Evidence for the Formation of Gas-Phase Inclusion Complexes with Cyclodextrins and Amino Acids

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Abstract: Experimental and theoretical evidence is provided that indicates the presence of inclusion complexes in the gas phase when cyclodextrin and amino acid mixtures are electrosprayed into a Fourier transform mass spectrometer. A guest exchange reaction that is enantiospecific is used to probe the structure of the gas-phase complex. Chiral selectivity is affected by both the size of the guest and the size of the cavity. These observations are based on a selected number of amino acids with various hosts. The experimental results are supported by molecular dynamics calculations. We further conclude that rather than nonspecific complexes, amino acid−cyclodextrin complexes produced in solution maintain the included structure even in the gas phase.

Introduction

The formation of inclusion complexes involving cyclodextrins (CDs) in solution is by now well documented.[1,2] Cyclodextrins are torus-like, cyclic sugars with an upper, wider rim and a lower, narrower one. The three most common are known as α-CD, which consists of six glucose units connected by an α-1,4-linkage, β-CD with seven glucose units, and γ-CD with eight. The internal cavity diameters are in the range of 6.0−6.5 for β- and 7.5−8.3 for γ-cyclodextrin.[2,3] The cavity serves as a convenient host for hydrophobic compounds such as those with pharmaceutical importance that have problems with solubility. The solubility of cyclodextrins can easily be varied by derivatization and often the included complex takes on the solubility of the host.

Studies of gas-phase host−guest complexes involving cyclodextrins have recently increased owing to the potential of mass spectrometry as a rapid diagnostic tool for determining inclusion. Because the ion production can be performed with a "soft" ionization method such as electrospray, it was at first believed that inclusion can be maintained through the ionization process. Furthermore, the presence of signals in the mass spectra with specific mass-to-charge ratios corresponding to the expected value for the inclusion complexes was thought to be a good indicator of solution-phase inclusion complexes.[4,5] A subsequent study by Prokai employing in-source CID produced results that were consistent with gas-phase inclusion complexes.[6] Varying degrees of dissociation were obtained when the gas-phase complexes were subjected to in-source CID that depended on the amino acid guest. Phenylalanine in particular had a higher dissociation energy than other amino acids that were not expected to produce inclusion complexes. Vouros and coworkers reported a study involving a greater number of guest molecules including amino acids with aromatic and nonaromatic side chains and peptides and came to the conclusion that the complexes observed in the gas phase were merely the product of electrostatic interactions and not inclusion.[7] Their conclusion was based on the presence of complexes in the mass spectra from guest molecules that they believed could not form inclusion complexes even in solution. Since then, other studies have been reported that attempted to differentiate between electrostatic interactions and inclusion. Most of these studies involved some kind of dissociation method such as collision-induced dissociation[8−11] (CID), heated capillary dissociation (HCD),[12] and blackbody induced radiation dissociation (BIRD)[13] with the belief that inclusion instilled additional favorable interaction above simple electrostatic.

The question of whether inclusion complexes are formed in the gas phase is a key question in the general understanding of gas-phase noncovalently bound complexes. The impetus for the formation of inclusion complexes in solution is the penetration of the hydrophobic part of the guest molecule into the cyclodextrin cavity and desolvation of the organic guest. This effect is most prominent in hydrophilic solvents, but it is unclear how this is manifested in the gas phase. For example, rather than contributing to additional attraction, inclusion may produce more repulsive interactions in the gas phase. Thus the use of dissociation methods to probe inclusion may be only marginally successful. A nondestructive method is preferable for determining gas-phase structures of noncovalently bound complexes. For this reason, Dearden et al. attempted to determine the presence

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of inclusion using H/D exchange reactions.[14] The lack of any specific trend in the reactivity of various alkylamine guests was thought to be due to the presence of gas-phase inclusion.

We reported a gas-phase, guest-exchange reaction with a cyclodextrin host that was sensitive to the chirality of the amino acid guest.[15] The amino acid guest (AA) is directly exchanged with a gaseous alkylamine as the complex is trapped in the analyzer cell of a Fourier transform mass spectrometer (eq 1).

$$[\mathrm{CD{:}AA} + \mathrm{H}]^+ + \mathrm{B} \xrightarrow{k} [\mathrm{CD{:}B} + \mathrm{H}]^+ + \mathrm{AA} \qquad (1)$$

Similar gas-phase reactions have more recently been reported in the study by Dearden with cyclodextrins;[14] earlier examples of guest-exchange reactions involving alkylamines with macrocyclic hosts were also reported by the same group.[16,17] In this report, we present theoretical and experimental evidence, based on the guest-exchange reactions, for the presence of gas-phase inclusion complexes.

