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Supramolecular Enhancement of Protein Analysis via the Recognition of Phenylalanine with Cucurbit[7]uril

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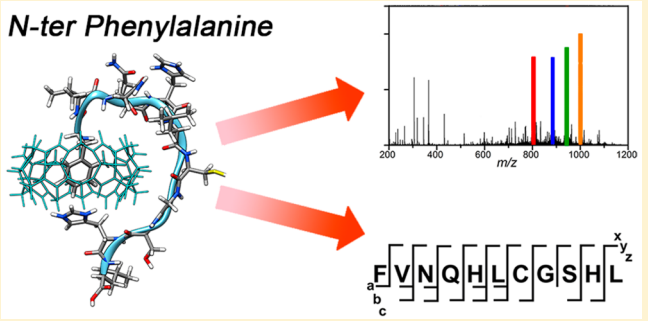
Supporting Information

ABSTRACT: Mass spectrometry (MS)-based analysis using enzymatic digestion is widely used for protein sequencing and characterization. The large number of peptides generated from proteolysis, however, suppresses the signal of peptides with low ionization efficiency, thus precluding their observation and analysis. This study describes a technique for improved analysis of peptic peptides by adding the synthetic receptor cucurbit[7]uril (CB[7]), which binds selectively to peptides with N-terminal aromatic residues. Capturing the N-terminal phenylalanine (Phe) of peptides using CB[7] enhances the peptide abundances both in electrospray ionization MS and in matrix-assisted laser desorption ionization MS. Moreover, collision-induced dissociation (CID) of the CB[7]·peptide complex ions generates b- and y-type fragment ions with higher sequence coverage than those generated with uncomplexed peptides. The signal enhancement mediated by CB[7] is attributed to an increase in the peptide proton affinities upon CB[7] complexation. The mechanistic details of the fragmentation process are discussed on the basis of the structures of the complex ions obtained from ion mobility (IM) measurements and molecular modeling. This study demonstrates a novel and powerful approach to the enhancement of protein and peptide analysis using a synthetic receptor, without the need for new instrumentation, chemical modifications, or specialized sample preparation. The simplicity and potential generality of this technique should provide a valuable asset in the toolbox of routine protein and peptide analysis.

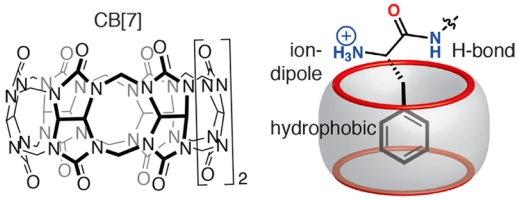


INTRODUCTION

The development of soft ionization techniques has made mass spectrometry (MS) the method of choice for protein sequencing and characterization.[1] In a typical procedure, proteins are first digested enzymatically and then analyzed by electrospray ionization MS (ESI-MS) or matrix-assisted laser desorption ionization MS (MALDI-MS).[1] The large number of peptides generated from the digest provides rich information on the primary structures of proteins (i.e., the amino acid sequences). Because of the variety of peptides competing for ionization, however, those with low proton affinities show low ionization efficiencies, which often precludes MS analysis and reduces sequence coverage.[2,3] Therefore, it is desirable to promote the ionization efficiencies of peptides of interest for efficient protein characterization. Derivatization[2,4] and instrumental modification[5] can improve ionization efficiency, but a convenient and widely accessible method has yet to be reported. Here, we present a novel and straightforward approach to improving protein analysis by simply adding the synthetic receptor cucurbit[7]uril (CB[7], Scheme 1).

Cucurbit[*n*]urils (CB[*n*]s, *n* = 5, 6, 7, 8, and 10), comprising *n* glycoluril ($C_4H_2N_4O_2$) repeat units and 2*n* methylene ($-CH_2-$) bridges, are neutral macrocyclic host molecules

Scheme 1. Structure of Cucurbit[7]uril



used in diverse fields of chemistry.[6−8] CB[*n*]s have a hydrophobic cavity that encapsulates nonpolar groups and two polar entrances lined with ureido carbonyl groups that can bind cationic groups. These interactions work together to enable CB[*n*]s to bind guests with high affinities.[9] Because of their excellent host properties, CB[*n*]s have been studied widely both in solution[10−15] and in the gas phase.[16−21] Among the family, CB[7] is soluble in water (~10^{-2} M),[8] and forms strong complexes with N-terminal Phe residues of peptides and proteins (K_a ~ 10^6−10^7 M^{-1}) in solution[10,13] due to inclusion

