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Chiral resolution of D- and L-amino acids by tandem mass spectrometry of Ni(II)-bound trimeric complexes

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Abstract

Mass-selected trimeric cluster ions, $[Ni^{II}(A)(ref^*)_2-H]^+$, where A designates the analyte amino acid and ref* designates the chiral reference amino acid, undergo competitive collision-induced dissociation to yield two dimeric clusters with a branching ratio dictated by the stereochemistry of both the analyte and the reference. This branching ratio (R) is related, using the kinetic method, to an enthalpic term, $\Delta Ni^{II}BDE$, the difference between the formation enthalpies of the two dimeric clusters. Chiral resolution of D- and L-amino acids is directly related to the relative stabilities of the two diastereomeric clusters formed by the dissociation of the Ni(II)-bound trimeric clusters. The dimeric product ions differ in enthalpy by only a few kilojoules per mole, but chiral recognition is achieved for all 19 naturally occurring chiral amino acids, using appropriate reference amino acids. Because there is evidence that the dissociating trimeric clusters may exist in different isomeric forms, the proportion of analyte versus reference amino acids in the mixture was examined to study the effect of this ratio on the success of chiral recognition. The effect was found to be negligible. This suggests that there is an equilibrium between these isomeric clusters, which is governed by thermochemical properties rather than the relative concentrations of the constituent amino acids. A linear correlation was observed between ln(R) and enantiomeric compositions of the analyte as expected from the kinetic method treatment, and the direct measurement of optical purity to within 3% enantiomeric excess was demonstrated. With this new chiral recognition technique, qualitative and quantitative chiral analysis of amino acids is achieved. (Int J Mass Spectrom 204 (2001) 159–169) © 2001 Elsevier Science B.V.

Keywords: Chirality; Amino acids; Tandem mass spectrometry; Kinetic method; Nickel complexes; Clusters; Enantiomeric excess

1. Introduction

The discovery of molecular asymmetry by Pasteur [1] in 1848 established the foundation for our understanding of molecular chirality. The importance of chiral purity of drugs is now widely recognized. For example, the infamous thalidomide tragedy (fetal malformations in pregnant women) of the 1960s and 1970s has been attributed to the levorotatory enantiomer of this compound, whereas all three forms (the racemic, L, and D forms) had roughly the same sedative activity, the property for which the drug was originally introduced. The accelerating development of chiral drugs [2–4], has already imposed increasing demands for accurate and rapid enantiomeric analysis, in areas as diverse as pharmacological and pharmacokinetic studies, clinical studies and analysis of synthetic combinatorial libraries [5].

Enantiomers can only be differentiated through multiple-point interactions with chiral agents—the

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famous "three-point rule" [6]. Chiral analysis is directly achieved with techniques such as polarimetry and circular dichroism, and by using a chiral reagent in chromatography [7], capillary electrophoresis [8], and nuclear magnetic resonance [9]. Concerns regarding the molecular specificity, speed, and sensitivity of these methods mean that there is an interest in their improvement and in alternatives.

Mass spectrometry [10] offers unparalleled sensitivity, specificity, and versatility to chemical analysis, but it does not always discriminate between geometrical and positional isomers and has not been widely used for chiral analysis. The earliest chiral distinction by mass spectrometry was made by Fales and Wright [11] in 1977. These authors showed that the chirality of dialkyl tartrates affects the relative abundance of the respective proton-bound dimers. Because most biologically important chiral compounds such as amino acids and sugars are nonvolatile, most chiral recognition studies [12–20] by mass spectrometry followed upon the introduction of fast-atom bombardment [21] and electrospray ionization (ESI) [22].

Because chiral recognition requires multiple-point interactions, it is no surprise that many studies utilize host–guest complexation. In these host–guest systems, the extent of chiral discrimination is determined by measuring the relative peak intensity where adduct ion abundance of the analyte with a host molecule is compared to that of a reference. The enantiomeric effect is then expressed as the ratio of relative peak intensities for the pair of enantiomers being examined [12,23,24]. However, this method is rather tedious because it requires that two successive measurements be made under the same conditions. Alternatively, a labeled form of one of the enantiomers can be used and this uses the same principle with an isotopically labeled enantiomer of the guest [17,25]. An obvious disadvantage is that the labeled guest is not always accessible. Similarly, reaction rates of guest exchange of a guest–host complex by a chiral reagent have been shown to be able to differentiate amino acids hosted by cyclodextrins [26], but quantitative analysis is yet to be shown. Instead of making a kinetic measurement, equilibrium constants can be deduced from ion abundance measurements and used for chiral recognition [27] and for the determination of enantiomeric composition [28]. The equilibrium method is confined to volatile chiral compounds at this stage. Equilibrium conditions allow the correlation of chiral distinction with free energy change in complexation, which reveals information on intrinsic chiral distinction. Tandem mass spectrometry was also used to achieve chiral recognition. For example, the kinetic method has been used to differentiate stereoisomers [18] by dissociation of proton-bound diastereomeric complexes and for chiral recognition of amino acids [29] through trimeric cluster dissociation by relating the fragment ion abundance ratio to fundamental thermochemical properties. Differences in kinetic energy release upon complex ion dissociation [13,20,30] can also be used to differentiate stereoisomers although extension of the method to chiral analysis has not been shown.

