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Dynamics of a Supramolecular Capsule Assembly with Pyrene

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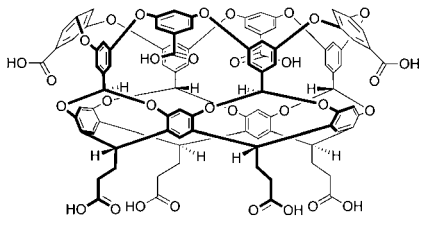
Supporting Information

ABSTRACT: Water-soluble octaacid cavitands (OAs) form dimeric capsules suitable for guest incorporation. Our studies reveal that the mechanism of pyrene (Py) binding involves the rapid (<1 ms) formation of the Py·OA complex followed by slower binding with the second OA. The dissociation of the capsular OA·Py·OA complex occurs with a lifetime of 2.7 s, which is 5 orders of magnitude slower than the microsecond opening/closing ("breathing") previously observed to provide access of small molecules to the encapsulated guest. These different dynamics of the capsules have a potential impact on how the chemistry of included guests could be altered.

Self-assembled container molecules provide ready access to a wide range of phenomena arising through controlled compartmentalization, including unusual chemical reactivity, separation technologies, and storage and/or transport. A growing focus of the field is the formation of containers in aqueous solution,[1−4] and in this regard, metal coordination[5−11] and the hydrophobic effect[12−16] have proven useful as methods to drive assembly. Regarding the latter, the Gibb group has developed deep-cavity cavitands that dimerize into capsules via the hydrophobic effect. These hosts, such as the so-called octa-acid (OA), possess water-solubilizing outer coats, deep hydrophobic pockets, and a hydrophobic rim of the cavity that promotes self-assembly.

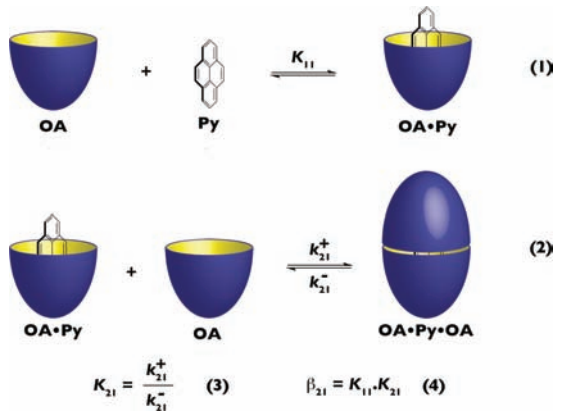


Octa acid (OA)

OA forms monodispersed and kinetically stable dimeric assemblies that—by dint of their water-free, low-polarity inner space[17,18]—can encapsulate molecules and act as yoctoliter reaction vessels for photochemical transformations,[17,19−22] bring about the separation of hydrocarbon gases,[13] engender molecular protection for the kinetic resolution of structurally similar molecules,[23] engender unusual self-sorting properties,[24] and lead to the electrochemical modulation of encapsulated guests.[25−27] Nevertheless weakening of the hydrophobic effect by the addition of cosolvents denatures these assemblies.[28]

In part, the properties of the supramolecular complexes formed by OA are dictated by the nature of the guest. An understanding of the complicated relationship between the host and guest can be garnered using many different physicochemical techniques. For example, the dynamics of encapsulated guest movement have been probed on both the millisecond (^{1}H NMR) and nanosecond (EPR) time scales.[29,30] Furthermore, a microsecond-time-scale opening of the dimeric capsule sufficient to allow access of small molecules without dissociation of one of the OAs or guest release has been inferred from excited-state quenching studies.[20,31] Nevertheless, missing from the current state of affairs are studies providing knowledge of the formation/dissociation dynamics of these encapsulation complexes. Indeed, the formation/dissociation dynamics of host−guest complexes in general has been underexploited because of the lack of suitable methodology.[32,33] Toward addressing this, here we measured in real time the kinetics of the formation of 1:1 and 2:1 host−guest complexes formed between OA and the guest pyrene (Py). Our results reveal the different rates of the processes shown in Scheme 1 and demonstrate for the first time that complex disassembly and concomitant guest release are kinetically very different from the "breathing" of the complex that allows small molecule entry and egress.

