproduct, derivation of overall binding constants and observed relaxation rate constants, typical binding isotherms, typical kinetic traces, fit of kinetic data to different models and titration of CB[7]. This material is available free of charge via the Internet at http://pubs.acs.org.

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