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Gas-phase fragmentation of host–guest complexes between β-cyclodextrin and small molecules

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ABSTRACT

Noncovalent interactions are ubiquitous in molecular interaction and supramolecular self-assembly. As a model system, host–guest complexes between β-cyclodextrin (β-CD) and small molecules have been extensively studied and widely used in a variety of application fields including drug transportation and fluorescence enhancement. However, details on how guest molecules interact with the β-CD hosts to demonstrate the observed effects still remain to be further studied. In this work, we report the study of gas-phase fragmentation of host–guest complexes formed between β-CD and small guest molecules by using collision induced dissociation (CID). The CID mass spectra of the complexes changed dramatically as the collision energy was increased. Fragmentation patterns of β-CD complexed with different small molecules were analyzed and the differences in the presence/absence of fragment ions from the β-CD were attributed to varied proton affinity of the small molecules. Furthermore, the CE_{50} values fitted from the fragmentation curves were used in the qualitative evaluation of interactions in noncovalent host–guest systems.

1. Introduction

The energy level of noncovalent interactions is typically less than 10 kJ/mol and one or two orders of magnitude less than the bond energy of common covalent bonds. However, they play a critical role in supramolecular self-assembly [1] through collective integration of individual interactions to form strong forces, which form the basis for molecular recognition. Examples of noncovalent interactions include hydrophobic interactions [2–5], π–π interactions [6–10], multiple-hydrogen bonding [11–14] and charge-transfer interactions [15,16]. Among these, the model system formed from β-cyclodextrin (β-CD) and a series of small molecules have been extensively studied and widely used in a variety of application fields such as drug transportation [17,18] and fluorescence enhancement [19,20].

The existing methods for the mechanistic study of noncovalent complexes include atomic force spectroscopy (AFM) [21], nuclear magnetic resonance (NMR) [22], X-ray diffraction (XRD) [23] and spectroscopic techniques [24–26]. NMR and XRD are suitable for the three dimensional structural analysis of biomolecules and their corresponding complexes in aqueous and solid phases, respectively. However, these two methods are time-consuming and complicated sample preparation procedures are needed. In addition, to perform NMR analysis a large amount of analyte is required. XRD analysis requires that analytes are crystals of high purity, which are sometimes difficult or impossible to form. While spectroscopic methods can provide information with respect to structural changes, little or no information on molecular weights and stoichiometric ratio of complexes can be retrieved.

In contrast, mass spectrometry (MS) offers a number of advantages over the above-mentioned techniques in that: (1) only very small amounts of analytes were needed for MS to carry out analyses; (2) it offers great sensitivity and selectivity; (3) compared with AFM and XRD, sample preparation for MS is very simple; (4) MS directly measures the molecular weights of host–guest complexes and its fragments, thus no labeling is necessary as needed in spectroscopic techniques. Up to now, there have been a few published reports of mechanistic study of host–guest complexes by MS, especially by electron capture/transfer dissociation (ECD/ETD) MS. Loo and co-workers reported top-down electrospray ionization (ESI)–ECD MS to localize the ligand binding site in the noncovalent α-synuclein/spermine complex [27]. The unique advantage of ECD is that the noncovalent bond between α-synuclein and spermine is unaffected while the covalent bonds within the α-synuclein backbone are cleaved, as a result of which the binding site information is preserved. This group also successfully applied the same

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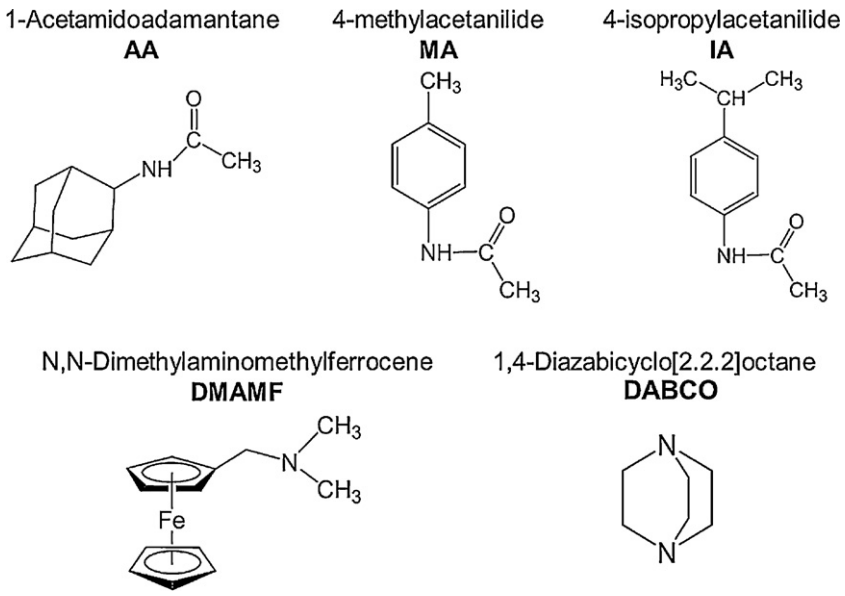