Recently, Yao et al. [31] showed that the dissociation of proton-bound trimeric clusters using non-amino acid compounds as references promise chiral recognition of amino acids. Previously, we had demonstrated that the dissociation kinetics of transition metal ion Cu(II)-bound trimeric complexes of amino acids, generated by electrospray ionization, can be used to distinguish the enantiomers of most of the natural amino acids [32]. This approach has potential for enantiomeric composition analysis [32]. In this article, we explore whether this approach can be applied using a different transition metal ion; we have selected nickel for this purpose.

The transition metal ion Ni(II)-bound complexes are generated by electrospraying an aqueous mixture of $NiCl_2$, the analyte amino acid and a chiral reference compound (an L-amino acid in this work). The mass-selected trimeric clusters dissociate to form two dimeric clusters exclusively, without other competitive or consecutive fragmentations, as shown by

$$[Ni^{II}(A)(ref^*)_2 - H]^+ \begin{cases} \xrightarrow{k_A} [Ni^{II}(A)(ref^*) - H]^+ + ref^* \\ \xrightarrow{k_{ref^*}} [Ni^{II}(ref^*)_2 - H]^+ + A \end{cases} \quad (1)$$

The branching ratio (R) can be represented by the fragment ion abundance ratio,

$$R = k_A/k_{ref*} = [Ni^{II}(A)(ref*) - H]^+/[Ni^{II}(ref*)_2 - H]^+ \quad (2)$$

where A is the analyte amino acid and ref* is the reference amino acid. Assuming the kinetic method treatment [32,33] holds, the natural logarithm of the ratio R will be proportional to the difference between the enthalpies of formation of the two dimeric products,

$$\ln(R) = \frac{\Delta Ni^{II}BDE[(A)(ref*)] - \Delta Ni^{II}BDE(ref*)_2}{\mathbf{R}T_{eff}} \quad (3)$$

where $\mathbf{R}$ is the gas constant, T_{eff} is the effective temperature of the dissociating complex, and $\Delta Ni^{II}BDE[(A)(ref*)]$ is defined as the enthalpy change for the reaction

$$A + ref* + Ni^{2+} \rightarrow [Ni^{II}(A)ref*) - H]^+ + H^+ \quad (4)$$

The reaction involves both deprotonation and binding to Ni^{2+}. The kinetic method treatment given here is based on energies but an analogous treatment using face energies is also possible. For a pair of enantiomers, given as A_D and A_L, the branching ratio is given as

$$R_D = [[Ni^{II}(A_D)(ref*) - H]^+]/[[Ni^{II}(ref*)_2 - H]^+] \quad (5)$$

$$R_L = [[Ni^{II}(A_L)(ref*) - H]^+]/[[Ni^{II}(ref*)_2 - H]^+] \quad (6)$$

The chiral resolution factor is then simply defined as

$$R_{chiral} = \frac{R_D}{R_L} = \frac{[[Ni^{II}(A_D)(ref*) - H]^+]/[[Ni^{II}(ref*)_2 - H]^+]}{[[Ni^{II}(A_L)(ref*) - H]^+]/[[Ni^{II}(ref*)_2 - H]^+]} \quad (7)$$

The enthalpic difference, $\Delta(\Delta Ni^{II}BDE)$, is related to the experimentally measured ratio, R_{chiral}, by the following equation:

$$\Delta(\Delta Ni^{II}BDE) = \Delta Ni^{II}BDE(A_D)(ref*) - \Delta Ni^{II}BDE(A_L)(ref*) = \mathbf{R}T_{eff}\ln(R_{chiral}) \quad (8)$$

Therefore, a nonzero $\Delta(\Delta Ni^{II}BDE)$ or a nonunit R_{chiral} value indicates successful chiral differentiation.

