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Thermal dissociation of protonated cyclodextrin-amino acid complexes in the gas phase

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Abstract

Dissociation temperatures of protonated gas-phase amino acid-cyclodextrin complexes were determined by producing the complexes with electrospray ionization and passing them through a resistively heated capillary. The dissociation temperatures of the complexes increased with the number of hydrogen bonding interactions and the ability of the cyclodextrin host to sterically lock the amino acid guest in place. There was little correlation between the gas-phase basicity of the amino acid and the dissociation temperature. Molecular modeling was used to better understand the nature of the interactions. (Int J Mass Spectrom 210/211 (2001) 215–222) © 2001 Elsevier Science B.V.

Keywords: Cyclodextrin; Inclusion complex, Amino acid

1. Introduction

Cyclodextrins are cyclic oligosaccharides with the most common containing six (α), seven (β), and eight units (γ) [1]. In solution, the oligosaccharides behave as host receiving compounds that are more hydrophobic than the cyclodextrins into the central cavity. The resulting inclusion complexes have long been known and have found applications in a wide variety of commercial products [1,2].

To gain further understanding of the inherent nature of the interactions, there have been several attempts to characterize the strength of the interaction in the gas phase. These methods have included collision-induced dissociation (CID) [3], heated capillary dissociation (HCD) [3], and blackbody infrared radiation dissociation (BIRD) [4,5]. In CID, the translationally excited complex is collided with gaseous molecules to produce fragmentation. With heated capillary dissociation, the complexes are passed through a resistively heated capillary tube to produce fragmentation whereas BIRD is used to photodissociate the ions. These methods were used initially to determine if the gas-phase complexes were indeed inclusion complexes with the notion that inclusion instilled some special stability to the complex. However, these methods were found to be too insensitive to the presence of inclusion. Instead, a gas-phase chiral-specific guest exchange reaction was used to obtain evidences for the presence of gas-phase inclusion complexes [6].

The first HCD studies of gas-phase inclusion complexes involved peptides with β-cyclodextrin hosts

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Dedicated to Professor Nikko Nibbering for over 30 years of research in ion/molecule chemistry. Nikko continues to be a good friend and mentor.

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[3]. A resistively heated capillary was designed with two independently temperature controlled regions that allowed us to show that the complexes dissociated in the gas phase rather than in the solution phase (or in droplets). The resistively heated capillary was similar in design to that introduced by Chait and co-workers to desolvate ions produced by electrospray ionization (ESI) [7]. HCD or thermal dissociation had been used previously to dissociate gas-phase ions. Smith had used a similar setup to obtain activation energies for the dissociation of multiply charged ions produced by ESI [8,9]. Smith and co-workers were also the first to use HCD to study noncovalent interactions in the streptavidin tetramer [10]. Wysocki and co-workers used heated capillary dissociation to obtain activation energies of peptides and peptide dimers [11].

We recently showed that HCD provides relative activation energies for compounds with similar structures but may fail for compounds that are too structurally dissimilar [12]. The problem was mainly due to the difficulty in obtaining a "true" temperature inside the capillary as the temperature was measured on the outer surface. Nonetheless, it was shown that the dissociation temperature or T_d provides a method for obtaining relative strengths of interactions (or more specifically barriers of dissociation) for similar systems. In this study, we used HCD to probe the strengths of the interaction in cyclodextrin–amino acid complexes.

2. Experimental

All experiments were performed on a home-built external source ESI Fourier transform mass spectrometry (FTMS) equipped with a 5.2 T superconducting magnet (Oxford Instruments, Witney, UK) [13]. All reagents were obtained as analytical grades and used without further purifications. Details of the HCD experiments have been published elsewhere and only those relating specifically to the work performed here will be described [3]. The complexes were prepared in a 50/50 water/methanol solvent in 1:5 molar ratios (β-cyclodextrin:amino acid) with β-CD concentrations of approximately 1.0×10^{-5}M. Electrospray was performed by applying 3.0–3.5 kV to the needle. The flow rates were adjusted to about 1–2 mL/h.

HCD experiments were performed with a stainless steel capillary having two independently heated regions [12]. Heating was performed resistively. During the HCD experiments, the temperature of the first segment (nearest the entrance) was set so that the protonated amino acid–cyclodextrin complex was the dominant peak. This occurred at a temperature range of 90–200 °C. Above this range dissociation of the complex occurred while below this range poor signal-to-noise was obtained, probably due to poor desolvation of the complex.