Experimental Section

Materials. All D-amino acids, the heptakis-(2,3,6)-tri-*O*-methyl-β-cyclodextrin (β-CD), (2,6)-di-*O*-methyl-β-cyclodextrin (di-*O*-methyl-β-CD), γ-cyclodextrin, and *n*-propylamine were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. The (2,3,6)-tri-*O*-methyl-γ-cyclodextrin was prepared from γ-cyclodextrin using published procedures.[18] L-Amino acids were obtained from Research Plus Inc. (Denville, NJ).

Guest Exchange Reactions. The experiments were performed using a home-built external source Fourier transform mass spectrometer with an electrospray ionization source. The details of the experimental procedure for producing the ions and obtaining the rate constants (k_L and k_D) have been published elsewhere.[12,15,19] Briefly, the cyclodextrin and the amino acid are mixed in solution (1:10 cyclodextrin−amino acid) and electrosprayed into the vacuum chamber through a resistively heated stainless steel capillary. The temperature of the capillary, monitored on the outside surface, is maintained at 180 °C to dissociate the higher order complexes composed of combinations of multiple numbers of hosts and guests. The alkylamine is introduced into the analyzer chamber by a variable leak valve after several freeze−thaw cycles. The protonated cyclodextrin−amino acid complexes ([CD:AA + H]$^+$) are isolated and allowed to react with a background pressure (between 1×10^{-7} and 6×10^{-7} Torr) of alkylamine (B). The exchange product is monitored as a function of time and the rate constants are extracted from pseudo-first-order rate plots.

To avoid contamination between runs, the syringe, sample line, and the electrospray tip were disassembled and cleaned with solvent. The stainless steel sample line was flushed with several milliliters of solvent. The electrospray tip was also replaced with a new unused tip. The largest source of error in the determination of absolute rate constant was the measurement of the pressure. The ion gauge could not be calibrated using standard calibration reactions due to limitations in the data system. Attempts were made to calibrate the ion gauge using published proton transfer reactions with proteins, but this method is not highly reliable so that errors in the absolute rates may be large. Nonetheless, the rate constants are suitably precise with deviations of less than 10%, as determined by multiple determinations. Similarly, the deviation in the selectivity (k_L/k_D) is less than 10% based also on multiple determinations.

Molecular Modeling (MM) Simulations. Molecular Modeling simulations were performed with β-CD and the five guests, Val, Phe, Tyr, Leu, and Ile. The calculations were started with fully optimized CD and amino acid structures. During the simulation, the structures of both the amino acid and the CD were allowed to fully optimize.

All molecular dynamics calculations were carried out using the consistent valence force field (CVFF) as part of the Insight II program. This model has been used successfully to model several cyclodextrin systems in the past.[20−22] Although there have been several computational studies involving cyclodextrins, we are not aware of any involving protonated guests. For a recent review of computational methods employed on cyclodextrins, the reader is referred to Lipkowitz.[23]

The complexes were pre-minimized, using the default settings with no cross terms and no Morse function, to ensure that they did not exist in highly unstable states. This is essential, as the cross terms may become unstable when the molecules are far from a minimum. The atomic coordinates may overlap when the two molecules are merged and cause the program to crash. Likewise, the Morse function may allow the bonded atoms to drift far apart producing unrealistic bond lengths if the molecule is in a high energetic state. Minimization was carried out in two steps. The first step involved the use of the steepest descent algorithm. Steepest descent is often used when the gradients are large and the configurations are far from the minimum. The drawback to this method is that convergence becomes extremely slow near the minimum as the gradient approaches zero. To overcome this problem the conjugate gradient algorithm (Polak-Ribiere method) is introduced in the second step, when the structure is closer to a minimum. Although the time per iteration is longer for the conjugate gradient method than for steepest descent, this is more than compensated by the more efficient convergence to a minimum achieved by conjugate gradient. Convergence was achieved when the gradient root-mean-square was below 10^{-3} kcal/(mol·Å). Throughout the simulations, all force field calculations assumed a dielectric of 1.0 and no cutoffs of any kind were used. The structures were heated and annealed as explained in the following paragraph.

