Chiral Recognition in High Performance Liquid Chromatography/Atmospheric Pressure Chemical Ionization Mass Spectrometry

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The stereospecific ion-molecule reaction between optically resolving agents (a pair of enantiomers of mandelic acid, methyl mandelate, and 1-phenylethylamine) and several enantiomers of amino acids were investigated by high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (HPLC/APCIMS). On the APCI mass spectra, the 1 : 1 adduct ion $[M\cdots H\cdots X]^+$ that the amino acid molecule $[M]$ reacted with the protonated optically resolving agent in mobile phase $[XH]^+$ has been prominently observed. The results show that the relative abundances of 1 : 1 adduct ions $[M\cdots H\cdots X]^+$ formed from samples (amino acids) and reagents (resolving agent) of the same configuration (*homo-chiral*) are much higher than those of different configurations (*hetero-chiral*). To support this hypothesis, we carried out X-ray crystallographic analysis on diastereomeric complexes of (*S*)-phenylalanine with (*R*)- and (*S*)-mandelic acid for a resolving agent as a model compound. The crystal structure obtained was in accordance with the stereochemical effects observed in APCIMS.

1. Introduction

One of the most important problems in mass spectrometry (MS) is chiral discrimination as stereochemical methodology. The chiral discriminations in MS have been the subject of intense scrutiny in decade year.^{1)~10)} It is noteworthy that chiral recognition in chemical ionization mass spectrometry (CIMS) has been performed under chiral conditions using an optically resolving agent as CI reactant.^{1)~5)} The basic principle of chiral recognition in CIMS based on the differentiation of these diastereomeric adduct ions. High performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometry (HPLC/APCIMS) has been found to be one of the most powerful techniques for structural analyses of pharmaceuticals and natural products, further more stereochemical studies. In the APCIMS under chiral conditions, the 1 : 1 adduct ion $[M\cdots X\cdots H]^+$ has been assumed to form stereoselective hydrogen bonded association complex at appropriate conditions. We have tried to carry out this stereospecific ion-molecule reaction between optically resolving agents (a pair of enantiomers of mandelic acid, methyl mandelate, and 1-phenylethylamine) and several enantiomers of amino acids (Scheme 1).

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Scheme 1

It is predicted based on previous papers^{3), 4)} that the relative abundances of 1 : 1 adduct ions $[\text{M}\cdots\text{H}\cdots\text{X}]^+$ formed from samples (amino acids) and reagents (resolving agent) of the same configuration (*homo-chiral*) are much higher than those of different configurations (*hetero-chiral*). Chiral recognition mechanism at the interface between crystal and solution had been studied by mainly X-ray crystallographic analysis.^{11), 12)}

We suggest that chiral sample and the resolving agent form an adduct ion through diastereoselective hydrogen bonding. To support this hypothesis, we carried out X-ray crystallographic analysis on a complex of an amino acid and a resolving agent as a model compound. This method would be expected to investigating chiral recognition in MS. This paper describes the chiral recognition through ion-molecule reaction between optically resolving agents with optically active amino acids in APCIMS.

2. Experimental

2.1 HPLC/APCI mass spectra

All mass spectra were measured with a Hitachi M 1000 LC/API mass spectrometer combined with an L-6200/L-6000 high performance liquid chromatograph system. A pump (L-6200 system; Hitachi, Japan) was used to deliver methanol as the mobile phase to the column (ODS, 10 mm length $\times$ 4.6 mm i.d.) at a flow rate 0.9 ml/min. Forty mm methanol solution of an optically resolving agent at flow rate 0.1 ml/min was delivered by post column addition using a pump (L-6000 system). Then, this two flows were mixed with a tee joint and introduced to the mass spectrometer. An aliquot of 5 μ l amino acid solution (10 mm methanol solution) was injected each time. Each sample was run three or five times under constant condition. The intensities of characteristic ions were determined on the peak area. Typical conditions of the LC/APCIMS were as follows; nebulizer temp. 200°C, vapourizer temp. 380°C, and drift volt. 100–120 V.

2.2 Chemicals

The optically resolving agents and amino acids were purchased from Wako Pure Chemical Industries Ltd. (Japan). D-(–)- and L-(+)-alanine: (R)-Ala and (S)-Ala (MW=89), D-(+)- and L-(–)-phenylalanine : (R)-Phe and (S)-Phe (MW=165), D-(–)- and L-(+)-phenylglycine: (R)-Pgly and (S)-Pgly (MW=151), D-(–)- and L-(+)-methionine: (R)-Met and (S)-Met (MW=149), D-(–)- and L-(+)-tryptophan: (R)-Try and (S)-Try (MW=204), D-(–)- and L-(+)-2-aminobutyric acid: (R)-Aba and (S)-Aba (MW=103) were chromatographically pure. The optically resolving agents were redistilled and/or recrystallized and determined the optical rotation as shown following; (R)-1-phenylethylamine; (R)-PEA: $[\alpha]^{20} = +36.5^\circ$, (S)-1-phenylethylamine; (S)-PEA: $[\alpha]^{20} = -36.5^\circ$, (R)-mandelic acid; (R)-MA: $[\alpha]^{20} = -153.0^\circ$, (S)-mandelic acid; (S)-MA: $[\alpha]^{20} = +153.0^\circ$, (S)-methyl mandelate;

(S)-MAM: $[\alpha]^{20} = +144.0^\circ$. Other chemicals were used HPLC grade.