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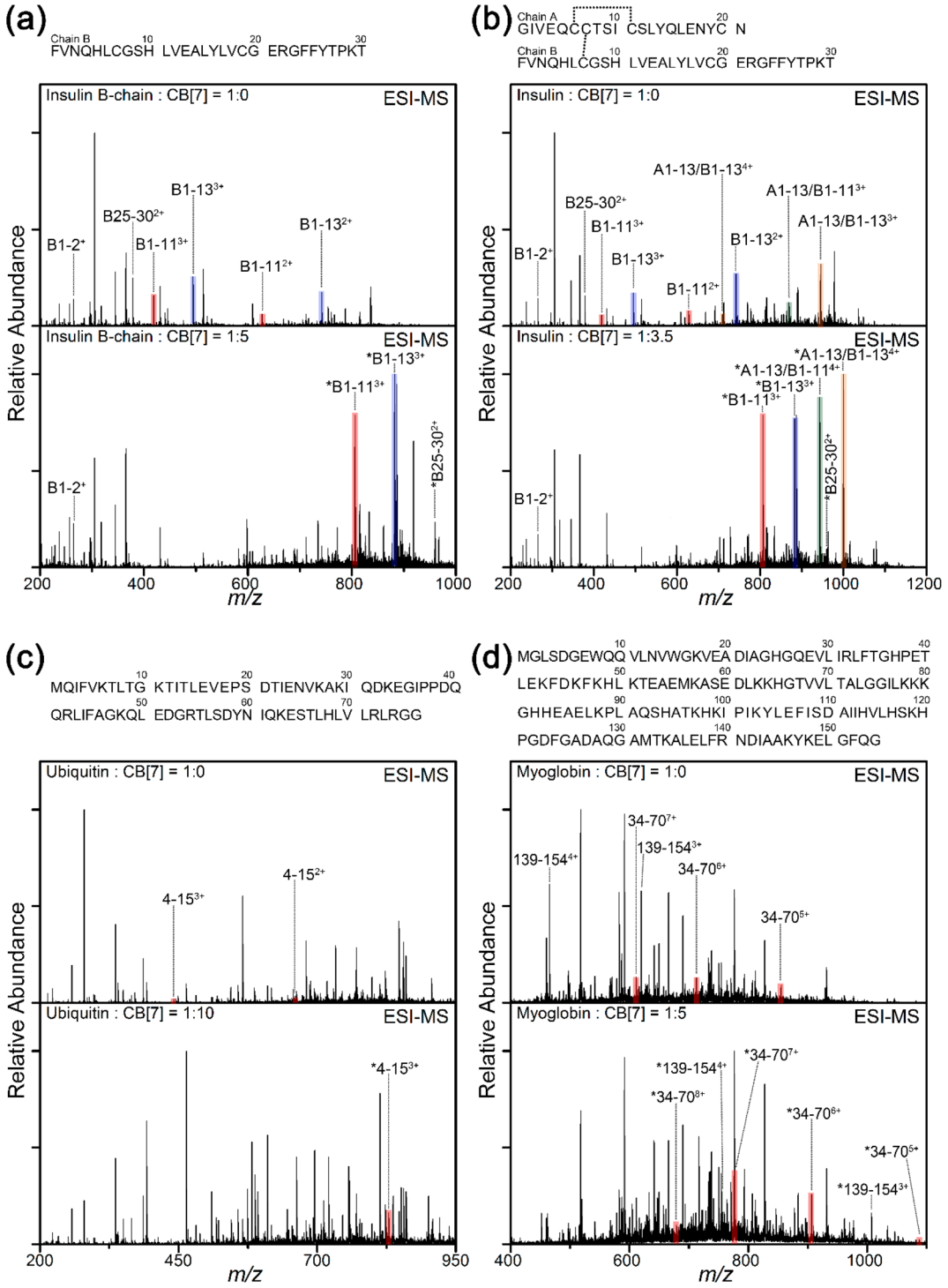

Figure 1. ESI mass spectra of peptic-digested (a) InsB, (b) Ins, (c) Ubq, and (d) Myb without CB[7] (top), and with CB[7] (bottom). The peak numbers of the peptides indicate the first and the last residues of the intact protein sequence. Full assignments are available in Figures S1−S4 and Tables S1−S4 in the Supporting Information.

of the hydrophobic side chain into the CB[7] cavity and ion-dipole interaction of the N-terminal ammonium group with the carbonyl oxygens of CB[7] (Scheme 1).[13,20] The interaction between CB[7] and Phe is preserved even when the complex is transferred to the gas phase via ESI or MALDI.[10,20,22] On the basis of previous studies on CB[6] and lysine-containing peptides,[16,17,19] in which the host facilitated interesting chemistry of the peptides, we were curious about the influence of CB[7] on the analysis of peptides and proteins using MS.

