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Gas phase studies of ammonium–cyclodextrin compounds using Fourier transform ion cyclotron resonance

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Abstract

Cyclodextrins (CDs) are cyclic oligosaccharides composed of 6, 7, or 8 glucose molecules (α-, β-, or γ-cyclodextrin, respectively) which are used widely in industry due to their ability to form inclusion complexes with a variety of molecules in aqueous solution. Much speculation has been made as to whether inclusion complexes form as a result of hydrophobic interactions between guest molecules and the inner hydrophobic cavity of the CDs in water. Fourier transform ion cyclotron resonance (FTICR) mass spectrometry was used to study adducts of cyclodextrins with various amines in the gas phase. Protonated cyclodextrins were generated using electrospray ionization, and were allowed to react with neutral amines. Adducts of each amine studied were observed to form with all three cyclodextrins. Equilibrium constants were measured for the exchange of neutral amines on protonated CD molecules. Size and shape dependent trends, especially with bulkier amines, suggest inclusion complex formation. Molecular modeling studies also support the formation of inclusion complexes rather than nonspecific adducts, and suggest that solvation of the charged guest by the CD host provides a large driving force for the formation of inclusion complexes, which are then stabilized by van der Waals interactions between the host and the guest. A second series of experiments was performed using gas phase hydrogen/deuterium exchange of protonated cyclodextrins and cyclodextrin–amine complexes with D_2O. The protonated cyclodextrins have a rapid rate of exchange that slows by more than a factor of 10 when an amino guest is added. The amino groups of the guests are expected to have significantly higher gas phase basicities than the hydroxyl sites on the cyclodextrins or the deuterating agent, accounting for the observed decrease in exchange rates for cyclodextrin–amine complexes. Observed differences in the α- versus β- versus γ-cyclodextrin exchange rates suggest an exchange mechanism dependent upon the size of the cyclodextrin ring and its gas phase conformation. (Int J Mass Spectrom 193 (1999) 181–195) © 1999 Elsevier Science B.V.

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1. Introduction

Over the past two decades, there has been much interest in the study of cyclodextrins. The popularity of the cyclodextrin molecules arises from physical properties that allow for their use in the cosmetics, agriculture, food, and pharmaceutical industries as well as their applications in chemical separations and enzyme modeling [1]. Although the cyclodextrins have been characterized in a great variety of crystal and solvated forms—it is estimated that by 1995, over

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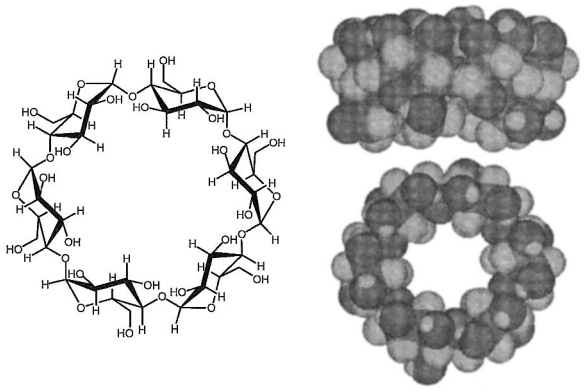

Fig. 1. Structural drawing and space-filling views of α-cyclodextrin. In both space-filling views, the secondary rim is shown on top.

1200 publications dealing with cyclodextrin research had been submitted [2]—little work has been done in the gas phase. This article will partially characterize cyclodextrins in the gas phase and answer some questions concerning the fundamental binding properties of these molecules.

Cyclodextrins consist of six, seven, or eight glucose monomers bound together via α-1,4-linkages to form a ring (Fig. 1), and are commonly referred to as α-, β-, and γ-cyclodextrin, respectively. Crystallographic data indicate that all the secondary hydroxyl groups are situated on one edge of the cone whereas the primary groups are all situated on the other [1]. The cavity diameter is slightly larger for the secondary hydroxyl rim, hereafter referred to as the upper rim, than for the primary hydroxyl, or lower rim. Thus, in condensed media cyclodexrins generally have the shape of a truncated cone, with a hydrophobic interior and a hydrophilic exterior. Cyclodextrin cavity dimensions are listed in Table 1.

This unique structure enables guest compounds, including those that are hydrophobic or apolar, to become included within the cyclodextrin cavity and remain bound there by noncovalent interactions. The guest molecules remain included once the complex has been crystallized from solution, hence the common use of cyclodextrins in the encapsulation of drugs and coloring or flavoring agents. Despite the vast number of studies on the inclusion properties of cyclodextrin molecules—approximately 85% of the studies published in 1995 dealt either directly or indirectly with inclusion properties and inclusion complexes [2]—the driving force for inclusion within the cyclodextrin cavity is not completely understood. Many factors are believed to play a role in this process, most notably van der Waals interactions, release of CD ring strain upon complexation, and hydrogen bonding (where applicable) [3]. However, the extent to which each of these factors helps to drive complexation is not yet known.

Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) offers many unique advantages to the study of these compounds. First, compounds may be studied in the gas phase without the complicating effects of solvent, which can also affect selectivity [4]. Second, ions may be trapped in the ICR cell for a period of time ranging from seconds to hours, depending upon instrument conditions, while reactions proceed between the trapped ions and neutral background molecules.

Electrospray ionization (ESI) has been shown to be a useful tool in the analysis of large molecules and is a popular method for the analysis of cyclodextrins. Unlike other ionization methods, ESI can produce ions in the gas phase without the requirement that the sample be volatile and without the use of an interfering solvent matrix. In addition, ESI can allow the study of the intact molecule without significant fragmentation [5,6] and can produce ions stable enough

Table 1
Physical dimensions of cyclodextrin molecules [1]

Cyclodextrin	Inner diam. of 2° rim (Å)	Outer diam. (Å)	Cavity volume (mL/mol)	Height (Å)
α-CD	5.7	13.7	174	7.8
β-CD	7.8	15.3	262	7.8
γ-CD	9.5	16.9	472	7.8

for characterization by mass spectrometry [7–9]. We have used FTICR coupled with ESI to study gas phase cyclodextrin complexes by two different techniques: equilibrium studies of the exchange of neutral amines on protonated cyclodextrins, and gas phase hydrogen/deuterium (H/D) exchange of protonated cyclodextrins and cyclodextrin–amine complexes. Each of these techniques has been used by this as well as other research groups to elucidate structural and thermodynamic information for various systems and will be discussed later [10–13].

The goals of this study are twofold: to determine whether cyclodextrins form adducts with guest molecules in the gas phase, and to determine whether the complexes formed are inclusion complexes rather than nonspecific adducts.