Scheme 1. Guest molecules used in this work.

methodology in the elucidation of the binding site in the non-covalent protein-ATP complexes [28]. More recently, using ETD, collision induced dissociation (CID) and ion mobility MS Williams and co-workers successfully identified the drug metalation site on a neurotransmitter peptide called Substance P [29].

In this work, we employed CID-MS to study the gas-phase fragmentation of host–guest complexes formed from β-CD and small guest molecules. Protonated host–guest complexes were generated by conventional electrospray after mixing β-CD with small guest molecules. The complex ions were then isolated and fragmented by using CID. The effects of guest molecules on the fragmentation patterns of the studied complexes were studied, discussed and finally explained from the perspective of the guests' proton affinities.

2. Experimental procedures

2.1. Chemicals

All chemicals used in this work, shown in Scheme 1, were purchased from Beijing Sinopharm Chemical Reagent Co., Ltd. (Beijing, China) and used without further purification. β-CD and 4-methylacetanilide (MA), 4-isobutylacetanilide (IA), acetylamantadine (AA), N,N-dimethylaminomethylferrocene (DMAMF), and 1,4-diazabicyclo[2.2.2]octane (DABCO) were mixed together, at a molar ratio of 1:10, and the dissolved in methanol/water/acetic acid (49.5/49.5/1, v/v/v) solution to reach a concentration of 10^{-5} mol/L (β-CD). Prior to mass spectrometric analysis, the above solutions were put under room temperature for more than 0.5 h to reach equilibrium.

2.2. Mass spectrometry and data processing

All experiments were performed on an Agilent 6500 series accurate-mass quadrupole time-of-flight (Q-TOF) mass spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA). A conventional ESI source was used to spray the as-prepared mixture solutions for ion generation. The solution flow rate was set to 5 μL/min and an external DC power was in electric contact with the solution via attachment of the electrode to the 1.0 mL Hamilton syringe tip. The DC voltage used was 3500–4000 V. High-purity nitrogen gas (Beijing Ruyuanruquan Technical Co. Ltd., 99.999%) was used as the nebulizer gas for ESI and its pressure was set to be 35 psi. MS/MS experiments were performed by isolating the complex ions of interests and fragmented using different CID energies. The ion acquisition time was set to be 500 ms. Nitrogen gas was also used as the collision gas in the collision cell. All data acquired was processed by Agilent MassHunter Qualitative Analysis software.

3. Results and discussions

The complex ions generated by ESI were isolated and subjected to CID under different CEs. Fig. 1 shows the CID mass spectra of [1-acetylamantadine+β-CD+H]$^+$ ([AA+β-CD+H]$^+$) complex ions obtained at CEs of 5, 10, 15, 20, 25, and 30 (a.u.). At the collision energy of 5, protonated 1-acetylamantadine can be clearly observed and ions related to β-CD or its fragments were not detected. As the CE was gradually increased, fragment ions of β-CD appeared and their abundance increased as CE increased. These fragment ions, spaced 162 Da (one sugar unit) from one another, include m/z 1135.16, 973.29, 811.23, 649.19, 487.15, 325.11, and 161.10, among which ions of m/z 1135.16 were identified as protonated β-CD.

For complexes formed between β-CD and IA/MA, similar MS2 mass spectra were acquired, as shown in Fig. 2. A very interesting experimental result to note is that in the case of DABCO and DMAMF as guest molecules, no protonated β-CD and its fragment ions were observed, even in the high CE regime (Fig. 3). Their CID mass spectra are very clean with only two or three major peaks, which is in great contrast with the CID mass spectra illustrated in Fig. 2. In Fig. 3A, the peak at m/z 113.07 was assigned to [DABCO+H]$^+$, while in Fig. 3B the peaks at m/z 244.02 and 198.97 were assigned to [DMAMF+H]$^+$ and [DMAMF+H-NH(CH_3)$_2$]$^+$, respectively. The fragment peak of [DMAMF+H-NH(CH_3)$_2$]$^+$ was possibly due to the low stability of DMAMF under the specific collisional condition.