In the present study, we report a method to enhance the MS analysis of poorly detected peptides via the selective non-covalent interaction between CB[7] and peptides with N-terminal Phe residues. This method for supramolecular signal enhancement is applicable to both ESI-MS and MALDI-MS and is especially significant for protein analysis involving pepsin digestion because pepsin favorably cleaves nonpolar sites, including Phe.[23] The signal enhancement effect of CB[7] is demonstrated for the analysis of the insulin B-chain (InsB; 3.4

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Table 1. Ratios of the Relative Abundances (RAs) of Peptide Peaks in the Presence and Absence of CB[7][a]

protein	sequence (residue)	peptide m/z (charge state)	CB[7]-peptide complex m/z (charge state)	RA ratio
InsB	FVNQHLCGSHL (1−11)	418.77 (+3)	806.58 (+3)	4.98
	FVNQHLCGSHLVE (1−13)	494.81 (+3)	882.63 (+3)	3.93
Ins	FVNQHLCGSHL (1−11)	627.81 (+2)	806.72 (+3)	10.23
	FVNQHLCGSHLVE (1−13)	741.88 (+2)	882.75 (+3)	2.85
	GIVEQCCTSICSL/FVNQHLCGSHL (A1−13/B1−11)	869.40 (+3)	943.21 (+4)	7.19
	GIVEQCCTSICSL/FVNQHLCGSHLVE (A1−13/B1−13)	945.45 (+3)	1000.23 (+4)	3.14
Ubq	FVKTLTGKTITL (4−15)	441.25 (+3)	829.11 (+3)	8.91
Myb	FTGHPETLEKFDKFKHLKTEAEMKASEDLKKHGTVVL (34−70)	611.16 (+7)	906.46 (+6)	1.92

[a]The abundances of the highest intensity uncomplexed and complexed peaks were compared, with the exception of Ubq4-15^{2+} and *Myb34-70^{7+} because their peaks overlapped with other peptide peaks.

kDa), insulin (Ins; 5.8 kDa), ubiquitin (Ubq; 8.6 kDa), and myoglobin (Myb; 17.0 kDa). Furthermore, improvement in the sequence coverage of CB[7]-bound peptides was observed in tandem MS experiments. We discuss the mechanistic basis for signal enhancement and improved sequence coverage by CB[7] on the basis of its charge stabilizing property. Our study shows that targeting and capturing specific residues in peptides can be an efficient approach to improving their analysis by MS, and it suggests novel mechanistic pathways for host-induced fragmentation of polypeptides.

EXPERIMENTAL SECTION

Chemicals and Reagents. InsB was purchased from Anygen (Gwangju, Korea). Human recombinant Ins, Ubq from bovine erythrocytes, Myb from equine heart, pepsin from porcine gastric mucosa, and formic acid were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mutants of InsB1−11 were synthesized using the standard Fmoc chemistry on Rink amide resin and purified by HPLC. HPLC-grade water and acetonitrile were purchased from Avantor Performance Materials, Inc. (Center Valley, PA, USA) and used as solvents. CB[7] was purchased from CBTECH (Pohang, Korea), and stock solutions (1 mM) were prepared by dissolving CB[7] in water. Pepsin digests were prepared by incubating protein (100 μM) with pepsin (0.24 mg/mL) in water containing 1% v/v formic acid at 37 °C for 15 min. Peptic digest solutions for ESI-MS were diluted 10-fold. CB[7] was then added to the peptic digest solutions, and the solutions were subjected to analysis by ESI-MS. The concentrations of CB[7] were optimized as 50, 35, 100, and 50 μM for 10 μM of InsB, Ins, Ubq, and Myb, respectively.

Electrospray Ionization Mass Spectrometry and Collision-Induced Dissociation. A Waters Synapt G2 HDMS quadrupole time-of-flight (Q-TOF) mass spectrometer (Waters, Manchester, UK) equipped with a Z-spray ESI source was used for MS experiments in the positive ion mode. A capillary voltage of 3.50 kV, sampling cone voltage of 10 V, extraction cone voltage of 5 V, source temperature of 100 °C, and desolvation temperature of 150 °C were set as parameters.

A Thermo Scientific LTQ Velos dual ion trap mass spectrometer (San Jose, CA, USA) was utilized in the positive ion mode for the collision-induced dissociation (CID) experiments. A source voltage of 3 kV and capillary temperature of 200 °C were set as parameters for ESI. Each spectrum was averaged from 200 scanned spectra obtained using the enhanced scan mode. The nomenclature for the parent and fragment ions was adopted from Roepstorff and Fohlman.[24] The left asterisk (*) superscript for the digested peptides and fragment ions refers to the presence of CB[7]. For example, a b_n^{2+} fragment ion complexed with CB[7] is referred to as $*b_n^{2+}$.

Matrix-Assisted Laser Desorption Ionization Mass Spectrometry. The Waters Synapt G2 HDMS mass spectrometer equipped with a MALDI source utilizing a 355 nm Nd:YAG laser (Waters, Manchester, UK) was used for MALDI-MS experiments. The MALDI laser firing rate and energy were 1000 Hz and 350 (arbitrary units), respectively. The matrix solution was 20 mg/mL α-cyano-4-hydroxycinnamic acid (CHCA) in 77% acetonitrile, 22.9% water and 0.1% formic acid (v/v). The concentration of proteins for peptic digestion was 20 μM, with peptic digestion conditions identical to those used in the ESI-MS experiments. The final concentrations of CB[7] after addition to the digested samples were optimized as 20 μM for InsB and 40 μM for Ubq. Then, the sample solutions for MALDI-MS were prepared by mixing the matrix and the analyte solutions at a 1:1 ratio by volume. The sample solutions (2 μL) were spotted on a sample plate for analysis.

