Host–Guest Chemistry

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Manifesting Subtle Differences of Neutral Hydrophilic Guest Isomers in a Molecular Container by Phase Transfer

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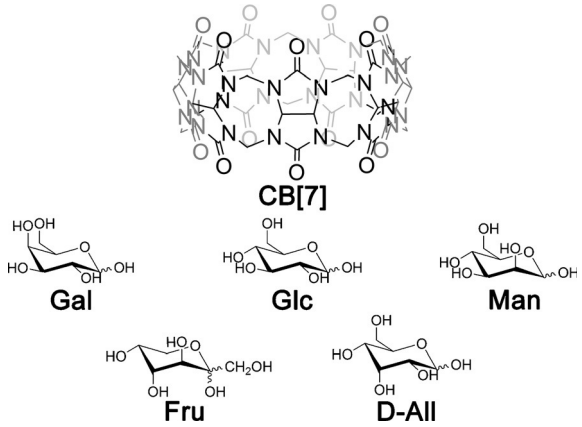
Abstract: *Achieving strong host–guest interactions between synthetic hosts and hydrophilic guests in solution is challenging because solvation effects overwhelm other effects. To resolve this issue, we transferred complexes of cucurbit[7]uril (CB[7]) and monosaccharides to the gas phase and report here their intrinsic host–guest chemistry in the absence of solvation effects. It was observed that effective host–guest interactions in the gas phase mediated by ammonium cations allow the differentiation of the monosaccharide isomers in complex with CB[7] upon vibrational excitation. The potential of the unique observation was extended to a quantitative supramolecular analytical method for the monosaccharide guests. The combination of host–guest chemistry and phase transfer presented in this study is an effective approach to overcome current limitations in supramolecular chemistry.*

Development of artificial host–guest systems with high selectivity is important for both fundamental studies and applications.[1] Consequently, artificial host–guest systems with remarkable binding properties have been developed.[1] However, achieving selective recognition of hydrophilic molecules is still challenging.[2] In particular, effective differentiation of hydrophilic guest isomers remains a significant challenge.[2] Essential requirements for such hosts are high affinities toward guests and the capability to recognize subtle differences in guest structures. However, these requirements are difficult to fulfill in aqueous solution because effective solvation of hydrophilic guests prevents their interactions with host molecules.[2–3] Optimized charged functional groups can drive host–guest interactions,[2,4] but strong Coulombic interactions can reduce the contribution from subtle structural effects on complexation. Thus, new approaches are necessary to utilize intrinsic binding properties of neutral hydrophilic guests and achieve selective recognition of their isomers.

A number of studies have reported distinctive host–guest chemistries in the gas phase that are unseen in solution.[3,5] Notably, removal of solvent was found to enhance noncovalent interactions and drive complexation.[3,5a] Utilizing this characteristic, we investigated the complexes of monosaccharide isomers and a synthetic host in the gas phase to examine their intrinsic binding properties in the absence of solvation effects. Monosaccharides are the most common neutral hydrophilic biomolecules, but artificial recognition systems effectively targeting them are limited.[6] The lack of such systems necessitates enzymatic methods to selectively recognize them in solution,[7] but they are influenced by analysis conditions such as temperature and pH.[8] The synthetic host molecule, cucurbit[7]uril (CB[7], Scheme 1),



Scheme 1. Structures of CB[7] and D-forms of five monosaccharides investigated in the present study.

has an adequate size for encapsulation of monosaccharides.[4b] However, interactions between CB[7] and neutral monosaccharides are expected to be weak in aqueous solution owing to the lack of stabilizing ion–dipole interactions. Herein, we show that the weak interactions between neutral monosaccharide guests and CB[7] in solution are promoted by their transfer to the gas phase. In addition, we demonstrate that in the gas phase, CB[7] can recognize subtle structural differences of isomeric guests to yield distinct dissociation patterns upon collisional activation. We characterized this unique chemistry with ion mobility mass spectrometry (IM-MS) and computational approaches, and present its utility as a supramolecular analysis method for isomeric guests.