Two types of initial geometries were used. In one set of calculations, the amino acid was placed near the upper, wider rim of the CD molecule (nonincluded complex). The initial geometry was 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. All structures within 5 kcal/mol of the lowest energy structures were examined. In every case, these structures yielded very similar features. In this paper, only representative structures are provided. The calculations were repeated with the amino acids placed inside the CD cavity as the starting geometry (included complex). Both approaches also yielded structurally similar results which tend to support the notion that the minima are representative of global minima.

Results and Discussion

Chiral Selectivity with Permethylated β-Cyclodextrin Hosts

Table 1 summarizes the rate constants (k) of various amino acid complexes reacting with *n*-propylamine for two fully methylated CD hosts. The size of the R group on the amino acids increases from Ala to Tyr in Table 1. Similarly, chiral selectivity (defined here by the ratio k_L/k_D) increases from Ala (1.6) to Leu (3.6) but decreases significantly for Phe and Tyr. The k_L/k_D ratios for Phe and Tyr are 0.82 and 0.67, respectively. The results are presented again graphically in Figure 1 to illustrate the anomalous behavior of both Phe and Tyr. Note that the selectivity increases linearly from Ala to Leu and Ile;

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Table 1. Rate Constants[a] for the Guest Exchange Reactions

amino acids		tri-O-met-β-CD	tri-O-met-γ-CD
Ala	k_L	2.4	1.02
	k_D	1.5	1.34
	k_L/k_D	1.6	0.76 (1.3)
Val	k_L	3.1	0.78
	k_D	1.0	1.1
	k_L/k_D	3.1	0.71 (1.4)
Ile	k_L	1.0	0.089
	k_D	0.27	0.203
	k_L/k_D	3.8	0.44 (2.3)
Leu	k_L	0.50	0.14
	k_D	0.14	0.10
	k_L/k_D	3.6	1.4
Phe	k_L	1.4	0.05
	k_D	1.7	0.09
	k_L/k_D	0.82 (1.2)	0.56 (1.8)
Tyr	k_L	0.019	–
	k_D	0.029	–
	k_L/k_D	0.67 (1.5)	–

[a] $k/(\times 10^{-11}$ cm^3/molecule s). Values in parentheses are the inverse of k_L/k_D.

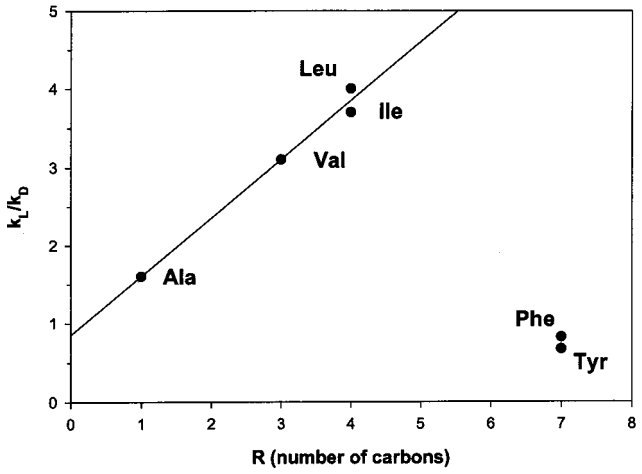

Figure 1. Chiral selectivity (k_L/k_D) as a function of the number of carbons on the side chain (R) of the amino acids. Chiral selectivity tends to increase with increasing number of carbons on the side chain of the alkyl amino acids. Phe and Tyr, however, do not follow this trend.

however, both Phe and Tyr have values that are too low, relative to the other amino acids. Values greater than 5.0 are expected if Phe and Tyr are to behave like the other amino acids.

To observe chiral selectivity in these host−guest systems, the incoming *n*-propylamine has to be in close proximity to the guest for the proton to be transferred. This is achieved by the amine approaching the protonated amino acid through either the upper or lower rim of the CD. The presence of chiral selectivity in the guest exchange reactions is by itself consistent with the presence of gas-phase inclusion complexes, although the coordination of the protonated amino acid to the exterior of the CD cannot be totally ruled out. Kitae et al. have claimed that inclusion is a necessary prerequisite for chiral separation in liquid chromatography.[24] The three-point interaction model,[25,26] often used to describe chiral selectivity, operates better when the guest is inside the cavity rather than in the rim or the outside surface. Lipkowitz et al. have found that the most enantiodifferentiating regions of permethylated β-cyclodextrin are in the interior rather than the exterior of the macrocycle.[27]