Ion Mobility Mass Spectrometry and Computational Modeling. The Waters Synapt G2 HDMS instrument was used for the ion mobility MS (IM-MS) experiments. The IM measurements were conducted with three instrumental parameters. The gas flows for the helium cell, TWIMS wave velocity and wave height were fixed at 180 mL min^{-1}, 300 m s^{-1} and 20.0 V, respectively, while the gas flows for the drift cell were 50, 60, or 80 mL min^{-1}. Calibration of experimental arrival times into collision cross-section (Ω_D) values was performed using previously reported Ω_D values for polyalanine,[25] melittin,[26] and denatured Ubq.[27] The calibrated Ω_D values from three measurements were averaged.

Computational modeling of CB[7]-peptide complexes was performed using GROMACS 4.5.5.[28] The CHARMM force field[29,30] and CHARMM general force field[31] were used for the simulations. Random coil structures of peptides were initially generated using Hyperchem 7.0 (Hypercube Inc., Gainesville, FL. USA). The peptide models were each merged with a CB[7] molecule and subjected to 500 cycles of simulated annealing using the following profile: heating from 300 to 800 K for 50 ps, constant temperature simulation at 800 K for 50 ps, cooling from 800 to 300 K for 25 ps, and equilibration at 300 K for 25 ps. The structures from the last frame of each annealing cycle were extracted and considered as candidate structures. The five structures with the lowest potential energies were selected as representative structures, and their Ω_D values were calculated with the projection approximation model[32] implemented in MOBCAL (http://www.indiana.edu/~nano/). For ions with two conformations, only one of the two conformations was represented by the lowest-energy structures. Thus, the lowest-energy structures among the models with theoretical Ω_D values in agreement with the experimental Ω_D values (±3%) were selected as representative structures for the other conformation. Simulation was not performed for uncomplexed peptides and the Myb34−70 peptide because they have large degrees of freedom and require long simulation time scales.

RESULTS AND DISCUSSION

Complexation with CB[7] Enhances ESI-MS Signals of Peptic Peptides with N-Terminal Phe. Figure 1 displays the mass spectra of peptic peptides with and without the addition of CB[7]. Figure 1a shows four peptides with N-terminal Phe from InsB, but their abundances are low prior to the addition of CB[7]. Among these peptides, addition of CB[7] did not generate complexes of B1−2, and B25−30 showed negligible difference in its abundance. Surprisingly, with the addition of

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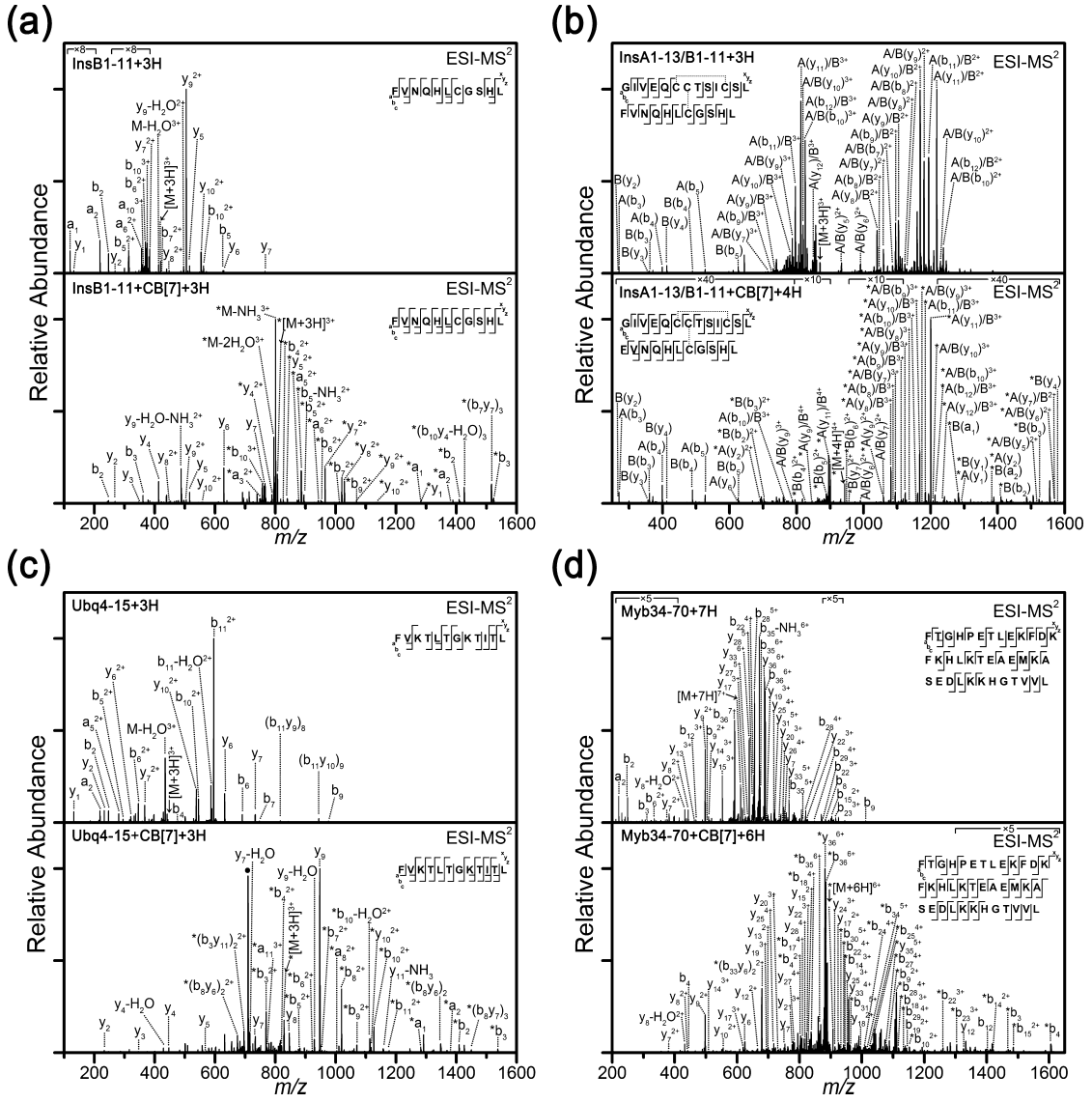