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We investigated the four most common monosaccharides (Scheme 1), D-galactose (Gal), D-glucose (Glc), D-mannose (Man), and D-fructose (Fru), among which Gal, Glc, and Man are aldoses, and Fru is a ketose. The negligible heating required to dissociate the complex during isothermal titration calorimetry (ITC) suggests that interactions between CB[7] and monosaccharides are weak in aqueous solution (Figure S1). In contrast, complex peaks of CB[7] and the monosaccharides were observed in their mass spectra (Figure S2). The exact mass values of the ions from high resolution Orbitrap MS confirmed that they are diammoniated CB[7]–monosaccharide complexes (observed: m/z 689.2372; calculated: m/z 689.2373). This result demonstrates that the phase transfer to the gas phase facilitated complex formation. The complexes were found to exist as doubly charged forms with ammonium or alkali cations, depending on the salts added to the solutions (Figure S2).

To understand the structures of monosaccharide complexes, IM experiments were performed (Figure 1). The IM technique separates ions based on their collisions with neutral gas molecules, allowing the assessment of their structural features.[3,5a,9] The experimental collision cross-section ($\Omega_{D,exp}$) value of uncomplexed, diammoniated CB[7] ions ($[CB[7]+2NH_4]^{2+}$) was 209.4 Å^2, which is highly similar to the $\Omega_{D,exp}$ values of monosaccharide complexes (Figure 1).

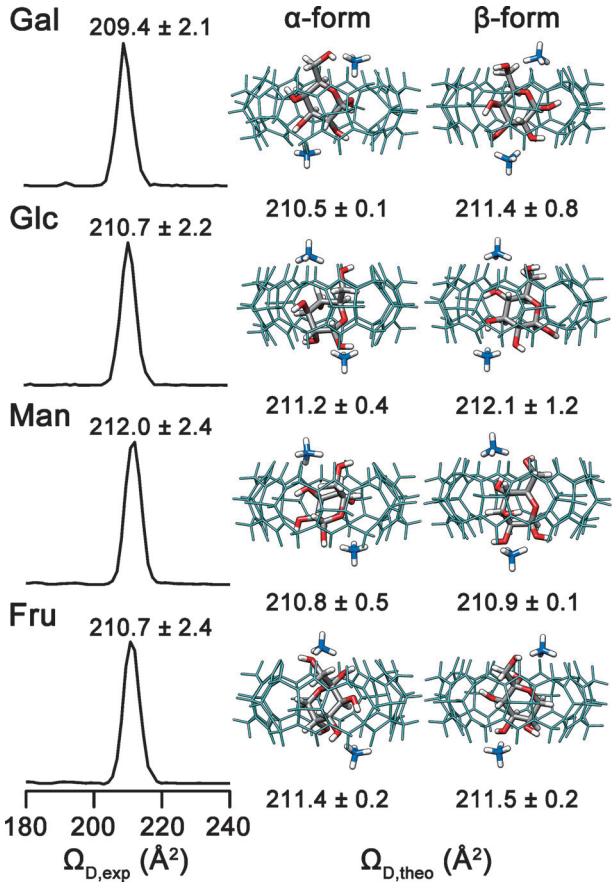

Figure 1. IM distributions, theoretical structures, and $\Omega_{D,theo}$ values of ammoniated monosaccharide complex ions. The $\Omega_{D,exp}$ value of $[CB[7]+2NH_4]^{2+}$ is 209.4 ± 2.2 Å^2.

The similarity indicates that the monosaccharides were internally bound to CB[7]. To further characterize the complex ions, computational modelling of the complexes was performed. Modelling was performed for both anomeric states of the monosaccharides, α and β, that co-exist in aqueous solutions. Although it is difficult to conclude in which states the monosaccharides exist inside CB[7], the monosaccharides were found to be internally bound to CB[7] regardless of their anomeric states (Figure 1). Moreover, the $\Omega_{D,exp}$ values showed good agreement with the theoretical collision cross-section ($\Omega_{D,theo}$) values of the models (Figure 1). On the other hand, externally-bound models of monosaccharide complexes showed an average $\Omega_{D,theo}$ value of 223 Å^2 (Figure S3), which is clearly greater than the experimental value. These results support the internal complexation of monosaccharides to CB[7] in the gas phase.

