Self-Inclusion and Dissociation of a Bridging \(\beta\)-Cyclodextrin Triplet

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**ABSTRACT:** To understand the self-inclusion and the dissociation in a branched \(\beta\)-cyclodextrin (CD) system, we designed and synthesized a \(\beta\)-CD trimer in which each CD group is connected to one of bridging arms of a planar triphenylbenzene core through a CuAAC click reaction. Only one rather than two or all of the three host CDs was demonstrated to be in a self-including state in water, while no self-inclusion was observed to occur in dimethylsulfoxide (DMSO) via the characterization of \(^1\)H and NOESY NMR spectra. The configuration structures of the CD groups in the self-included state were evaluated, and the dissociation to free state in water was investigated under various conditions like heating, increased acidity, and discharging versus the addition of competitive guests. While raised temperature and increased acidity did not break the self-inclusion, two adamantane guest molecules were found to show capability in driving the equilibrium to get back to free state against the self-inclusion. The inclusion process of the added guests was believed to involve in the dissociation of the self-inclusion and the occupation of the guests in CD cavity. The results of host–guest interaction study indicated that the stable combination of guests was favorable for blocking the structural overturning of glucose toward trapping the bridging group into the cavity.

**INTRODUCTION**

Cyclodextrins (CDs) are a series of cyclic oligomers consisting of \(\alpha\)-\(\omega\)-glucopyranose monomers that connect each other through 1,4-positions. While inheriting the biocompatibility and solubility in water from glucopyranose, the CDs are widely used as a supramolecular host and display useful potentials in biomaterials.

Because of the intramolecular hydrogen bond network formed from OH-2 and OH-3 groups as well as the \(4C_1\) chair conformation of glucopyranose units, the formed remarkable structural rigidity comprises a truncated conical hollow structure.

The H-3 and H-5 inside the CDs contribute to the hydrophobic cavity with different sizes depending on the number of glucopyranose groups, which provides an opportunity to interact with varied forms of guest molecules through hydrophobic and van der Waals interactions.

In contrast to the properties of the isolated state, the bridged CDs not only possess all of the advantages of isolated ones but also bring about extended utilities and synergistic effects toward new host–guest inclusion properties.

For example, a bridging CD dimer interacted with zinc-porphyrin sulfonate guest through a co-inclusion of two CD cavities, yielding an ultrasensitive detector of electrochemical luminescence.

Multiple bridging CDs were used as building blocks to assemble with other molecular units for the fabrication of multifunctional structures through multiple host–guest interactions. However, the linking groups often cause an unexpected structural state due to the inevitable self-inclusion deriving from a conformation transformation, especially when the linker is a triazole group that is formed from a commonly used “click reaction” for coupling two groups.

The self-inclusion for single decorated \(\beta\)-CD has already been studied carefully, in which one of glucose units was proposed to rotate 360\(^\circ\), allowing the linker to get into the cavity and the multibridging CDs undergo the similar way.

The understanding for the self-inclusion of bridging CDs, however, is still not clear enough because the known outline limited to that the occurred self-inclusion was closely relied on the length and hindrance of the linkers. For instance, no self-inclusion occurs when the linker of a bridging system is shorter, and the phenomenon is observed only when the linker is longer enough for allowing the overturning of a glucopyranose group.

In a tetra-bridging system, CDs are found in a self-included state, but the number and position of CDs is still unknown and up to date, the self-inclusion of tribridging CDs was not involved.

A general method overcoming the self-inclusion in all bridged CD systems has been established through fixing the primary face of the bridged CD and restraining the rolling of the glucose unit that grafts a connecting group, but the method is pretty hard in synthesis.
During developing building units comprising of tribridging CDs for host–guest cross-linked self-assemblies, understanding the possible self-inclusion and finding out effective approach for avoiding such an unwanted occupation become very important. Therefore, in this work, we present the self-inclusion and dissociation of a triplet-arms-bridging β-CDs with a planar triphenylbenzene core (tri-CD) in water. The obtained results confirm that only one β-CD unit is in a self-included state. Different methods are employed to break the self-inclusion and two types of adamantane derivatives that have strong interaction with β-CD are found to be able to resist the self-inclusion in a large extent. We believe that the included system can interact with other building blocks through electrostatic interaction for further assembly, and the broadened use of bridging CDs can be envisioned with these guests.

