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Insight into Unusual Downfield NMR Shifts in the Inclusion Complex of Acridine Orange with Cucurbit[7]uril

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This paper describes the structure and properties of the complex of acridine orange (AO) with cucurbit[7]uril (CB[7]) in aqueous solutions, studied by ^{1}H NMR spectroscopy, matrix-assisted laser desorption/ionization time-of-flight (MALDI–TOF) mass spectrometry, UV/Vis spectroscopy, and fluorescence spectroscopy with the aid of competitive binding methods. It was found that AO was included in the CB[7] cavity through strong ion–dipole interactions and hydrogen bonds. Theoretical studies based on calculations performed from first principles further confirmed this binding mode. Unusual downfield NMR shifts of the AO proton resonances in the presence of CB[7] were observed, in sharp contrast with cases of inclusion of other organic molecules in the CB[7] cavities, which resulted in upfield shifts of their proton resonances. The downfield shifts of the proton resonances of the AO·CB[7] complex were found to be the net result of small upfield shifts arising from the inclusion of AO in the CB[7] cavity and large downfield shifts resulting from the deaggregation of the AO aggregates, which were readily formed even at low concentrations in aqueous solutions. Further theoretical calculations explained the unusual downfield NMR shifts well. These research results are of importance for understanding of the structures and properties of complexes of related organic molecules with macrocyclic hosts.

Introduction

Cucurbit[*n*]urils (CB[*n*]s, *n* = 5–10) are pumpkin-shaped and highly symmetrical macrocycles with extremely nonpolarizable cavities.[1] A CB[*n*] consists of *n* glycoluril units joined by pairs of methylene bridges to form a rigid and highly symmetric structure with an annular and hydrophobic cavity and two highly polar carbonyl portals.[2–5] Out of the family, CB[7] has attracted considerable attention because of its superior solubility (20–30 mM) in aqueous solution[2,6–8] as well as by being of a size comparable to that of β-cyclodextrin (β-CD).

In general, binding by CB[*n*]s is driven by hydrophobic interactions with the inner cavities and ion–dipole interactions and hydrogen bonds with the carbonyl groups lining the portals.[9–12] There are a number of reports describing the formation of host–guest complexes between CB[*n*]s and organic molecules accommodated in the CB[*n*] cavities.[6–13] Fluorescence enhancements of emission bands upon encapsulation of dyes with nonfluorescent CB[*n*]s in aqueous solutions were first observed and documented by Wagner and co-workers,[14–16] and enhanced fluorescence emissions of other dyes in the presence of CB[*n*]s were then reported by different research groups.[17–22] These fluorescence enhancements are attributed to the inclusion of dye molecules within the hydrophobic CB[*n*] cavities and to the suppression of dye aggregation and have potential applications in a variety of fields.[23,24] The complex of rhodamine 6G (R6G) with CB[7] exhibited significantly enhanced fluorescence and acted as a superior probe for use in single-molecule detection.[17] A fluorescence switch from green to blue was produced through the inclusion of protonated 2-aminoanthracene with CB[7].[6] The complex of Dapoxyl with CB[7] has been used for continuous assays of amino acid decarboxylases based on the protolytic fluorophore displacement principle by measurement of changes in fluorescence.[18] On the other hand, it should be noted that the modes of inclusion or association between fluorescent dyes and CB[7] are not accurately known in most cases.[19] In the absence of structural data from X-ray crystallography, the geometry of a supramolecular complex can be deduced from induced shifts in NMR spectra, molecular mechanics calculations, and symmetry considerations.[25] ^{1}H NMR spectroscopy is one of the most effective tools for studies of complex formation between CB[*n*]s and organic molecules.[26–30] In general, downfield shifts of the resonances of guests result from the deshielding effects of the carbonyl groups of the CB[*n*]s when the portions of the guests are

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located in the portals, whereas upfield shifts indicate that the guests are encapsulated in the shielding hydrophobic cavities. However, low dye concentrations commonly prevent detailed NMR characterization.[19]

Acridine orange (AO) is a cationic fluorescent dye widely used for cell staining[31,32] and as a DNA intercalator[33] and transmembrane pH-gradient probe,[34] and its complex with CB[7] was reported very recently by three research groups.[35–37] Mohanty and Pal,[35] as well as Zhou,[36] found fluorescence enhancement of AO by CB[7] with the formation of a 1:1 complex, and an inclusion mode was deduced only from fluorescence and UV/Vis spectral studies. Garcia and co-workers[37] investigated the complexes of AO and its analogues (tricyclic condensed heterocycles with amino substituents) with CB[7] and CB[8] by a few methods. The formation of the complexes led to increases in fluorescence quantum yields in the case of CB[7], whereas the dimeric or trimeric dyes encapsulated in CB[8] were significantly less fluorescent than the same dyes in dilute solutions.[37] Although no NMR spectrum of the complex of AO with CB[7] was shown, the NMR spectra of the complexes of thionine (TH) and methylene blue (MB) with CB[7] and CB[8], respectively, were presented.[37] The resonances of the TH and MB protons underwent downfield shifts in the presence of CB[7] but were shifted upfield in the case of CB[8].[37] However, the authors were unable to give a definite explanation for the unusual downfield NMR shifts of the proton resonances of TH and MB, assuming that each of them was included in the CB[7] cavity. Here we give a deeper insight into the unusual downfield NMR shifts of the proton resonances of AO with CB[7] with the aid of competitive binding of bis(cyclopentadienyl)cobalt(III) hexafluorophosphate (Cob^+), which has been demonstrated to encapsulate inside the cavity of CB[7].[38] A combination of the experimental data and theoretical calculations (calculations based on first principles rather than semiempirical ones) not only verifies the formation of an inclusion complex of AO with CB[7] but also reveals the origin of the unusual downfield NMR shifts for the complex. These research results should be helpful for the understanding of the structures and properties of complexes of related organic molecules with macrocyclic hosts.