We then performed low-energy collision-induced dissociation (CID) to explore the chemistry of monosaccharides inside the CB[7] cavity. Simple displacement of the monosaccharide guests was observed with activation of alkali-metallated complex ions (Figure S4). By contrast, activation of diammoniated complex ions produced complex ions of monosaccharide fragments and CB[7] (Figure 2). The major dissociation products were complex ions that underwent ammonia or water losses. Interestingly, the dissociation patterns were different depending on the monosaccharides encapsulated in CB[7] (Figure 2 and Table S1). For example, the m/z 662.8 peak was most prominent in the case of Glc, whereas the m/z 653.8 peak was most abundant for Fru. The CID of monosaccharide complexes in three different types of mass spectrometers (ion trap, Orbitrap, and Q-TOF) all generated fragmentation patterns that were distinct for different monosaccharide complex ions, illustrating the generality of the observation (Figures 2 and S5). In contrast to the complexed ions, uncomplexed monosaccharide ions did not produce clearly distinguishable fragmentation patterns, with Gal and Man, and Glc and Fru, respectively displaying similar patterns that cannot be used for rigid analysis (Figure S6). These results show that specific interactions of CB[7] with monosaccharide isomers drive the formation of distinct dissociation products during CID. We further tested the influence of anomeric distribution of monosaccharides in solution on the dissociation patterns of their complexes, and observed that the dissociation patterns are not affected by the anomeric distributions (see the Supporting Information).

We performed theoretical studies to understand the binding properties of monosaccharides inside the CB[7] cavity. The PC values, the volume of guests divided by the volume of the host cavity, of monosaccharides (61–62 %) were found to exceed the previously reported PC range (30–50 %) for chemical reactions of guests to occur inside a host (Table S2).[9b] This is an interesting observation because displacement of guests with high PC values was reported to be preferred over reactions inside the host cavity, because some void space is necessary for efficient reactions.[9b] It is notable that CB[7] and monosaccharides are bound by relatively weak interactions such as dipole-dipole and van der Waals interactions. It can be suggested that the absence of strong interactions allows freer motion of monosaccharides

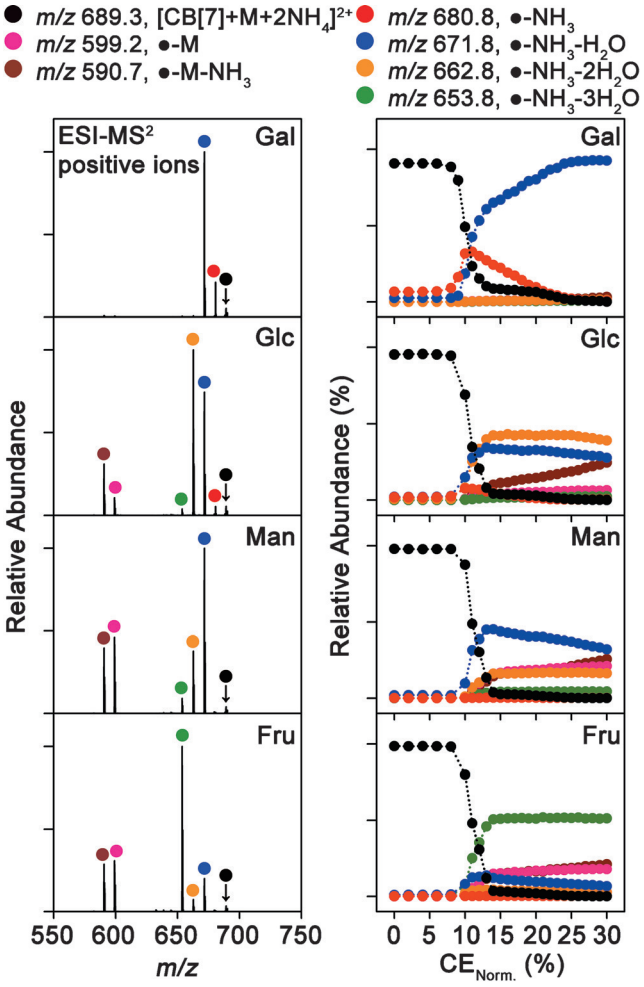

Figure 2. MS^2 spectra of diammoniated monosaccharide (M) complex ions with CB[7] at a normalized collision energy ($CE_{Norm.}$) of 15% and their survival yields depending on the $CE_{Norm.}$ ranging from 0 to 30%.