2. Experimental

All experiments were performed using a Bruker (Billerica, MA) APEX 47e Fourier transform ion cyclotron resonance mass spectrometer equipped with a 4.7 tesla magnet and external electrospray ionization source with a hexapole ion guide (Analytica; Branford, CT). Our instrument and general experimental approach have been detailed in a previous work [14] and will not be described here.

2.1. Equilibrium

Equilibrium experiments were performed by introducing neutral amines into the ICR cell via variable leak valves (Varian; Palo Alto, CA). The amines were first degassed using a minimum of three freeze–pump–thaw cycles, then leaked into the cell, with the partial pressure of each amine between 1.0×10^{-8} and 5.0×10^{-8} mbar. The pressure of the first amine was allowed to stabilize before the addition of the second. In each case but one, *n*-propylamine (Aldrich) was placed in one leak valve with one of the following placed in the second leak valve: *n*-butylamine (EM Science), *sec*-butylamine (Matheson), *iso*-butylamine (Baker), *tert*-butylamine (Kodak), or cyclohexylamine (Baker). All amines were used without further purification. In the final case, cyclohexylamine was placed in the first leak valve with methylbenzylamine (Fluka) in the second.

The cyclodextrins were obtained from Sigma, and used without further purification. Each was first dissolved separately in high performance liquid chromatography (HPLC) grade water (Baxter), then diluted into a solution of water/methanol/acetic acid (48.5:48.5:3) to give a spraying solution containing all three cyclodextrins at a final concentration of 100 μg/mL each.

Once cyclodextrin–amine adducts had formed in the cell, the adducts of one amine with each cyclodextrin were isolated by ejecting adducts of the second amine using a home-built implementation of the stored waveform inverse Fourier transform (SWIFT) technique [15]. Protonated cyclodextrins were then allowed to react with the neutral amines until equilibrium was reached, with reaction delay times typically up to 30 s. Adducts of the other amine with all three cyclodextrins were then ejected and treated in the same manner, and the results were compared to ensure equilibrium conditions were reached for all species present.

2.2. H/D exchange

For H/D experiments, deuterium oxide (Cambridge) was leaked into the ICR cell using a variable leak valve (Varian) following multiple freeze–pump–thaw cycles and allowed to passivate cell surfaces overnight. The cell pressure was then adjusted to between 7.0 and 8.5×10^{-9} mbar and allowed to stabilize. A solution of α-, β-, and γ-cyclodextrin was then prepared as described for the equilibrium experiments at a final concentration of 100 μg/mL α-cyclodextrin, 150 μg/mL β-cyclodextrin, and 150 μg/mL γ-cyclodextrin in methanol/water/acetic acid (48.5:48.5:3). This solution was electrosprayed into the ICR cell and the protonated cyclodextrins were trapped for up to 120 s while the proton exchange reaction was monitored. The experiment was then repeated for a series of similar cyclodextrin solutions where one of the following amines had been added at a concentration of 3 μL/mL of solution: *n*-butylamine, *tert*-butylamine, cyclohexylamine, or methylbenzylamine [R-(+)]. All experiments were performed at ambient temperature (~298 K).

Table 2
Rate constants for the formation of cyclodextrin-amine complexes

	Rate const. $\times 10^{-9}$ (cm^3 $molecule^{-1}$ s^{-1})		
Amine	$k_{(\alpha)}$	$k_{(\beta)}$	$k_{(\gamma)}$
n-Butylamine	2.49 ± 0.39	3.64 ± 0.37	2.84 ± 0.44
Sec-butylamine	1.11 ± 0.31	1.21 ± 0.32	1.76 ± 0.30
Iso-butylamine	3.10 ± 0.82	2.65 ± 0.17	1.74 ± 0.77
Tert-butylamine	2.08 ± 0.38	1.86 ± 0.46	2.18 ± 0.58
Cyclohexylamine	1.25 ± 0.22	2.06 ± 0.25	1.46 ± 0.24

2.3. Molecular modeling

All calculations were carried out using SPARTAN 4.0 (Wavefunction, Inc.; Irvine, CA). Starting geometries were obtained using the SPARTAN builder, one conformer beginning with the amine R group included in the cyclodextrin cavity, and the other beginning with the amine rotated 180°. No conformational searching was used. Energies were minimized using the MMFF molecular mechanics package included in SPARTAN, then full geometry optimizations were carried out at the PM3 semiempirical level.

3. Results

3.1. Rate of adduct formation

The rate constants for the formation of cyclodextrin–amine adducts in the gas phase are listed in Table 2 [16]. In all cases, the rate of adduct formation was considerably more rapid than that of proton transfer to the neutral amine, which was not observed. All of the results are within an order of magnitude of the collision rate, and for most systems, $k_\alpha \approx k_\beta \approx k_\gamma$. This implies that the mechanism of binding is similar for all of the systems tested.

3.2. Rate of guest exchange

The rate constants for displacement of one guest by another during the equilibrium experiments are listed in Table 3. Exchange reactions involving the smaller amines are all rapid, approaching the collision rate. However, the rate constants for exchange involving the release of larger amines, namely *tert*-butylamine, cyclohexylamine, and methylbenzylamine, decrease by 1–2 orders of magnitude relative to the exchange rates involving release of small amines.

3.3. Equilibrium

Adducts (1:1) with each of the amines were observed for all three cyclodextrins. In contrast to previous studies [17,18], there was no evidence of clusters formed from multiple cyclodextrin molecules or of multiple amines attached to a single cyclodextrin in any of our experiments, suggesting that either the cluster species formed in solution or were artifacts of the electrospray process. In addition, protonated cy-

Table 3
Rate constants for the exchange of amines by cyclodextrin-amine adducts during the equilibrium experiments[a]

	α-CD		β-CD		γ-CD	
Amine pair	k_1	k_2	k_1	k_2	k_1	k_2
nba/npa	2.49 ± 1.5	1.48 ± 0.55	1.64 ± 0.45	0.74 ± 0.41	1.96 ± 0.53	0.87 ± 0.35
sba/npa	2.25 ± 0.59	0.83 ± 0.3	2.19 ± 0.97	0.70 ± 0.3	0.23 ± 0.2	0.96 ± 0.1
iba/npa	1.91 ± 1.0	2.16 ± 1.0	1.74 ± 0.78	0.98 ± 0.5	1.55 ± 0.47	1.00 ± 0.41
tba/npa	1.50 ± 0.70	1.46 ± 0.10	2.90 ± 1.0	0.78 ± 0.3	3.78 ± 3.0	0.39 ± 0.3
cha/npa	1.21 ± 0.65	0.37 ± 0.3	6.09 ± 4.0	0.44 ± 0.2	2.79 ± 2.3	0.35 ± 0.3
mba/cha	0.63 ± 0.2	5.35 ± 2.4	0.09 ± 0.07	8.98 ± 8.0	0.05 ± 0.02	6.27 ± 2.2

[a] All values $\times 10^{-9}$ in units of (cm^3 $molecule^{-1}$ s^{-1}). Abbreviations as follows: npa is *n*-propylamine, nba is *n*-butylamine, sba is *sec*-butylamine, iba is *iso*-butylamine, tba is *tert*-butylamine, cha is cyclohexylamine, and mba is methylbenzylamine. Here, k_1 refers to the formation of the adduct containing the larger guest and k_2 refers to the formation of the adduct containing the smaller guest (usually *n*-propylamine).