To obtain T_d values, the temperature of the second segment was raised incrementally from an initial temperature equal to that of the first segment to a temperature (T_d) where the relative abundance of the complexed ion goes to zero (threshold value of 1% relative to the $[CD+Na]^+$ complex). Care was taken to ensure that the temperature of the capillary remained constant during the acquisition of the spectra. More acquisitions were also collected near the T_d to improve the signal-to-noise ratio of the spectra. This allowed us to determine the T_d values more accurately. Nozzle-skimmer dissociation between the capillary and the skimmers was also a concern. Although we tried to minimize this process, it is possible that the internal energy of the ion may have been increased thereby affecting the final T_d values. For this reason, the tuning parameters such as the electrode voltages in the source and the ion transport region were maintained throughout all the experiments. Experiments were performed either with single amino acids or a mixture involving two or three. Either way, the experiments produced similar T_d values.

The molecular modeling (MM) calculations were carried out using the Insight II/Biosym program. The structures were minimized through several heating and annealing cycles. The structure with the lowest energy was selected in each case. The cyclodextrin and amino acids structures were first constructed and optimized using the Insight II builder module. The protonated complexes were formed by merging the respective hosts and protonated amino acid guests. In the first set of calculations, the amino acids were

placed initially near the upper, wider rim of the CD molecule (noninclusion complex). In the second set of calculations, the amino acids were placed initially inside the CD cavity (inclusion complex). In both cases the complexes were heated to 600 K for 400 ps. At every 8 ps, a structure from the trajectory was captured and annealed in steps of 100 to 0 K. This resulted in 50 annealing simulations with a corresponding number of structures. Generally, several structures with very similar energies were obtained. During the simulation, the structures of both the amino acids and the hosts were allowed to fully optimize. Only the lowest energy structure of each enantiomer is presented. However, all the structures within 5 kcal/mol of the lowest energy structure were examined and found to share the same structural features.

3. Results

3.1. Heated capillary dissociation of selected amino acids complexed to tri-O-methyl-β-cyclodextrin

The host tri-O-methyl-β-cyclodextrin (TMCD) has three methyl groups on each glucose unit resulting in 21 methyl groups, 7 in the narrow rim and 14 in the wider rim. The thermal stability of permethylated β-cyclodextrin:amino acid complexes was probed in the gas phase by heated capillary dissociation.

Fig. 1 shows a representative set of spectra used to determine the dissociation temperature of a protonated cyclodextrin–amino acid complex. At T = 282 °C the protonated cyclodextrin–Val complex $[CD:Val+H]^+$ was a major peak and about 50% as intense as the base peaks of $[CD + Na]^+$ and $[CD:NH_3 + H]^+$. Ammonia was an impurity of the samples and the corresponding cyclodextrin complex was often found in the spectra. As the temperature of the capillary was heated from 282 °C to 378 °C, the amino acid complex steadily decreased in intensity. During this period, the intensity of the sodium $[CD + Na]^+$ and the potassium coordinated cyclodextrin species remained essentially the same. These complexes dissociated at significantly higher temperature (>500 °C). For this reason, the intensities of the amino acid complexes were referenced to the intensity of the alkali-metal-coordinated complex. Note also that the ammonium complex $[CD:NH_3 + H]^+$ decreased in intensity as the temperature increased albeit the complex persisted to higher temperatures.

The temperature was measured by welding a thermocouple to the outside surface of the heated capillary. We attempted to measure the internal temperature by mounting a thermocouple directly in the outflow of the capillary. It was found that the temperature of the flow varied by as much 50 °C below the value measured on the external capillary surface [12]. The difference was not constant and was greatest at the highest temperature.

A plot of the normalized (to the Na^+ complex) intensity of the Val complex $[CD:Val + H]^+$ as a function of temperature is shown in Fig. 2. Based on this plot, we assigned the dissociation temperature for the Val complex. The T_d values of all the other amino acids were performed in the same manner. Table 1 lists the dissociation temperatures and the gas-phase basicities of several naturally occurring amino acids. Some of the amino acid complexes could not be produced and were not examined in this study. The standard error of each measurement was ±5 °C, based on multiple determination. It must be emphasized that T_d varied with the tuning of the source voltages due to nozzle-skimmer activation, which increases the internal energy of the complex and under certain conditions even dissociates the complex. Therefore, the T_d values should be used merely for comparison and no physical significance should be assigned to the absolute numbers.