The substantial difference between these two sets of small molecules is not entirely unexpected. Because the complex ions have only one positive charge (proton), during their fragmentation the guest molecule and the β-CD molecule/fragment will compete for the proton. Guest molecules with higher proton affinity will hold the proton more tightly, which suppresses other ionization channels of forming protonated β-CD or β-CD fragment ions. Indeed, in line with the observed complex fragmentation patterns, the proton affinities of DABCO and DMAMF are found to be 958 kJ/mol and 930–950 kJ/mol [30], which are much more higher than those of

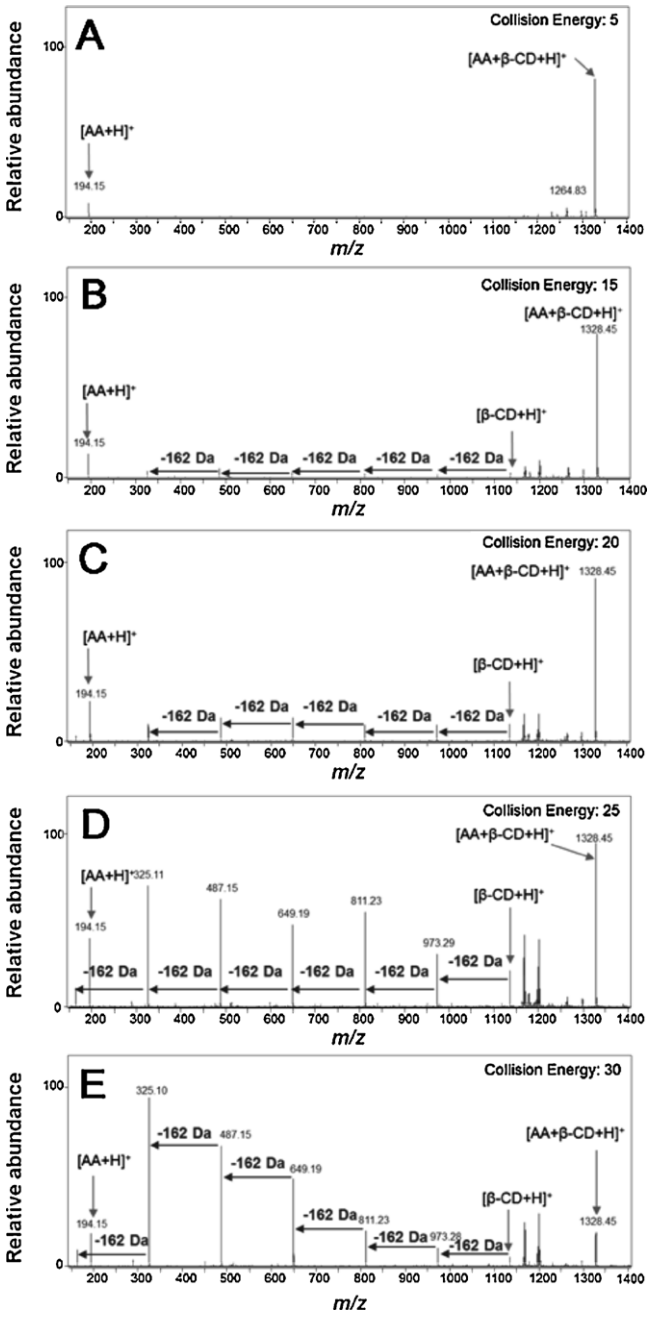

Fig. 1. MS2 mass spectra of $[AA+\beta\text{-CD}+H]^+$ (AA: 1-acetylamantadine) complex ions at the (A) collision energy of 5, (B) collision energy of 15, (C) collision energy of 20, (D) collision energy of 25, and (E) collision energy of 30.