Scheme 1. Cartoon Representation of the Formation of OA·Py (Equation 1) and OA·Py·OA (Equation 2) and Definitions of K_{21} and β_{21} (Equations 3 and 4)



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Previous ^{1}H NMR experiments showed that OA forms a 2:1 host–guest complex with Py.[17,18] Py encapsulation leads to marked red shifts (5–6 nm) in its excitation (Figure 1) and

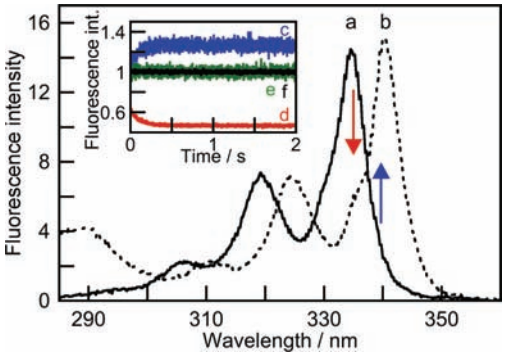

Figure 1. Fluorescence excitation spectra for Py (0.2 μM, λ_{em} = 390 nm) in the absence (a, solid line) and presence (b, dashed line) of OA (4 μM) at pH 8.9 (10 mM borate buffer). The arrows show the direction of the intensity change when Py is encapsulated within OA. The inset shows the kinetics for mixing of Py (0.2 μM) with OA (3 μM) upon excitation at 340 nm (c, blue) or 335 nm (d, red). The intensities for the mixing of Py with buffer (e, green, λ_{ex} = 340 nm; f, black, λ_{ex} = 335 nm) were normalized to 1. The higher signal-to-noise ratio for excitation at 335 nm reflects the higher intensity of the Hg–Xe excitation lamp at this wavelength.

absorption spectra (Figure S1 in the Supporting Information). Theoretically, such spectral changes could be due to the formation of Py dimers.[34] However, there was no evidence of accompanying broadening of the absorption/excitation spectra nor detection of any excimer emission (Figures S1 and S2). Red shifts of 3–8 nm between the Py absorption in homogeneous solution and when bound to hosts as a monomer were previously observed for Py binding to bile salt aggregates[35] and a macrocycle.[36] A consequence of the large absorption shift observed for OA-encapsulated Py is that depending on the excitation wavelength either a positive or negative change in the fluorescence is observed (Figure 1 inset).

Time-resolved fluorescence measurements provided further evidence of monomeric Py complexation, its isolation from the aqueous phase in the dimeric OA capsule, and the absence of any significant equilibrium amount of the 1:1 complex. The fluorescence lifetime of 361 ± 1 ns for Py (0.5 μM) in the presence of OA (10 μM) was much longer than that in aqueous borate buffer (130 ± 1 ns) and is similar to that previously reported.[18] Furthermore, the iodide anion (I^-) quenching rate constant for singlet-excited-state Py in the capsule was measured to be (5.9 ± 0.7) × 10^5 M^{-1} s^{-1} (Figure S3), a value much lower than that in aqueous buffer [(1.0 ± 0.2) × 10^9 M^{-1} s^{-1}]. Both of these metrics confirm the efficient protection afforded by the capsule. Additionally, no other Py emission with a lifetime intermediate between those of free and encapsulated Py was observed, indicating that the 1:1 complex was not present in any appreciable amount.

The iodide quenching rate constant for singlet-excited Py encapsulated in OA·Py·OA is similar to that previously determined for oxygen quenching of encapsulated triplet-excited Py (5 × 10^5 M^{-1} s^{-1}).[31] This similarity supports the interpretation that the quenching rate constant for an excited guest in the capsule is determined by a partial opening (or "breathing") of the capsular complex that allows small guests to enter and egress the complex.[31] Such a mechanism predicts similar rate constants for both different quenchers and different excited states of the same guest.