The gas-phase basicity (GB) of the amino acids relative to the amine reagent may contribute to both the absolute rates and the selectivity (Table 1). Since the guest-exchange reaction is effectively a proton transfer from the protonated amino acid to *n*-propylamine, it is expected that the reaction will be affected by the relative gas-phase basicity of the amino acid and the alkylamine. However, a close inspection of Table 1 indicates that there is no specific dependence between gas-phase basicities and reactivities for this group of amino acids. For example, Val, which is significantly more basic (GB = 876.7 kJ/mol) than Ala (GB = 867 kJ/mol), has a greater k_L value (3.1×10^{-11} and 2.4×10^{-11} cm^3 molecules^{-1} s^{-1}, respectively). Phe, which is also more basic than either Leu or Ile, has rate constants that are greater than those for both Leu and Ile. Therefore, although basicity may be a factor in influencing the rates of the reactions, structural factors play a similarly important role.

The lowest energy structures resulting from the molecular modeling (MM) simulations of complexes of Val, Phe, and Tyr are presented in Figure 2. The 50 structures for each set of calculations were examined and we found general similarities with structures that are within 5 kcal/mol of the lowest energy structures. Furthermore, both sets of MM calculations, i.e., beginning with the included and nonincluded complexes, also produced similar structures. In every case, inclusion of the protonated amino acid was the most stable species. These results are in line with several earlier MM simulations that also predict the stability of gas-phase inclusion complexes.[20,23,28,29]

Figure 2A shows the most stable conformations for D- and L-Val complexed to permethylated β-CD. For convenience, the cyclodextrin hosts are oriented as similarly as possible, with the wider rim on top. For L-Val, both the N- and the C-termini interact primarily with the lower rim via hydrogen bonding. Note that the alkyl side chain is also oriented toward the upper rim. For D-Val, the N-terminus interacts again with the lower rim, while the C-terminus interacts with the upper rim. The alkyl side chain is now oriented toward the inner wall of the host cavity. It is the differences in the interaction between the amino acid and the cyclodextrin host that account for a large part of the selectivity.

The lowest energy structures of the Phe and Tyr complexes (Figure 2, B and C) share similar features that are consistent with the experimental behavior of the respective complexes. However, unlike Val where distinct interactions are observed for each enantiomer, the two enantiomers of Phe and Tyr interact with the host in very similar ways. Visual inspection of Figure 2B,C shows that in both sets of enantiomers the phenyl group is oriented toward the upper rim. The large size of the phenyl group and its associated steric interactions must be reconciled with the hydrogen bonding interactions of both N- and C-termini. Thus, the N- and C-termini are forced to interact primarily with the lower rim. The similarities in the binding, therefore, produce similar rates for both enantiomers.

It should be noted that Tyr reacts significantly slower than Phe. From the structure in Figure 2, the hydroxyl phenyl group appears to hydrogen bond strongly with the oxygen atoms in