Results and Discussion

The emission band of AO underwent a significant enhancement and blueshift from 537 to 520 nm upon addition of CB[7] to aqueous AO solution at a concentration of 3.2×10^{-7} mol L^{-1} (Figure 1). The association constant (K) of AO with CB[7] could be obtained by nonlinear least-squares fitting (details in the Supporting Information) to the fluorescence titrations with the assumption that AO formed a 1:1 complex with CB[7]. A K value of $(5.6 \pm 0.3) \times 10^5$ L mol^{-1} was calculated from the plot (inset of Figure 1). The estimated K value is consistent with those reported recently,[35,37] which have ranged from 1.5×10^5 to 3.6×10^6 L mol^{-1} depending on the exact natures of the aqueous solutions used (presence of metal cations, concentration, pH). All these data indicated that a stable 1:1 complex was formed between AO and CB[7]. The complex stoichiometry was further confirmed by the MALDI-TOF spectrum, with the appearance of a major peak at m/z = 1429, corresponding to the AO·CB[7] complex (Figure S1 in the Supporting Information).

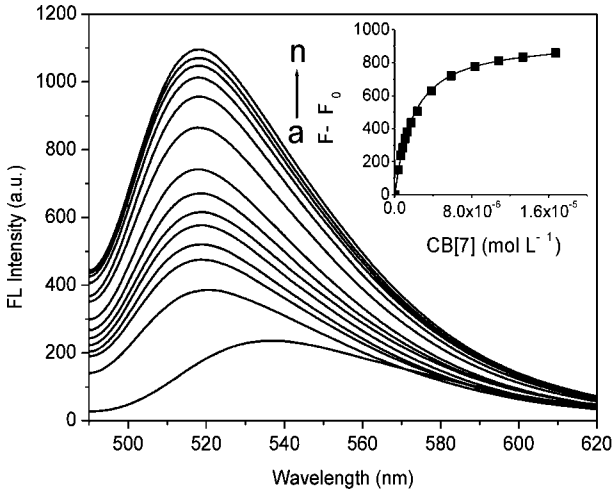

Figure 1. Fluorescence emission spectra of AO [3.2×10^{-7} mol L^{-1}, phosphate-buffered saline (PBS) at pH = 7.5] in the absence and in the presence of CB[7] at different concentrations. CB[7] (10^{-7} mol L^{-1}): (a) 0.0, (b) 3.5, (c) 6.3, (d) 8.0, (e) 10.8, (f) 12.5, (g) 16.9, (h) 23.3, (i) 38.3, (j) 58.3, (k) 83.3, (l) 108.3, (m) 133.3, and (n) 166.7. The inset shows the plot of nonlinear least-squares fitting used for the association constant.

To elucidate the binding mode between AO and CB[7], a competitive binding method was used. Cob^+ had been reported to encapsulate inside the cavity of CB[7] to form a highly stable 1:1 inclusion complex with CB[7], and the K value of the inclusion complex had to be at least 10^6 L mol^{-1}.[38] Note that metal cations (Na^+ or K^+) would be able to bind to the carbonyl portals of CB[7], which would have a small influence on the measured association constant of the complex of AO with CB[7]. When Cob^+ was added to a system of AO and CB[7], the fluorescence intensity of the AO gradually decreased, and the band shifted from 516 to 537 nm with increasing Cob^+ concentration (Figure 2), indicating that the AO·CB[7] complex was dissociated by the competitive binding of Cob^+. Cob^+ is a cationic complex without fluorescence emission and displayed only a slight influence on the fluorescence emission of aqueous AO solution (Figure 2). These results indicate that the addition of Cob^+ drove the originally included AO out of the CB[7] cavity, because of Cob^+'s strong binding ability for CB[7]. It can thus be inferred that an inclusion complex was originally formed between AO and CB[7]. Consideration of the internal cavity of CB[7] – it is approximately 7.3 Å across at the equator, 5.4 Å across at both carbonyl portals, and 9.1 Å in height – shows that it is not big enough to accommodate an entire AO molecule. It is most

likely that AO was partially encapsulated inside the CB[7] cavity; the partial inclusion complex of AO with CB[7] and the competitive binding of Cob^+ is schematically illustrated in Figure 3. The AO remained in its symmetrically protonated form (pK_a = 10.5)[39] in neutral aqueous solution. The position of the central N protonation for AO was confirmed by ^1H NMR at neutral pH.[39,40]