The T_d values depended generally on the functional group of the side chain. The amino acids with alkyl and aromatic side chains have the lowest dissociation temperatures. The majority of the T_d values lies in a range spanning nearly 30 degrees from the lowest belonging to Ile to Ala. Gly and Pro, the smallest amino acids, have higher T_d, with 415 and 413 °C, respectively, or nearly 20 degrees higher than Ala. The amino acids with basic side chains have the highest dissociation temperatures. Arg has the lowest T_d in the group, which is 40 degreees larger than Ala.

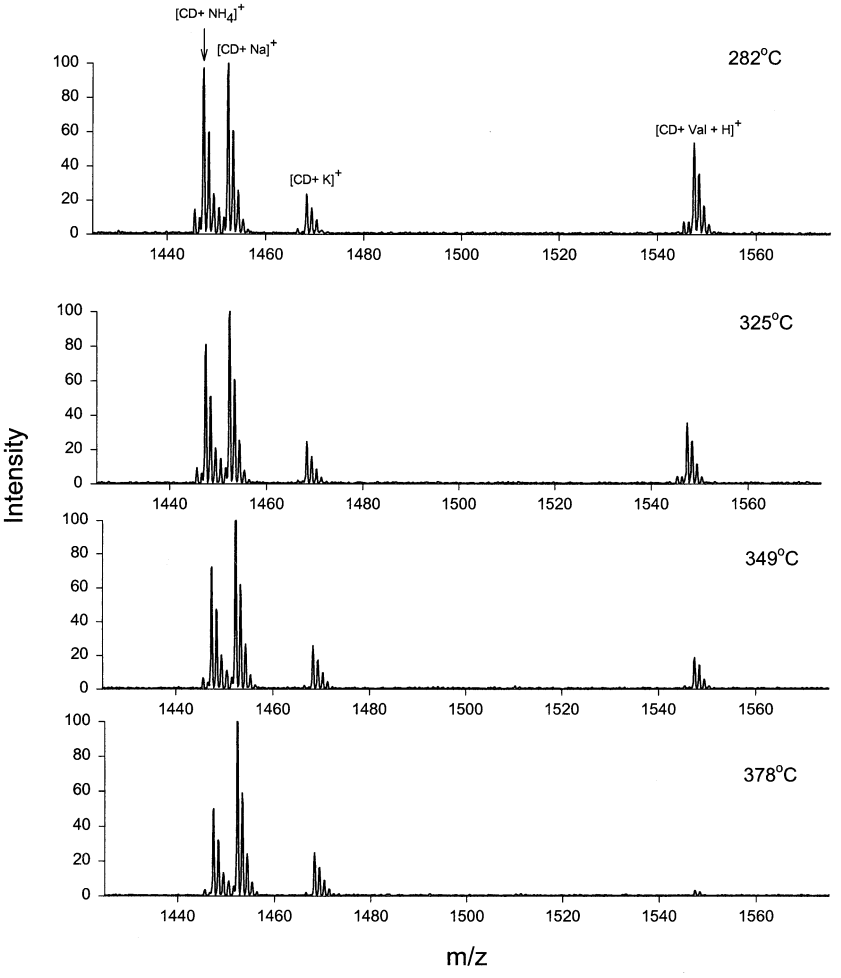

Fig. 1. ESI FTMS spectra of solution containing permethylated β-cyclodextrin (TMCD) and valine. The 1:1 complex is observed between *m/z*1540 and 1560. The base peaks correspond to TMCD complexes of NH_4^+ and Na^+. As the temperature is increased from 282 to 378, the intensity of the amino acid complex is decreased. The alkali metal complexes ($[CD+Na]^+$ and $[CD+K]^+$) remain relatively unchanged. = s, 11p

Amino acids with functional groups of intermediate polarity (–OH and $-CONH_2$) fall in between the two groups with values ranging from 403 °C for Thr to 413 °C for Ser.

3.2. Heated capillary dissociation of selected amino acids complexed to di-O-methyl-β-cyclodextrin

For comparison di-O-methyl-β-cyclodextrin (DM-CD)was also examined as a host. Complexes of DMCD have been studied earlier in solution and in the gas phase (vide infra). This compound is a partially methyl-derivatized β-cyclodextrin with 14 methyl groups (seven on each narrow and wide rims). Table 2 lists the T_d of a selected group of amino acid complexed with di-O-methyl-β-CD and tri-O-methyl-β-CD for comparison. The T_d for DMCD follows the order Gly<Val<Tyr<Phe<Trp indicating that Gly is the least strongly bound while Trp is the most strongly bound. The order of the dissociation temperature for DMCD matches that of TMCD except for one amino acid. With TMCD, Gly has the highest T_d of the