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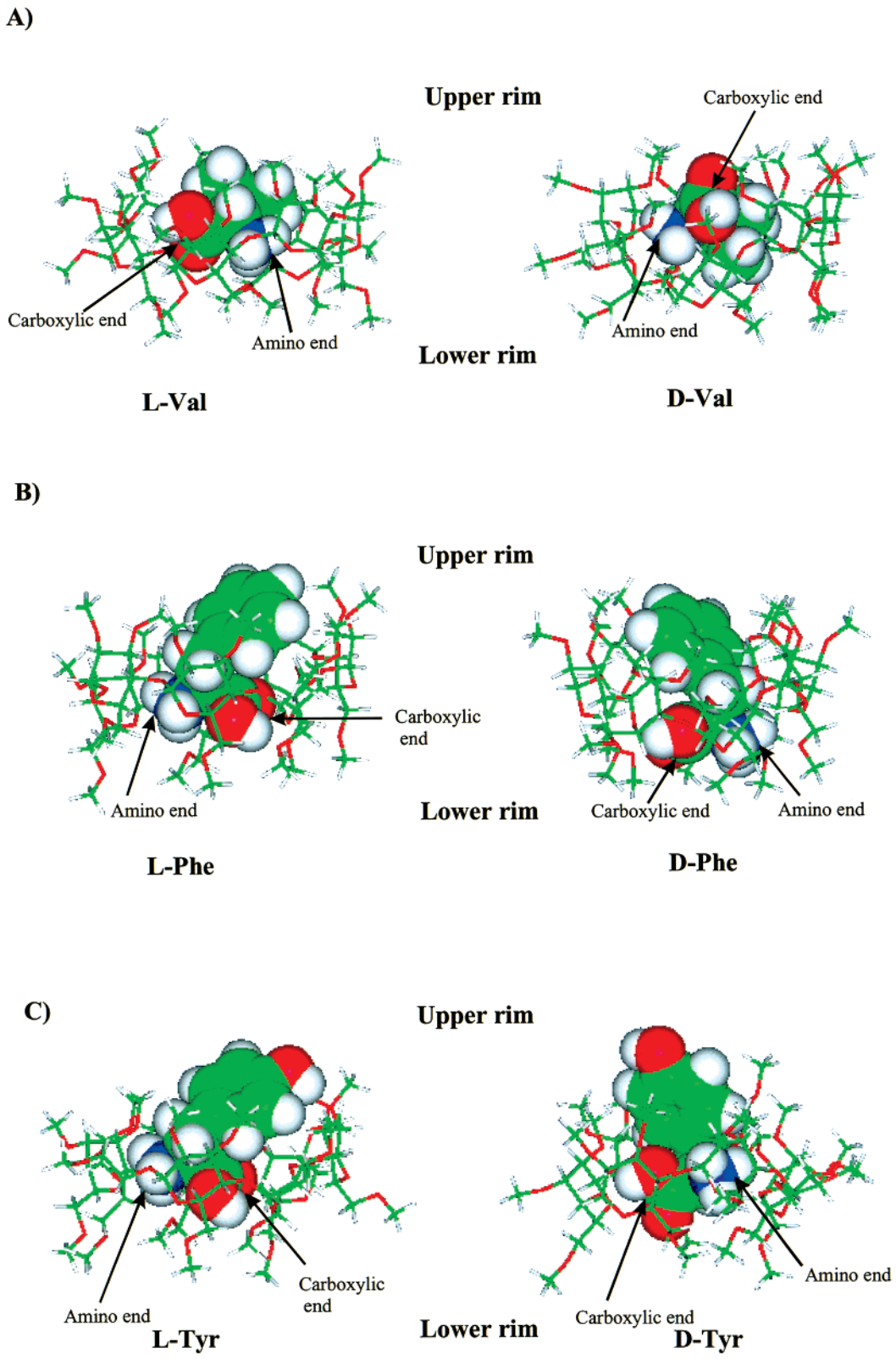

Figure 2. Low energy structures for the enantiomeric pairs of Val, Phe, and Tyr. Structures were calculated using the Insight II/Biosym package. The structures clearly show that L- and D-Val have different coordinating interactions leading to chiral selectivity. Phe and Tyr, on the other hand, show very similar interactions and consequently have low chiral selectivity.

the upper rim (vida supra). A strong interaction between the side chain and the cyclodextrin rim may hinder the approach of the incoming *n*-propylamine thereby decreasing the rates of exchange. Another contributing factor is probably the relative gas-phase basicities of Tyr and *n*-propylamine. Nearly all the amino acids have gas-phase basicities (GB) less than *n*-propylamine with the exception of Tyr which is 3.1 kJ/mol more basic the *n*-propylamine.

The trend in chiral selectivity of the other amino acids can be explained by the combination of inclusion and a "cavity size"

