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Chiral recognition of non-natural α-amino acids

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On the occasion of Prof. J.L. Beauchamp's 60th birthday. Jack—live long and prosper.

Abstract

The gas-phase guest exchange reactions of a number of non-natural α-amino acids complexed to permethylated β-cyclodextrin were examined with Fourier transform mass spectrometry. The enantioselectivity of the reactions were determined. Molecular modeling calculations were performed to support the experimental results. The amino acids included homoserine, *cis*-4-hydroxyproline, allo-threonine, and allo-isoleucine. Results from molecular modeling calculations suggest that enantioselectivity is governed by differences in the binding interaction between the amino acid host and the permethylated β-cyclodextrin guest. (Int J Mass Spectrom 222 (2003) 259–267)

Keywords: Chiral recognition; Amino acids; Cyclodextrin; Host–guest complexes

1. Introduction

Chiral recognition has long been an area of considerable interest in mass spectrometry. The challenge has been that both enantiomers have identical masses and physical characteristics under mass spectrometry. Enantiomers differ primarily by how they interact with other chiral compounds (selectors). In this regard, chiral differentiation methods that employ mass spectrometry are akin to the chromatographic method where the analyte is allowed to interact with a chiral selector. In mass spectrometry, the nature of these interactions can be made to manifest themselves under various ionization and ion dissociation conditions. The advantage of mass spectrometry, however, is speed, sensitivity, and structural information.

The properties of host–guest complexes involving the analyte (guest) and the selector (host) have been examined for their potential utility for chiral analysis. The earliest studies have involved the use of dialkyltartrates as host molecules. Complexes of protonated dimers produced by chemical ionization were examined by Fales and Wright with enantioselectivity observed from the relative abundances of the complex ions [1]. The use of alkyl tartrates was expanded in subsequent studies by Nikolaev and co-workers [2–6]. Host–guest complexes were produced from the condensed phase with softer ionization methods such as fast atom bombardment and electrospray ionization. The relative abundances during the ionization event were observed to be enantiospecific by Sawada and co-workers [7–15]. These studies have exploited a number of host molecules including cyclodextrins and various modified crown ethers. Quantitation was made

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possible by isotopically labeling one enantiomer and observing the relative intensity in a 1:1 mixture of enantiomers [11]. In addition to proton bound dimers, metal bound complexes have also been examined for enantiospecificity. For example, Leary and co-workers employed cobalt complexes with alkyl tartrate selectors for distinguishing mixtures of enantiomeric compounds [16]. Enantioselective ion/molecule reactions of host–guest complexes have also been discovered by Dearden and co-workers [17,18] and by our laboratory.

In recent studies, the chiral differentiation of amino acids has received attention. Vekey and Czira determined that the dissociation products of amino acid dimers were enantiospecific and depended on the chirality of the amino acid [19]. Cooks and co-workers' examinations of copper complexes of amino acid by the kinetic method yielded a quantitative method for determining enantiomeric excess [20,21]. Wan and co-workers employed electrospray ionization and amino acid derivatives as selectors to examine the chiral recognition of amino acids [22,23].

We have used ion/molecule reactions to examine the enantioselectivity of guest exchange reactions involving α-amino acids [24,25]. In this reaction, host–guest complexes of derivatized cyclodextrins (CDs) and protonated amino acids are reacted with neutral alkyl amines [24]. The amino acid (AA) is displaced by the amine (B) in a guest exchange reaction to produce a new protonated complex $[CD{:}B + H]^+$ (Scheme 1). Enantioselectivity is obtained in the rates of the exchange reactions. These reactions have been used to determine enantioselectivity in amino acids and chiral drugs [26,27]. Methods have also been developed with these reactions to determine enantiomeric excess.

There have been recent interests in incorporating uncommon amino acids in proteins [28–30]. The goal is to produce new enzymes with new activities, enhanced specificities, and even the formation of new

$$[CD{:}AA + H]^+ + B \longrightarrow [CD{:}B + H]^+ + AA$$

Scheme 1. Gas-phase guest exchange reaction of amino acid (AA) and an alkyl amine (B) with β-cyclodextrin host.