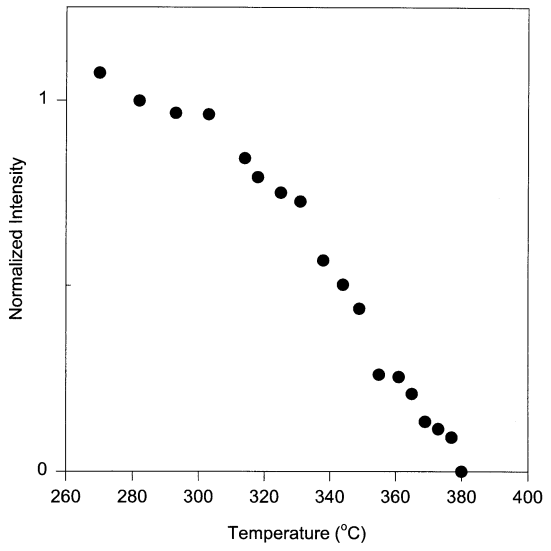

Fig. 2. Intensity of the [CD:Val + H]$^+$ complex relative to the [CD + Na]$^+$ complex as a function of temperature. The T_d of the complex is extrapolated to 378 °C.

group, whereas with DMCD it has the lowest value. The order (minus Gly) follows generally the order of capacity factors obtained by Corradini et al. using high performance liquid chromatography [14], with the exception that Tyr and Phe are reversed,

Table 1
Dissociation temperatures (T_d) of various protonated amino acids complexed to tri-*O*-methyl-β-cyclodextrin; the gas phase basicity (GB) and proton affinity (PA) were obtained from the literature [26]

Amino acid	T_d (average) °C	GB (kJ/mol)	PA (kJ/mol)
Lys	460	951	996
His	457	950.2	988
Arg	441	1006.6	1051
Gly	415	852.2	886.5
Pro	413	886	920.5
Ser	413	880.7	914.6
Gln	406	900	937.8
Trp	403	915	948.9
Thr	403	888.5	922.5
Ala	396	867.7	901.6
Tyr	392	892.1	926
Phe	386	888.9	922.9
Leu	386	880.6	914.6
Val	378	876.7	910.6
Ile	368	883.5	917.4

Table 2
Dissociation temperatures (T_d) of selected protonated amino acids complexed with di-*O*-methyl-β-cyclodextrin (DMCD) and tri-*O*-methyl-β-cyclodextrin (TMCD)

Amino acid	DMCD (°C)	TMCD (°C)
L-Trp	395	404
L-Tyr	381	392
L-Phe	330	386
L-Val	324	378
L-Gly	240	414

Val<Tyr<Phe<Trp. Gly was not examined in the original study. The order in T_d values also follows that reported by Ramanathan and Prokai for values of dissociation threshold of the protonated gas-phase complexes [15]. They found that collision-induced dissociation values obtained between the capillary and the skimmer region followed the same ordering with Val having the lowest dissociation threshold and Trp the highest. The similarity between the results of the three experiments is significant and suggests that the intrinsic binding interactions play a major role in the selectivity and separation of these compounds in liquid chromatography. The results further suggest a link between gas-phase and solution-phase behavior.

4. Discussion and molecular modeling calculations

The T_d values obtained by HCD give indications of the height of the activation energy for the dissociation reaction. Although the absolute values cannot be obtained with the HCD, we have shown that for similar systems they provide a relative ordering of the activation energies [12]. To understand the relative ordering of the cyclodextrin–amino acid complexes, we need to identify the interactions that may contribute to the dissociation barrier.

The major interactions between the protonated amino acid guests and the cyclodextrin host involve ion/dipole interactions, hydrogen bonding, and steric factors. It has now been established that for amino acids with nonbasic side chains, the site of protonation is the terminal amine [16–23]. In the complex,

the site of protonation remains the terminal amine [24]. Molecular modeling calculations predict that the ammonium group interacts with the oxygens of the cyclodextrin molecules, primarily with the narrow rim and, in some cases, the glycosidic bond in cyclodextrin [6,25]. Additional hydrogen bonding occurs with the carboxylic acid group. For amino acids with polar side chains, additional stabilization of the complex is obtained via hydrogen bonding interactions between the side chains and the cyclodextrin hosts. However, for amino acids with large, nonpolar alkyl groups, the side chain interacts with the cyclodextrin hosts repulsively resulting in low T_d values. Indeed, amino acids with hydrogen bond donors in the side chains, such as –OH and $-NH_2$, produced T_d values generally larger than those with only alkyl groups. Tyr has a higher T_d value than the comparably size Phe due to the hydroxyl group. Similar behavior is observed with Ser and Ala. When the side chain functional group is an amine, the number of hydrogen bonding interactions increases even further. Although the site of protonation in Lys, His, and Arg are more ambiguous, the relative positions of the two amino and the carboxylic acid groups make extensive hydrogen bonding between the guest and the host possible. For this reason, these amino acids have the highest T_d.