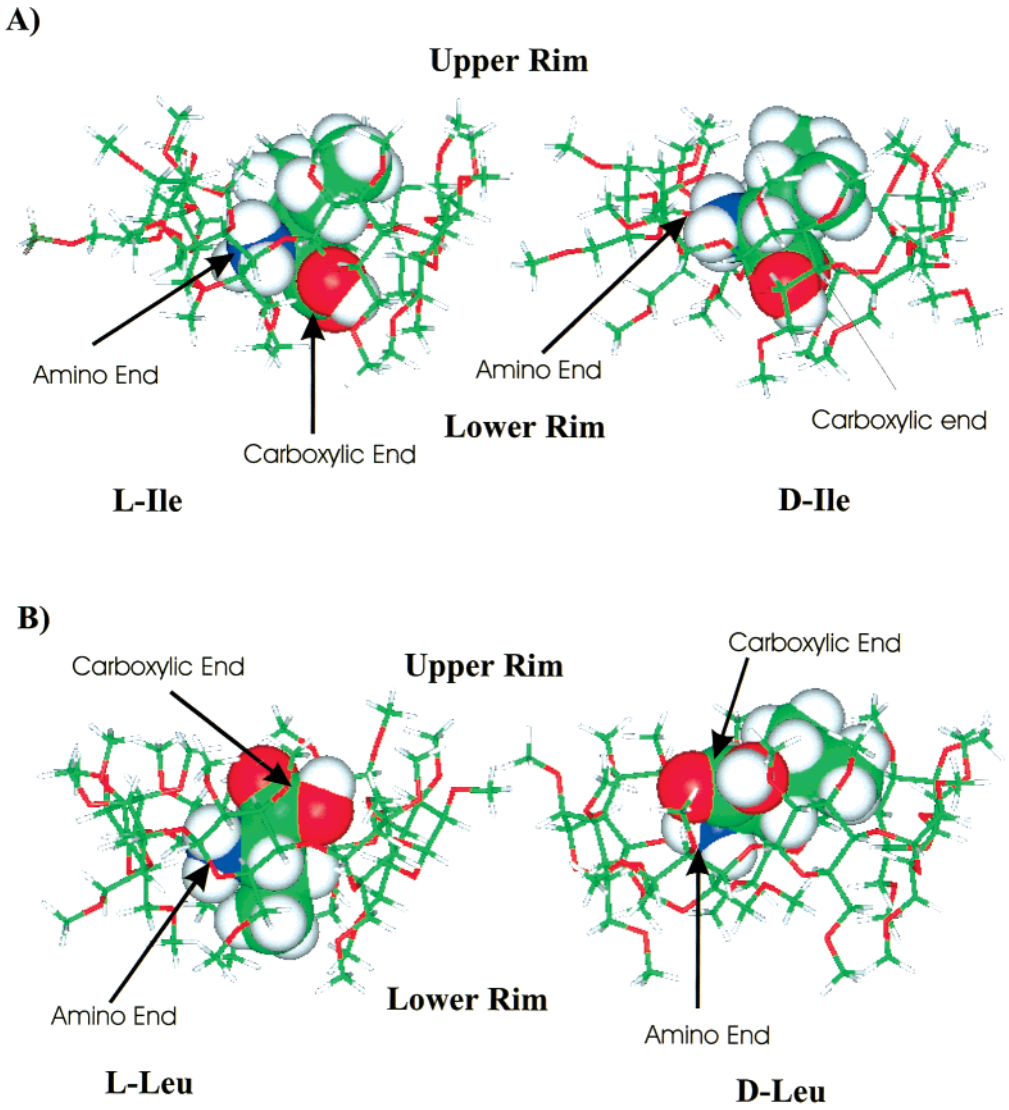

Figure 3. Low energy structures for the enantiomeric pairs of Leu and Ile. Structures were calculated using the Insight II/Biosym package. The structures clearly show that the L- and D-isomers have different coordinating interactions leading to chiral selectivity. Also note the compact structures of the branched alkyl amino acids, compared to the aromatic amino acids in Figure 2.

effect. There exists an optimal size where chiral selectivity is favorable. For small amino acids with small side chains such as Ala, even the β-CD cavity is too large. Both enantiomers of Ala can assume numerous types of coordination. Several of these would probably be similar, thereby decreasing selectivity. As the size of the side chain is increased, a complementary size is encountered that provides some limitation in the number of different complex structures but still allows the enantiomers to find favorable but distinct interactions. This situation is obtained with Ile and Leu.

One may argue that the side chains of Ile and Leu are bulkier than the planar side chains of Phe and Tyr. However, MM calculations show that the side chains of Ile and Leu are more flexible and are readily included inside the cavity of β-CD. Figure 3 shows the lowest energy structures of the enantiomers of Ile (**a**) and Leu (**b**). MM calculations predict that both Leu and Ile form compact structures that can fit almost completely inside the β-CD cavity. Inspection of the structures also shows that the D- and the L-isomers adopt distinct conformations that lead to chiral selectivity. The phenyl side chains, on the other hand, are not as flexible. The planar benzene ring is forced to lie through the upper rim allowing the amino and carboxyl termini to be included inside the β-CD cavity. Thus, the rigidity and the planarity of the aromatic amino acids lead to greater steric interactions compared to the flexible structures of Leu and Ile.