Table 1
Reaction selectivity of common amino acids and uncommon amino acids complexed to permethylated β-cyclodextrin

Amino acids	k_L	$S(k_L/k_D)$
Serine (Ser)	0.64	1.2
Homoserine (HSer)	0.35	2.2
Proline (Pro)	1.2	1.5
cis-4-Hydroxyproline (HPro)	0.031	1.4
Threonine (Thr)	0.12	0.59
Allo-threonine (AThr)	0.18	22
Isoleucine (Ile)	1.0	3.7
Allo-isoleucine (AIle)	1.9	4.1
Leucine (Leu)	0.52	4.0

Rate constants for L-enantiomers are $k_L \times 10^{-11}$ cm^3/molecule s.

and unique life forms. In this research, we examine the use of the guest exchange reaction to illustrate that enantioselectivity in the exchange reactions is also present for uncommon α-amino acids (Table 1).

2. Experimental

2.1. Materials

All amino acids, heptakis-(2,3,6)-tri-*O*-methyl-β-cyclodextrin (β-CD), and *n*-propylamine were obtained from Sigma Chemical Co. (St. Louis, MO) and used without further purification. The silica tubing used to manufacture the microspray tips was purchased from Polymicro Technologies Inc. (Phoenix, AZ).

2.2. Guest-exchange reactions

Guest-exchange experiments were performed on a home-built external source ESI-FTMS equipped with a 4.7 T superconducting magnet (Oxford Instruments, Witney, England). The details of the instrument have been published elsewhere [31,32]. The solutions were electrosprayed into the vacuum chamber by applying a voltage (1.5–2.5 kV) at the liquid junction on the base of the microspray tip. The typical flow rates ranged from 10 to 15 μL/h. The microspray tips were manufactured from silica tubing with an o.d. of 150 μm and an i.d. of 25 μm.

Stock solutions of amino acids and oligosaccharides (0.01 M) were prepared in a 50/50 (v/v) water/methanol solution. The complexes were prepared by mixing the cyclodextrin solution with a 10–50-fold excess of the desired amino acid solution. The resulting solution, with final concentration of cyclodextrin approximately 1.0×10^{-5} M, was directly electrosprayed into the mass spectrometer.

The isolation of specific ions was performed using a series of rf bursts and sweeps at frequencies corresponding to the unwanted masses. The isolated complex ion was then allowed to undergo a guest-exchange reaction with an amine that was previously leaked into the analyzer cell. Gaseous amines were first purified on the vacuum manifold by several freeze–thaw cycles and then leaked into the analyzer cell to final pressures between 1 and 6×10^{-7} T, as determined by an uncalibrated ion gauge. The appearance of the exchange product was monitored as a function of time. Rate constants (k) were obtained from the slopes of the pseudo-first-order rate plots ($\ln I/I_0$ vs. t, where I is the intensity of the complex at time t and I_0 is the sum of the intensities of the product and starting complex). The largest source of error in determining rate constants was in the accurate determination of the pressure. Our current data system has limited access to the very low mass range prohibiting us from performing the standard pressure calibration reactions involving methane. We have performed pressure calibration with published deprotonation reactions of proteins, however the consistency was not satisfactory. We therefore caution the reader that the absolute rates will not be accurate. However, the important number in this study is the selectivity, which we defined as the ratio k_L/k_D. In this ratio, any deviation in pressure from the "true" value was completely eliminated. The uncertainty in selectivity values was less than 10% as determined from multiple determinations.

2.3. Molecular modeling

The permethylated β-cyclodextrin and amino acids structures were constructed and optimized using the Insight II builder module. The protonated oligosaccharide:amino acid complexes were formed by merging the respective sugars and protonated amino acids AAH^+. Calculations of the complexes were started with fully optimized oligosaccharide host and AAH^+ (amino acid) structures. During the simulation, the structures of both the AAH^+ (protonated amino acids) and the hosts were allowed to fully optimize. The protonated amino acid (AAH^+) was placed near the wide rim of the CD molecule and the complex 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. The heating/annealing cycles helps avoid local minima and provides the best solution to the global minimum. This resulted in 25 annealing simulations with a corresponding number of structures. Generally, several structures with very similar energies were obtained. 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. Gas-phase guest-exchange reaction