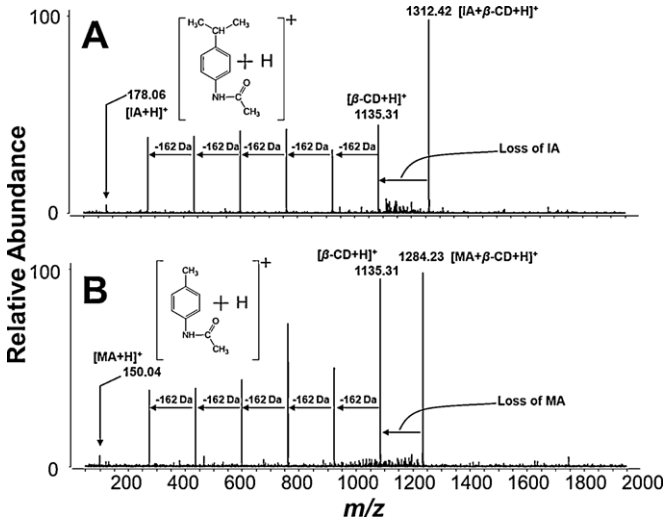

Fig. 2. MS2 mass spectra of host–guest complex ions formed from (A) β-CD and IA and (B) β-CD and MA. The collision energies used are 22 and 17, respectively.

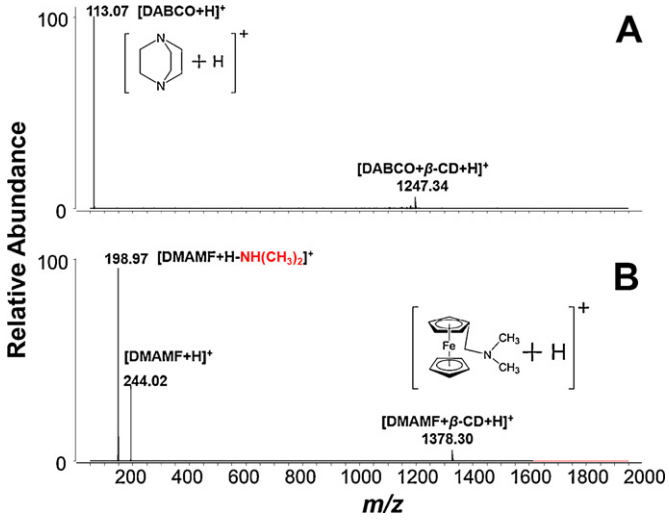

Fig. 3. MS2 mass spectra of host–guest complex ions formed from (A) β-CD and DABCO and (B) β-CD and DMAMF. The collision energies used are both 30.

AA, MA and IA. Therefore, based on these interesting experimental results it can be concluded that the affinity of the guest molecule is the predominant factor for the distribution of protonated guest molecules, protonated β-CD and β-CD fragment ions in the pool of ions after fragmentation.

It should also be noted that in Figs. 1–3, no ions due to the combination of the guest molecule with any possible fragment of β-CD were observed, no matter what CE (0–40) was used. This is corroborative evidence that all these guest molecules can form complexes only with intact β-CD, which has a hydrophobic cavity within which the guest was accommodated. Once the β-CD backbone is broken up, the interaction between the guest molecule and β-CD will be lost. From the specific host–guest systems chosen in this work, however, no information on which part of the guest was contained in the β-CD cavity can be acquired because we did not find any ions due to the combination of the guest fragment and β-CD.

3.1. Comparison with interaction forces measured by AFM

In an attempt to make a comparison among the interactions in the host–guest systems studied, the intensity of complex ions was plotted against the collision energy to find out CE_{50} values. To define CE_{50}, the percentage of complex ions fragmented should be defined first, which is expressed as:

$$\frac{A_0 - A_{CE}}{A_0},$$

in which A_0 represents the intensity of complex ions as the collision energy was set to 0, and A_{CE} represents the intensity of complex ions as the collision energy was increased to CE (>0). CE_{50} is defined as the collision energy required to fragment 50% of the complex ions, which is expressed as:

$$\frac{A_0 - A_{CE_{50}}}{A_0} = 50\%.$$

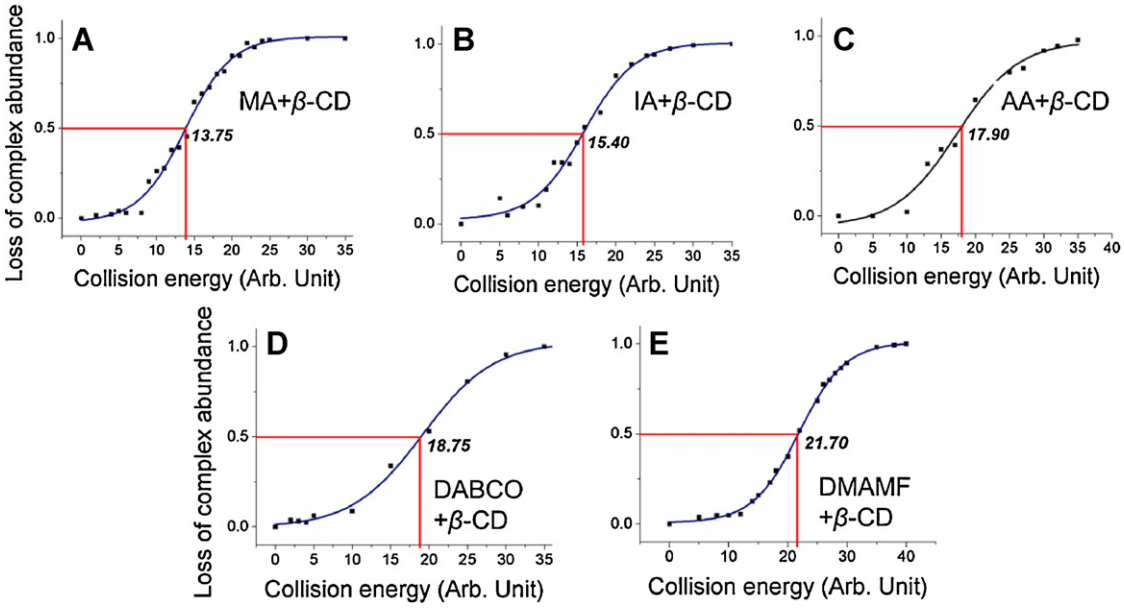

Fig. 4. Fitted fragmentation curves of the five host–guest systems in this work. Besides the point of intersection of the two red lines are the corresponding CE_{50} values. (A) MA + β-CD, (B) IA + β-CD, (C) AA + β-CD, (D) DABCO + β-CD, and (E) DMAMF + β-CD.

Each fitted fragmentation curve turns out to be S-shaped. The reason why we selected CE_{50} rather other CE_n is that the points where CE_{50} reside have the largest slope, and a slight uncertainty in complex abundance would not result in a large shift in CE. As such, CE_{50} is the appropriate parameter to reflect the host–guest interactions. CE_{50} values of the five systems in this study are shown in Fig. 4, and these systems have previously been investigated by other research groups using AFM [31,32]. It is surprising to find out that the CE_{50} values obtained are consistent with the interactions measured for each system. As shown both in Vancso's and Miyake's published results and in this study, DABCO and DMAMF interacts more strongly with β-CD than the other three guest molecules; and within the group of these three guest molecules, their respective CE_{50} values are also consistent with the corresponding interaction forces (45 ± 15 pN, 89 ± 15 pN, 102 ± 15 pN). However, CID-MS and AFM are two essentially different techniques, at least in that AFM analysis is carried out in a unidirectional approach by pulling the two interacting components [33] while as CID is performed the target analytes are impacted from all possible directions. It is therefore not unexpected that we failed to correlate these two sets of results. Nevertheless, in consideration of limited sample pretreatment and short analysis time it offers, this methodology can still be potentially used for the qualitative determination of noncovalent interactions.

4. Conclusions

In this paper we have applied CID-MS to investigate the gas-phase fragmentation of host–guest complexes formed between β-CD and guest molecules. It has been found that using guest molecules with different proton affinities, the fragmentation patterns of the complex ions turned out to be quite different. For those three guests with lower proton affinities, protonated β-CD and its fragment ions were clearly observed, while for the other two guests with higher proton affinities the above-mentioned ions were not observed at all. It is therefore concluded that the only proton in a host–guest complex ion will be more localized on the guests with higher proton affinities. In all five host–guest systems $[\beta\text{-CD+G}]^+$ instead of $[\beta\text{-CD fragment+G}]^+$ were observed, which indirectly proves that the interaction exists only between whole β-CD and guests. In addition, by plotting the intensity of complex ions vs. collision energy the CE_{50} values for each host–guest system can be obtained, and the two sets of experimental results were found to be consistent with each other. The present method can therefore be potentially used in the rapid qualitative determination of the interactions in noncovalent host–guest systems.

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