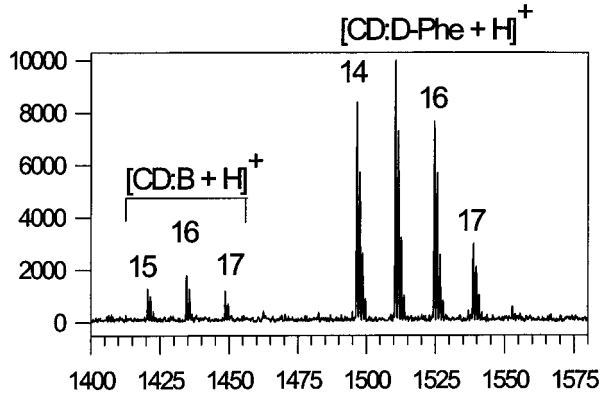

Figure 4. A typical mass spectrum for the guest exchange reaction of di-O-met-β-CD. The different peaks correspond to CD with 14−17 methyl groups, complexed to Phe ([CD:Phe + H]$^+$). The corresponding exchanged products, [CD:B + H]$^+$, are observed after a suitable delay time. The exchange reaction with *n*-propylamine (B) takes place inside the analyzer cell at a pressure of 3.0−5.0 × 10^{-7} Torr.

The electronic character of the aromatic amino acids differs from those of the aliphatic amino acids but that should not produce any specifically favorable or unfavorable interaction with the host. The effect of decreasing selectivity with Phe and Tyr is a size effect, that is, the β-CD cavity is too small to allow maximum chiral selectivity. When the optimal size of a specific cavity is exceeded, the large bulky groups make it difficult for each enantiomer to find distinct interactions so that chiral selectivity is decreased. By this reasoning, decreasing or increasing the size of the cavity should also affect the chiral selectivities of the amino acids. Both possibilities were investigated and the results are presented below.

Despite the utility of MM calculations, they yield little information regarding the mechanism of the reaction. The calculations help predict when the host−guest interactions are different which translates to differences in reactivities. However, the calculations provide neither the nature of the selectivity (whether k_L/k_D is greater than or less than 1.0) nor the absolute magnitude of the selectivity. Therefore, the question of why Phe and Tyr, for example, do not have identical values although the interactions of the guest with the host appear the same cannot be precisely answered at this time. Undoubtedly, the hydroxyl group on the side chain of Tyr contributes to favor one enantiomer over the other.

The Effect of Increasing Cavity Size on Chiral Selectivity: Reactions with γ-CD as Host. The chiral selectivities of a group of amino acids with γ-CD as hosts are tabulated (Table 1). Nearly all the rate constants for the γ-CD complexes are smaller than that for the β-CD complexes owing possibly to the increase in size and bulkiness of the larger host. Note that the selectivities for Ala, Val, Ile, and Leu all decrease. For example, Val and Leu decrease from 3.1 to 0.71 (or inverse 1.4) and 3.6 to 0.44 (or inverse 2.3), respectively. Indeed, the selectivity for Phe increases slightly($1/[k_L/k_D] = 1.2$ to 1.8). A nonincluded complex should not be affected by the size of the host. For β-CD, the size of Val and Leu complements the size of the cavity so that large chiral selectivities are produced. Increasing the size of the cavity further disrupts the preferred fit thereby decreasing chiral selectivity. For Phe, the reverse is true. With β-CD, Phe is too large to obtain large selectivities, but when the cavity size is increased, the selectivity is improved because Phe now has more space to allow each enantiomer to find favorable and more distinct orientations. Furthermore, the change in the rate constants with the increasing size of the host is further evidence for the presence of inclusion.

Because the specific details of the mechanism are still unknown, we have no explanation at this time for why the selectivity reverses for some of the amino acids, for example, Ala, Val, and Ile. The main point of these experiments is to show that chiral selectivity varies with the size of the cavity. There are several factors to consider when developing a mechanism for the exchange. First, knowing the differences in the host−guest interaction is only the beginning. How the amine approaches the protonated amino acid, i.e., via either the upper or lower rims, is a question that needs to be further explored. Second, because of the nature of the host−guest interaction, a single distinct complex structure probably does not exist for either the D- or the L-isomer. Rather, the complex is composed of a population made up of several distinct structures, some of which may have slightly different reactivities. This is particularly true when the sizes of the host and the cavity are not complimentary.