3.1.1. Serine and proline analogs

The exchange reaction of homoserine and *cis*-4-hydroxyproline was performed. Homoserine is one carbon longer on its side chain than serine (Fig. 1). The mass spectra, after various reaction times, in the reaction of the cyclodextrin–homoserine complex with *n*-propylamine, are shown in Fig. 2. The reaction of homoserine (HSer) was slightly slower than serine (Ser) ($k_L = 0.35$ and 0.64×10^{-11} cm^3/molecule s, respectively). The magnitude of the rate constant was consistent with its size, being slightly larger than Ser. The enantioselectivity for HSer was larger than Ser (2.2 vs. 1.2 for serine), again commensurate with its size. We have shown that there is an optimal size for selectivity with permethylated-β-cyclodextrin. The maximum selectivity occurred with compounds as large

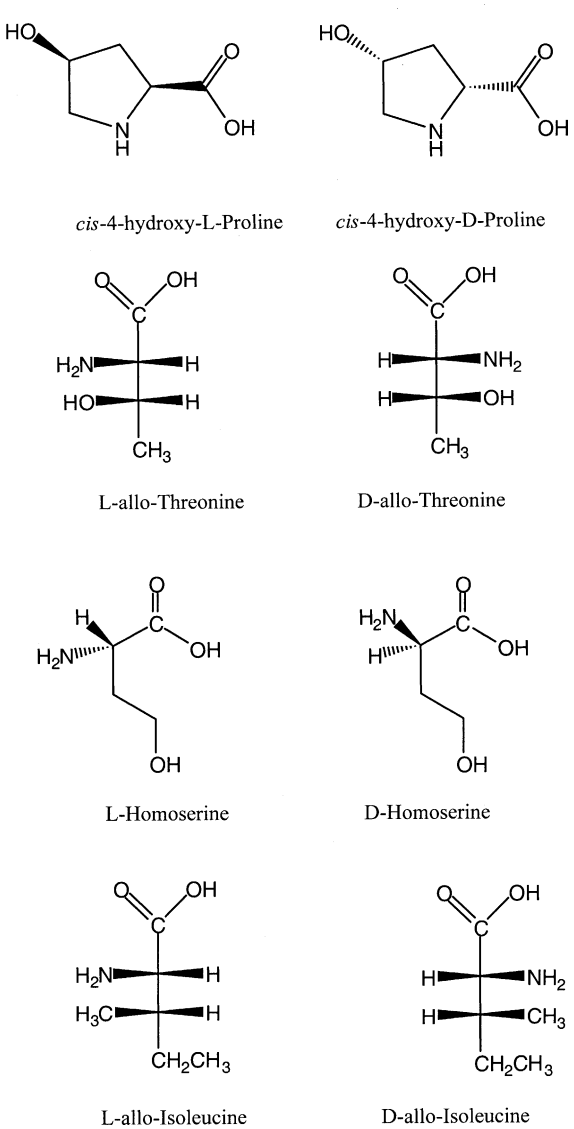


Fig. 1. Structures of non-natural amino acids.

as leucine and decreased with both increasing and decreasing side chain size. Thus phenylalanine and valine both with larger and smaller alkyl side chains, respectively, had lower selectivity than Leu.

The reaction of *cis*-4-hydroxyproline was considerably slower than proline. The rate constant for the L-enantiomer was nearly two orders of magnitude smaller. The differences in rate constants was consistent with the larger side chain of HPro and the presence of an additional hydrogen bonding interaction between the hydroxyl side chain and the oxygen atoms on the cyclodextrin. Both work to decrease the overall rates of the reactions. Interestingly, the enantioselectivity of HPro was nearly that the same as Pro. The size of HPro was only slightly greater than Pro indicating that size is a greater determining factor in the enantioselectivity, than additional attractive interactions. Similar observations were made with the natural amino acids [25].