Figure 2. Low-energy collision-induced dissociation (CID) (ESI-MS^2) spectra of (a) the InsB1−11 peptide ion, (b) the InsA1−13/B1−11 peptide ion, (c) the Ubq4−15 peptide ion, and (d) the Myb34−70 peptide ion, without CB[7] (top) and with CB[7] (bottom). The peak annotated with a black dot for the Ubq4−15 ion could not be assigned.

CB[7], the abundances of the InsB1−11 (FVNQHLCGSHL) and the InsB1−13 (FVNQHLCGSHLVE) peptides increased by factors of ∼5 and ∼4, respectively (Table 1), and the CB[7]·peptide complex peaks became dominant in the mass spectrum (highlighted in Figure 1a). In addition to the InsB1−11 and InsB1−13 peptides, peptic digestion of full-length Ins generated InsA1−13/B1−11 (GIVEQCCTSICSL/FVNQHLCGSHL) and InsA1−13/B1−13 (GIVEQCCTSICSL/FVNQHLCGSHLVE) peptides, both of which contain an N-terminal Phe (Figure 1b). The abundances of these two peptide ions also increased following CB[7] addition by factors of ∼7 and ∼3, respectively.

An analysis of a larger protein, Ubq, shows that the signals for peptides with N-terminal Phe are initially barely detectable (Figure 1c). The low signal of these peptide peaks is problematic for further confirmation and characterization of their sequences using tandem MS. Upon addition of CB[7], the peak for the Ubq4−15 peptide (FVKTLTGKTITL) shows an increase in the relative abundance by a factor of ∼9, and becomes significant for analysis. A similar signal enhancement effect was also observed in the case of Myb (Figure 1d), with a 2-fold increase in the abundance of Myb34−70 (FTGHPETLEKFDKFKHLKTEAEMKASEDLKKHGTVVL) peptide. The consistent signal enhancement effect observed for four different proteins indicates that binding peptic peptides with CB[7] is an effective technique that can be applied broadly.

In addition to the peptides with N-terminal Phe, CB[7] also formed complexes with peptides with N-terminal Tyr. For example, Figure S2 shows the presence of *InsB26−30 and *InsA14−21/B17−25 complex ions. However, their uncomplexed forms also exist in the spectrum, indicating that binding of CB[7] to N-terminal Tyr of peptides is significantly weaker than to N-terminal Phe.[20] This result demonstrates that CB[7] selectively interacts with and affects the mass spectra of peptides with N-terminal Phe.

Enhanced Sequence Coverage of CB[7]·Peptide Complexes in CID. Analysis of fragment ions using tandem MS is an important procedure for the accurate characterization of peptide ions. Therefore, we tested the application of tandem MS for further characterization of CB[7]·peptide complex ions

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(Figure 2). We also compared the sequence coverage and percentage of complementary b/y ion pair detection of the complexed ions with those of the uncomplexed ions, both of which are important for reliable analysis using tandem MS. The most abundant peptide ions whose peaks do not overlap with other peaks were compared, and the results are summarized in Table 2.