The simplest form of kinetic method treatment predicts that the logarithm of the fragment ion abundance ratio for dissociation of trimeric clusters constituted from a given enantiomer is linearly proportional to the energy change of the two competitive channels leading to the corresponding dimeric clusters as shown in Eq. (1). Given that the respective dissociation processes for a pair of enantiomers have different energy changes, the energy change from a sample that is enantiomerically impure should be linearly proportional to the enantiomeric composition of the analyte in the sampled trimeric clusters. Therefore, the logarithm of the fragment ion abundance ratio obtained from the sample should be linearly proportional to the enantiomeric composition of the sample. The expected linear relationship between $\ln(R)$ and the enantiomeric composition (molar fraction of an enantiomer) of the sample was demonstrated experimentally as is discussed later. An alternative method [33] of quantitative analysis of the enantiomeric composition of an analyte requires two independent experiments. One is performed with D-enantiomer of the reference compound and the other with L-enantiomer. In this method, the trimeric cluster ion $[Ni^{II}(A)_2(ref*) - H]^+$ instead of $[Ni^{II}(A)(ref*)_2 - H]^+$ is isolated for the collision-induced dissociation (CID) experiments. Based on an analogous derivation, a linear relationship is expected between the logarithm of the ratio of the fragmentation ion abundance ratio (R'_{chiral}) and the enantiomeric composition of the analyte. Although this approach was not explored here, it has been shown to be a feasible alternative [33].

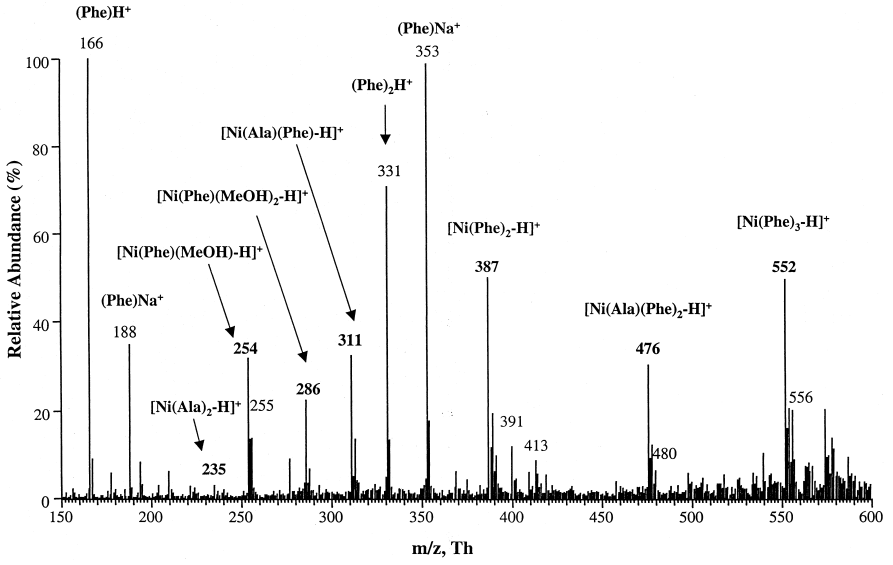


Fig. 1. Electrospray mass spectrum of a sample containing L-Ala (1.33×10^{-4} M), L-Phe (1.33×10^{-4} M) and $NiCl_2$ ($3.33\times10^{\times5}$ M) in a 1:1 water/methanol solution.

2. Experimental

All experiments were performed using an LCQ ion trap mass spectrometer (ThermoQuest, San Jose, CA) equipped with an electrospray ionization source and a syringe pump. For the ion trap mass analyzer, the AGC settings were 5×10^7 counts for a full-scan mass spectrum and 2×10^7 counts for a full-scan product ion spectrum with a maximum injection time of 200 ms. The spectra shown represent the average of about 30 scans where each scan is an average of five individual microscans. Operating conditions in the ESI source were as follows: spray voltage, 4.50 kV; capillary voltage, 3 V; heated capillary temperature, 150 °C; tube lens offset voltage, 20 V; sheath gas (N_2), 30 units (roughly 0.45 L/min). All experiments were performed in the positive ion mode. The sample was infused via the syringe pump at a flow rate of 1.0 μL/min. The Mathieu q_z values used for resonance excitation and ejection were 0.25 and 0.83, respectively, whereas isolation was achieved by the stored waveform inverse Fourier transform method through broadband excitation of the undesired ions. Fragmentation was achieved by collisions of the isolated clusters with buffer gas (He) during an excitation period of 30 ms. Data were obtained at a collision energy chosen such that the total fragment ion abundances amounted to about 20% of that of the selected precursor ions. Mass/charge ratios (m/z) are reported in Thomson (Th) where 1 Th=1 Da/unit charge [34].

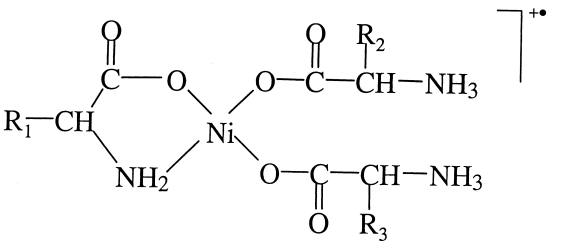
Optically pure amino acids and nickel (II) chloride were purchased from Sigma Chemical Co (St. Louis, MO) and used as received. Methanol (HPLC grade) was obtained from Mallinckrodt Baker Inc. (Paris, KY). Stock solutions of the amino acids (4×10^{-4} M) and $NiCl_2$ (1×10^{-4} M) were prepared in a 50:50 water/methanol mixture. The samples used for the experiments were made by mixing 1.0 mL each of the stock solutions of analyte amino acid, reference amino acid, and $NiCl_2$.