Gas-phase basicity (GB) is often an indicator of strong hydrogen bonding interactions. The large basicities associated with Lys, His, and Arg, are the result of interactions between the amine side chain and the amine terminus. However, we find no simple correlation between GB and T_d (Fig. 3). When GB is plotted as a function of T_d, a scatter plot is produced with Arg, His, and Lys grouped together in one area, while the other amino acids are grouped in another. The lack of a simple correlation is consistent with the complexity of the interaction in the protonated complex and suggests that hydrogen-bonding interaction is not the only contributing factor to T_d.

Molecular modeling calculations were performed to provide additional insight into the interaction of the protonated amino acids with the cyclodextrin hosts. The inclusion structure is the preferred state for the complexes. Molecular dynamics calculations initiated with inclusion and noninclusion structures yielded

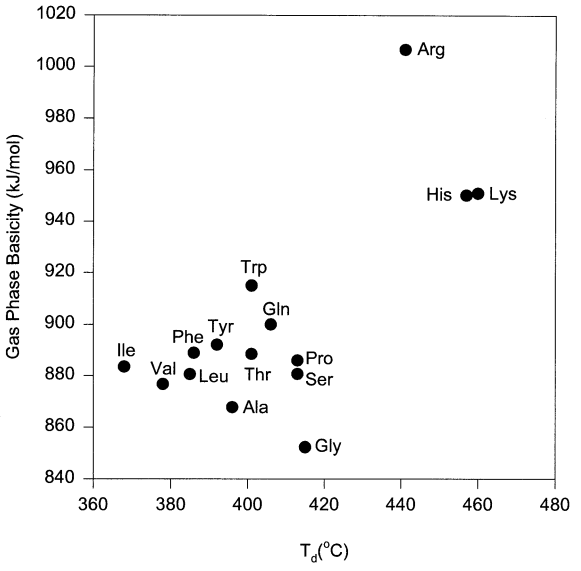

Fig. 3. Plot of the gas-phase basicity of various amino acids as a function of their T_d when complexed to permethylated β-cyclodextrin (TMCD).

similar results. That is, the inclusion structure is the energetically most favorable [25]. Experimental and theoretical evidences for the presence of gas-phase inclusion complexes have recently been provided [6].

The optimized structure for protonated Arg, with protonation on the side chain, complexed to TMCD shows interaction of the ammonium group with the narrow (lower) rim while the molecule extends through the cavity allowing the N-terminus and the carboxylic acid to interact intimately with the upper rim (Fig. 4). Similar results were obtained with Arg containing peptides and with the amino acid Lys [4]. This structure optimizes hydrogen-bonding interactions and accounts for the high T_d obtained with the amino acids with basic side chains.

The T_d of Gly appears anomalous until the size of the amino acid is considered. The lowest T_d belongs to amino acids with large alkyl side chain such as Ile, Val, and Leu. Presumably, repulsive interactions between the alkyl side chain and the inner cavity of the cyclodextrin destabilizes the resulting complexes relative to the smaller amino acids. Indeed, Ala has a high T_d whereas Gly and Pro, both with small,