2.3 X-Ray crystallographic analysis

X-Ray crystallography was performed on 1 : 1 diastereomeric complexes; (S)-MAM $\cdots$ (R)-Phe and (S)-MAM $\cdots$ (S)-Phe as the model compounds of 1 : 1 adduct ion for the enantio-selective reaction in APCI-MS using a AFC-5R Four Circle Diffractometer (Rigaku Denki) equipped with NEWS-3860 (Sony). X-Ray of Cu- $K_\alpha(\lambda) = 1.5418\text{\AA}$ was used. The structural analysis were performed using SIR 85. The crystal data: (S)-MAM $\cdots$ (R)-Phe; $a = 19.895\text{\AA}$, $b = 5.557\text{\AA}$, $c = 17.095\text{\AA}$, $\beta = 123.83^\circ$, $U = 1570.1\text{\AA}^3$, monoclinic C2, $Z = 4$, final $R = 0.035$. (S)-MAM $\cdots$ (S)-Phe; $a = 19.389\text{\AA}$, $b = 5.725\text{\AA}$, $c = 15.784\text{\AA}$, $\beta = 115.25^\circ$, $U = 1581.1\text{\AA}^3$, monoclinic C2, $Z = 4$, final $R = 0.035$.

3. Results and Discussion

The stereospecific ion-molecule reaction in LC/APCIMS between optically resolving agents (a pair of enantiomers of MA, MAM, and PEA) and several enantiomers of amino acids were investigated. The stereochemical effect could be observed from the diastereomeric adduct ions reacted with chiral molecule and chiral reactant ion. The formation of the adduct ion is especially affected nebulizer temperature and drift voltage. The most suitable condition for appearance of the remarked adduct ion was required that protonated molecule $[XH]^+$ of MA, MAM, and PEA as reactant ion is much prominent. These ions $[XH]^+$, MA: m/z 153, MAM: m/z 167, and PEA: m/z 122 should keep the original chirality. Therefore, the adduct ion would be formed diastereomeric hydrogen-bonded association complex by stereospecific ion-molecule reaction with one of enantiomer of amino acid as target molecule (M). The reaction pathway for formation of the characteristic ions, 1 : 1 adduct ion $[M\cdots H\cdots X]^+$ and protonated molecule $[MH]^+$, would be suggested to be competing reaction as shown in Scheme 2. The reactions are most likely (1) association for formation of adduct ion, (2) proton transfer for $[MH]^+$, and (3) fragmentation for MH^+ .

The relative peak abundances of characteristic ions were different, depending on the absolute configuration of M and X. The ease of formation of the characteristic ions are contributed by proton affinity (PA) of M with X. Since a pair of enantiomers is equivalent PA, it is reasonable to assume that such different relative peak abundances under same condition reflect the different stability of the diastereomeric adduct ions. It is considered that the stability closely related the stereochemical structure of the adduct ions reacted by enantioselectivity. Typical mass spectra of an amino acid with an optically resolving agent under the appropriate condition in each case were shown in Fig. 1. These mass spectra were demonstrated good reproducibilities as shown in Fig.

Scheme 2.

Fig. 1. Typical mass spectra of (S)-phenylalanine measured with (R)- and (S)-optically resolving agent. a-1) (S)-Phe with (R)-MA; a-2) (S)-Phe with (S)-MA; b-1) (S)-Phe with (R)-MAM; b-2) (S)-Phe with (R)-MAM; c-1) (S)-Phe with (R)-PEA; c-2) (S)-Phe with (R)-PEA. The operated conditions are shown in experimental.

Fig. 2. The reproducibilities of mass chromatograms and mass spectra (S)- and (R)-2-aminobutyric acids measured with (S)-mandelic acid. (S)-Aba with (S)-MA (upper); (R)-Aba with (S)-MA (bottom). The operated conditions are shown in experimental.