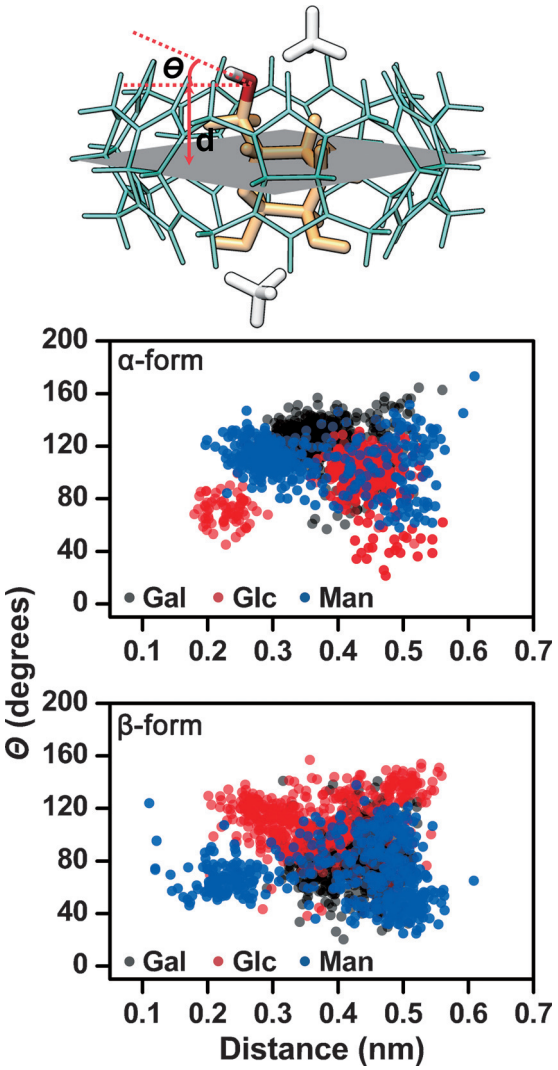

Figure 3. Geometries of the C6 hydroxyl of the monosaccharides (α and β) inside the CB[7] cavity. Fru was not compared as it is structurally different from the other three aldoses.

inside the CB[7] cavity to cause internal chemical reactions upon collisional activation. Although weakly interacting guests generally favor simple departure from the host upon collisional activation,[3] covalent bond cleavages observed from ammoniated complex ions show that ammonium cations facilitate the internal reaction of monosaccharides. Ammonium cations exert a capping effect on both CB[7] portals through strong hydrogen bonds with both the host and the guests to stabilize the complex. In addition, ammonium cations can donate protons to promote fragmentation of the monosaccharides inside the host.

Interestingly, we also observed dissimilarities in the geometries inside the CB[7] cavity in the theoretical models of the complexes (Figure 3). Although such dissimilarities may be the cause of the differences in their CID spectra, the theoretical models represent the ground state structures of the complex ions before collisional activation. Vibrational excitation of ions by conversion of their translational energy into internal energy is an important part of low-energy CID.[10] Therefore, high-temperature molecular dynamics (MD) simulations were used to model vibrationally excited states of the complex ions in vacuo. Figure 3 displays plots of the distance (d) and angle (θ) between the C6 hydroxy groups of the monosaccharides and the central plane of CB[7]. It was observed that the C6 hydroxy groups of different monosaccharides occupy different regions of the conformational space, regardless of their anomeric states. In addition, the relative positions of most other hydroxy groups with respect to CB[7] were different depending on the monosaccharides (Figure S7). These results suggest that CB[7] can recognize subtle structural differences between monosaccharide isomers under vibrationally excited conditions, and produce disparate potential energy landscapes of the complex ions to yield distinguishable fragmentation patterns.

The unique chemistry of CB[7] in the absence of water has the potential for an analytical application for monosaccharide isomers. Although many studies have used MS to examine host–guest interactions,[5d,11] identical m/z values of isomeric compounds make their MS analysis difficult. Furthermore, while a number of studies applied host–guest chemistry and tandem MS to distinguish isomeric compounds,[12] the exten-

sion to quantitative analysis is limited. For such applications, a strong relationship should be established between the relative abundances of host–guest species in MS and their concentrations in solution. However, in general, the abundances of noncovalent complexes from electrospray ionization (ESI) are nontrivially determined by their binding properties, both in solution and the gas phase, and are difficult to predict.[3,5a] On the contrary, because the monosaccharides have negligible interactions with CB[7] in solution, it can be expected that the abundances of their complex ions in the gas phase will have a relatively simple relationship with their concentrations in solution. This makes quantification of monosaccharides possible because the fragmentation pattern of a mixture of CB[7]–monosaccharide complex ions can be decomposed into a linear combination of the fragmentation patterns of individual complex ions. Specifically, the gas-phase fractions of monosaccharide complex ions in a mixture follow Equation (1):

