Gas-Phase Chiral Differentiation of Amino Acid Guests in Cyclodextrin Hosts

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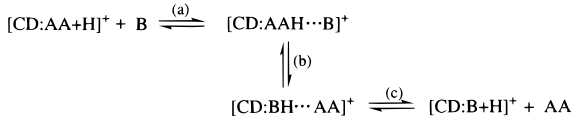
Protonated cyclodextrin-amino acid complexes react with alkylamines by exchanging the amino acid for the alkylamine. β-Cyclodextrin (2,3,6-tri-*O*-methyl-β-cyclodextrin or CD) incorporates an amino acid (AA) guest in solution to form a complex that persists when the solution is electrosprayed into the heated capillary and transported into the analyzer cell of a Fourier transform mass spectrometer. In the analyzer cell with a background pressure of $1-6 \times 10^{-7}$ Torr of *n*-propylamine (B), an exchange reaction occurs (Scheme 1). The amino acid guest is replaced by the *n*-propylamine. The exchange rates are measured and found to differ according to the chirality of the amino acid. With alanine, the naturally occurring L-enantiomer is 1.6 times more reactive than the D; with valine the L-enantiomer is 3.1 times more reactive, while with phenylalanine the L-enantiomer is only 0.8 times as reactive. The rate constants of racemic mixtures of alanine and valine are approximately the averages of the rate constants of pure L and pure D suggesting that this method may be useful for determining the enantiomeric excess of amino acids.

Chiral differentiation of amino acids is of immediate analytical importance. Currently, cyclodextrins are used in conjunction with HPLC to separate and quantify enantiomeric mixtures of amino acids. The interaction of amino acids and cyclodextrins produces a host−guest complex that can be chromatographically separated.[1−3] Several examples of chiral recognition in mass spectrometry have been reported.[4−26] The majority of these studies takes advantage

Scheme 1



of the excess energy produced during ionization to differentiate enantiomers. For example, the population of complexes formed with FAB have a high metastable component. A fraction of these species dissociate before detection. However, because the complexes formed between chiral hosts and guests are diastereomeric, they have unique stabilities and rates of dissociation. The resulting mass spectrum contains different relative abundances that reflect these differences and provides a method for monitoring enantiomeric excess. Examples of chiral recognition that specifically involve gas-phase ion/molecule reactions is not as common. These have involved dimerization reactions, dissociation or decomposition reactions, ligand exchange of proton-bound dimers, and gas-phase deprotonation reactions.[27−30] There have been no reported reactions, chiral or otherwise, dealing with guest exchange of cyclodextrin hosts in the gas phase. We present a novel reaction involving the guest exchange of cyclodextrin hosts in the gas phase. In addition, we show chiral specificity in the exchange reactions of the amino acid guests.

Experiments are performed using external source Fourier transform mass spectrometry (FTMS).[31,32] Ions are produced from a solution of 1×10^{-5} M cyclodextrin and a 10-fold excess of amino acid. The amine, purified on the vacuum manifold with several freeze−thaw cycles, was introduced into the analyzer chamber using a variable leak valve with pressures between 1 and 6×10^{-7} Torr. Under these conditions, CD coordinated to various cations including NH_4^+, Na^+, and K^+ are observed (spectra not shown). Some guest-exchanged product $[CD:B+H]^+$ is observed almost immediately (as soon as 300 ms after ion injection) that we have determined to come from the rapid exchange of $[CD:NH_3+H]^+$ with B. The desired complex $[CD:AA+H]^+$ is observed in large abundance. Typically, the NH_4^+ and Na^+ complexes are the most abundant. The amino acid complex is 50% as abundant, while the K^+ species is about 10% as abundant. To simplify the data analysis, all ions other than $[CD:AA+H]^+$ are ejected. Some residual $[CD+K]^+$ is retained for use as an internal standard. This ion is unreactive under the experimental conditions. Identical reaction conditions are employed for the enantiomeric pairs.

The series of spectra shown for L- and D-alanine with β-cyclodextrin in the figures illustrates the typical behavior of the complexes under the reaction conditions. After isolation (Figure 1a, 1.1 s reaction time), the spectra show the amino acid complexes (the precursor ions $[CD:Ala + H]^+$) as the most intense

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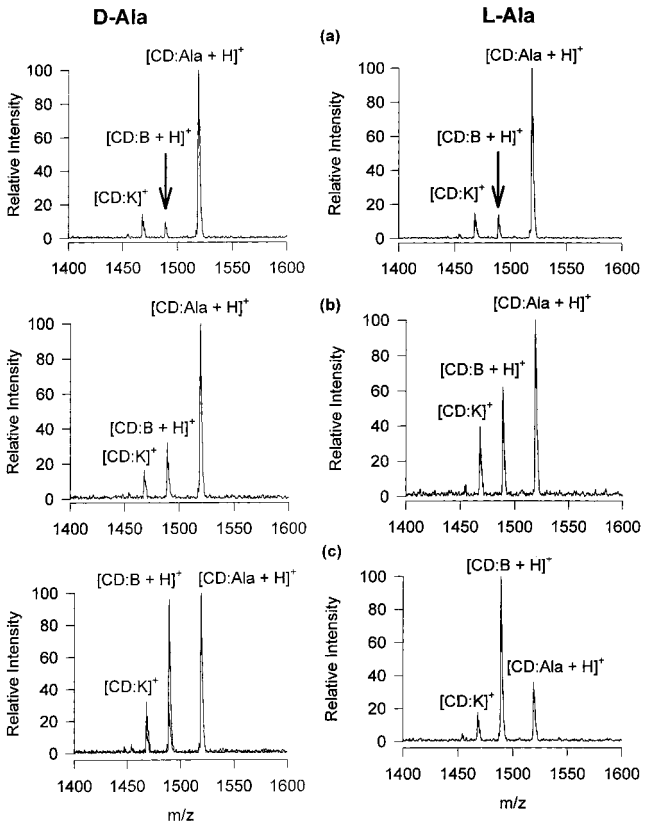