**RESULTS AND DISCUSSION**

**Synthesis and Structural Characterization.** The synthetic route of the tri-CD is shown in Chart 1a and the detailed procedures are summarized in the Experimental Section and Electronic Supporting Information (ESI, Figures S1–S6). The precursor 4-propynyl-acetophenone and 1,3,5-triphenylbenzene core decorated with alkynyl were prepared according to the procedures in the literature. The propynylxoyacetophenone was prepared from hydroxyphenyl-ethanone and bromopropyne through Williamson’s reaction. The triphenylbenzene was synthesized from the condensation of acetophenone compound. As for the final product tri-CD, it was prepared from the Cu(I)-catalyzed alkyne–azide cycloaddition (CuAAC) with azido-modified β-CD. The 1H NMR and 13C NMR spectra of the target product tri-CD in DMSO-d6 confirm the agreement with the target chemical structure (Figures S4 and S5). The proton peaks at downfield are well corresponding to the triazole and triphenylbenzene rings, while the peaks at upfield are consistent with the bridged CDs in literature. The MALDI-TOF MS spectrum gives a peak referring to the mass of tri-CD+Na+ (Experimental Section and Figure S7). The elemental analysis indicates that the chemical composition is corresponding to tri-CD·9H2O, in which each CD contains three water molecules averagely, as described in Experimental Section.

**Self-inclusion and Its Solvent Dependence.** The 1H NMR spectrum of tri-CD in DMSO-d6 shows that only four proton peaks corresponding to triazole and triphenylbenzene rings, marked with H(a*, b*, c*, and d*) (Chart 1a), appear in the downfield in Figure 1a, indicating identical chemical environment of the groups. With the addition of D2O up to the volume ratio of 1:1, some additional proton peaks emerge at downfield but move slightly toward higher fields with respect to each of corresponding protons H(a*, b*, c*, and d*) and can be attributed to the protons of triazole and triphenylbenzene groups starting to locate at different chemical environments. Further increasing D2O volume ratio to 7:3, the proton peaks ascribing to H(a*, b*, c*, and d*) change to another

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**Figure 1.** (a) 1H NMR spectra of tri-CD in the D2O solutions with different DMSO-d6 volume percentage, where the peaks H(d and d*) are set as references, (b) the chemical shift change (Δδ) plots of the protons referring to their positions in D2O shown in (a), and (c) the difference plots of Δδ values from each proton at different state vs the increase of DMSO-d6.
state almost completely, implying that the identical three CD groups have deviated to their initial environment. The proton peaks evolve gradually into two different states and some of CD groups can be assigned to undergo a self-inclusion process by referring to the published results. At the state of full D$_2$O, a complete self-inclusion of one CD while the other two CD groups at free state can be confirmed based on the ratio of integral values. Clearly, the protons H(a, b, c, and d) that change regularly following the gradual decrease of DMSO-d$_6$ volume percentage can be definitely ascribed to the protons at noninclusion state because of their clear evolution from H(a*, b*, c*, and d*), owing to the difference of shielding effect between the self-inclusion and noninclusion CD units. The other peaks H(a′, b′, c′, and d′) that emerge at new positions can be attributed to the groups connecting to the self-inclusion unit. The integral area calculation further points out that the self-inclusion only occurs in one of three CDs, due to the 2:1 area ratio of H(a, b, c, and d) to H(a′, b′, c′, and d′).

By taking the tri-CD in D$_2$O as the initial state, we can outline more about the opposite process of self-inclusion-dissociation by evaluating the peaks’ position change of guest groups on the linking arm versus the addition of DMSO-d$_6$ up to 40% (Figure 1b). When using the proton H(d) far from inclusion position at free state for reference, the movement of guest groups toward rolling over can be deduced to experience a conformation change from self-inclusion to noninclusion. It is seen that proton H(d′) shifts toward lower field and finally disappears, indicating its going into the deep cavity of CD and out of inclusion. The consistent phenomenon occurs on proton H(c′) and further confirms the structural change during polarity decrease. For other protons H(a′, b′), they shift to higher field with the addition of DMSO-d$_6$ (Figure 1c), revealing the move away from the interaction with CD. From the analysis of the chemical shift change of protons H(a′, b′, c′, and d′) at inclusion state relevant to the protons H(a, b, c, and d), we also observe the larger movement of corresponding groups out of cavity at inclusion state after the addition of DMSO-d$_6$.