An understanding of the kinetics of assembly and disassembly of the host–guest complex requires knowledge of the types of complexes present at equilibrium and their respective equilibrium constants. Measurements of the fluorescence intensity change as a function of OA concentration were carried out to determine the overall binding constant (Figure S4). The resulting binding isotherm (Figure 2 and

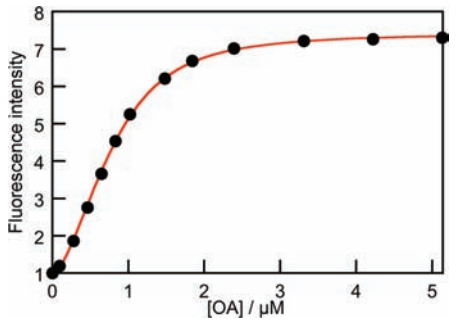

Figure 2. Binding isotherm for the complexation of Py (0.2 μM, λ_{ex} = 340 nm) with OA obtained from steady-state fluorescence experiments. The data were fit to the sequential model shown in Scheme 1.

Figure S5) fit the expected 2:1 binding model and gave an overall β_{21} value of (3.19 ± 0.06) × 10^{12} M^{-2}.

Because of the transient nature of the 1:1 complex, the equilibrium constant for this species (K_{11}) can be determined only from kinetic studies. Stopped-flow experiments revealed that the kinetics for complex formation followed a two-phase behavior (Figure 3). First, an initial offset between the intensity

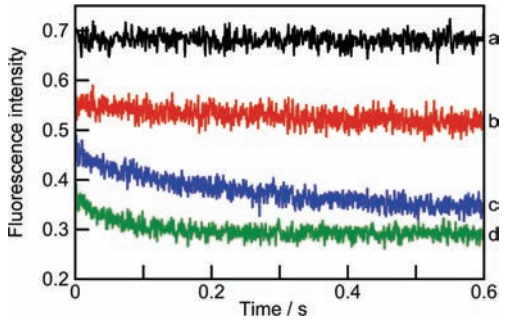

Figure 3. Kinetics for Py (0.2 μM, λ_{ex} = 335 nm) upon mixing with (a) buffer or (b–d) OA at a concentration of (b, red) 1 μM, (c, blue) 2 μM, or (d, green) 4 μM.

for Py in water (black line) and the intensities for Py in the presence of OA (colored lines) was observed. This result indicates that a reaction occurred within the 1 ms mixing time of the stopped-flow experiment. Furthermore, the magnitude of this offset intensity increased as the concentration of OA was raised. Consequently, a series of stopped-flow experiments in which the initially measured fluorescence intensity was plotted against [OA] revealed a 1:1 binding isotherm (Figure S6). This fit to a 1:1 model confirmed that the initial fast reaction was the binding of Py to OA and revealed an equilibrium constant for this process of K_{11} = (4.5 ± 0.6) × 10^5 M^{-1}. With K_{11} and β_{21} in hand, a K_{21} value of (7 ± 1) × 10^6 M^{-1} was calculated using eq 4 in Scheme 1. The determination of both K_{11} and K_{21} for the guest Py represents the first glimpse of the effect of

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desolvating the rim defined by the "uppermost" aromatic rings around the portal of the host (see the structure shown above). In the formation of the 1:1 complex, one hydrophobic pocket and half a guest are desolvated. In the capping of the 1:1 complex to form the capsular 2:1 complex, there is this same desolvation, plus the additional desolvation of the rims as the hosts clamp together around the guest. This additional factor means that the assembly demonstrates positive cooperativity ($K_{11} < K_{21}$) and explains why the 1:1 complex is not present at equilibrium in any appreciable amount.

Although the kinetic experiments shown in Figure 3 revealed the value of K_{11}, they could not provide any kinetic information on the formation of OA·Py because the formation of the 1:1 complex is faster than the time resolution of the stopped-flow experiments.