Figure 1. The reaction of D and L-alanine with *n*-propylamine (B) at 1.0×10^{-7} Torr with reaction times of (a) 1.1 s, (b) 3.6 s, and (c) 9.6 s. The L-isomer is more reactive, although the rate constant for the L-enantiomer is only 60% larger than that observed for the D-enantiomer. The spectra readily show the chiral specificity in the relative abundances of product and precursor ions.

peaks. At this time, some of the exchange products (the product ions $[CD:B + H]^+$) are already observed. The products of an alternative reaction that involves extraction of the amino acid by the base to form a protonated amino acid − *n*-propylamine dimer $[AA\cdots H\cdots B]^+$ were not observed. These were initially the expected products as hydrogen bonding is strengthened when the two heteroatoms have similar intrinsic basicities.[33,34] An N−H···N interaction is, therefore, expected to be stronger than an N−H···O. Evidently, the protonated amino acids are multiply coordinated to CD in the complex creating significantly stronger interactions.

After a reaction time of 3.6 s (Figure 1b), the $[CD:BH]^+$ product is about 70% as abundant as the precursor for the L-enantiomer, while the product is only 30% as abundant as the precursor for the D. After 9.6 s (Figure 1c), the product and precursor ions with D-alanine are of nearly equal abundances, while the L yields product ions that are three times more intense than the precursor. Visual inspection of the spectra in the figures clearly shows that L-alanine exchanges more rapidly than D-alanine.

Rate constants were determined for the reaction of three amino acids, alanine, valine, and phenylalanine, with *n*-propylamine and 2-butylamine using procedures described earlier.[30] The results are tabulated with each rate constant determined over several days from triplicate runs with pressures ranging from 1 to 6×10^{-7} Torr. The deviation in the rate constants is less than 10%. The rate constants reflect the low efficiency of the exchange reactions with values relative to ADO theory[35] of about 1%. In contrast,

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

amino acid	*n*-propylamine (GB 217.9)[b]					2-butylamine (220.5)[b]		
	GB[c]	k_L	k_D	k_L/k_D	k_{D+L}	k_L	k_D	k_L/k_D
Ala	213.6	2.4	1.5	1.6	1.9	1.6	1.3	1.2
Val	215.7	3.1	1.0	3.1	2.0	1.5	1.1	1.4
Phe	216.6	1.4	1.7	0.8		0.16	0.17	0.9

[a] $k/(\times 10^{-11}$ cm^3/molecule s). [b] Reference 39. [c] Reference 40.

proton-transfer reactions involving the same amino acids and *n*-propylamine typically have unit efficiencies. Valine is the most reactive and shows the greatest selectivity. The L-enantiomer is three times more reactive than the D. Similarly, alanine shows enhanced reactivity for the L-isomer by a factor of 1.6. Phenylalanine behaves unlike the other two amino acids. It is the least reactive and shows the least selectivity, even favoring the D-enantiomer slightly by a factor of 1.2. For comparison, the mixtures of D- and L-amino acids were prepared and analyzed in the same manner. The rate constants of the racemic mixtures are nearly equal to the average of the rate constants for the enantiomerically pure compounds (Table 1). The differences in the behavior of Phe compared to the other amino acids are intriguing. We expected all amino acids with nonpolar side chains to behave in the same or similar manner. One may posit that the differences are somewhat related to the way the amino acids are included into the cyclodextrin host. With Phe, the phenyl side chain is most likely included in the hydrophobic cavity with the remainder of the amino acid positioned to interact with the hydrophilic rim. Conversely, both Gly and Ala are sufficient small to be completely included in the cyclodextrin cavity.

The more basic compound, 2-butylamine, is generally less reactive than *n*-propylamine. The racemic mixture was used for the experiment. The selectivity is further diminished with each amino acid. The lower reactivity suggests that steric interactions (2-butylamine is a branched monoalkylamine) are important and, in this case, more important than intrinsic basicity in the guest exchange reaction.

The nature of the specificity is not immediately evident. In the reaction scheme depicted above, chiral specificity may take place during steps (a), (b), or (c). Chiral specificity is typically a kinetic phenomenon associated with the rate-limiting step. Step (a) is an exothermic reaction and is generally barrierless. Furthermore, the association is the result of long-range ion/dipole interactions that occur at distances greater than those involved in steric interactions. Steps (b) and (c) are the more likely candidates for chiral specificity. Intracomplex proton transfer (b) may be decreased by steric interaction. This phenomenon is well documented and has been demonstrated in this and other laboratories.[36−38] Another possibility is that step (c) is the rate-limiting step. In this scenario, proton transfer takes place and the amino acid leaves. If the amino acid is included in the cyclodextrin cavity, then escape may be the rate-limiting step. The cyclodextrin rims with the methyl derivatives are chiral and escape may favor one enantiomer over the other. Both possibilities are currently the subject of ongoing studies.

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