As the used tri-CD sample solid in the above investigation comes from the evaporation of the tri-CD water solution, the obtained solid should be in a self-included state. To clarify the influence of the initial solid on the self-inclusion, the NMR spectra are carried out within 2 min after adding the solid tri-CD into DMSO-d$_6$, and only noninclusion structure is observed. The result means the conformation transformation is very fast in DMSO, indicating that the initial history does not affect the succedent structure understanding. We also prepared a noninclusion solid via the precipitation of tri-CD from DMSO by the addition of acetone. When this solid dissolves in D$_2$O, only a self-included structure is detected from NMR measurement within 2 min, revealing that the reverse conformation transformation is very fast as well.

2D NMR NOESY Spectra of Self-Inclusion. To further confirm the assignment of the proton peaks sourcing from the bridging groups and the self-inclusion with the interaction between protons from host and guest groups in space, two-dimensional nuclear overhauser effect spectroscopy (2D NOESY) of the tri-CD is characterized. Based on the principle of 2D NOESY, correlation peaks in 2D spectrum occur on the protons closing to each other with the spatial distance less than 5 Å. The NOESY spectrum in DMSO-d$_6$ (Figure S8) shows that the proton peak at 8.16 ppm correlates with the protons of methenyl, phenyl ring, and CD and can be clearly assigned as H(d*). Yet, the other singlet peak at 7.81 ppm does not show such correlation with methenyl and CD and thus it can be attributed to H(a*). The doublet peak at 7.18 ppm that has an apparent correlation signal with H(d*) should be H(c*) due to the close distance from it, while the rest doublet peak at 7.76 ppm that has no correlation with H(d*) can be ascribed to H(b*) because of the longer distance from it. The apparent correlations of protons H(a*, b*) with H(c*) but not H(d*) support the assignment for these protons. The 2D NOESY spectrum in D$_2$O (Figure S9) and D$_2$O mixture with DMSO-d$_6$ (20%) (Figures 2 and S10) show similar correlation relationships among protons H(a, b, c, and d) and among H(a′, b′, c′, and d′) to those among H(a*, b*, c*, and d*) in DMSO though the assignment for the latter is difficult due to the heavier overlap of the proton peaks. It is interesting to see that the protons H(c and d) show no correlation with all protons H(a′, b′, c′ and d′) that are ascribed to the groups in the self-inclusion state, yet the protons H(a and b) display obvious correlation with H(a′ and b′). According to the position labeling, it is rational for the protons of 1,3,5-triphenylbenzene, especially for those near the central phenyl group to show correlations even if a part of it is at the state of self-inclusion. The protons H(a′, b′, c′, and d′) exhibit distinct correlation with the protons of H-3 and H-5 in the cavity of CD part, further identifying the conformation overturn and the self-inclusion. In contrast to this, the protons H(d and c) perform light correlation with the protons from CD while the protons H(b and a) display a bit stronger correlation. Because of the heavier overlap and the comp lexation for the assignment of protons in CD, it is impossible to discern the one at the inclusion state from the other ones at free state for a precise clarification. However, based on the data here, we ascribe the former case to the close distance of the protons from the CD at free state while the latter case to the influence of self-inclusion to the central phenyl group. Thus, a self-inclusion structure is proposed as presented in Chart 1a.

Temperature Influence on the Self-Inclusion. The host–guest inclusion of CD is sensitive to the environment, and the interaction gets weak with the increase of temperature. By taking self-inclusion as a kind of host–guest interaction of a complex bearing the two parts, we evaluate the temperature dependence of tri-CD in aqueous solution. As
shown in Figure 3, with the increase of temperature, both the ratios of integral areas between the proton peaks at free and self-included states maintain constant while no additional peaks appear under heating. The result demonstrates that the self-inclusion is not destroyed during the temperature increasing up to 80 °C. Since the chemical shift of HDO species (halt deuteration of water, normally setting as reference in D₂O) moves a lot upon heating, it becomes no longer applicable and therefore we select peak H(d) as the reference due to its position far from the self-inclusion site, as used in NMR measurement (Figure 1) in the mixture solvent. As the proton peaks at the included state always emerge at higher field in comparison to those assigned at free state, the relative moving of the proton chemical shifts for all protons from the bridging group at high temperature can be ascertained to derive from the local position change with respect to the host–guest inclusion. It is seen that the proton peaks from H(a, b, and c) locating at free side and the proton peaks from H(a′, b′, and c′) inside of CD move to lower field (Figure 3b), indicating the groups getting rid of the influence of self-inclusion. In contrary to this, the proton peak of H(d′) shifts to higher field, implying it getting closer to inner position of the CD cavity. By further comparing the shifting of proton peaks between the groups at free state and at inclusion state (Figure 3c), H(d′) shows largest change with the temperature rising up, while H(c′) displays a larger shifting than those of H(a′, b′) which show smaller shifting, indicating the order for the moving from inclusion position. The positive values imply the protons H(c′, d′) getting closer to the cavity and the negative values reveal the protons H(a′, b′) getting out of the cavity. Because the dissociation has to encounter the reverse path of the self-inclusion to make the triazole group going out of the cavity, the observed state at raised temperature should be at the actuated stage for overturn of the glucopyranose unit. As expected, the inclusion position change of host and guest groups with each other versus the temperature increasing is in complete agreement with the solvent polarity change before the ratio of DMSO no higher than 40%.