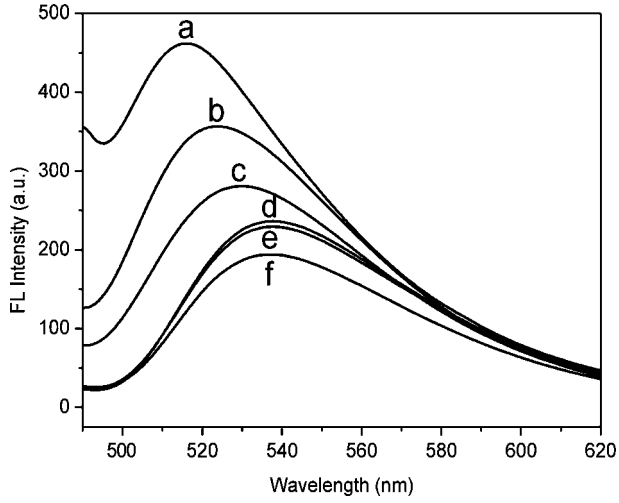

Figure 2. Fluorescence emission spectra of AO (3.0×10^{-7} mol L^{-1}) and CB[7] (6.0×10^{-7} mol L^{-1}) in the absence and presence of Cob^+ at different concentrations. Cob^+ (10^{-7} mol L^{-1}): (a) 0, (b) 2.0, (c) 4.0, and (d) 6.0. (e) AO. (f) AO (3.0×10^{-7} mol L^{-1}) and Cob^+ (8.0×10^{-7} mol L^{-1}).

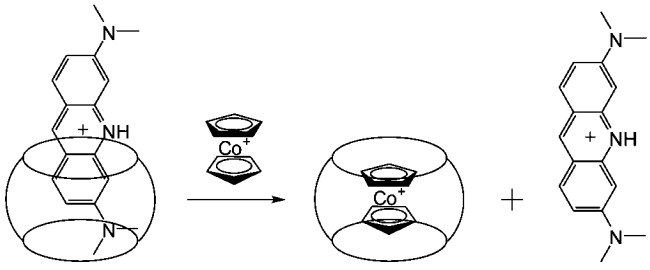

Figure 3. Schematic illustration of competitive binding between Cob^+ and AO for CB[7].

On the other hand, the interaction of AO with CB[7] was also validated by theoretical calculations. From Figure 4 it can be clearly seen that the interaction energies of AO with CB[7] inside and outside the cavity of CB[7] were 40.6 and 35.0 kcal mol^{-1}, respectively. In addition, the dipole–dipole interaction energy of the encapsulated AO inside the CB[7] cavity was also larger than that outside the cavity. These results indicate that AO preferred to be accommodated inside the cavity of CB[7]. To make the interaction between host and guest molecules clearer, the structure of the AO·CB[7] complex was investigated, and some geometric details are presented (Figure 4). AO inside the CB[7] cavity formed six hydrogen bonds through its hydrogen atoms and the carbonyl oxygen atoms of CB[7], including one strong N–H···O [$R_{H(N)\cdots O}$ = 2.10 Å] and five weaker C–H···O [$R_{H(C)\cdots O}$ = 2.44–2.80 Å] bonds. In comparison, in the case of AO outside the CB[7] cavity, there were only one N–H···O [$R_{H(N)\cdots O}$ = 1.92 Å] and three C–H···O [$R_{H(C)\cdots O}$ = 2.40–2.84 Å] bonds.

The ^1H NMR spectra of AO and the AO·CB[7] complex in D_2O are shown in Figure 5. Upon addition of CB[7] (1.0 equiv.) the resonances of the AO ring protons became too broad to be distinguished clearly, whereas the resonance of the methyl protons shifted downfield and became broad. Note that these induced NMR shifts of the AO protons in the presence of CB[7] were in sharp contrast with those associated with the inclusion of other organic molecules in the CB[7] cavity, with upfield shifts of their proton resonances.[26–30] It was known that Cob^+ could be completely included in the CB[7] cavity with an upfield shift of its proton resonance.[38] Upon addition of Cob^+ to the AO·CB[7] complex, the NMR spectrum of the aqueous AO solution was completely recovered, along with an upfield shift of the Cob^+ proton resonance. These observations further confirmed the occurrence of the quantitative displacement of the included AO out of the CB[7] cavity by the competitive binding of Cob^+; but what is the reason for the unusual downfield NMR shifts of the proton resonances of AO observed upon inclusion in the CB[7] cavity?

It is well known that the sensitivity of NMR spectroscopy is much weaker than those of fluorescence and UV/Vis spectroscopy. Some (planar) organic dye molecules readily aggregate in aqueous solutions even at low concentrations. It is most likely that these dye molecules form different types of aggregates beneath the NMR detection limit. Individually dispersed AO molecules would have a different NMR spectrum from the AO aggregates, with downfield shifts. Upon addition of CB[7], the aggregated AO molecules in the aqueous solution were deaggregated to enter the CB[7] cavity in the single-molecule state. On one hand, the AO deaggregation gave rise to a downfield shift of the AO proton resonances; on the other, the AO inclusion caused an upfield shift relative to single AO molecules. The concurrence of the AO deaggregation and inclusion resulted in the observed unusual NMR shifts of the AO·CB[7] complex. From this point of view, the NMR spectroscopic data for the inclusion complexes of TH and MB with CB[7] and CB[8] reported by Garcia and co-workers[37] can be satisfactorily explained. It is most likely that TH and MB were in the dimeric, trimeric, oligomeric, and polymeric states in the aqueous solutions. The downfield shifts of the proton resonances seen with inclusion of the dyes (TH and MB) in CB[7][37] were very similar to those observed in our AO case. In the presence of CB[8], the dyes were encapsulated inside the CB[8] cavities in the dimeric state, and the upfield NMR shifts of the inclusion complexes mostly resulted from the shielding hydrophobic cavity. If the dye molecules were initially monodispersed in the aqueous solution, larger induced resonance shifts would be observed when they were encapsulated within the CB[8] cavities in the dimeric state,