3. Results and discussion

3.1. Formation and dissociation of Ni(II)-bound cluster ions

Abundant clustering was observed in the ESI mass spectrum of mixtures of amino acids and $NiCl_2$. A

typical electrospray mass spectrum of such a mixture, that of L-alanine (L-Ala) and L-phenylalanine (L-Phe), is shown in Fig. 1. The spectrum is comprised of several types of ions, including relatively abundant protonated and sodiated amino acids and the corresponding homo and heterodimeric clusters. The presence of sodiated clusters is due to adventitious sodium. The absence of $(Ala)H^+$ and $(Ala)_2H^+$ is ascribed to the lower proton affinity of L-Ala (901.6 kJ/mol) as compared to L-Phe (922.9 kJ/mol) [35]. Similarly, the greater cation affinity of methanol compared to water results in the formation of solvated ions containing methanol. For example, singly charged Ni(II)-bound heteroclusters containing solvent molecules are observed at *m/z* 254 and 286, as evidenced by the presence of the characteristic Ni isotope ions. This is in agreement with observations made previously with Cu(II) [32] and also with Ni(II) data reported by Lavanant et al. [36]. The most interesting classes of ions are the singly charged Ni(II)-bound amino acid dimers and trimers formed by way of deprotonation of one of the constituent amino acids while the remaining amino acids bind to Ni(II) covalently. The latter bond is either through the neutral amino nitrogen atom or through the carboxylate group of the zwitterionic form of the amino acid. The trimeric clusters are believed to assume one of the two structures illustrated in Scheme 1, the distinction between which is not important to this study. The trimers were further examined by tandem mass spectrometry (MS/MS) as detailed in the following. Assignment of these electrospray-generated ions is also detailed in Table 1.



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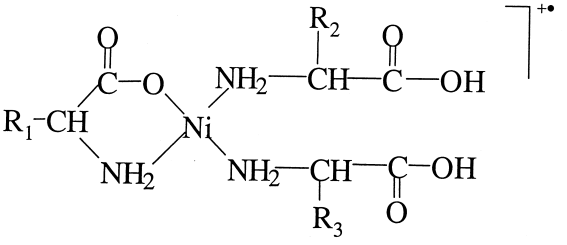

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Scheme 1. Possible structures of a Ni(II)-bound trimeric cluster ion composed of two reference amino acid and one analyte amino acid.

The dissociation of representative ions of each type discussed previously was examined by CID in the quadrupole ion trap. The protonated amino acids, as expected, lose ammonia. The protonated amino acid dimers, on the other hand, exhibit competitive fragmentation by loss of intact neutral amino acids, to an extent which correlates with their relative proton affinities [37–39]. The Ni(II)-bound amino acid dimeric and trimeric clusters behave quite differently to the simple proton-bound dimers and trimers. The dimeric clusters fragment by loss of amino acid-derived small molecules CO_2 and H_2O. This behavior is similar to that of the corresponding Cu(II)-bound dimeric clusters which show CO_2 loss. The Ni(II)-bound trimeric amino acid clusters dissociate via competitive losses of intact amino acid molecules and this dissociation behavior provides the basis for enantiomeric distinction.

The product ion spectra of the diasteriomeric complexes $[Ni^{II}(D\text{-Val})(L\text{-Phe})_2-H^+$ and $[Ni^{II}(L\text{-Val})(L\text{-Phe})_2-H]^+$ are shown in Fig. 2. The difference in the branching ratios for the two trimeric clusters is obvious, i.e. the R values, $[Ni^{II}(Val)(L\text{-Phe})-H^+/\ [Ni(L\text{-Phe})_2-H^+$, are different for D- and L-Val. The corresponding R_D and R_L values for the respective valine clusters are 0.51 and 0.32 and the indicator of chiral resolution, the ratio of the ratios ($R_{chiral}=R_D/R_L$), is 1.6 for this enantiomeric pair. This ratio is related by kinetic method arguments, given previously, to the parameter, $\Delta(\Delta Ni^{II}BDE)$, which is