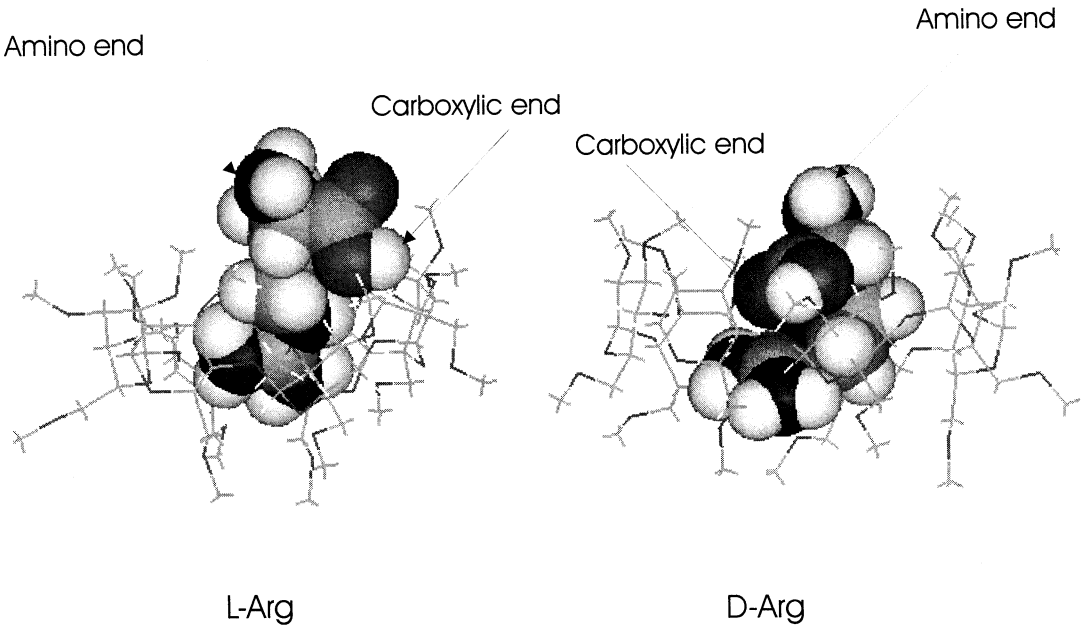

Fig. 4. Lowest energy optimized structure of protonated arginine complexed to TMCD. The cyclodextrin host is depicted in stick form while the amino acid is in space filling. The proton is placed on the side chain, the most basic site on the molecule. The protonated amine coordinates with the lower (narrow) rim while the molecule threads through the cyclodextrin cavity allowing the C- and N-termini groups to interact with the upper (wider rim).

compact structures have T_d that are even higher than amino acids with polar side chains.

Molecular modeling calculations predict that Gly is fully included in TMCD (Fig. 5). The top view of the complex in the space-filling mode shows that the methyl groups fully encapsulate the protonated amino acid. For small amino acids, the methyl groups create a "steric lock" that keeps the amino acid inside the

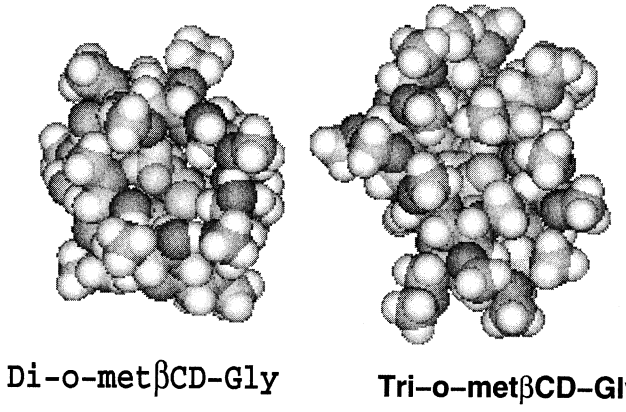

Fig. 5. Lowest energy optimized structure from molecular modeling of protonated glycine complexed to DMCD and TMCD. The structures are presented in the space-filling mode. In both hosts the protonated glycine forms an inclusion complex with the host. TMCD has seven more methyl groups in the upper rim than DMCD. In TMCD the methyl derivatives in the upper rim block the escape of the glycine more effectively than in DMCD giving a higher dissociation temperature.

cavity. This was also observed with Pro, which because of its cyclic structure makes the compound highly compact.

The notion of the steric lock is supported by the T_d with DMCD host. DMCD has seven instead of 14 methyl groups in the upper rim. This opens the upper rim allowing Gly to escape more easily thereby decreasing T_d. Amino acids with large alkyl side chains already penetrate the upper rim and are not affected as strongly by the change in methylation. Therefore, the relative stability of the Gly complex is lower with DMCD than with TMCD.

5. Conclusion

Two major forces strengthen gas-phase inclusion complexes, hydrogen bonding interaction and steric locking. Increasing the number of hydrogen bonding interactions in turn increases the dissociation temperature. Steric locking also plays a role where methyl derivatives on the rim of cyclodextrin block the escape of the amino acids. Therefore, small amino acids with small number of potential hydrogen bonding sites may have large dissociation temperatures because they are constrained from leaving by the derivatives on the rim of the cyclodextrin host.

Acknowledgement

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