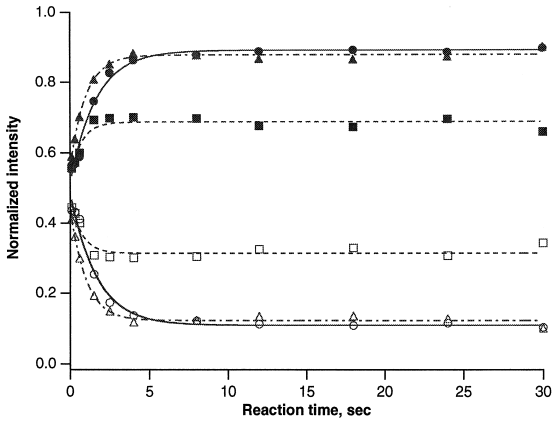

Fig. 2. Exchange of *tert*-butylamine and *n*-propylamine on cyclodextrins. The *n*-propylamine adducts were initially isolated in these experiments. Open symbols represent complexes of *n*-propylamine, whereas filled symbols represent *tert*-butylamine complexes. Circles = α-, squares = β-, and triangles = γ-cyclodextrin. Lines are exponential fits to the data. Note that the same product ratios are reached as in Fig. 3, where the other adduct was initially isolated.

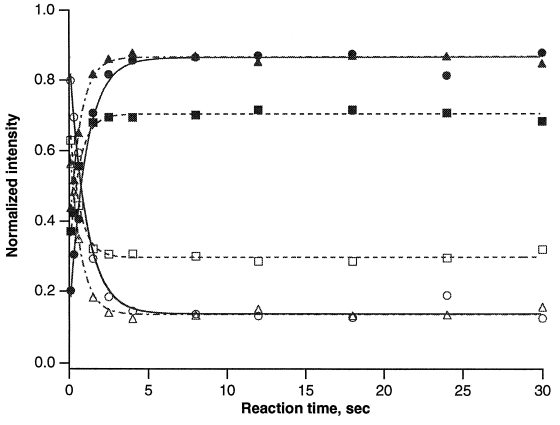

Fig. 3. Exchange of *tert*-butylamine and *n*-propylamine on cyclodextrins. The *tert*-butylamine adducts were initially isolated in these experiments. Symbols are the same as in Fig. 2. Lines are exponential fits to the data. Note the similarity to Fig. 2.

clodextrin and cyclodextrin–amine complexes were observed in the 1^+ charge state only. When two amines were in the cell simultaneously, adducts of each cyclodextrin were observed with each amine.

Equilibrium was generally attained rapidly, with the normalized adduct peaks reaching a constant ratio approximately four seconds following ion isolation, as shown in Figs. 2 and 3.

To ensure that the reaction reached equilibrium, two procedures were followed. First, data were collected until a change in the ratio of the two adduct species was no longer observed. Second, ion ejection was performed in both directions; that is, adducts of one amine were ejected and allowed to reform, then the procedure was repeated for the other amine. Equilibrium constants were calculated relative to the formation of protonated cyclodextrin·(*n*)-propylamine adducts according to the following equation:

$$\mathrm{CDH}^+ \cdot \mathrm{N} + n\text{–propylamine} \rightarrow \mathrm{CDH}^+ \cdot n\text{–propylamine} + \mathrm{N} \quad (1)$$

where

$$K_{\mathrm{eq}} = \frac{[\mathrm{CDH}^+ \cdot npa]P_N}{[\mathrm{CDH}^+ \cdot N]P_{npa}} \quad (2)$$

for the exchange of *n*-propylamine (npa) and a neutral amine, *N*, on α-, β-, or γ-cyclodextrin. The concentrations of the cyclodextrin—amine adducts were assumed to be proportional to the normalized peak intensities of the adducts of interest [19]; neutral pressures were obtained from the observed partial pressures of the two amines. Observed K_{eq} values are summarized in Table 4 along with standard deviations from replicate measurements.

Due to the nature of FTICR instrumentation, it is difficult to obtain an accurate measurement of the pressure in the ICR cell. In fact, the pressure measurement may be off by as much as a factor of 5 [20]. Because the equilibrium constants are pressure dependent, they contain an inherent error that makes direct comparison of absolute K_{eq} values difficult; however, because all three cyclodextrins were analyzed simultaneously, the comparison of K_{eq} for α- versus β- versus γ-cyclodextrin for a given pair of amines can be made with relatively high confidence. A value of K_{eq} greater than 1 indicates a preference for *n*-propylamine, whereas a value less than 1 indicates a preference for the other amine. These ratios are more easily observed in the bar graph in Fig. 4. Comparison of the ratios for the three cyclodextrins with each

Table 4
Equilibrium constants for the exchange of neutral amines on protonated cyclodextrins[a]

Amine	K_{eq}		
	α-CD	β-CD	γ-CD
n-Butylamine	0.589 ± 0.070	0.282 ± 0.088	0.377 ± 0.11
Sec-butylamine	0.333 ± 0.024	0.290 ± 0.017	0.470 ± 0.057
Iso-butylamine	1.22 ± 0.18	0.519 ± 0.13	0.663 ± 0.16
Tert-butylamine	0.089 ± 0.020	0.273 ± 0.021	0.093 ± 0.013
Cyclohexylamine	0.246 ± 0.045	0.097 ± 0.093	0.104 ± 0.14
Methylbenzylamine	2.05 ± 0.07	9.64 ± 3.4	1.40 ± 0.30

[a]All values reported as means with standard deviations from replicate measurements. All are equilibria with respect to *n*-propylamine formation [reaction (1)].

amine pair shows some small reproducible differences, but no clear size-dependent trends. Most constants are similar, varying by no more than a factor of 10, and often two of the three constants for a given amine pair are within statistical limits of one another.