**pH Influence on the Self-Inclusion.** The triazole group in the bridging arm can be protonated under acidic environment and the formed positive charge is unfavorable for the host–guest interaction with CD. We evaluate the self-inclusion change of tri-CD by adding deuterated hydrochloric acid (DCl) into deuterated aqueous solution. As shown in Figure 4a of tri-CD solution (0.5 mmol·L⁻¹), no additional peaks are observed and the integral values of those peaks ascribing to the protons at free state maintain the ratio of 2:1 to those from the protons at included state, demonstrating that the self-inclusion is not broken, even the concentration of DCl reaches to 1.8 mol·L⁻¹. Under the circumstances of normalized chemical shift of proton H(c), the movement of other protons' chemical shifts discloses the structural change induced by acidity. Apparently, the proton peaks from H(d′, d) exhibit a bit higher moving lower field, and the shifting for the latter even much larger than that for the former indicates the protonation-induced influence on the hydrogen atom of triazole ring. Within the range of used acidity, the proton H(d) still shows tendency toward lower field, so that the protonation seems not complete. By taking the peak shifts of H(d and d′) as the reference of protonation level at 1.8 mol·L⁻¹ DCl, we can figure out the percentage of the protonated triazole group only ca. 15% and ca. 10% of the triazole group at pH In 1 of DCl (pH < 1). That means, in a pH = 1 solution, the protonated triazole group is ca. 5% and tri-CD is hard to protonate under this condition. It should be noted that the

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**Figure 3.** (a) Partial NMR spectra of tri-CD in D₂O at different temperatures with the peak H(d) as reference, (b) the chemical shift change (Δδ) plots of the protons referring to their positions at 25 °C shown in (a), and (c) the difference plots of Δδ values from each proton at different state vs the temperature increase.

**Figure 4.** (a) Models of tri-CD in acidic environment; (b) ¹H NMR spectra of tri-CD (0.5 mmol·L⁻¹) in D₂O with the addition of DCl at the concentration shown in the spectra, and the peak H(c) is set as the reference, (c) the chemical shift change (Δδ) plots of the protons referring to their positions at no DCl shown in (b), and (d) the difference plots of Δδ values from each proton at different state vs the DCl concentration increase.
chemical shifts of other protons on bridging groups (Figure 4b) move on contrary to the cases adding DMSO and increasing temperature. All of the proton peaks of H(a and b) and H(a’ and b’) shift to higher field, illustrating that they are getting closer or inside of the CD cavity. In accordance with this, the peak of proton H(c’) shifts lower field, suggesting that the proton tends to leave the cavity. The relative movement of protons at free and inclusion states shown in Figure 4c supports the inclusion groups going out toward the triazole group. With these changes, we conclude that there is a pulling effect of the guest into CD cavity accompanied by the protonation of triazole group in acidic solution due to the repelling force of the hydrophilic protonate position to the hydrophobic cavity.

