Evidence for an Intermolecular Proton-Transfer Reaction Induced by Collision in Gas-Phase Noncovalently Bound Complexes

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A novel endothermic proton-transfer reaction is observed when low-energy collision-induced dissociation (CID) of a complex composed of oligosaccharides and protonated amino acid is performed. Protonated oligosaccharide fragments resulting from glycosidic bond cleavages are observed. This corresponds to a proton transfer from the more basic amino acid to the less basic cyclodextrin. Furthermore, the resulting cyclodextrin fragments suggest that fragmentation and dissociation of the fragments are fast processes, faster than the reverse proton transfer to the amino acid. Experiments with the methyl ester and betaine indicate that the proton originates from the ammonium group rather than the carboxylic acid. Dissociation threshold experiments further support the origin of the proton. When the gas-phase basicity (GB) of the amino acid is increased, the relative dissociation threshold increases. This system is ideal for observing proton transfer in low energy CID experiments. In addition, due to specificity in oligosaccharide−amino acid interactions, this reaction may provide the possibility of site-selective bond cleavage reactions that are specified by the coordination.

Proton-transfer reactions in gas-phase complexes bound by ion−dipole interactions are the central steps in a wide variety of ion−molecule reactions including gas-phase basicity measurements, hydrogen−deuterium (H−D) exchange, and peptide fragmentation reactions.[1−3] Exothermic proton-transfer reactions are readily observed and are the basis for the determination of important gas-phase basicity values. Similarly, endothermic proton-transfer reactions are also relatively common and are employed specifically in H−D exchange and peptide fragmentation. For example, the mechanism often invoked in the H−D exchange of amino acids and peptides involves several endothermic proton-transfer reactions involving the ion−dipole complex.[4−10] The fragmentation of peptides and proteins under conditions of low-energy collisions is believed to be facilitated by similar proton-transfer reactions.[11−16] Although the complete detail of the mechanism is still not understood, sequence specific fragment ions are believed to be due to the direct cleavage of the peptide bond preceded by the protonation of the amide nitrogen. However, the most favorable site of protonation is not always the site of fragmentation. For example, in small peptides where protonation occurs on the N terminus, fragmentation occurs along the peptide bond requiring the protonation of a less basic nitrogen-amide site. The reaction corresponds to an endothermic (∼+20 kcal/mol)[17−21] proton transfer occurring between the N terminus and the amide of the peptide bond. The reaction is further supported by the dissociation studies of Wysocki and co-workers that show the presence of Arg (a highly basic residue) increases the calculated activation thresholds for dissociation of the protonated peptide.[22,23] Endothermic proton-transfer reactions were also the basis of several studies by Fenselau involving the collisions of protonated peptides with amine and methane gases.[24,25] These reactions produced fragmentation of the peptides as well as peptide adducts of the collision gas. It was suggested that the collision complexes were relatively long-lived to allow the reaction to occur. Although endothermic proton-transfer reactions in complexes are often proposed in these and other systems, there has been little or no direct evidence for the existence of this important process.

Complexes of cyclodextrin and protonated amino acids are produced from a solution of 1×10^{-5} M cyclodextrin and a 10-fold excess of amino acid. Experiments are performed using external source Fourier transform mass spectrometry (FTMS).[26,27] Under these conditions, CD coordinates to various cations including NH_4^+, Na^+, and K^+.[28] The desired ion $[CD{:}AA + H]^+$ is isolated using an arbitrary waveform generator (Ionspec, Irvine, CA). Collision-induced dissociation (CID) is performed using sustained off-resonance excitation.[29] This involves a 1000-ms excitation pulse at 800 Hz lower than the cyclotron frequency. During this time, two nitrogen pulses are fired to produce a nearly constant background pressure of 10^{-5} Torr during the CID event.

The resulting product ions are fragments of the cyclodextrin (Figure 1). The amino acid is dissociated from the complex as a neutral species. The fragments correspond to losses of methanol $[CD + H - 32]^+$, one and two monosaccharide residues ($[CD + H - 32 - 204]^+$ and $[CD + H - 32 - 2 \times 204]^+$, respectively). Experiments were performed with Gly, L- and D-Phe, Ile, Ala, Leu, Lys, and His. The results are similar for all of the amino acids. The same products are observed with slight variations in intensities. The net reaction in each case is a proton transfer from the amino acid to the cyclodextrin with charge being retained on the less basic (cyclodextrin) fragments. The complex of Arg was

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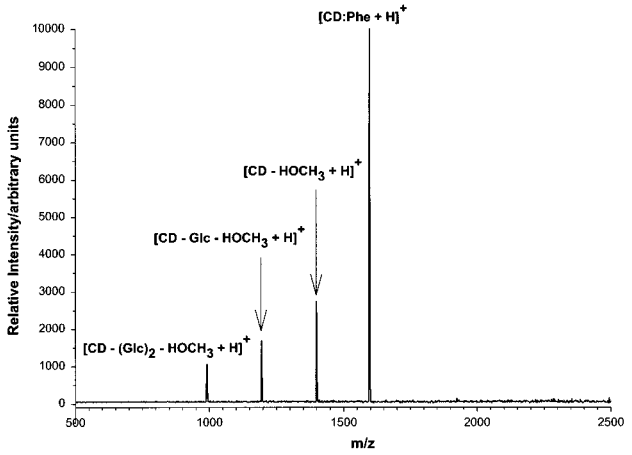

Figure 1. Collision-induced dissociation of complex consisting of protonated alanine and permethylated β-cyclodextrin. The products correspond to cyclodextrin fragments only.