3.1.2. Threonine and its analog

Threonine (Thr) has two chiral centers. The L and D designations refer to the chirality of the α-carbon. The non-natural form, allo-threonine (AThr), is depicted in Fig. 1. The reaction rate constants of the exchange reaction for the L-enantiomer of both threonine and AThr were nearly the same ($k_L = 0.12$ and 0.18×10^{-11} cm^3/molecule s, respectively). These values were only slightly less than that of Ser—consistent with the larger sizes of the amino acids. However, the D-enantiomer of AThr reacted considerably slower than Thr yielding significantly larger enantioselectivity for the latter ($S = 21.6$) compared to the former ($S = 0.59$). This represented the largest selectivity for the guest-exchange reaction measured so far.

3.1.3. Leucine and its analogs

Among the naturally occurring amino acids, both leucine and isoleucine have the highest selectivity with the permethylated-β-cyclodextrin host (4.0 and 3.7, respectively). The high selectivity suggests that the size of the compound and the dimensions of the permethylated-β-cyclodextrin cavity complement to yield the highest selectivities.

Isoleucine has a second chiral center on the alkyl side chain. Allo-isoleucine (AIle) is the non-natural enantiomer at the second chiral center. The reactivity of AIle was similar in every respect to that of Ile. The absolute rate constants are slightly higher. The L-enantiomer of AIle reacts nearly twice as fast as Ile. However, the selectivity is nearly identical with values of 3.7 for Ile and 4.1 for AIle.