**Influence of Competitive Guests on the Self-Inclusion.** Considering the activity similar to that of DMSO, competitive guests are believed to dissociate the self-inclusion. Three adamantane derivatives with different substituted groups (Chart 1c), N,N,N-trimethyl-adamantan-1-aminium iodide (ada1), 2-adamantanacycloxy-N,N,N-trimethyl-ethan-1-aminium iodide (ada2), and sodium adamantane-1-formate (ada3), are used to evaluate their influence on the self-inclusion of tri-CD. From the $^1$H NMR spectra in Figure 5, it is seen that beside the unchanged signals ascribing to the initial self-included state, three additional broad peaks emerge. Definitely, the CD groups in tri-CD become different from self-inclusion state after the addition of competitive guests and the additional proton signals can be attributed to the inclusion effect of ada1, ada2, and ada3, from which the self-inclusion has been dissociated. By excluding the disturbance of self-inclusion species via a deconvolution technique through a peak fitting, the integral area calculation gives a molar ratio of 3:9:6, just corresponding to the $H(\text{d}^\bullet)/[H(\text{a}^\bullet) + H(\text{b}^\bullet)]/H(\text{c}^\bullet)$ of tri-CD in the state that all of its CDs are identical. According to the integral area calculation, ada2 displays the strongest capability in breaking self-inclusion and utmost 94% of self-inclusion have been relieved. ada3 decomposes 75% of self-included species under the same condition and ada1 makes 53% of self-including species transform into the nonincluding state even if its concentration becomes twice of ada2 and ada3.

$^1$H NMR titration experiment is performed to monitor the dissociation of self-inclusion and consecutive host–guest inclusion process of tri-CD in the presence of guest molecules in water. By taking ada2 as an example, its gradual addition to tri-CD solution is conducted at a fixed tri-CD concentration of 1.0 mmol·L$^{-1}$. As shown in Figure 6a, because two of CDs in tri-CD always maintain at the nonincluded state, the addition of ada2 until twice of the tri-CD’s concentration leads to an underlying host–guest inclusion and the fast exchange of protons results in a mean peak H(d). However, when the concentration of ada2 reaches the three-fold of tri-CD, the full host–guest inclusion state starts to be observed. The result also indicates that the dissociation of self-inclusion is sourced from the competitive inclusion of ada2. In a proposed route of competitive host–guest interaction in Figure 6b, $K_d$ is defined as the association constant of one guest with one CD unit; $K_d^1$ is dissociation constant of the self-inclusion. For process-2, it can be regarded as the combination of dissociation of self-inclusion and the binding of external guest. In addition, the apparent association constant can be defined as $K_a \times K_d^1$ based on the inclusion equilibrium.

**Analysis of Fast and Slow NMR Exchange.** From the analysis of spectra in Figures 5a–d and 6a, we can separate the process of proton changes into two different types in the NMR study along the adding of guests, fast and slow exchange processes. The protons H(a, b, c, and d; a’, b’, c’, and d’) show a fast exchange after adding of guests because the shifted amalgamative signals appear in the spectra while the protons H(∗a, b∗, c∗, and d∗) display slow proton exchange as the separated proton signal pairs in which one belong to the unchanged state maintains at the initial position, and the other attributing to the changed state emerges at a different chemical shift. For a NMR fast exchange process, corresponding protons belonging different species give one merged peak. Here, the protons in the aromatic core in free tri-CD and H-G2 complex have the similar chemical structure and thus give a merged peak whose position relies to the ratio of free tri-CD and H-G2 complex, which shifts with the addition of the adamantane guest. When the formed H-G2 complex includes the third ada2 to form a H-G3 complex, a typical slow NMR exchange denoted as process-2 (Figure 6b) is observed because the process undergoes a dissociation of self-inclusion and then subsequent inclusion of guest. As a result, the proton in the aromatic core in tri-CD of H-G3 is no more similar to the one of uncombined tri-CD and the new peaks of H(∗a, b∗, c∗, and d∗) appear and the integral areas increase, indicating the formation of H-G3 along with the addition of ada2. As for the peak broadening, it maybe because the free space and hydrophobic interaction deriving from released self-inclusion.

**Figure 5.** Partial NMR spectra of (a) tri-CD solution, (b) tri-CD solution (3 mmol·L$^{-1}$) with ada1 (18 mmol·L$^{-1}$, 6 equiv), (c) tri-CD solution (4 mmol·L$^{-1}$) with ada2 (12 mmol·L$^{-1}$, 3 equiv), and (d) tri-CD solution (4 mmol·L$^{-1}$) with ada3 (12 mmol·L$^{-1}$, 3 equiv), green peaks are the linear fitting curves, the three integral areas are the areas of three broad peaks apart from the small peaks, (e) NMR process of fast and slow exchange.
results in the triphenylbenezene core’s aggregation. The peak broadening of noninclusion tri-CD appears at 35% DMSO solution, as shown in Figure 1a. Further increasing the content of DMSO leads to the decrease of solution polarity, leading to the aggregation unpacking and the peak broadening disappearance.