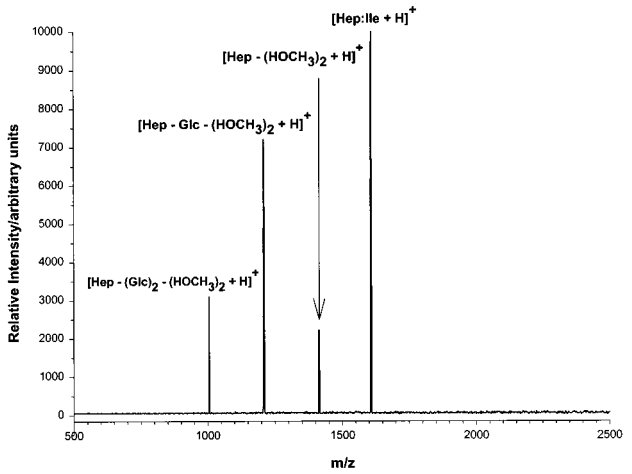

Figure 2. Collision-induced dissociation of complex consisting of protonated alanine and permethylated maltoheptaose. Maltoheptaose is the linear analogue of β-cyclodextrin. The products correspond to fragments of maltoheptaose only.

also examined but did not yield fragment ions; only loss of the parent ions was observed. This result suggests that either the complex was too strong and did not dissociate or the products were too small to be observed. We suspect the former, that is, no proton transfer is observed at the maximum kinetic energy and only ejection of the precursor ions from the analyzer cell is observed.

For comparison, CID of protonated amino acids complexed to the linear analogue of cyclodextrin, permethylated-maltoheptaose (MH), was also performed (Figure 2). The complexes $[MH:AA + H]^+$ were formed under the same conditions. All the CID products correspond to the loss of a neutral amino acid. In addition, losses of two methanols $[MH + H - 2 \times 32]^+$, a glucose and two methanol $[MH + H - 2 \times 32-204]^+$, and two glucoses and two methanols $[MH + H - 2 \times 32-2 \times 204]^+$ are the three dominant products. The CID of maltoheptaose typically produce cleavages at every glycosidic bonds. The lack of other fragments suggests a specific interaction between the amino acid and the oligosaccharide resulting in a few distinct products. The losses of one or two residues are consistent with cleavages near the terminal position (whether reducing or nonreducing ends). Because the glycosidic bonds are nearly identical and would be expected to have similar proton affinities, the preference for the cleavage at the terminal positions suggests a specific coordination between the amino acid and maltoheptaose at those positions. These interactions may provide a method for affecting cleavages at specific positions where the amino acid may be favorably coordinated.

Table 1

amino acid	proton affinity[33]	multicollisional dissociation threshold E_{com} (eV)
D-Phe	216.5	1.8 ± 0.2
L-Phe	216.5	1.6 ± 0.2
D-Ala	215.7	1.7 ± 0.2
L-Ala	215.7	1.7 ± 0.5
L-Lys	238.3	2.6 ± 0.2
L-His	236.4	2.6 ± 0.6

Multicollisional dissociation threshold (MCDT) measurements were performed by varying the translational energy of the ion and monitoring the fragment ions. The values for center-of-mass collision energies are obtained using a fit of an empirical equation[30] and extrapolating the intensity of the fragment to zero.[31] The summary of the results is tabulated (Table 1). We find a correlation between proton affinity and dissociation threshold; increasing the proton affinity of the amino acid increases the dissociation threshold. The complex containing Phe and Ala dissociates around 1.8 eV (COM energy), while the more basic Lys and His require around 2.6 eV for dissociation. The results are consistent with those obtained in the CID of the Arg complex. This amino acid is so basic that proton transfer does not occur even at the highest effective collision energies. Furthermore, although chiral specificity has been observed in similar systems using ligand-exchange reactions, the results show no such specificity.[32] The differences in binding of the Ala and Phe enantiomers are evidently too small to be measured with this method.

For amino acids with alkyl side chains, the protonated amino acid may donate one of two types of protons, that from the ammonium group or the carboxylic acid. Experiments were performed with the methyl ester of Phe. CID of the resulting complex under similar conditions produces the same cyclodextrin fragments, suggesting that the protonated amine and not the acid is involved in the proton-transfer reaction. These results are further supported by CID of the complex of betaine. This compound, an *N*,*N*,*N*-trimethylammonium glycine, does not produce fragments; only loss of signal is observed. These results suggest that proton transfer occurs between the protonated amine and the cyclodextrin, rather than the carboxylic acid.

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