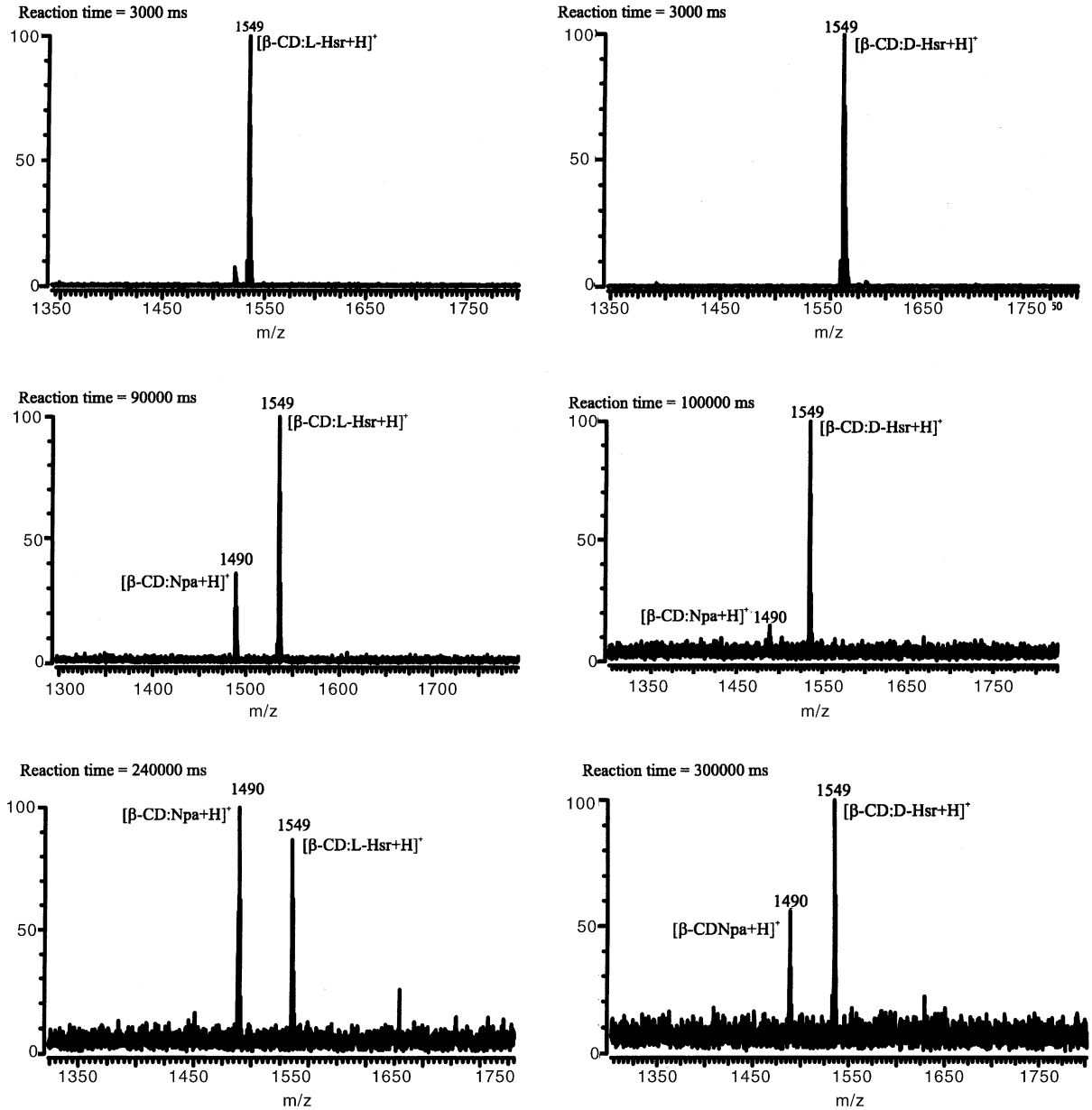


Fig. 2. Representative FT mass spectra of the homoserine complex $[CD:HSe + H]^+$ reacting with *n*-propyl amine. The L-enantiomer (left column) reaction times are 3, 90 and 240 s. The D-enantiomer (right column) reaction times are 3, 100 and 300 s.

The three sets of compounds are useful for relating rate constants with the different structural features. For example, the ratio of the rate constants for Ile and Leu (k_{Ile}/k_{Leu}) is 1.9, indicating that the two isomers can be differentiated by this method.

3.2. Molecular modeling of gas-phase complexes

The lowest energy structures of the protonated complexes belonging to the amino acids are provided. In every case, we assumed that the site of protonation

was the amine terminus. The amine group is the most basic site for α-amino acids containing alkyl and hydroxyl side chains. Because finding the global minima of large molecules is always difficult, we examined all structures within 5 kcal/mol of the most stable (lowest energy structure) to ensure that the major structural features are similar and represented in the lowest energy structure.

The positions of the protonated ammonium group, the carboxylic acid group, and the side chains were noted relative to the narrow (pictured toward the bottom of the page) and wide rims (toward top). The wide rim was composed of (methylated) oxygens on Carbon 2 (C2) and Carbon 3 (C3) of glucose while the narrow rim was composed of oxygens on Carbon 6 (C6). Each glucose unit was linked by an oxygen via the