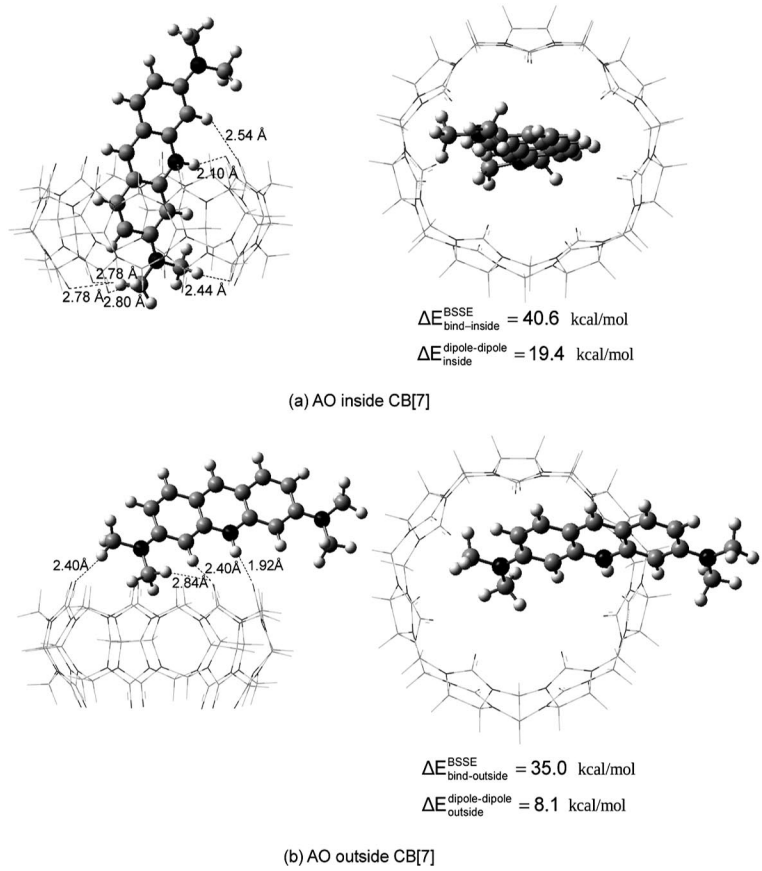

Figure 4. Side and overhead views of AO (a) inside and (b) outside the CB[7] cavity, calculated by the B3LYP/6-31G(d) method. The interaction energies of AO inside and outside the cavity of CB[7] with basis set superpostition error (BSSE) and their dipole–dipole interaction energies are also shown.

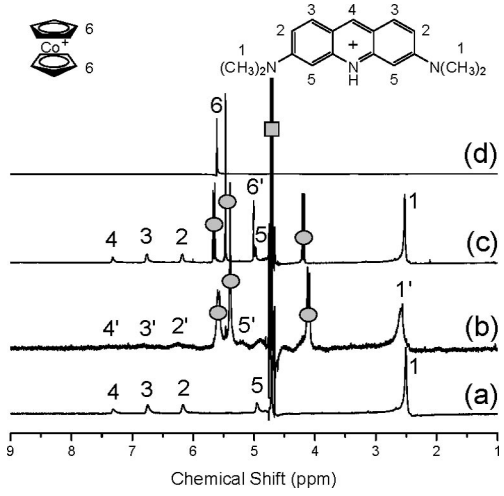

Figure 5. ^{1}H NMR spectra of: (a) AO, (b) AO and CB[7] (1.0 equiv.), (c) AO, CB[7] (1.0 equiv.), and Cob^+, and (d) Cob^+. AO 1.0×10^{-3} mol L^{-1}; CB[7] proton resonances labeled with cicles; HOD proton resonance labeled with squares.

because both dye aggregation and dye inclusion would lead to upfield NMR shifts of the corresponding proton resonances.

To provide a better understanding of the downfield NMR shifts of the inclusion complex of AO with CB[7], the ^{1}H NMR spectra of AO in D_2O at different concentrations and in the absence of CB[7] were first investigated (Figure 6). The resonances of the AO ring and methyl protons were observed to shift markedly to low fields and to become narrower upon dilution. A plausible explanation would involve deaggregation of polymeric AO species (the planes of the AO rings were stacked in a nearly parallel array) into oligomeric, dimeric, and monomeric forms.[41,42] In the case of MB, similar phenomena were also observed in aqueous solutions (Figure S2 in the Supporting Information).

The ^{1}H NMR spectra of AO at a relatively low concentration (3.0×10^{-4} mol L^{-1}) and of the corresponding AO·CB[7] complex in D_2O were further investigated (Figure 7). Upon addition of CB[7] (0.4 equiv.) the resonances of the AO protons shifted slightly to lower fields and became broad, and these induced shifts were even more pronounced upon addition of CB[7] (1.0 equiv.). However, only one set of broad peaks of the AO proton resonances was observed irrespective of the number of equivalents of CB[7] added. These NMR spectroscopic data mean that the intermolecular complexation–decomplexation process between AO and CB[7] was fast on the NMR timescale, similarly to

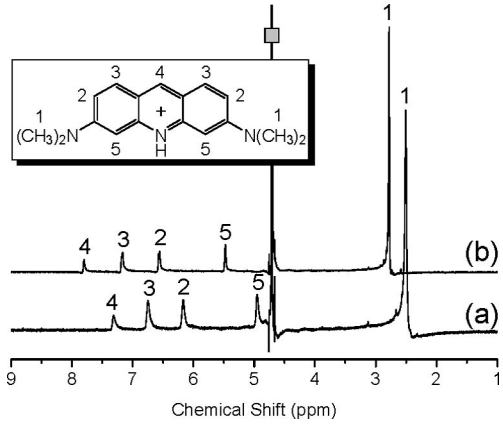

Figure 6. ^{1}H NMR spectra of aqueous AO solutions at different concentrations, with HOD proton resonance labeled with squares: (a) 1.0×10^{-3} mol L^{-1}; (b) 3.0×10^{-4} mol L^{-1}.