For *n*- and *iso*-butylamines, α-cyclodextrin shows slightly less preference for the butylamine relative to *n*-propylamine than the larger cyclodextrins, with β- and γ-cyclodextrin within error limits of one another. For *sec*-butylamine, the picture changes slightly, with γ-cyclodextrin showing a slightly higher preference for the propylamine and α- and β-cyclodextrin within error limits of one another. The most drastic differences in ratios are seen in the *tert*-butylamine, cyclohexylamine, and methylbenzylamine sets. In each of these sets, one constant is significantly greater in magnitude than the others, and the remaining two are approximately within error limits of one another. In the case of *tert*-butylamine, the β-cyclodextrin constant is three times the others; for cyclohexylamine the α-cyclodextrin constant is two and a half times larger than the others; and for methylbenzylamine, the β-cyclodextrin constant is more than four times the others. In this experiment, methylbenzylamine was measured versus cyclohexylamine, then the results were converted to results relative to *n*-propylamine for comparison with the other results. This set is interesting for two reasons: first, the cyclohexylamine versus *n*-propylamine data set shows that β- and γ-cyclodextrin prefer cyclohexylamine so strongly that the equilibrium constants are very small and error limits are as large or larger than the K_{eq} values themselves, making the error bars appear very large for this data set; second, although both α- and γ-cyclodextrin appeared to prefer methylbenzylamine over cyclohexylamine, β-cyclodextrin very strongly preferred cyclohexylamine over methylbenzylamine. In fact, the β-cyclodextrin·methylbenzylamine adduct peaks were just above the noise level, regardless of the relative background amine pressures. This may give the false appearance of a vast preference of β-cyclodextrin for *n*-propylamine over methylbenzylamine, which may or may not in reality exist. In addition, it raises a question as to what would cause a reversal of this nature for two amines of such similar size and functionality.

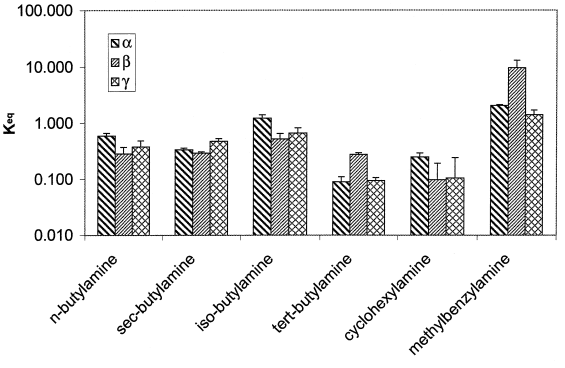

Fig. 4. Equilibrium constants, K_{eq}, for the exchange of various amines with *n*-propylamine in adducts with α-, β-, and γ-cyclodextrin. Values greater than 1 indicate equilibrium favors the *n*-propylamine adduct; values less than 1 indicate the other amine is favored.

3.4. H/D exchange

Exchange of deuterium for hydrogen was observed to proceed at a very rapid rate for protonated cyclo-

dextrins. Qualitatively, we expected to observe seven exchangeable protons on α-cyclodextrin, eight on β-cyclodextrin, and nine on γ-cyclodextrin, corresponding to the hydroxyl hydrogens on the primary rim plus the charge-carrying proton for each cyclodextrin molecule. The hydroxyl hydrogens on the secondary rim experience a fairly high degree of intermolecular bonding, the strength of which is believed to increase with ring size [21], and would not be expected to exchange as rapidly as the primary rim. This appears to be true for α-cyclodextrin, but not for β- and γ-cyclodextrin. In order to avoid possible problems arising from translational heating of the ions during isolation, naturally occurring ^{13}C isotope peaks were not ejected prior to the reaction delay, and likely account for all of the peaks above M + 7 in α-cyclodextrin. However, this does not account for all of the peaks above M + 8 for β-cyclodextrin or above M + 9 for γ-cyclodextrin, indicating that H/D exchange in these molecules is not limited to the primary hydroxyl rim. Both of the larger cyclodextrins exhibited sixteen exchanges or more, but both the β- and γ-cyclodextrin signals deteriorated significantly before the full extent of the exchange could be determined.

The ICR leak valves and cell were allowed to passivate with D_2O prior to running the experiment; however, some H_2O was still likely to be present, and back reactions must be accounted for in rate calculations, although the reaction can be treated as pseudo first order. If we plot the normalized signal intensity of the M + 1 (cyclodextrin + proton) peak over time, we see that for the protonated cyclodextrins, exchange is very rapid, and is in fact very near the collision rate. Qualitatively, $k_\gamma > k_\beta \gg k_\alpha$ for multiple exchanges on the protonated cyclodextrins (see Fig. 5).

Examination of the rate constants for the first H/D exchange of cyclodextrin–amine complexes shows that the exchange is more than ten times slower than for protonated cyclodextrins (see Fig. 6). This is true for all the guest amines examined. During the 2 min duration of each experiment, only one proton exchanged observably, as opposed to six or more for the protonated cyclodextrins. Representative plots of the normalized peak intensity over time for a protonated cyclodextrin and some cyclodextrin-amine complexes are shown in Fig. 7. Again, results were similar for all cyclodextrin–amine complexes tested. In addition, these complexes demonstrated significantly lower decay of signal at longer trapping times than their protonated counterparts. This signal loss is most likely attributed to proton transfer from the analyte molecule to a neutral species [22]. Consistent with this, our results suggest that proton transfer to a neutral species is greatly reduced when a cyclodextrin–amine complex is formed. This topic will be addressed again in Sec. 4.

3.5. Molecular modeling

The molecular modeling calculations were aimed at determining whether inclusion of the amine R group in the cyclodextrin cavity is preferred. Because extensive conformational searches were not carried out, the results (Table 5) represent only a small sample of the possible conformers. However, from these limited results it is clear that there is a significant enthalpic driving force for inclusion of guests small enough to enter the cyclodextrin cavity.

4. Discussion

4.1. Inclusion versus non-specific adduction

An inclusion complex is one where a host molecule containing a cavity forms an adduct with a guest in such a way that the guest is situated in the cavity, and is held there by strictly noncovalent forces [3]. Traditional mass spectrometry measures mass-to-charge ratio only and gives structural information based on fragmentation patterns. Under the conditions of our experiments, electrospray ionization does not produce fragmentation. How then can we determine whether the observed compounds are nonspecifically bound adducts formed as an artifact of the electrospray or selectively bound inclusion complexes?