$$R_{\mathrm{Mix}} = R_{\mathrm{A}}\alpha_{\mathrm{A}} + R_{\mathrm{Ref}}\alpha_{\mathrm{Ref}} = R_{\mathrm{A}}\alpha_{\mathrm{A}} + R_{\mathrm{Ref}}(1-\alpha_{A}) \quad (1)$$

where R_{A}, R_{Ref}, and R_{Mix} represent the intensity ratios of two select fragment peaks of the analyte complex ions, reference complex ions, and the mixture of monosaccharide complex ions, which are directly obtainable from their tandem mass spectra. The variables α_{A} and α_{Ref} represent the fractions of the analyte and reference monosaccharide complex ions, respectively, in the gas phase. The α values are not directly comparable with their concentrations in solution, c_{A} and c_{ref} [Eq. (2)], because efficiencies of complexation with CB[7] during transfer to the gas phase can be different depending on the monosaccharide. We assume a simple relationship between α and c values for this system:

$$\frac{\alpha_{\mathrm{A}}}{\alpha_{\mathrm{Ref}}} = m \times \frac{c_{\mathrm{A}}}{c_{\mathrm{Ref}}} \quad (2)$$

We obtained the m values of monosaccharides by performing tandem MS experiments of various solutions containing a monosaccharide and a reference monosaccharide. D-allose (D-All) was utilized as the reference monosaccharide because it is an unnatural monosaccharide not present in humans, and its fragmentation pattern was most different with those of the natural monosaccharides (Figure S8). It was found that the ratio of α_{A} to α_{Ref} was proportional to the ratio of c_{A} to c_{ref}, indicating that Equation (2) holds (Figure 4). The m values of Gal, Glc, Man, and Fru with D-All were 0.489, 0.345, 0.631, and 0.189, respectively, and showed excellent linearity ($R^2 = 0.999$). Quantification of monosaccharides using these relationships provided a limit of detection (LOD) and limit of quantification (LOQ) of 2.55–5.00 μM and 7.72–15.10 μM, respectively (Table S3), showing that this method is promising for determining the concentrations of small amounts of monosaccharides in the human body ($\approx$20 μM).[13] It is also possible to extend to this method to quantify monosaccharides in a binary mixture using a standard addition method (details available in the Supporting Information, Figure S9). Using this method, the concentrations of monosaccharides in binary mixtures were obtained with good accuracy (Table S4). For example, the concentrations of Gal (20 μM) and Glc (5 μM) in their mixture were obtained as 18.8 ± 0.4 μM and 5.1 ± 0.1 μM, indicating that this method can be an effective analytical method for isomeric mixtures that does not require sample derivatization or a lengthy analysis time.

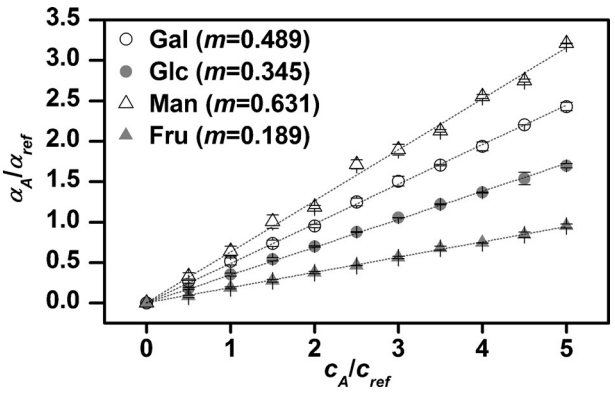

Figure 4. Calibration curves of four monosaccharides with D-All for quantification of single monosaccharide solutions.

In summary, we showed that combination of host–guest chemistry and gas-phase chemistry is a powerful approach to overcome the current limitations in supramolecular chemistry from solvation effects. Transfer to the gas phase promotes host–guest complexation and allows the differentiation of isomeric guests based on small differences in their structures. Application of this chemistry to analysis of monosaccharides and their mixtures demonstrates the value of exploring the molecular recognition of weak binding guests in the absence of water, and provides a new perspective to the study and application of molecular recognition.

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