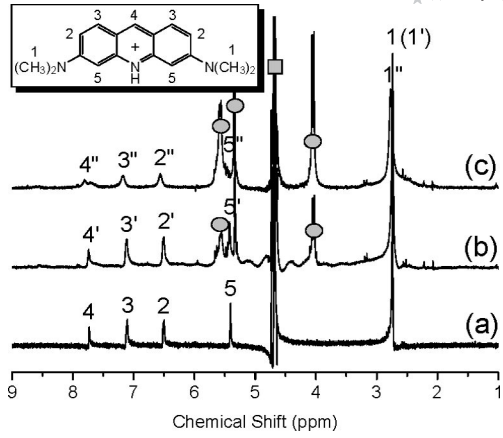

Figure 7. ^{1}H NMR spectra of: (a) AO, (b) AO and CB[7] (0.4 equiv.), and (c) AO and CB[7] (1.0 equiv.). AO 3.0×10^{-4} mol L^{-1}; CB[7] proton resonances labeled with circles; HOD proton resonance labeled with squares.

the case of the complex of 1-(5-carboxypentyl)-1′-ethyl-4,4′-bipyridinium with CB[7] reported by Kaifer and coworkers.[43] Rapid exchange was occurring between AO in the aqueous solution and AO encapsulated inside the CB[7] cavity. The fast intermolecular exchange process averaged out the differences in proton resonance not only between the AO·CB[7] complex and AO in the aqueous solution but also between the partially included moiety of AO in the CB[7] cavity and the extended one of AO outside the cavity. In view of the C_{2v} symmetry of AO it is most likely that one half of the AO was dissociated from CB[7] and the other half might be included rapidly (the two halves are innately indistinguishable), so that only one set of broad peaks was observed. Obviously, the downfield shifts of the proton resonances of the AO·CB[7] complex were the net result of upfield shifts arising from the inclusion of AO in the CB[7] cavity and downfield shifts resulting from the deaggregation of the AO aggregates. From Figures 5b and 7c, it can be seen that the magnitudes of the downfield shifts of the inclusion complex of AO at a high concentration with CB[7] were larger than those for the corresponding complex at a low AO concentration, because the downfield shifts resulting from the deaggregation of AO at a high concentration were larger than those at a low concentration.

DFT calculations of ^{1}H NMR spectra also clearly showed that the proton resonances of AO dimers (Figure 8a) and of AO inclusion complexes inside the CB[7] cavity (Figure 8c) were shifted upfield relative to those of AO monomers (Figure 8b). The shifts of the former were larger than those of the latter. Direct comparison of spectrum *a* with spectrum *c* explained well the unusual downfield NMR shifts of the inclusion complex. In our NMR spectral experiments, the inclusion complex of AO with CB[7] corresponded to the deaggregation of AO aggregates, resulting in a downfield shift of the AO proton resonances, followed by the encapsulation of AO in the shielding hydrophobic CB[7] cavity, leading to an upfield shift of the AO proton resonances. The inclusion-induced upfield shifts were overwhelmed by the deaggregation-induced downfield ones.

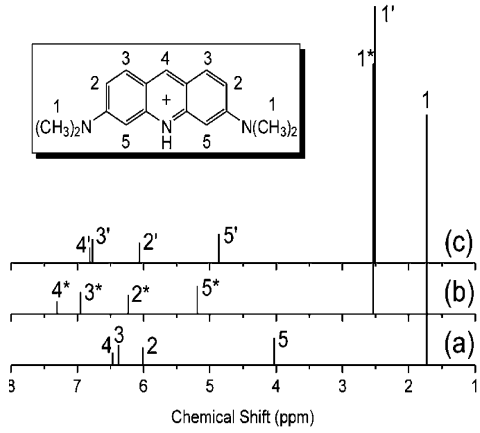

Figure 8. Theoretically predicted ^{1}H NMR spectra of: (a) AO dimer, (b) AO monomer, and (c) AO inside the CB[7] cavity. The calculations were carried out at B3LYP/6-31G(d) level.

UV/Vis spectroscopy was further used to study the deaggregation of AO aggregates when they were included in the CB[7] cavities. Two absorption maxima at 267 and 490 nm corresponded to the AO monomers at a low concentration of 3.0×10^{-6} mol L^{-1} (spectrum *a* in Figure S3 in the Supporting Information). With increasing AO concentration in the aqueous solution, a peak at ca. 470 nm grew progressively, so that the peak intensity reached an absorbance comparable to that at 490 nm due to the AO monomers. These spectral changes were generally attributed to the aggregation of the dye molecules into dimers, trimers, and high oligomers as the concentration was increased.[44–46] It is obvious that the AO molecules were aggregated when the dye concentration of AO was at 3.0×10^{-5} mol L^{-1} (spectrum *d* in Figure S3 in the Supporting Information). Upon addition of CB[7] to the aqueous AO solution, the absorption peak of the AO monomers was increased in intensity together with a slight blueshift, and the peak at 470 nm disap-