Table 1
Observed ions[a] in the ESI mass spectrum of a mixture L-Ala, L-Phe, and $NiCl_2$

m/z	Type of ion	Composition
166	Protonated	$(Phe)H^+$
188	Sodiated	$(Phe)Na^+$
210	di-sodiated	$(Phe - H + Na)Na^+$
235	Ni bound	$[Ni^{II}(Ala)_2 - H]^+$
254	Ni bound	$[Ni^{II}(Phe)(MeOH) - H]^+$
255	Protonated	$(Phe)(Ala)H^+$
277	Sodiated	$(Phe)(Ala)Na^+$
286	Ni bound	$[Ni^{II}(Phe)(MeOH)_2 - H]^+$
311	Ni bound	$[Ni^{II}(Phe)(Ala) - H]^+$
331	Protonated	$(Phe)_2H^+$
353	Sodiated	$(Phe)_2Na^+$
387	Ni bound	$[Ni^{II}(Phe)_2 - H]^+$
476	Ni bound	$[Ni^{II}(Ala)(Phe)_2 - H]^+$
552	Ni bound	$[Ni^{II}(Phe)_3 - H]^+$

[a] Not reported are background ions at *m/z* 391 and 413, *m/z* 480, the adduct ion of *m/z* 391 with a molecule of alanine, and *m/z* 556, the adduct ion of *m/z* 413 with a molecule of phenylalanine.

indicative of chiral recognition. Using an effective temperature of 350 K, a value taken from a study of lithium and sodium ion binding energies of N-terminal modified amino acids [40] by the kinetic method in an ion trap mass spectrometer, the $\Delta(\Delta Ni^{II}BDE)$ is estimated to be 1.3 kJ/mol. Higher values of T_{eff} would make this difference even smaller and we conclude that the two $\Delta Ni^{II}BDE$ values differ by less than 2 kJ/mol. In spite of this small value, the chiral distinction is readily made.

3.2. Selection of reference amino acids

It has been shown in the previous study using Cu(II) complexes of amino acids, that optimal resolution is obtained when the reference amino acid contains an aromatic side chain, as in the case of phenylalanine. Because of the differences in the electronic structures of Ni(II) and Cu(II), the effect of the choice of chiral reference amino acid has been re-examined in the present study. For each analyte amino acid of interest, selected reference amino acids were examined for chiral recognition. Note that if the difference in metal ion affinities between the analyte and the reference is too large, the dissociation of the

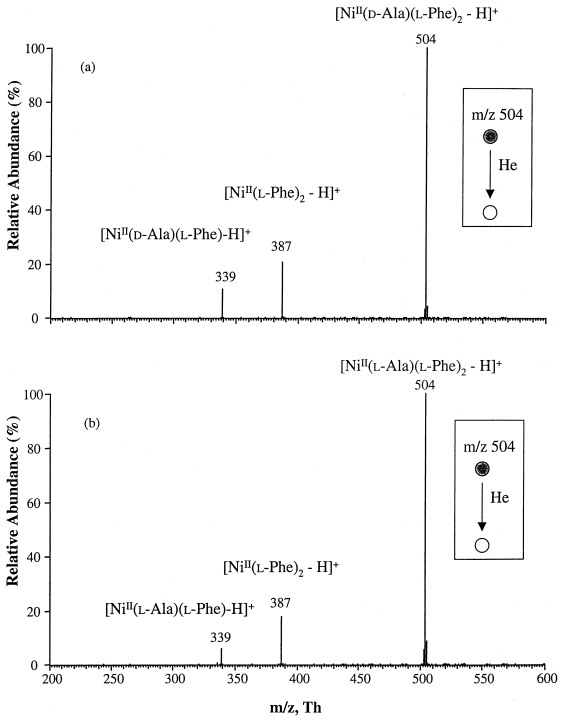

Fig. 2. Tandem mass spectrometric product ion spectra of (a) $[Ni^{II}(D\text{-}Ala)(L\text{-}Phe)_2 - H^+$ and (b) $[Ni^{II}(L\text{-}Ala)(L\text{-}Phe)_2 - H^+$. The data were recorded in the full-scan MS/MS mode employing an isolation width of 4 Th and a nominal collision energy of 10% CID (250 mV zero-to-peak ac excitation).

trimeric cluster predominantly favors only one fragmentation channel and this prevents accurate measurement of the abundance of the other fragment ion. Hence, it is essential to select a reference amino acid of appropriate metal ion affinity.

The results for the cases examined are tabulated in Table 2. They reveal the following points (1) the presence of an aromatic side chain enhances chiral resolution as evidenced by the data obtained with L-Phe and L-Trp as references for all four analytes. (2) A hydroxyl group in the side chain appears to have a negative effect on chiral resolution, as indicated by the results using L-Ser and L-Thr as references—almost no chiral resolution was observed in the cases of leucine and threonine and a much smaller resolution in the case of tyrosine. This effect is further exhibited in the case of serine, where L-Tyr failed to allow chiral resolution, implying that the presence of

Table 2
Chiral recognition of selected amino acids using various reference compounds