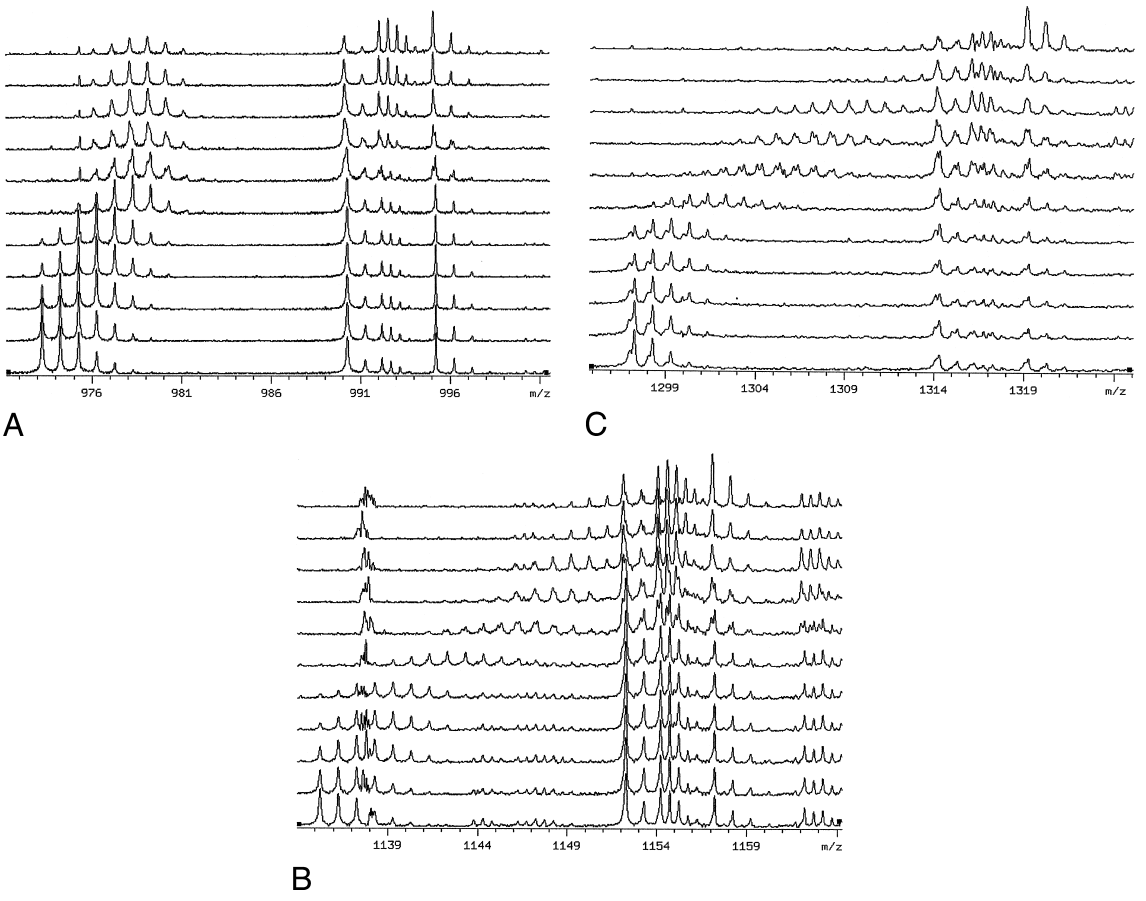

Fig. 5. Mass spectra of protonated (a) α-, (b) β-, and (c) γ-cyclodextrins as a function of reaction time with D_2O in the ICR cell. Shortest reaction times are at the bottom of the plot, and the longest reaction times are at the top.

4.2. Equilibrium

The use of FTICR should eliminate the type of false positive results seen in previous studies [17] for the following reasons. In the equilibrium experiments, adducts are formed in the gas phase, not in solution. This ensures that binding of amine guests to cyclodextrin hosts is not an artifact of the electrospray process. The use of ion ejection demonstrates that adducts re-form quickly and that for an amine pair, the same relative concentrations of cyclodextrin–amine adducts are reached regardless of which one is ejected. This indicates that adducts form with at least some degree of selectivity.

We analyzed a series of amines with varying degrees of size and steric bulk (Table 4) with the expectation that some would fit inside all three cyclodextrins, whereas others would fit only inside the larger β- and γ-cyclodextrins. Instead, we found that in all but one case, the equilibrium constants were very similar, and even in the exceptional case—methylbenzylamine versus cyclohexylamine—the largest and most sterically hindered molecule showed a preference not only for the larger γ-cyclodextrin, but also for α-cyclodextrin. If a guest were binding outside the cavity, we would expect to see clearly defined trends in the equilibrium exchange of one amine for another as the size and bulk of the amines changed, but such trends are not evident. This suggests that the binding process is more complex than