Table 2. Sequence Coverage and the Percentage of Complementary Ion Pair Detection for Uncomplexed and Complexed Peptide Ions

ion		sequence coverage (%)	percentage of b/y complementary ion pairs (%)
uncomplexed	InsB1−11^{3+}	90	40
	InsB1−13^{3+}	83	50
	InsA1−13/B1−11^{3+}	86	36
	InsA1−13/B1−13^{3+}	83	38
	Ubq4−15^{3+}	73	45
	Myb34−70^{7+}	61	22
complexed	*InsB1−11^{3+}	100	80
	*InsB1−13^{3+}	100	67
	*InsA1−13/B1−11^{4+}	100	55
	*InsA1−13/B1−13^{4+}	92	54
	*Ubq4−15^{3+}	100	73
	*Myb34−70^{6+}	75	47

CID spectra of the uncomplexed InsB1−11 ion show b- and y-type fragment ions, which are fragments typically observed in low-energy CID (Figure 2a, top).[33] Similarly, the complexed InsB1−11 ion also dissociates into b- and y-type fragment ions (Figure 2a, bottom), providing further sequence information. Interestingly, full sequence coverage was achieved with CID of the complexed InsB1−11 ion, whereas the uncomplexed ion showed negligible fragmentation between Gly8 and Ser9 (insets of Figure 2a). Furthermore, a greater number of b/y complementary ion pairs was present in the case of the complexed ion due to the observation of additional *b_3, *b_4^{2+}, *b_{10}^{3+}, and *y_4^{2+} ions. This result implies that CB[7] complexation could improve the tandem MS analysis of peptides. CID of other peptide ions and their complexes also generates b- and y-type fragment ions with more extensive cleavages (Figure 2b−d and Table 2). For example, 19 of 22 amide bonds were cleaved and 8 b/y complementary ion pairs were present for the uncomplexed InsA1−13/B1−11 ion (Figure 2b, top). For the complexed form, all amide bonds were cleaved, and 12 b/y complementary ion pairs were present (Figure 2b, bottom). The Ubq4−15 peptide ion also exhibited complete sequence coverage and a greater number of b/y complementary ion pairs in the complexed form (Table 2). The sequence of the complexed Myb34−70 ion was not completely covered due to its large size, but more extensive cleavage was nonetheless observed (61−75%, Table 2). Table 2 and Figure S5 show that the complexed InsB1−13 and InsA1−13/B1−13 ions also exhibit increased sequence coverage (83−100% and 83−92%, respectively) and greater percentages of b/y complementary ion pairs (50−67% and 38−54%, respectively). The consistent improvement in fragmentation efficiency observed for diverse peptides indicates that complexation of peptides with CB[7] can promote a wider range of backbone cleavages than is typically possible in the absence of CB[7], without the need for covalent peptide modification or special instrumentation. Moreover, the signal enhancement effect by CB[7] can contribute additionally to the enhanced tandem MS analysis of low-abundant peptides, such as Ubq4−15 (Figure 1c).

Complexation with CB[7] Enhances MALDI-MS Signals of Peptic Peptides with N-Terminal Phe. The signal

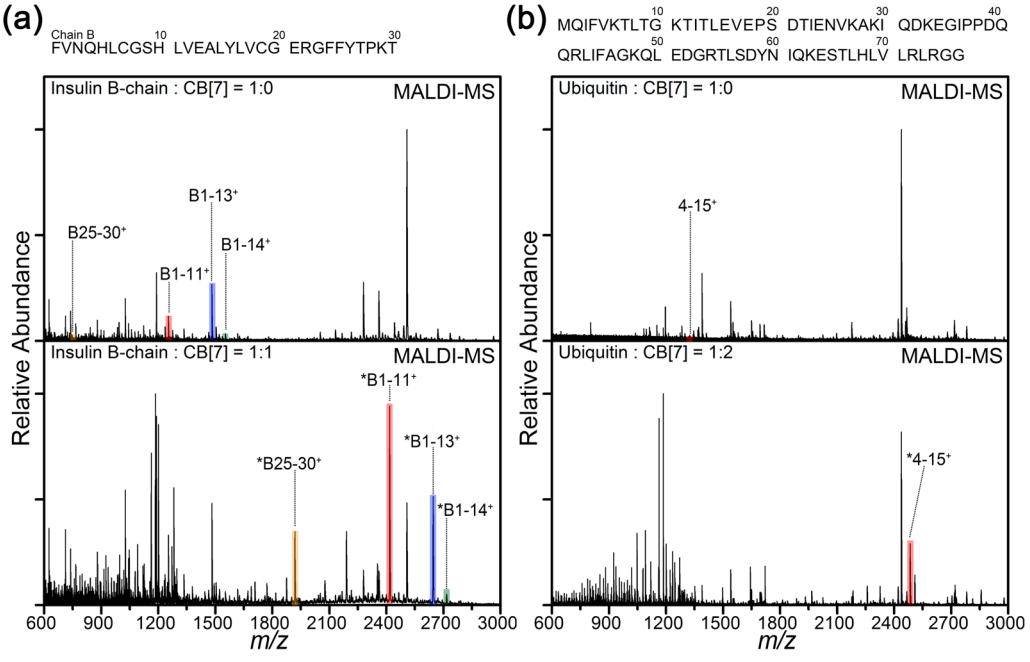

Figure 3. MALDI mass spectra data for peptic-digested (a) InsB, and (b) Ubq, without CB[7] (top), and with CB[7] (bottom). Full assignments are available in Figures S6−S7 in the Supporting Information.