Analyte	Reference	Ratio of products[a]		R_{chiral}	Δ(PA)[b] (kJ/mol)	Δ(ΔNi^{II}BE) (kJ/mol)
		R_D	R_L			
Leucine	L-Val	2.49	2.50	1.00	4.0	0.00
	L-Ser	2.05	2.08	0.99	4.0	−0.03
	L-Pro	0.0880	0.096 4	0.91	−5.9	−0.27
	L-Phe	0.715	0.533	1.34	−8.3	0.85
	L-Tyr	0.312	0.220	1.42	−11.4	1.02
Threonine	L-Ser	23.0	23.1	1.00	7.9	0.00
	L-Ile	1.34	1.15	1.16	5.1	0.43
	L-Pro	0.323	0.283	1.14	2.0	0.38
	4-OH-L-Pro	0.233	0.215	1.08	…	0.22
	L-Phe	1.51	1.21	1.25	−0.4	0.65
	L-Tyr	0.719	0.619	1.16	.5	0.43
Serine	L-Ala	25.6	24.4	1.05	13.0	0.14
	L-Pro	0.078	0.084 3	0.925	−5.9	−0.23
	L-Phe	0.259	0.247	1.05	−8.3	0.14
	L-Tyr	0.115	0.115	1.00	−11.4	0.00
	L-Trp	0.0122	0.010 1	1.21	−34.3	0.56
Tyrosine	L-Ile	40.1	17.4	2.30	8.6	2.42
	L-Thr	2.95	2.41	1.22	3.5	0.58
	L-Phe	9.05	4.33	2.09	3.1	2.14
	L-Asn	0.0032	0.001 44	2.22	−3.0	2.32
	L-Trp	0.161	0.535	0.30	−22.9	−3.50

[a] Data were collected at a nominal CID energy of 10% with an isolation width of 4 Th.
[b] Values are calculated from proton affinities obtained from [35].

OH negated the effect of the aromatic ring. (3) There appears to be no correlation between the proton (or cation) affinity difference and the ease of chiral recognition in the cases examined here. (4) Serine, threonine, and aliphatic amino acids show the smallest degree of chiral resolution, which is most readily attributed to the lack of interaction between the analyte and reference amino acids. In these cases, compounds other than amino acids might be effective references.

3.3. Chiral recognition of other amino acids

Based on the results obtained above, appropriate reference amino acids were selected for each amino acid on a case by case basis, as summarized in Table 3. As the Ni(II) affinities of the amino acids are unknown, the choice of the reference amino acid was made, in part, by considering the proton affinity differences between the analyte and reference compounds. However, proton affinity is not always a consistent measure of the metal ion affinity [41]. For example, the acidic amino acids aspartic acid and glutamic acid, whose proton affinities [35] (908.9 and 913 kJ/mol) are both lower than phenylalanine (922.9 kJ/mol), bind much more strongly to Ni(II) than does phenylalanine. As a result of this, when L-Phe was used as the reference amino acid for chiral recognition of these two acidic amino acids, the branching ratios of the corresponding trimeric clusters are far greater than expected from the proton affinity difference. Such cases were also found in other analyte/reference combinations such as Pro/Phe, Cys/Asn, Asn/Gln, Lys/His, and Arg/Lys, as shown in Table 3. Among other considerations, therefore, a major criterion for the reference selection is that the resulting R values for the fragment abundance ratio should allow accurate measurement of the abundances of both fragment

Table 3
Chiral distinction observed with selected reference compounds

Analyte,[a] A	Reference,[a] ref*	Ratio of products[b]		R_{Chiral}	$\Delta(\Delta Ni^{II}BDE)$ (kJ/mol)
		R_D	R_L		
Valine	L-Phenylalanine	0.509	0.322	1.58	1.33
Leucine		0.600	0.496	1.21	0.56
Proline		20.5	11.6	1.77	1.66
Alanine		0.0427	0.0351	1.22	0.58
Isoleucine		1.21	0.733	1.65	1.46
Aspartic acid		15.3	6.44	2.38	2.19
Glutamic acid		22.2	8.38	2.65	2.84
Serine		0.323	0.292	1.11	0.30
Threonine		1.51	1.28	1.18	0.48
Tyrosine		8.98	3.82	2.35	2.49
Phenylalanine	L-Tryptophan	0.0881	0.0290	3.04	3.26
Tryptophan	L-Asparagine	1.10	0.140	7.86	6.00
Glutamine		17.5	6.64	2.64	2.82
Cysteine		0.610	0.786	0.78	−0.72
Asparagine	L-Glutamine	5.69	2.21	2.57	2.75
Methionine		0.194	0.144	1.35	0.87
Lysine	L-Histidine	0.114	0.134	0.85	−0.47
Arginine	L-Lysine	0.288	0.425	0.68	−1.14
Histidine	L-Arginine	0.0848	0.117	0.73	−0.94

[a] For a trimeric cluster ion, $[Ni^{II}(ref^*)_2(A) - H]^+$, ref* designates the reference amino acid and A the analyte.
[b] Refers to the branching ratio of $[Ni^{II}(A)(ref^*) - H]^+/[Ni^{II}(ref^*)_2 - H]^+$. Collision is caused by dipolar ac excitation in He buffer gas, and the voltage used ranges from 238 to 263 mV to ensure fragmentation of ~20% of parent ions.

ions. All 19 enantiomeric pairs of amino acids were measured and they showed chiral resolution factors (R_{chiral}) ranging from 1.11 to 7.86 (Table 3). Because the choice of reference for a given amino acid is not limited to those examined here, it is likely that the chiral resolution achieved in these experiments is not the best obtainable. It is advisable that the reference for a particular amino acid be re-evaluated to ensure optimal results.