The Effect of Decreased Cavity Size on Selectivity: Reactions with Partially Methylated β-CD Hosts. Another method for changing the size of the cavity is by varying the amount of methyl derivatives on the CD rims. 2,6-Di-*O*-methyl-β-cyclodextrin is obtained commercially as a mixture of CDs differing only in the number of methyl groups in the upper, wider rim. A fully methylated β-CD has 21 methyl groups, three for each glucose unit. The commercially available compounds have major components consisting of 14 to 17 methyl groups, although as many as 19 methyl groups are often observed in the mass spectra. The lower rim, which accounts for seven methyl groups, is completely methylated in all cases as the 6-hydroxyl is the most reactive. The second group of seven methyls occupies the 2-position in the upper rim. The last group of seven occupies the least reactive site, namely the 3-position. As the number of methyl groups in the upper rim increases between 14 and 21, it effectively reduces the size of the cavity as the additional methyl groups are oriented toward the center of the rim.

Table 2. Rate Constants[a] for Di-O-met-β-CD with Varying Number of Methyl Groups

amino acids		14-met	15-met	16-met	17-met	21-met
Ala	k_L	ND	1.7	2.2	2.1	2.4
	k_D	1.4	3.1	3.0	1.6	1.5
	k_L/k_D	**ND**	**0.6 (1.7)**	**0.7 (1.4)**	**1.3**	**1.6**
Val	k_L	1.1	1.1	1.0	1.9	3.1
	k_D	0.2	0.2	0.4	0.6	1.0
	k_L/k_D	**5.5**	**5.5**	**2.5**	**3.1**	**3.1**
Phe	k_L	ND	0.02	0.03	0.04	1.4
	k_D	ND	0.01	0.02	0.03	1.7
	k_L/k_D	**ND**	**2.0**	**1.5**	**1.3**	**0.8**

[a] $k/(\times 10^{-11}$ cm^3/molecule s). Values in parentheses are the inverse of k_L/k_D. ND = not determined.

A typical mass spectrum is shown in Figure 3. β-CDs having 14−17 methyl groups are shown complexed to D-Phe ([CD:D-Phe + H]$^+$). The corresponding exchange products are also observed ([CD:B + H]$^+$). Table 2 summarizes the rate constants and the chiral selectivity obtained for three representative amino acids. The 14-methyl complex was very unreactive and no significant exchange product was observed. Increasing the number of methyl groups generally increases the reactivity with the exception of the D-enantiomer of Ala. The increased reaction rates associated with greater methylation may be attributed to the loss of intramolecular hydrogen bonding in the rim as the CD is further methylated. The loss of H-bonding allows easier access for the incoming amine. The greater degree of methylation also decreases the chiral selectivity for the amino acids with large alkyl side chains such as Val (5.5 to 3.1) and Phe (2.0 to 0.8). Ala, with a small alkyl side chain, is unaffected.

These results are consistent with selectivity based on the size of the cavity. The trend is readily evident for Val and Phe, that is, as the degree of methylation increases, the selectivity decreases. The trend in reactivity is consistent with the notion that selectivity is governed by the size of the cavity. The behavior of Ala appears anomalous as the selectivity reverses for the 15- and 16-methyl derivatives. Again, the complexity of the interaction and the lack of knowledge regarding the reaction mechanism prohibit us from making inferences based on the absolute selectivity. The partially methylated systems are further complicated by the uncertainty in the location of the methyl derivative. With sixteen methyl groups on β-CD, there are seven 3-carbon positions that two methyl groups can occupy resulting in nine isomeric structures. The interaction of the guest and the reactivity of the complex may vary for each isomer and this is particularly true for small guests that can take on numerous modes of interactions. What is important is

that the selectivity for Ala is generally unaffected because its size is too small even for the fully derivatized β-CD host.

Conclusions

The results strongly point to the existence of inclusion complexes in the gas phase, and argue against the formation of nonspecific adducts. We believe that the inclusion complexes are formed in solution and are stabilized primarily by hydrogen-bonding interactions. The hydrophobic effect is believed to be a major driving force in the formation of inclusion complexes in solution. However, even in the absence of solvent, inclusion is still favorable. The molecular modeling results suggest that rather than being hydrophobic, the cavity of cyclodextrins is in fact highly polar and well suited for coordinating with the ammonium and the carboxylic groups of amino acids. The relatively high hydrophilicity of the β-cyclodextrin cavity, particularly in the permethylated form, has been suggested by Lipkowitz.[23] All the amino acids we examined including Ala, Val, Ile, Leu, Phe, and Tyr are all included in the gas-phase complex. Furthermore, relatively bulky amino acids, such as Tyr and Phe, also appear to favor inclusion.

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