peared with increasing CB[7] concentration (Figure 9). Because CB[7] itself does not absorb in the visible region, the spectral changes observed upon addition of CB[7] were due to the deaggregation of AO aggregates and the inclusion of single AO molecules in the CB[7] cavities. These results are consistent with the corresponding NMR spectra. Upon addition of Cob^+ to a system of the AO·CB[7] complex, the peak at 486 nm due to the inclusion of a single AO molecule in the CB[7] cavity decreased, accompanied by the reappearance of the peak at 470 nm due to the AO aggregates with increasing Cob^+ concentration (Figure 10). The last UV/Vis spectrum measured was identical to that observed prior to addition of CB[7]. This means that the single AO molecules included in the CB[7] cavities were replaced by the competitive binding of Cob^+, followed by the formation of aggregates in the aqueous solution. The competitive binding process could be directly observed from the color changes of the system (Figure S4 in the Supporting Information). The aqueous AO solution showed a yellow color and became green upon addition of CB[7], and the recovery of the yellow color followed after the addition of Cob^+. These results provided further evidence for the encapsulation of AO in the CB[7] cavity.

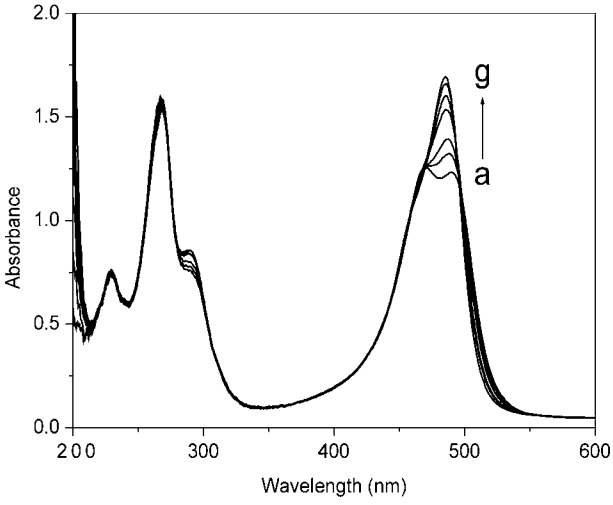

Figure 9. Absorption spectra of AO (3.0×10^{-5} mol L^{-1}, Tris-HCl at pH = 7.0) in the presence of CB[7] at different concentrations. CB[7] (10^{-5} mol L^{-1}): (a) 0, (b) 0.5, (c) 1, (d) 3, (e) 5, (f) 8, and (g) 12.

Further UV/Vis spectral studies indicate that the above spectral changes resulted from the competitive binding of Cob^+, and there was no interaction between Cob^+ and AO. The UV/Vis spectra of (a) CB[7], (b) Cob^+, (c) AO, (d) a mixture of Cob^+ and CB[7], and (e) a mixture of Cob^+ and AO are compared in Figure S5 in the Supporting Information. It is obvious that spectrum *c* was essentially the sum of spectra *a* and *e*. This indicates that no interaction occurred between Cob^+ and AO and that Cob^+ had no influence on the AO absorbance in the visible region, whereas the addition of CB[7] to the aqueous Cob^+ solution gave rise to an obvious decrease in intensity in the band at 261 nm, which verified the formation of the inclusion complex of Cob^+ with CB[7].

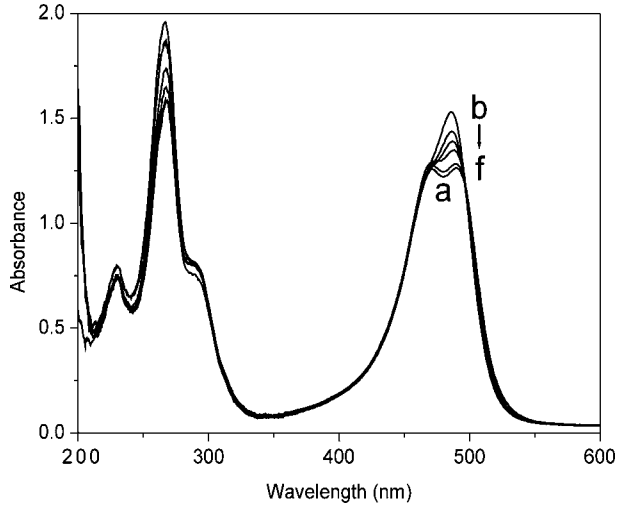

Figure 10. Absorption spectra: (a) of AO (3.0×10^{-5} mol L^{-1}, Tris-HCl at pH = 7.0), and (b)–(f) of the complex of AO (3.0×10^{-5} mol L^{-1}) and CB[7] (3.0×10^{-5} mol L^{-1}) in the presence of Cob^+ at different concentrations. Cob^+ (10^{-5} mol L^{-1}): (b) 0, (c) 0.5, (d) 1, (e) 2, (f) 3.5.

Conclusions

The formation of the inclusion complex of AO with CB[7] has been confirmed by a combination of a number of techniques and with the aid of competitive binding of Cob^+ and theoretical calculations based on first principles. Unusual downfield NMR shifts of the proton resonances of included AO (relative to AO) were observed in aqueous solutions, with aggregation easy even at low concentrations. The process of complex formation might correspond to the deaggregation of AO aggregates followed by the inclusion of single AO molecules in the CB[7] cavities. The intermolecular complexation/decomplexion processes between the AO·CB[7] complex and AO in the aqueous solution and between the partially included moiety of AO in the CB[7] cavity and the extended one outside the cavity were fast on the NMR timescale. The downfield shifts of the proton resonances of the AO·CB[7] complex were the net result of small upfield shifts arising from the inclusion of AO in the CB[7] cavity and large downfield shifts resulting from the deaggregation of the AO aggregates. Further theoretical calculations explained the unusual downfield NMR shifts well. These research results should be very helpful for understanding of the structures and properties of complexes of related organic molecules with macrocyclic hosts.