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enhancement effect of CB[7] was also observed in MALDI spectra. Three peptides containing N-terminal Phe from the digestion of InsB revealed increases in their relative abundances when complexed with CB[7] by factors of ~2 to ~8 (Figure 3a, Table S5). Remarkably, the initially undetectable InsB25−30 peptide became clearly observable following the addition of CB[7]. Similarly, the Ubq4−15 peptide, which was not detectable by MALDI-MS, was highly abundant when CB[7] was added (Figure 3b). Thus, the signal enhancement effect of CB[7] is not limited to ESI-MS, but is also applicable to MALDI-MS and can yield information that is not easily obtainable in the absence of CB[7]. Furthermore, the complex ions yielded the typical a-, b-, and y-type ions in CID, which provide additional information for further characterization of the ions using tandem MS (Figure S8 in the Supporting Information).

Understanding the Enhanced MS Signal and Fragmentation Mediated by CB[7]. It has been shown previously that ionization of CB[7] yields doubly charged ions, even when CB[7] is complexed with a guest that is typically present in the gas phase as a singly charged ion.[20] This is because positively charged functional groups of guests are stabilized by strong ion-dipole interactions with the carbonyl-lined portals of CB[7], thus diminishing the electrostatic repulsion between the two cationic groups.[20,21] The strong ion-dipole interactions even allow guests to remain bound to CB[7] under activation conditions in which covalent bonds dissociate.[20] A similar charge enhancement effect was also observed in the present study. For example, the most abundant charge states of B-chain Ins peptides (B1−11 and B1−13) and full-chain Ins peptides (A1−13/B1−11 and A1−13/B1−13), all containing an N-terminal Phe, are +2 and +3, respectively, in the absence of CB[7], but +3 and +4, respectively, when CB[7] is added (Figure 1b). In case of the Ubq4−15 peptide, the addition of CB[7] made the +2 charged ions undetectable, and only the +3 charged ions were observed (Figure 1c). These results clearly show that CB[7] has a charge enhancement effect on its guests, which has also been observed for other hosts.[19,34] In addition, previous studies had demonstrated similar effects in solution; binding guests by CB[7] induces substantial increases in their pK_a values due to increased proton affinities.[20,35,36] High proton affinity is one of the most important factors for efficient ionization by ESI and MALDI[2,37] because a large number of analytes compete for protons during the ionization process. Thus, the enhanced protonation during the ionization process can be inferred as the cause of the observed signal enhancement effect of CB[7]. Small complexation effects observed for InsB25−30 and Myb139−154 support this hypothesis; these two peptides did not show charge enhancement upon complexation (Figure 1), which indicates that they have relatively high proton affinities in the uncomplexed forms.

To understand the improved peptide fragmentation efficiency upon CB[7] binding, we first studied the fragmentation mechanism of CB[7]-bound peptides using model peptides (FGGGG and KGGGG, Figure S9 in the Supporting Information). The results from these experiments show that the fragmentation of CB[7]-bound peptides can proceed via both the "b_x−y_z" peptide fragmentation pathway and the diketopiperazine peptide fragmentation pathway[33] (see Schemes S1−S2 and Discussion in the Supporting Information). Both pathways require the transfer of "mobile protons"[38] to the amide backbone, suggesting that CB[7] promotes the transfer of protons to various sites to enhance backbone dissociation during CID.

For a more detailed understanding of the CB[7]·peptide complex ions, we investigated the structure of the CB[7]·peptide complex ions using IM-MS (Figure 4 and Figure S10 in the Supporting Information), which can provide global structural information on ions in the gas phase, combined with computational modeling. The IM distributions showed that both the uncomplexed and the complexed peptide ions generally exist in one or two conformations (Figure S10). The theoretical Ω_D values of the lowest energy structures of the complex ions generally showed good agreement with the experimental values, with relatively small errors observed (~3%, Table S6). The theoretical models suggest that CB[7] is bound to the N-terminal Phe of the peptides, as can be expected based on its binding properties in solution.[10,13] Interestingly, the models also suggest that CB[7] attracts other positively charged residues toward the other carbonyl portal to induce "peptide tweezer" structures in the gas phase (Figure 4). These additional residues for interaction are His10 and Lys8 for the InsB1−11 and Ubq4−15 complex ions, respectively. Other peptide complexes also showed compact structures with the peptide chains wrapping around CB[7] (Figure S11). This previously unreported structural behavior of peptides induced by CB[7] demonstrates that CB[7] has an exceptional charge stabilizing capability in the gas phase.