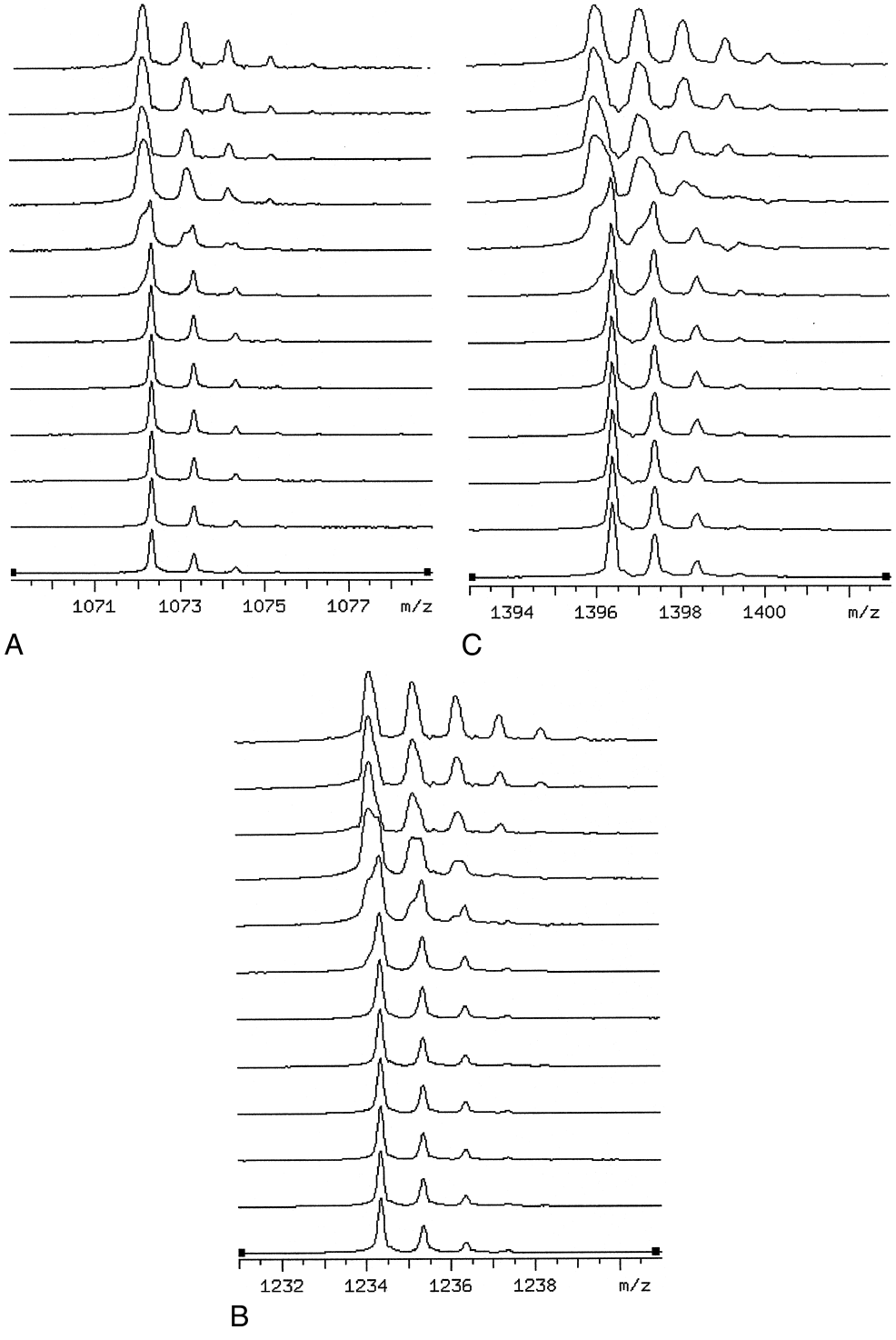

Fig. 6. Mass spectra of cyclodextrin–cyclohexylamine complexes for (a) α-, (b) β-, and (c) γ-cyclodextrins as a function of time in the ICR cell. Shortest reaction times are at the bottom of the plot, and longest reaction times are at the top.

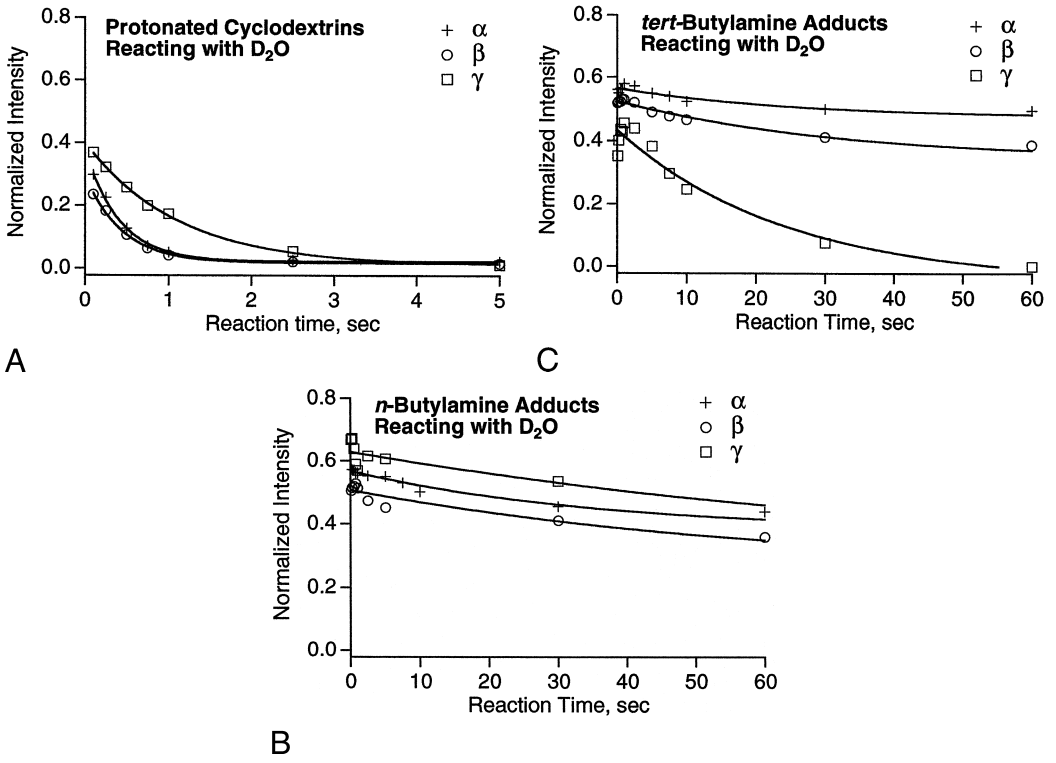

Fig. 7. Relative intensity of the all-^{12}C peak as a function of reaction time with D_2O for (a) protonated cyclodextrins, (b) *n*-butylammonium adducts of the cyclodextrins, and (c) *tert*-butylammonium adducts of the cyclodextrins. Lines are exponential fits to the data. These plots follow the progress of the first H/D exchange as a function of time.

would be expected if nonspecific, exterior adducts of the amine with the cyclodextrins were forming.

Semiempirical calculations performed by our group on a limited number of conformers (Table 5) show the included guest to be energetically favored over the nonincluded guest for all of the butylamine isomers except *tert*-butylamine (and more recent calculations with more thorough conformational searching indicate even this guest is probably included in α-cyclodextrin) [23]. Calculations performed by other groups have also shown guest binding inside the cavity to be favored over binding outside for guests less polar yet more bulky than the amines used in this study [24–26]. Another series of computational studies concluded that the most important noncovalent forces involved in the binding of cyclodextrin host–guest complexes are the short-range dispersion forces, and these are generally one to two orders of magnitude smaller than hydrogen binding-type forces [27]. Although amines were not used in either study, there does seem to be a consensus among computational chemists that cyclodextrins prefer to include guests, and that solvent effects are not as important as specific interactions between the guest molecules and the cyclodextrin [4,24,28].