Experimental Section

CB[7] was synthesized by the reported procedure.[47,48] AO was purchased from Shanghai Reagent Co. (China). Cob^+ was purchased from Aldrich. PBS (pH = 7.5) and Tris-HCl (pH = 7.0) solutions at pH = 7.5 were used in the experiments. NMR samples were prepared in D_2O (Beijing Chongxi High-Tech Incubator Co., Ltd.; 99.9% minimum in D). All chemicals used were of analytical grade, and water used was double-distilled.

Steady-state fluorescence spectra were recorded with an F-4500 fluorescence spectrometer (Hitachi, Japan) with a 5.0 nm bandwidth for excitation and emission. The excitation wavelength was fixed at 485 nm. The UV/Vis spectra were recorded with a U-3010 spectrometer (Hitachi, Japan). All fluorescence and absorbance measurements were carried out with aqueous solutions in quartz cells of 10 mm path length. ^{1}H NMR measurements were carried out with a Bruker DRX 500 spectrometer, and MALDI-TOF mass spectra were recorded with an Autoflex time-of-flight instrument (Bruker, Germany) with α-cyano-4-hydroxycinnamic acid as the matrix. The pH measurements were made with a model pHS-3C pH-meter (Shanghai, China). All measurements were performed under air at ambient temperature.

Density functional theory (DFT) was applied to investigate the electronic structures of the complex of AO with CB[7]. The hybrid functionals, B3LYP,[49–51] with the 6-31G(d) basis set were employed in DFT calculations. The interaction energies between AO and CB[7] were calculated as the difference in electronic energies between the AO·CB[7] complex and the two monomers. Because of incompleteness of the basis set, one often encounters basis set superposition error (BSSE)[52] in the evaluation of interaction energies. The Boys and Bernardi counterpoise method[53] was employed to lessen the BSSE. All calculations were performed with the Gaussian03 program[54] in parallel mode on a Linux workstation housed at Nanjing University.

Supporting Information (see footnote on the first page of this article): Equation for nonlinear least-squares fitting of association constant, mass spectrum of AO with CB[7], absorption spectra of aqueous AO solutions of different concentrations, absorption spectra of CB[7], Cob^+, AO, and their mixtures, color changes of aqueous AO solutions in the absence and presence of CB[7] and Cob^+, and NMR spectra of aqueous MB solutions with different concentrations.