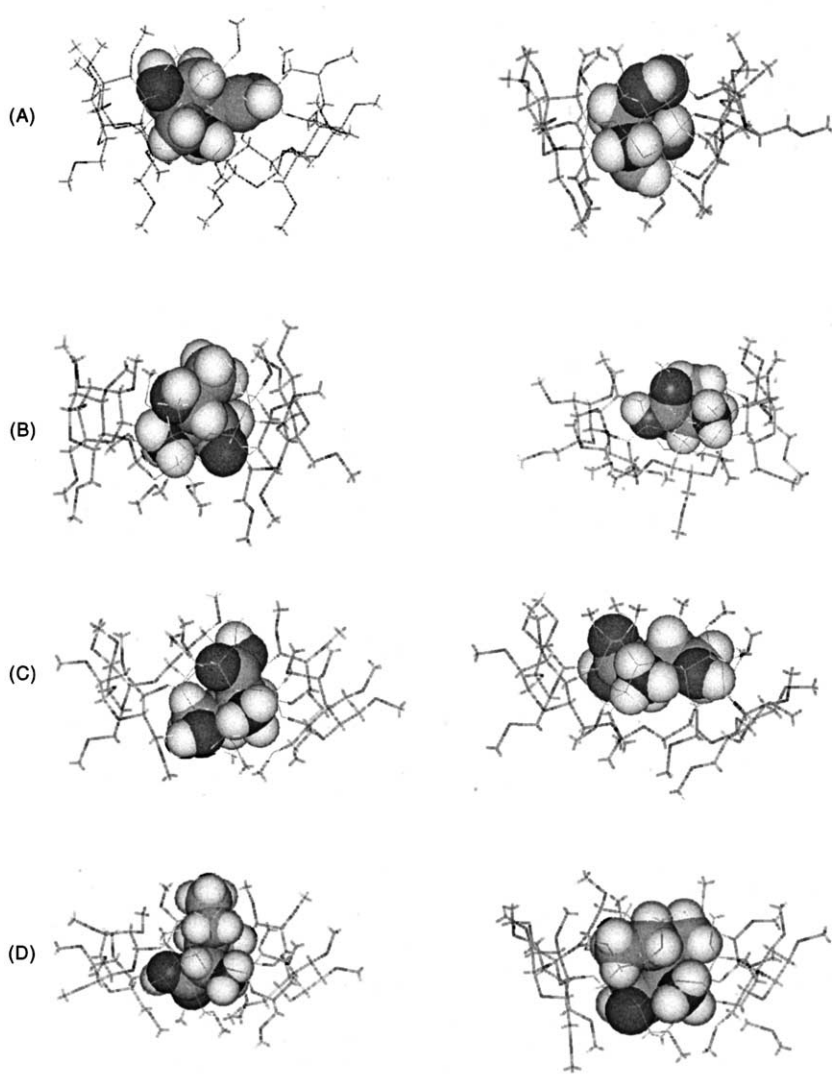


Fig. 3. Lowest energy structures from molecular modeling calculations of protonated amino acid complexes with permethylated-β-cyclodextrin. The L-forms are on the left. (A) *cis*-4-Hydroxyproline, (B) allo-threonine, (C) homoserine, (D) allo-isoleucine.

glycosidic bond. The oxygen and its lone pairs were available for interactions with compounds included in the cyclodextrin cavity. The interactions of the carboxylic and the ammonium groups to the cyclodextrin involved primarily hydrogen bonding and ion/dipole. In previous studies, we noted that the position of the N- and C-termini relative to the rims were indicative of the enantioselectivity. In situations where both enantiomers interacted similarly, the enantioselectivity was small. When the interactions of either enantiomer were distinct, the enantioselectivity was large.

The lowest energy structures of permethylated-β-cyclodextrin complexes with *cis*-4-hydroxy-L-proline are shown in Fig. 3A (the L-form is on the left). For the L-enantiomer the protonated ammonium coordinated primarily with the narrow rim with N–H≡O distances of 3.1 Å for the narrow rim oxygen and 2.8 Å for the glycosidic bond oxygen. The carboxylic acid interacted with the wide rim oxygen (–CO_2–H≡O, 1.8 Å) while the hydroxyl on the side chain interacted with the glycosidic bond oxygen (–O–H≡O, 3.2 Å). The D-enantiomer behaved in a similar manner; the ammonium group interacted with the narrow rim and the glycosidic bond oxygen (3.5 and 3.4 Å, respectively), while the carboxylic acid interacted with the wide rim (4.1 Å). The hydroxyl group of the side chain interacted with the glycosidic bonds (3.45 Å). The similarity in the bonding interactions between D- and L-enantiomer was consistent with the relatively low selectivity observed for involving *cis*-4-hydroxy-L-proline.

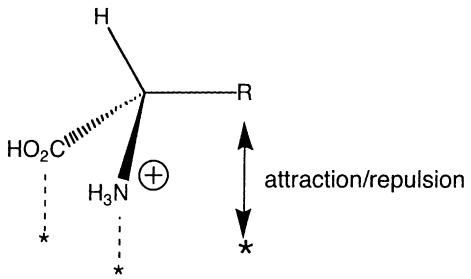
Enantiomers of allo-threonine exhibited greater differences in their interaction with the cyclodextrin host (Fig. 3B). In the L-form the ammonium group yielded hydrogen-bonding interactions with the narrow rim, and one with the glycosidic bond oxygen (1.9, 2.1 and 2.4 Å, respectively), while the carboxylic groups interacted primarily with the glycosidic bond oxygen (2.3 Å). The hydroxyl group of the side chain interacted primarily with the glycosidic bond oxygen (3.2 Å). The D-form exhibited interaction of the ammonium group with the narrow rim (1.9 and 1.9 Å) and the glycosidic bond oxygen (2.3 Å). The carboxylic group interacted with the wide rim (2.0 Å). The hydroxyl on the side chain interacted with the glycosidic bond oxygen (2.1 Å). The differences in the coordination of the L- and D-forms were manifested in the interaction of the carboxylic acid. For the L, this involved mainly the glycosidic bond, while for the D it involved the wide rim. In the previous case, the carboxylic acid groups both interacted primarily with the upper rim. The larger variation in the two complex structures is consistent with a higher enantioselectivity for allo-threonine but perhaps not with the large magnitude of the value ($S = 21.6$). There is a stronger interaction between allo-threonine and the cyclodextrin host than *cis*-4-hydroxy-L-proline as evidenced by the generally shorter distances in the former.