**Calculation of Association Constant for Competitive Guests.** The proposed process-2 is set as an equivalent route including a dissociation of self-inclusion by forming an unstable intermediate and then a recombination with the guest (Figure 6b). Thus, the plots of integral area of proton peaks H(d) (Sd) and H(d*) (Sd*) versus the concentration of the added guest are obtained (Figure 6c). Here, the total integral area of peaks H(d, d*, and a) is fixed at 3 because with this reference, the peaks of H(a) and H(d*) and the sum of peaks of H(d, d, and d*) will have the same integral area. Based on the fact that [H-G2] is in its stabilized state, when the concentration of ada2 is over 2 mmol-L\(^{-1}\), further addition of ada2 leads to the formation of H-G3. By taking Sd* as the concentration of H-G3, the plot of [H-G3] versus [ada2] is in the accordance with 1:1 host–guest interaction regulation (Figure 6d). Through a nonlinear fitting method, \(K_s\) and \(K_d\) value for ada2 is calculated to be \(2.23 \pm 0.17\) \(\times 10^4\) mol\(^{-1}\)-L. In contrast, \(K_s\) and \(K_d\) value for ada3 is calculated to be \(2.64 \pm 0.49\) \(\times 10^3\) mol\(^{-1}\)-L. (Figures S11 and S12), further revealing its weaker host–guest interaction with tri-CD. Although ada1 interacts with self-included \(\beta\)-CD partially, as described in the former discussion about Figure 5d, the host–guest interaction is obviously too weak to destroy the self-inclusion in the NMR titration study at such concentration, even the binding constant could not be calculated due to no apparent proton peak H(d*) emerging.

The host–guest interaction of proposed process-1 versus the change of [tri-CD] can be calculated under the fixed [ada2]. The nonappearing of proton peak H(d*) in the \(^1\)H NMR titration experiment (Figure S13) indicates that the self-included CD unit does not take part in the host–guest interaction when tri-CD is excess. A 1:1 inclusion ratio of host–guest interaction of added guests for each CD unit demonstrates that one free CD interacts with one guest molecule and the self-included CD does not interact with guest under this condition. In a simplified model, the two noninclusion CD units in tri-CD can be regarded as isolated CDs to have the same ability to include guest molecules because they are separated from each other completely. Thus, the calculation based on 1:1 nonlinear fitting method on the plots (Figure S13c,e,g) gives the \(K_s\) values 1.41 \(\pm 0.53\) \(\times 10^4\), 1.57 \(\pm 0.45\) \(\times 10^3\), and 7.28 \(\pm 0.03\) \(\times 10^2\) mol\(^{-1}\)-L for ada2, ada3, and ada1, which show the same order to those of apparent association constants.

With the \(K_s\) and \(K_d\) value in process-2 and \(K_s\) value in process-1, we estimate the \(K_s\) value to be ca. 1.58 \(\times 10^2\) for ada2 and ca. 1.68 \(\times 10^{-2}\) for ada3. As the process is almost irrelevant to the added guest molecules, the average value 1.63 \(\pm 0.05\) \(\times 10^2\) is deduced. When it was used as the \(K_d\) on ada1, the association constant \(K_s\) value for process-2 of ada1 is calculated to be 12.15 mol\(^{-1}\)-L, which is too small to be deduced from an NMR titration technique directly as those of other added guests conducted in process-2.

**Free-Energy Analysis of tri-CD under External Conditions and Inclusion Intensity of Guests.** Based on above self-inclusion and its dissociation results under different conditions, we proposed free-energy plots with regard to the status of tri-CD (Figure 7a–d). By adding DMSO to water solution of tri-CD, the free energy of noninclusion tri-CD decreases with its volume ratio increase (Figure 7a). Rising the temperature accelerates the thermal motion of molecules, the self-inclusion tends to be unstable yet the dissociation does not occur due to the lower energy (Figure 7b). When the glucose unit in self-inclusion rotates \(90^\circ\) in acid solution, the triazole group gets into the cavity (Figure 7c), leading to a difficult dissociation. On the other hand, when adding guests less than

![Figure 6](https://dx.doi.org/10.1021/acsomega.0c00363)

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twice of tri-CD in water solution, the H-G2 complex forms first and the system free energy does not decrease to the lowest level due to undisturbed self-inclusion. When excess guest is added, the energy gets lower by breaking self-inclusion and forming new host–guest inclusion. However, the formation of H-G3 complex without self-inclusion strongly depends on the energy compensation from the host–guest inclusion of the added guests (Figure 7d).