Table 5
Semiempirical (PM3) heats of formation of cyclodextrin–ammonium complexes

	ΔH_f° (PM3, kJ mol^{-1})		
Host/guest R group	R_{in}	R_{out}	$R_{in} - R_{out}$
α-CD/*n*-propyl	−4742.6	−4716.6	−25.9
α-CD/*iso*-butyl	−4776.5	−4746.7	−29.7
α-CD/cyclohexyl	−4828.2	−4791.4	−36.8
α-CD/methylbenzyl	−4575.2	−4548.2	−27.0
α-CD/*tert*-butyl	−4809.7	−4824.4	14.6
β-CD/*tert*-butyl	−5687.7	−5707.1	19.4
γ-CD/*tert*-butyl	−6580.9	−6572.3	−8.6

In solution, the driving force for inclusion complex formation is believed to be a combination of four effects. These are polar–apolar interactions between an apolar guest and a polar solvent, such as water, in the presence of the apolar cyclodextrin cavity; release of ring strain upon complexation; van der Waals interactions; and hydrogen bonding. In the gas phase, no solvent interactions are present. The ring strain argument applies only to α-cyclodextrin, which is distorted in its hydrated form. However, there is no evidence that ring strain exists in the gas phase molecule and even in solution, this is not the most significant effect [3]. This leaves van der Waals interactions and hydrogen bonding to account for differences in selectivity.

The tendency in nature is to solvate a bare charge. Therefore, the charged proton on the cyclodextrin molecule would have a tendency to migrate to the inside of the cavity where it can be better solvated by the host. This would provide a driving force for the amines to enter the cavity where they would be held by a combination of van der Waals forces and hydrogen bonding.

The only amines that appeared to demonstrate a significant preference for one cyclodextrin over another (Table 4) were *tert*-butylamine, cyclohexylamine, and methylbenzylamine. These are also the most sterically bulky compounds used in our study. van der Waals forces exhibit the greatest stabilizing effect on an inclusion complex when the guest fits snugly into the cyclodextrin cavity [29,30]. If we can assume that the degree of hydrogen bonding is similar for all of the compounds studied, a difference in the degree of van der Waals stabilization could account for the difference in selectivity.

All of the amines used in these experiments are simple, primary amines capable of forming three hydrogen bonds. There is no significant difference in the electronegativity of the substituents bound to the amine nitrogen, or in the hydroxyl hydrogens forming the hydrogen bonds with each amine. Ignoring steric effects on the hydrogen bonding, it is reasonable to assume the degree of hydrogen bonding is similar for all of the amine compounds used here.

Based on size alone, many compounds will fit comfortably inside more than one of the cyclodextrin cavities. However, we expect that in the gas phase the cyclodextrin cavity that exhibits the tightest fit with the guest, achieving the most van der Waals contact without inducing large amounts of steric strain, should form the more stable complex.

In our study, *tert*-butylamine showed a great preference for α-cyclodextrin, and cyclohexylamine showed nearly equal preference for β- and γ-cyclodextrin when compared to *n*-propylamine, but a significant preference for β-cyclodextrin when compared to methylbenzylamine (Table 4). Calculations indicate that based on the diameters of the molecules, all three of these compounds should fit inside the wide rims of all three cyclodextrins [23]. However, the sterics surrounding the amino groups are quite different. If hydrogen bonding is taking place between the amino groups and the hydroxyl hydrogens on either rim, the amines with appropriately sized substituents near the nitrogen would fit more snugly against the walls of the cyclodextrin cavity and experience greater stabilization from van der Waals forces when the amines bind inside the cavity. This might explain the relatively high affinity of *tert*-butylamine for α-cyclodextrin.

Why then would cyclohexylamine, which has a diameter very close to that of *tert*-butylamine, prefer β-cyclodextrin over α-cyclodextrin? van der Waals interactions can be described as temporary attractions between adjacent noncovalently bound molecules due to momentary induced dipole–dipole interactions. Greater stabilization by van der Waals forces should occur in the guest which has the greatest amount of three-dimensional contact space with the host and not just the largest diameter. *Tert*-butylamine possesses threefold symmetry about a central carbon, which is also bound to the amino group. α-Cyclodextrin is composed of six glucose monomers, and also has a threefold symmetry axis, unlike either β- or γ-cyclodextrin. This may explain why α-cyclodextrin would form a better "fit" with *tert*-butylamine than with cyclohexylamine, while β-cyclodextrin shows just the opposite. Recent studies support the idea that in some container-type molecules, guest selection is based

upon how well the guest literally "fills out" the host molecule cavity [31].

4.3. H/D exchange

H/D exchange has long been used in mass spectrometry to probe structure. Early work on the subject suggests that deuterium exchange occurs sequentially and that exchange may be enthalpically driven [11,32]. The proposed mechanism involves the formation of an intermediate complex containing HD_2O^+ where proton transfer occurs.

We are aware of no measurements of the gas phase proton affinity or basicity of any of the cyclodextrins. Therefore, we cannot say whether the differences in basicity between the deuterating agent and the cyclodextrins are driving the exchange reactions. Our results do support the idea of a sequential exchange, as evidenced by the change in peak distribution over time.

Protonated cyclodextrin molecules have only one type of functional group (hydroxyl) that could undergo exchange. This would not allow for the same type of accelerating effect seen in protein studies [12,33], which could indicate that either cyclodextrin proton affinity is within approximately 25 kcal/mol of that of D_2O (because it is generally believed that proton affinities must be similar for exchange to occur [34]) or that some other force is driving the exchange.

A recent study of H/D exchange in solid phase cyclodextrins both with and without included guests found that when cyclodextrin crystals were exposed to D_2O vapor over a long period of time (up to two weeks) exchange did occur in both protonated cyclodextrins and cyclodextrin–guest complexes. However, in contrast to our results, the rate of exchange in α-cyclodextrin was observed to be slower than that of β-cyclodextrin, and exchange occurred much more rapidly when a benzaldehyde guest was included in the α-cyclodextrin cavity [35]. γ-Cyclodextrin was not included in the aforementioned study. It is not particularly surprising that the gas and solid phase results differ, because diffusion through the crystals is probably rate-limiting in the latter. It is worth noting that H/D exchange in the solid phase occurs on the order of days, whereas H/D exchange in the gas phase occurs at close to the collision-limited rate.

Lebrilla has proposed the presence of a proton-bridged intermediate in the mechanism for gas phase H/D exchange [36,37]. This differs from the proposed mechanism of exchange in crystalline cyclodextrins where exchange occurs on the shell of water molecules hydrating the rim of the cyclodextrins, which then exchange the hydroxyl hydrogens of the cyclodextrin itself [35]. Once this hydration shell is removed, the structural rigidity that slows exchange in α-cyclodextrin is also removed and H/D exchange may become at least partially dependent upon the number of exchangeable protons, where we would expect $k_{(\gamma)} > k_{(\beta)} > k_{(\alpha)}$, as observed.