The fast initial intensity offset observed in the stopped-flow experiments was followed by a further slow decrease in the Py emission intensity (Figure 3). The kinetics leveled off within 10 s (only 0.6 s are shown in Figure 3; see Figure S7 for the complete data set). The normalized intensity change measured at equilibrium in the kinetic experiment was the same as the intensity obtained from the steady-state experiments (Figure S8), showing that the kinetics were complete within 10 s. This result eliminated the possibility of any change in the assembly occurring over longer periods of time. The kinetics of this slower process followed a monoexponential decay (Figures S9 and S10) that was assigned to the formation of OA·Py·OA via the bimolecular reaction between OA·Py and OA. Overall rate constants for relaxation processes always correspond to the sum of the rate constants for the forward and backward reactions, where an increase in the observed rate constant is expected when the concentrations of reagents involved in the bimolecular process are raised.[32,33] The faster decay of the kinetics observed at higher OA concentrations (Figure 3) confirmed that this process corresponds to a bimolecular reaction involving OA.

A global analysis method was employed in which kinetic traces at different OA concentrations were simultaneously fit to two models based on eqs 1 and 2, where in both cases the value of K_{11} was fixed. In one model, the dissociation rate constant was included, while in the second model, this rate constant was considered to be infinitesimally small. Both models produced the same value for k_{21}^{+} (Figure S11 and Table S1), supporting the conclusion that the contribution of the dissociation process to the observed rate constant is negligible. The average value for k_{21}^{+} determined from two independent experiments was $(2.6 \pm 0.2) \times 10^{6}$ M^{-1} s^{-1}, while a value for k_{21}^{-} of (0.37 ± 0.06) s^{-1} was calculated using eq 3 in Scheme 1. This latter rate constant corresponds to a lifetime of 2.7 s for the dissociation of the OA·Py·OA capsule.

As discussed above, the opening/closing of OA·Py·OA to provide access of small molecules (e.g., O_2 or I^-) to Py without release of Py was estimated to occur on the microsecond time scale. Hence, even for ill-fitting Py[37] the "breathing" dynamic is at least 10^5 times faster than the release of Py from the capsule. It is important to note that the k_{21}^{-} value for OA·Py·OA does not correspond to the dissociation of the guest-free OA·OA dimer, since there is no evidence to suggest that OA forms significant amounts of capsule in the absence of guest at such low concentrations of OA and Na^+.[14,29]

The association of 1:1 host–guest complexes where the guest fits into the cavity of the host is frequently fast and close to a diffusion-controlled process. For example, the association rate constant for guests binding to cyclodextrins[38] and for the binding of a napththalene derivative to cucurbit[7]uril[39] were determined to be $(4–10) \times 10^{8}$ M^{-1} s^{-1}. A similar rate constant for Py·OA formation would explain the fast (<1 ms) equilibration observed. The lower rate for the binding of the second OA is analogous to the lower rates observed for the formation of the 2:2 β-cyclodextrin:Py complex.[40] Similarly, the results presented here demonstrate that the dynamics of the OA capsule system is defined by the dissociation rate constant of the higher-order complex (i.e., OA·Py·OA). Where OA capsules differ from cyclodextrins is in the slow release of the guest from the OA capsule in comparison with the much faster "breathing" dynamics. These two kinds of opening processes suggest that selective enhancement of "bimolecular" reactions involving the ingress of small molecules while maintaining the general structure of the complex should be possible. Previous results showed that capsule confinement affects the bimolecular reactivity of guests,[41,42] and reactions with encapsulated guests can occur when small external molecules enter the capsule.[20] Our results suggest that it may be possible to filter out competing reactions with larger reagent molecules attempting to enter the complex. The origin of this separate dynamic "breathing" process resides in the fact that the OA capsule leads to true compartmentalization, a phenomenon that cannot occur with simple macrocyclic hosts. Consequently, the use of OA capsules as host systems offers more versatility to influence competitive reaction pathways of guests differentially. Future exploitation of the differences in the capsule dynamics will aid the rational design of functional OA supramolecular systems and are currently underway.

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, absorption and fluorescence spectra, quenching plots, models for binding isotherms and analysis of stopped-flow kinetics. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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