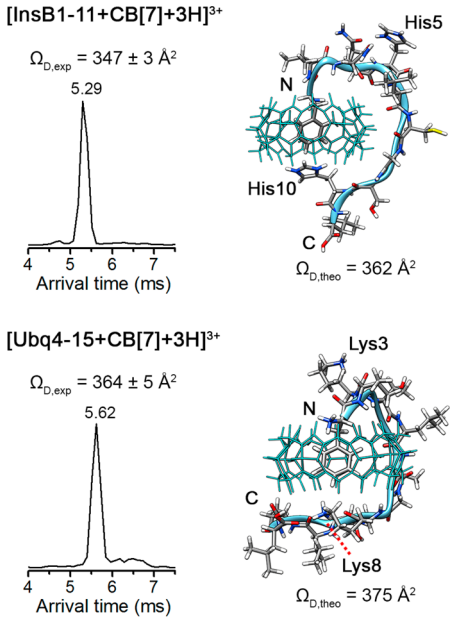

Figure 4. Ion mobility spectra and the lowest-energy theoretical structures of InsB1−11 and Ubq4−15 in complex with CB[7]. Small, nondominant peaks in the spectra arise from noise and less abundant overlapping ions. The structures of the other complexes are available in Figure S11.

On the basis of these results, we inferred that the effective interaction of CB[7] with charged functional groups creates new pathways for peptide fragmentation. For example, Figure 2c shows that cleavage at the C-terminal amide backbones of Lys residues does not occur upon CID of the uncomplexed Ubq4−15 peptide ions. This observation is in contrast to Lys facilitating cleavage of its C-terminal amide bond.[33] As cleavage of the amide bond C-terminal to a Lys residue should be

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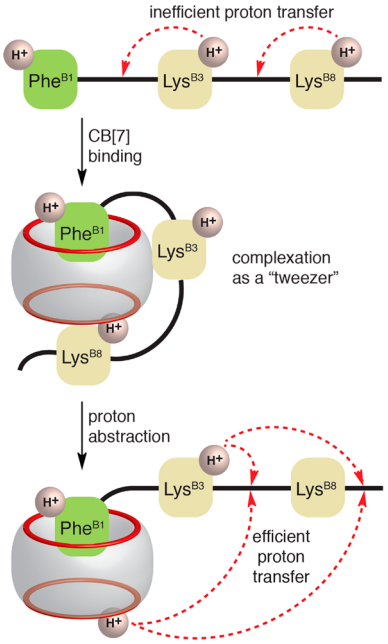
preceded by neutralization of the Lys side chain and transfer of a proton to the amide bond,[33] it can be speculated that these processes are unfavorable for the uncomplexed Ubq4−15 ion. By contrast, experimental results demonstrate that fragmentation at these sites is possible when the peptide is complexed with CB[7] (Figure 2c, bottom). This observation indicates that CB[7] can facilitate neutralization of a Lys residue and proton transfer to its C-terminal amide bond. Presumably, this process proceeds by transfer of a proton from a Lys side chain to CB[7] upon activation, followed by transfer of a proton on CB[7] or other charged groups to the amide bond C-terminal to the Lys residue (Scheme 2).

Scheme 2. Proposed Fragmentation Pathways of the Uncomplexed and Complexed Ubq4-15 Peptide Ion



It is possible that other factors may also promote diverse fragmentation pathways of CB[7]-bound peptides. Figure 2 shows that CB[7] can migrate to charged residues with which it did not interact prior to activation. For example, CB[7] is most likely to interact with His5 of the $*(b_7y_7)_3$ fragment ion from InsB1−11 (Figure 2a, bottom), whereas our computational modeling (Figure 4) and mutation studies (Figure S11 and Discussion in the Supporting Information) show that CB[7] originally interacts with the N-terminal Phe and His10 of the peptide prior to activation. This observation suggests that transfer of CB[7] occurs during activation.[39] Such a transfer of CB[7] to different sites in the peptide ion would change the conformation of the peptide and the potential energy surface of the fragmentation pathways, thus making different fragmentation pathways accessible during CID.

CONCLUSION

This work demonstrates a novel approach to the enhanced MS analysis of proteins via the increased signal abundance and sequence coverage of peptic peptides mediated by CB[7]. We have shown that capturing N-terminal Phe with CB[7] is a viable and general approach to selectively improving the MS analysis of peptic digests. The signal enhancement due to CB[7] addition can be attributed to an increase in proton affinity. The enhanced sequence coverage mediated by CB[7] can be attributed to the strong interaction of CB[7] with basic residues opening up diverse fragmentation pathways during CID. This technique does not require new instrumentation, chemical labeling, or specialized sample preparation. Rather, a small amount of commercially available CB[7] is simply added to samples to improve their analysis. The simplicity and potential generality of this technique should provide a valuable asset in the toolbox for routine protein analysis. We anticipate the expansion of this method to include other host−guest interactions that target a range of peptide sequence types for the efficient analysis of peptides and proteins.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.5b10648.

Additional discussions and additional experimental data. (PDF)

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Notes

The authors declare no competing financial interest.

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