Our results show the first exchange for γ-cyclodextrin to be considerably slower than that for either α or β, which would not be expected if exchange were dependent solely on the number of sites. The existence of a proton-bridged intermediate is partially inferred from the observation that sites immediately adjacent to each other do not exchange easily while those that could be spanned by a bridging proton exchange rapidly. The primary hydroxyl groups in all three cyclodextrins are close enough together for easy proton bridging. γ-Cyclodextrin, however, is much less structurally rigid than the other two mainly due to its larger size. This floppiness might account for a smaller degree of bridging in γ-CD, leading to slower exchange. Sterics might also play a role. If γ-cyclodextrin were to partially collapse on itself, attack on the hydroxyl groups by the deuterating agent could be sterically hindered and slowed enough to account for the observed reduction in exchange rate. This would not be as readily observed in solution, where water molecules surrounding the hydroxyl rims of the molecule would help to maintain the ring shape [3].

Why does the presence of a guest in the cyclodextrin decrease the rate of exchange in the gas phase but increase the rate in the solid phase? This could be due to differences in conformation once a guest is included which are likely to be observed in the solid phase but not in the gas phase. It was mentioned earlier that one of the arguments for driving an inclusion reaction was the release of ring strain from

the α-cyclodextrin molecule as a guest is included, but that this effect was only observed in the hydrated molecule. The solid phase H/D studies used hydrated α-cyclodextrin and indicate that with an apolar guest included a much less rigid ring structure exists, which could increase the proton transfer rate of the inclusion complex. If α-cyclodextrin does not exhibit ring strain in the gas phase, this increase in exchange rate would not be observed. A similar argument could be made for β-cyclodextrin.

Another explanation may have to do with the interactions between the cyclodextrin and the guest molecule. All of the guest compounds used in the solid phase study are apolar and would exhibit only van der Waals interactions with the cyclodextrin hosts, whereas the amines used in our study would be expected to experience a high degree of hydrogen bonding to the cyclodextrins. The H/D exchange rate constants have little or no dependence on which amine is bound, nor do the size or shape of the cyclodextrin appear to have a significant effect on the H/D exchange rate in the gas phase. If the amines were bound outside the cavity, we would expect steric hindrance to slow the rate of attack on the complex by D_2O molecules. Since no such trends are present, this would again argue for inclusion complex formation, although this result alone is not conclusive.

The gas phase proton affinities for all the amines studied are documented to be well above that of D_2O [38]. The gas phase proton affinity of methylbenzylamine is not known, but is expected to be near that of cyclohexylamine. If we assume that the rate of gas phase H/D exchange on cyclodextrins is proton affinity dependent, as has been suggested for other compounds, it appears that the binding of an amine inside the cyclodextrin cavity either inhibits the formation of a proton-bridged intermediate on the cyclodextrin or changes the gas phase proton affinity of the entire molecule. The latter is expected to happen if the proton is transferred to the guest in the complex.

Since α-cyclodextrin possesses sixfold symmetry, the hydrogen binding of a primary amine to the six hydroxyl groups of the lower cyclodextrin rim would be expected to have threefold symmetry, leaving no adjacent hydroxyl hydrogens capable of forming proton bridges. This would decrease the exchange rate as observed. However, in both β- and γ-cyclodextrin, two or more adjacent hydroxyl groups capable of bridging would be present with a hydrogen-bound amine guest, and would be expected to have faster rates of exchange than α-cyclodextrin–amine complexes. This was not observed. It was mentioned earlier that signal did not decay as rapidly with increasing trapping time for cyclodextrin–amine complexes as for protonated cyclodextrins and that in our case, the decay is most likely due to proton transfer from the cyclodextrin molecule to a neutral species. The amine guests are undoubtedly much more basic than the cyclodextrins, and are therefore much less likely to lose protons than are the protonated cyclodextrins. Past studies have shown that when protonated amino acid is complexed with a monosaccharide, the H/D exchange rates of the protonated amines decrease significantly [39]. If the complex is really an ammonium cation inside a cyclodextrin, it is not surprising that H/D exchange is suppressed: the proton affinity difference between water and any of the amines used in these studies is large enough that exchange is not observed between ammonium cations and D_2O. Exchange between an ammonium cation included in a cyclodextrin cavity and D_2O would be expected to be even less likely, due to the steric constraints imposed by the cyclodextrin.

5. Conclusions

The results presented here argue for the formation of cyclodextrin–amine inclusion complexes in the gas phase. Although the sizes and shapes of the guest molecules used in the equilibrium studies did not reveal any dramatic trends, the similarity of the equilibrium constants suggests that all of the guests used in our study are binding in similar ways, presumably inside the cyclodextrin cavities. Some minor trends were observed in the binding preference of certain amines by one cyclodextrin over the other, which can be explained by the role of van der Waals interactions as a stabilizing force in the formation of

inclusion complexes. These include the preference of α-cyclodextrin for *tert*-butylamine and that of β-cyclodextrin for cyclohexylamine.

The H/D exchange experiments also appear to support the formation of inclusion complexes in the gas phase. The fast rate of exchange seen in the protonated cyclodextrins compared to the much slower rate for the cyclodextrin–amine complexes suggests that adding an amine guest to the cyclodextrin cavity causes proton transfer to the highly basic amine group, which has a much greater gas phase proton affinity than the hydroxyl sites of the cyclodextrin or the deuterating reagent, D_2O. The fact that the rate of H/D exchange for a cyclodextrin–amine complex does not appear to be dependent upon the size of the guest or of the cyclodextrin–amine adduct also argues in favor of inclusion. The difference in exchange rates observed for α- versus β- versus γ-cyclodextrin may suggest a mechanism dependent upon the size of the cyclodextrin ring and its gas phase conformation.

Future studies. The equilibrium experiments were designed with the goal of probing differences in the binding characteristics of cyclodextrins as the size, substitution, and steric bulk of the amine increased. We saw only subtle trends we believe to be indicative of inclusion complex formation. We plan to perform a second series of equilibrium experiments with larger amines which are known (from condensed phase work) to be too large to fit inside either one or all of the cyclodextrin cavities. This will help define the differences in trends for guest binding inside and outside the cavities.

Varying the degree of substitution on the amine nitrogen would significantly change the sterics of complexation as well as lead to large changes in proton affinity. The differences between primary, secondary, tertiary, and quaternary amines would serve to give information about the role of van der Waals interactions versus hydrogen bonding. We expect to see in these instances a pronounced difference in both the equilibrium constants in absolute value as well as in the α- versus β- versus γ-cyclodextrin results.

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