3.4. Effects of mixing ratio of analyte versus reference on chiral distinction

Chiral discrimination achieved on the basis of the method proposed above involves Ni(II)-bound trimeric clusters which are proposed to have structures illustrated in Scheme 1. These suggested structures are based on data for the corresponding copper complexes [33,42]. In **1** the deprotonated amino acid is covalently bound to Ni(II) while the other two amino acids bind to Ni(II) through deprotonated carboxylate group of the zwitterions. Alternatively, binding is through the amino nitrogens of the neutral ligands in **2**. The distinction between **1** and **2** is not attempted. Because there are three amino acid molecules involved in the formation of this cluster, it is possible that different amino acids could exist in the deprotonated form in a set of isomeric structures. This might affect chiral recognition based on dissociation data.

As a further test of whether isomeric forms of the trimeric clusters are being sampled, a solution composed of three amino acids, L-valine, L-leucine, and L-alanine, was examined. A Ni(II)-bound trimeric cluster containing all three amino acids, $[Ni^{II}(Val)(Leu)(Ala) - H^+$ (*m/z* 394), was observed in the mass spectrum. When this cluster was subjected to CID, three different dimeric clusters were produced, as shown in Fig, 3 suggesting that the dissociating trimeric clusters do indeed exist in more than one isomeric form. Consequently, if the relative proportion of amino acids dictates the extent of formation of the different isomeric clusters, it will likely affect the

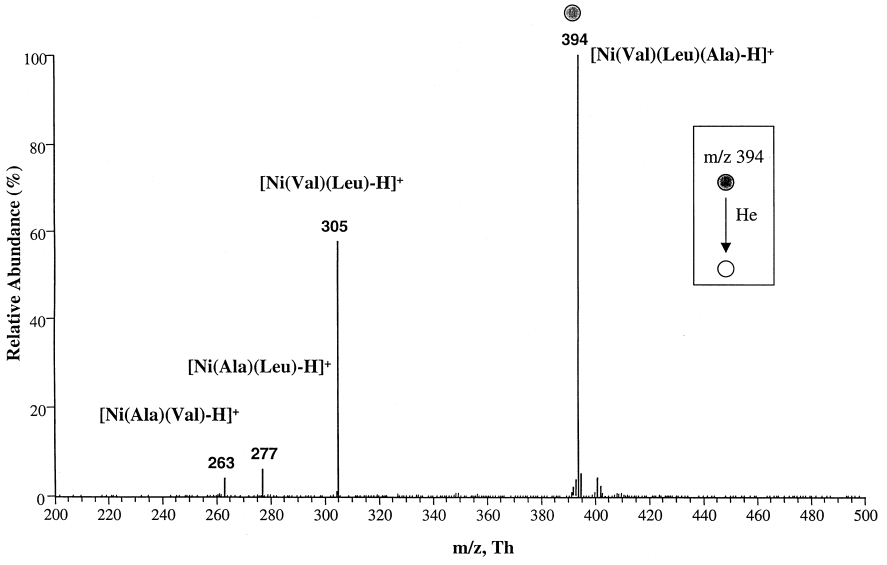

Fig. 3. Tandem mass spectrum of a Ni(II)-bound heterotrimeric cluster ions, $[Ni^{II}(Val)(Leu)(Ala)-H^+$, which contains three different L-amino acids and fragments to yield three different dimeric fragment ions. The data were recorded in the full-scan MS/MS mode employing an isolation width of 4 Th and a nominal collision energy of 9% CID (225 mV zero-to-peak ac excitation).

branching ratio unless interconversion between the isomers occurs. Evidence for such inter-conversion is supplied in the experiment now described.