Acknowledgments

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[1] C. Marquez, W. M. Nau, *Angew. Chem. Int. Ed.* **2001**, *40*, 4387–4390.
[2] J. Lagona, P. Mukhopadhyay, S. Chakrabarti, L. Isaacs, *Angew. Chem. Int. Ed.* **2005**, *44*, 4844–4870.
[3] K. Kim, N. Selvapalam, Y. H. Ko, K. M. Park, D. Kim, J. Kim, *Chem. Soc. Rev.* **2007**, *36*, 267–279.
[4] Y. Tan, S. W. Choi, J. W. Lee, Y. H. Ko, K. Kim, *Macromolecules* **2002**, *35*, 7161–7165.
[5] J. W. Lee, Y. H. Ko, S. Park, K. Yamaguchi, K. Kim, *Angew. Chem. Int. Ed.* **2001**, *40*, 746–749.
[6] R. Wang, L. Yuan, D. H. Macartney, *Chem. Commun.* **2005**, 5867–5869.
[7] J. W. Lee, S. Samal, N. Selvapalam, H.-J. Kim, K. Kim, *Acc. Chem. Res.* **2003**, *36*, 621–630.
[8] J. Mohanty, W. M. Nau, *Angew. Chem. Int. Ed.* **2005**, *44*, 3750–3754.
[9] D. Kim, E. Kim, J. Kim, K. M. Park, K. Baek, M. Jung, Y. H. Ko, W. Sung, H. S. Kim, J. H. Suh, C. G. Park, O. S. Na, D. K. Lee, K. E. Lee, S. S. Han, K. Kim, *Angew. Chem. Int. Ed.* **2007**, *46*, 3471–3474.
[10] I. Hwang, W. S. Jeon, H. J. Kim, D. Kim, H. Kim, N. Selvapalam, N. Fujita, S. Shinkai, K. Kim, *Angew. Chem. Int. Ed.* **2007**, *46*, 210–213.
[11] S. Moghaddam, Y. Inoue, M. K. Gilson, *J. Am. Chem. Soc.* **2009**, *131*, 4012–4021.
[12] S. Liu, P. Y. Zavalij, L. Isaacs, *J. Am. Chem. Soc.* **2005**, *127*, 16798–16799.
[13] Z. Miskolczy, L. Biczok, M. Megyesi, I. Jablonkai, *J. Phys. Chem. B* **2009**, *113*, 1645–1651.
[14] B. D. Wagner, S. J. Fitzpatrick, M. A. Gill, A. I. MacRae, N. Stojanovic, *Can. J. Chem.* **2001**, *79*, 1101–1104.
[15] B. D. Wagner, N. Stojanovic, A. I. Day, R. J. Blanch, *J. Phys. Chem. B* **2003**, *107*, 10741–10746.
[16] M. A. Rankin, B. D. Wagner, *Supramol. Chem.* **2004**, *16*, 513–519.
[17] T. A. Martyn, J. L. Moore, R. L. Halterman, W. T. Yip, *J. Am. Chem. Soc.* **2007**, *129*, 10338–10339.
[18] A. Hennig, H. Bakirci, W. M. Nau, *Nat. Methods* **2007**, *4*, 629–632.
[19] A. L. Koner, W. M. Nau, *Supramol. Chem.* **2007**, *19*, 55–66.
[20] Y. Ling, W. Wang, A. E. Kaifer, *Chem. Commun.* **2007**, 610–612.
[21] R. L. Halterman, J. L. Moore, L. M. Mannel, *J. Org. Chem.* **2008**, *73*, 3266–3269.
[22] M. Megyesi, L. Biczok, I. Jablonkai, *J. Phys. Chem. C* **2008**, *112*, 3410–3416.
[23] W. M. Nau, J. Mohanty, *Int. J. Photoenergy* **2005**, *7*, 133–141.
[24] J. Mohanty, H. Pal, A. K. Ray, S. Kumar, W. M. Nau, *ChemPhysChem* **2007**, *8*, 54–56.
[25] S. Liu, A. D. Shukla, S. Gadde, B. D. Wagner, A. E. Kaifer, L. Isaacs, *Angew. Chem. Int. Ed.* **2008**, *47*, 2657–2660.
[26] T. Ooya, D. Inoue, H. S. Choi, Y. Kobayashi, S. Loethen, D. H. Thompson, Y. H. Ko, K. Kim, N. Yui, *Org. Lett.* **2006**, *8*, 3159–3162.
[27] R. Wang, L. Yuan, H. Ihmels, D. H. Macartney, *Chem. Eur. J.* **2007**, *13*, 6468–6473.
[28] A. C. Bhasikuttan, J. Mohanty, W. M. Nau, H. Pal, *Angew. Chem. Int. Ed.* **2007**, *46*, 4120–4122.
[29] D. C. Sharmistha, M. Jyotirmayee, P. U. Hari, C. B. Achikanath, P. Haridas, *J. Phys. Chem. B* **2009**, *113*, 1891–1898.
[30] P. Montes-Navajas, L. Terurl, A. Corma, H. Garcia, *Chem. Eur. J.* **2008**, *14*, 1762–1768.
[31] S. A. Krolenko, S. Y. Adamyan, T. N. Belyaeva, T. P. Mozhenok, *Cell Biol. Int.* **2006**, *30*, 933–939.
[32] A. I. Kononov, *J. Phys. Chem. B* **2001**, *105*, 535–541.
[33] M. B. Lyles, I. L. Cameron, *Biophys. Chem.* **2002**, *96*, 53–76.
[34] S. Clerc, Y. Barenholz, *Anal. Biochem.* **1998**, *259*, 104–111.
[35] M. Shaikh, J. Mohanty, P. K. Singh, W. M. Nau, H. Pal, *Photochem. Photobiol. Sci.* **2008**, *7*, 408–414.
[36] Y. Zhou, H. Yu, J. Sun, L. Wu, Q. Lu, L. Wang, *J. Inclusion Phenom. Macrocyclic Chem.* **2008**, *61*, 259–264.
[37] P. Montes-Navajas, A. Corma, H. Garcia, *ChemPhysChem* **2008**, *9*, 713–720.
[38] W. Ong, A. E. Kaifer, *Organometallics* **2003**, *22*, 4181–4183.
[39] S.-H. Chou, M. J. Wirth, *J. Phys. Chem.* **1989**, *93*, 7694–7698.
[40] *Heterocyclic Compounds*; *V.9. Acridines* (Ed.: R. M. Acheson), 2nd ed., Interscience, New York, **1973**.
[41] D. J. Blears, S. S. Danyluk, *J. Am. Chem. Soc.* **1966**, *88*, 1084–1085.
[42] D. J. Blears, S. S. Danyluk, *J. Am. Chem. Soc.* **1967**, *89*, 21–26.
[43] V. Sindelar, S. Silvi, A. E. Kaifer, *Chem. Commun.* **2006**, 2185–2187.
[44] J. S. Tan, R. L. Schnelder, *J. Phys. Chem.* **1975**, *79*, 1380–1386.
[45] R. D. Falcone, N. M. Correa, M. A. Biasutti, J. J. Silber, *Langmuir* **2002**, *18*, 2039–2047.
[46] I. Fuyuki, K. Toshifumi, N. Toshihiko, *J. Phys. Chem. C* **2007**, *111*, 6983–6988.
[47] C. Marquez, F. Huang, W. M. Nau, *IEEE Trans. Nanobiosci.* **2004**, *3*, 39–45.
[48] J. Kim, I. S. Jung, S. Y. Kim, E. Lee, J. K. Kang, S. Sakamoto, K. Yamaguchi, K. Kim, *J. Am. Chem. Soc.* **2000**, *122*, 540–541.
[49] A. D. Becke, *Phys. Rev. A* **1988**, *38*, 3098–3100.
[50] C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B* **1988**, *37*, 785–789.

[51] B. Miehlich, A. Savin, H. Stoll, H. Preuss, *Chem. Phys. Lett.* **1989**, *157*, 200–206.
[52] B. J. Ransil, *J. Chem. Phys.* **1961**, *34*, 2109–2118.
[53] S. F. Boys, F. Bernardi, *Mol. Phys.* **1970**, *19*, 553–566.
[54] M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery Jr, T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, *Gaussian 03*, Revision D.01, Gaussian, Inc., Wallingford, CT, **2004**.

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