The association intensity of tri-CD interacting with the ionic adamantane derivatives is further characterized by 2D NOESY spectra (Figure 8a). The correlation signals indicate that the ionic head groups of all the guests are located at the secondary face of the CD unit, implying the irrelevance of the group orientation to the inclusion association. Considering the possible additional interaction of ionic head with CD unit, Fourier transform infrared (FT-IR) spectra (Figure 8b,c) are performed. The carbonyl stretching vibration band shifts from 1730 to 1722 cm⁻¹ for ada2 and from 1555 to 1544 cm⁻¹ for ada3 demonstrate the enhanced interaction of hydrogen bond. On the other hand, the nearer ionic heads close to the adamantane group performs inferior to the tight inclusion interaction with CD cavity. Therefore, it is obvious that ada2 has the strongest host–guest association intensity due to the strong hydrogen bond interaction and less repelling among the quaternary ammonium cation far from the CD cavity (Figure 8d).

Figure 7. Energy comparison of tri-CD (a) in water and DMSO, (b) at 25 and 80 °C, (c) in neutral environment and acid environment, (d) with different guests and (e) process of dissociation through rotating of glucose unit.

Figure 8. (a) Proposed inclusion structure of host–guest complex and 2D NOESY spectra of tri-CD with three guests, FT-IR spectra of tri-CD including complex with (b) ada2 and (c) ada3, (d) binding forms of ionic head groups in CD, and 2D NOESY spectra of tri-CD (e) in DMSO-d₆ (f) in D₂O without and with (g) excess ada2 and (h) position change of the triazole group before and after combination with guests.

observed (Figure 8e–g) implies that the triazole group is in the state very close to the primary face of CD and the bending of CD units can be expected (Figure 8h).

Self-Inclusion on One CD Unit. All observed pieces of evidence from the integral area ratio in 1D ¹H NMR, proton correlation in 2D NOESY NMR, and the combinative number of guests in two processes of host–guest interaction figure out that the self-inclusion occurs only on one CD unit of the tri-CD. Considering the size of both β-CD and branched bridging groups, the spacial hindrance is believed to play a key role in blocking the formation of multi-self-inclusion. In the case of one CD unit at self-included state (Chart 1a), the occupied space does not allow the other CDs to form self-inclusion.
the case of that three CD units of tri-CD were all at self-included state, the ideal closest distance between the core center to the edge of β-CD’s primary face should be more than 7.9 + 4.4 = 12.3 Å by taking account of the diameter of second face, where the former is the height of CD and the latter is the distance between the core center and CD (Chart 2). In the present bridging β-CD triplet, however, the largest distance between the core center to the edge of CD is ca. 10 Å, much shorter than the required distance for all three CD units in self-included state at the same time. Therefore, the first CD self-inclusion induced CD approaching to the bridging center makes the self-inclusion of the second CD become also impossible due to the space limit.

**CONCLUSIONS**

The structural change of a β-CD triplet bridging with a planar tri-CD via a click reaction in solutions is investigated, and one of the three CD units is demonstrated to be self-included in water. The organic solvent DMSO is found to drive the dissociation of self-inclusion completely and we quantify the conformation transformation during dissociation of self-inclusion in mixture DMSO/H$_2$O solutions with increased volume ratio of the organic solvent. The dependence of the self-inclusion state against heating or acidification is identified to be difficult in destroying the self-inclusion but can make the part of inclusion more or less deep inside or getting outside. Finally, we find out that the competitive guest molecules like some adamantane derivatives, which have additional interaction with the CD unit, are able to help to dissociate the self-inclusion in some extent. Based on the difference in host–guest interaction intensity, we confirmed that the ability of the competitive guests to break the self-inclusion is parallel to the dissociation constants for the self-inclusion in the presence of ada2 and ada3. The energy analysis points out that the strong binding energy of competitive guests and the weakening of self-inclusion interaction can restrict the conformation rolling-over of glucopyranose units in bridged CD triplet molecules. The present results also imply that the suitable design of the bridging group can be used to control the state of CD unit during applying such kinds of supramolecular hosts in self-assemblies with cross-linked structures.