The interactions of the homoserine enantiomers differed somewhat slightly, reflecting the moderate enantioselectivity of the exchange reaction ($S = 2.2$, Fig. 3C). In the L-form the ammonium interacted exclusively with the narrow rim (2.0, 2.2 and 2.3 Å), while the carboxylic acid group interacted exclusively with the wide rim (2.0 Å). The hydroxyl group of the side chain interacted with the narrow rim (1.7 Å). In the D-form, the ammonium hydrogens interacted with the narrow rim, a ring oxygen, and the glycosidic bond oxygen (1.8, 2.8 and 2.0 Å, respectively). The carboxylic acid group interacted with the glycosidic bond oxygen (2.2 Å), as did the hydroxyl group of the side chain (1.9 Å).

Allo-isoleucine showed moderate differences in the interaction of the two enantiomers with permethylated-β-cydodextrins (Fig. 3D). In the L-form the ammonium group interacted with the narrow rim (1.9 Å), the ring oxygen (2.7 Å) and the wide rim (2.0 Å). The carboxylic acid group interacted with a ring oxygen (1.9 Å). In the D-form, the ammonium group interacted primarily with the narrow rim (2.0, and 1.9 Å) and a glycosidic bond oxygen (2.4 Å). The carboxylic acid interacted primarily with the narrow rim (1.6 Å).

4. Discussion

The “three-point interaction” model is often used to understand enantioselectivity [33–36]. For amino



Scheme 2. The three-point interaction involving amino acid guests in a host molecule. Two points of attractions are due to hydrogen bonding between the host (asterisk) and the carboxylic and ammonium group. The third interaction is either attractive or repulsive depending on the functional group in R.

acids, the three-point interaction is summarized by the illustration in Scheme 2. The site of protonation is the N-terminus. Molecular modeling calculations in this study and those with natural amino acids [25] suggested that the resulting ammonium group interacts primarily with the narrow rim of permethylated β-cyclodextrin. The interaction is more favorable with the narrow rim as the C6-methoxyl groups are more flexible to allow better coordination with the ammonium group. The carboxylic acid groups may interact with either the narrow or wide rim depending on the constraints placed on R by the cavity wall. The differences in the interaction of the two enantiomers are reflected by the positions of the ammonium and carboxylic acid groups relative to the two rims. When the interactions of the enantiomers to the β-cyclodextrin are similar, the enantioselectivity is small.

For amino acids with R = alkyl, the interaction between R and the cavity is often repulsive. There is an optimal size for the amino acid that yields the maximum enantioselectivity [25]. For β-cyclodextrin, the optimal size corresponds to leucine and isoleucine. For this reason, isoleucine, leucine, and allo-isoleucine have high enantioselectivities. Alanine and valine, two smaller amino acids, have lower selectivities.

Even when the side chain is hydroxylated, which would make the interaction between the R group and the cavity favorable, an optimal size is still favored. Homoserine, threonine, and allo-threonine, amino acids with a larger hydroxylated side chains than serine, have higher selectivity. *cis*-4-Hydroxyproline has a low selectivity because the molecule is compact. The enantioselectivities of threonine and allo-threonine were unusual when compared to other amino acids. With threonine the D-enantiomer is favored by almost a factor of two while all other amino acids favored the L-enantiomer. Additionally, the enantioselectivity of allo-threonine was significantly larger than any other amino acids. It represents the largest enantioselectivity measured to date. The reason for the unique enantioselectivities of threonine and allo-threonine is not readily apparent from the molecular modeling simulations.

Acknowledgements

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