The dependence of the branching ratio on the relative concentrations of amino acids was studied by varying the concentrations of amino acids, L-Val and L-Leu. Both trimeric clusters $[Ni(L\text{-}Leu)_2(L\text{-}Val)-H^+$ and $[Ni(L\text{-}Val)_2(L\text{-}Leu)-H^+$ were examined in the range of amino acid concentration ratios between 4:1 to 1:4. In all cases, the formation of both trimeric clusters was favored at higher L-Val concentration, however, no measurable concentration effects were observed on the branching ratio. The measured ratios for R_L(Val) and R_L(Leu) are 1.74 ± 0.01 and 2.41 ± 0.02, respectively. Experiments using L-Ala and L-Tyr gave similar results. For the case of tryptophan as analyte and L-asparagine as reference, the measured R_D and R_L values were 1.15 ± 0.02 and 0.163 ± 0.008, respectively, when averaged over the concentration ratio range of 1:4 to 4:1. Therefore, it is concluded that relative concentrations of analyte versus reference do not affect the chiral recognition in these systems. It is likely that equilibrium exists between the dissociating clusters controlled by the nature of the constituent amino acids rather than being controlled by their proportions in the gas phase. This is a fortunate result since it allows quantitative analysis of unknown samples.

3.5. Enantiomeric purity measurement

According to the thermochemical treatment presented earlier, a linear relationship is expected between the enantiomeric composition of the analyte on the one hand, and the natural logarithm of *R*, the branching ratio for a Ni(II)-bound trimeric monodeprotonated complex comprised of two reference amino acids and one analyte amino acid, on the other. This was empirically demonstrated for the case where tyrosine is the analyte and L-phenylalanine is the reference. The experiments were performed using solutions having a constant reference concentration and also a constant analyte concentration, but with varying proportions of the D and L enantiomers. The mass-selected trimeric cluster ion $[Ni^{II}(Tyr)(L\text{-}Phe)_2-H^+$ was collisionally dissociated and the fragment ion abundances measured. The natural logarithm of $[Ni^{II}(Tyr)(L\text{-}Phe)-H^+$/ $[Ni^{II}(L\text{-}Phe)_2-H^+$ plotted

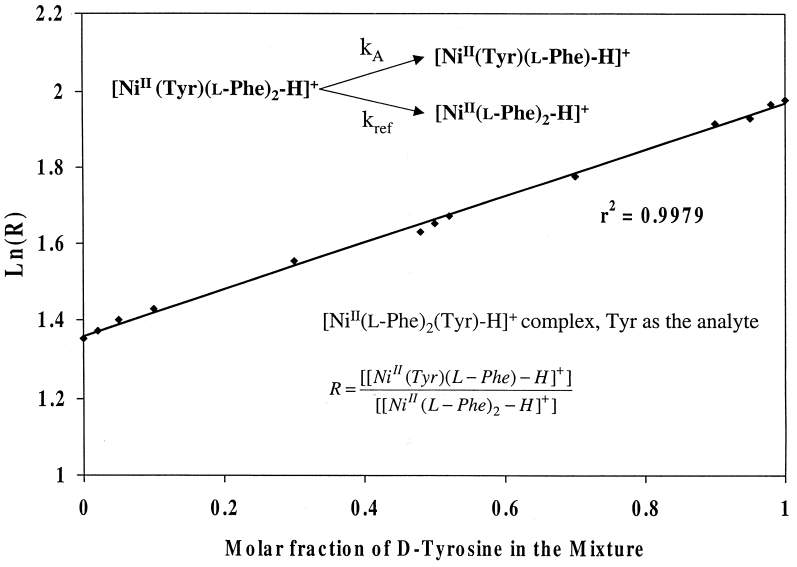

Fig. 4. Chiral analysis by the kinetic method. Mixtures containing tyrosine with varying enantiomeric compositions yield trimeric ions which dissociate competitively to show a linear relationship between the logarithm of the branching ratio, ln(*R*), and the molar fraction of D-tyrosine.

as a function of molar fraction of D-tyrosine in each sample is shown in Fig. 4. A linear relationship is clearly evident from the graph and the regression r^2 value is 0.9979. The ability to analyze samples with small optical purity differences by this method is noted. The data for the samples having nearly pure D and nearly pure L fall on the same straight line. The direct measurements at 3% ee (enantiomeric excess) and the good linear relationship suggest that this method is capable of measuring very small differences in enantiomeric composition. Note that the results shown in Fig. 4 come from a single set of measurements and the uncertainty associated with these measurements was estimated to be 3%.

Conclusion

Chiral distinction of all nineteen chiral amino acids is achieved by examination of the dissociation kinetics of their Ni(II)-bound, singly charged, trimeric complexes. The kinetic method provides the basis for understanding this method of chiral recognition in the gas phase. The ready oxidation of cysteine to cystine by Cu(II) precluded use of the copper ion but this reaction could be avoided by studying the Ni(II) complex. The relative proportion of analyte to reference in the sample solution does not appear to result in measurable effects on the chiral resolution factor, a result that is likely to be due to an equilibrium between the isomeric forms of the trimeric cluster ions being sampled. The linear relationship observed between ln(*R*) and enantiomeric composition suggests that this method can be used for quantitative measurements of enantiomeric composition of amino acids, as demonstrated with tyrosine, where 3% ee can be measured directly. Application of this method to other classes of compounds, including dipeptides, is currently under investigation.

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