**EXPERIMENTAL SECTION**

Tosyl chloride, β-CD, sodium ascorbate, 3-bromoprop-1-yl and 4-hydroxy-acetophenone were purchased from Aladdin (Shanghai, China). Deuterated water (D$_2$O) and dimethyl sulfoxide (DMSO-d$_6$) were products of CIL. Other reagents and chemicals were of analytical reagent grade. Water used was deionized. 4-(Prop-2-yn-1-yl oxy)-acetophenone, mono-6-OTs-β-CD, mono-6-N$_3$-β-CD, ada1, ada2, and ada3 were synthesized according to the literature studies $^{35,39-42}$.

**Synthesis of 1,3,5-Tris-(4-(prop-2-yn-1-yl oxy)phenyl)benzene.** Followed by a literature method, $^{36}$ 4-(prop-2-yn-1-yl oxy)-acetophenone (1 g, 5.74 mmol) was dissolved in absolute ethanol (30 mL) and silicon tetrachloride (4 mL, 34.8 mmol) was added dropwise to the solution under an ice-bath. The mixture was stirred at room temperature for 24 h and then quenched with water under an ice-bath. The ethanol was removed under reduced pressure and extract with dichloromethane and water. The organic solvent was dried and subjected to a silica-gel chromatography (petroleum ether/dichloromethane = 3/2). The product (760 mg) was obtained as a white solid after drying and the yield was 85%. $^1$H NMR (500 MHz, CDCl$_3$): δ 7.66 (s, 3H), 7.63 (d, $J = 8.53$ Hz, 6H), 7.09 (d, $J = 8.53$ Hz, 6H), 4.76 (d, $J = 2.40$ Hz, 6H), 2.55 (t, $J = 2.39$ Hz, 3H). $^{13}$C NMR (126 MHz, CDCl$_3$): δ 157.29, 141.73, 134.69, 128.40, 124.09, 115.28, 78.58, 75.71, 55.93. Maldi-tof MS, ca. for M$: 468.17$; found, 468.456.

**Synthesis of tri-CD.** By referring the typical method from the literature studies, $^{25}$ CuSO$_4$·5H$_2$O (0.85 g, 3.4 mmol) was dispersed in 50 mL DMSO containing 1,3,5-tris-(4-(prop-2-yn-1-yl oxy)phenyl)benzene (469 mg, 1 mmol) and mono-6-N$_3$-β-CD (3.9 g, 3.4 mmol). In addition, sodium ascorbate solution (1.3 g, 6.8 mmol in 10 mL water) was added under stirring. The solution was then heated to 80°C for 7 days and then poured into acetone after being cooled down. The formed precipitation was collected by filtration and purified over a silica-gel chromatography with i-propanol/H$_2$O/NH$_4$OH (80/15/5 to 60/35/5 in volume ratio) as the eluent, giving tri-CD (1.4 g) as a white solid in yield of 36%. $^1$H NMR (500 MHz, DMSO-d$_6$): δ 8.21 (s, 3H), 7.81 (d, $J = 8.18$ Hz, 6H), 7.76 (s, 3H), 7.18 (d, $J = 8.31$ Hz, 6H), 5.90–5.66 (m, 42H), 5.17 (s, 6H), 5.06–4.51 (m, 42H), 4.35 (t, $J = 5.62$ Hz, 3H), 4.02 (t, $J = 9.17$ Hz, 3H), 3.77–3.58 (m, 68H), 3.40–3.26 (m, 57H, overlapped with the DOH peak), 3.15 (d, $J = 9.58$ Hz, 3H), 2.93 (d, $J = 9.08$ Hz, 3H). $^{13}$C NMR (151 MHz, DMSO-d$_6$): δ 158.46, 142.97, 141.57, 133.41, 128.73, 125.98, 123.32, 115.55, 102.67, 102.48, 102.35, 101.74, 83.94, 82.51, 82.09, 81.98, 81.87, 81.43, 73.68, 73.60, 73.44, 73.32, 73.11, 72.90, 72.79, 72.65, 72.52, 72.22, 70.50, 61.52, 60.64, 60.49, 60.40, 60.33, 59.45, 50.89. MALDI-TOP MS, ca. for tri-CD + Na$: 3971.30$; found, 3971.180. Elemental analysis, ca. for tri-CD 9H$_2$O: C, 46.46%; H, 6.11%; N, 3.07%. Found C, 46.27%; H, 5.98%; N, 2.87%.
ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00363.

Characterizations of compounds, 2D NMR spectra in different solvents, and studies of host–guest interactions (PDF)

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Notes
The authors declare no competing financial interest.

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