2. The coefficient of variation (C.V. %) of these measurements was less than 10%. These stereochemical effects were compared with the relative peak abundances of $[M \cdots H \cdots X]^+$ / $[XH]^+$ on the mass spectra and summarized in Tables 1, 2, and 3. In all these cases, we have found the order of R - R adduct ion $> R$ - S or S - R adduct ion and S - S adduct ion $> S$ - R or R - S adduct ion. The results are shown a general rule that the relative abundances of 1:1 adduct ions $[M \cdots H \cdots X]^+$ formed from samples (amino acids) and reagents (resolving agent) of the same configuration (*homo-chiral*) are much higher than those of different configurations (*hetero-chiral*).

On the other hand, the optical resolution of racemic amino acid has been performed through diastereomeric crystallization and preferential crystallization was well known. MA is generally used as the acidic chiral resolving agent for the optical separation *via* diastereomeric salt.¹³⁾ MA is possible to separate, not only racemic organic amines but also some of racemic amino acids. When (S)-MA is used to the chiral separation of racemic amino acids, (S)-amino acid-(S)-MA 1:1 complex shows faster precipitation than its diastereomeric 1:1 complex in generally. This phenomenon is reflected the stability of salt crystal, it is seemed to be agreed with results of LC/APCI-MS through

Table 1. Relative Abundances of Characteristic Ions in the APCI Mass Spectra of α -Amino Acids with Mandelic Acid (MA) as Resolving Agent

α -Amino acid M	MA X	m/z (%)		A/B	$A/A^a)$	
		$[M\cdots H\cdots X]^+$ A	MH^+ B		1) $R-R/R-S$ 2) $S-S/S-R$	1) $R-R/S-R$ 2) $S-S/R-S$
Ala	<i>R</i>	<i>R</i>	242 (44.3)	90 (100)	0.44	
	<i>R</i>	<i>S</i>	(32.5)	(100)	0.33	1) 1.3
	<i>S</i>	<i>R</i>	(30.7)	(100)	0.31	
	<i>S</i>	<i>S</i>	(62.3)	(100)	0.62	2) 2.0
Phe	<i>R</i>	<i>R</i>	318 (75.3)	166 (100)	0.75	
	<i>R</i>	<i>S</i>	(53.0)	(100)	0.53	1) 1.4
	<i>S</i>	<i>R</i>	(45.6)	(100)	0.46	
	<i>S</i>	<i>S</i>	(84.6)	(100)	0.84	2) 1.8
Pgly	<i>R</i>	<i>R</i>	304 (75.3)	152 (100)	0.35	
	<i>R</i>	<i>S</i>	(24.0)	(100)	0.24	1) 1.5
	<i>S</i>	<i>R</i>	(22.6)	(100)	0.23	
	<i>S</i>	<i>S</i>	(39.4)	(100)	0.39	2) 1.7
Met	<i>R</i>	<i>R</i>	302 (67.3)	150 (100)	0.67	
	<i>R</i>	<i>S</i>	(42.4)	(100)	0.42	1) 1.6
	<i>S</i>	<i>R</i>	(43.3)	(100)	0.43	
	<i>S</i>	<i>S</i>	(58.2)	(100)	0.58	2) 1.7
Try	<i>R</i>	<i>R</i>	357 (33.3)	205 (100)	0.33	
	<i>R</i>	<i>S</i>	(13.8)	(100)	0.14	1) 2.4
	<i>S</i>	<i>R</i>	(31.0)	(100)	0.31	
	<i>S</i>	<i>S</i>	(60.5)	(100)	0.61	2) 2.0
Aba	<i>R</i>	<i>R</i>	256 (60.8)	104 (100)	0.61	
	<i>R</i>	<i>S</i>	(25.9)	(100)	0.26	1) 2.3
	<i>S</i>	<i>R</i>	(57.0)	(100)	0.57	
	<i>S</i>	<i>S</i>	(100)	(65.2)	1.53	2) 2.7

^{a)} Homo-chiral adduct ion/hetero-chiral adduct ion.

the enantio-selective reaction in the gaseous phase. Then, we performed the X-ray crystallography of 1 : 1 diastereomeric complex; Phe-(*S*)-MA as the model for the enantio-selective reaction of APCI-MS. Both diastereomeric complexes were recrystallized from aqueous solution. We showed the two crystal data described on this experimental. Both crystal are very similar to the point of lattice constants and space group except for the length of *c*-axis and β angle. (*Z* number means the number of the molecules in a unit cell.) Comparison with both crystal structures, the difference of the *c*-axis and β angle was revealed from the hydrogen bonding pattern caused by chiral discrimination. As shown in Fig. 3, the Packing diagrams of both crystals viewed along to the *b*-axis. Phe and (*S*)-MA are aligned parallel to the *a*-axis and formed infinite chain through the hydrogen bond. These hydrogen bonds were formed among the hydroxy functions of the mandelic acid, and carbonyl functions of MA and Phe. The infinite chains also

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Table 2. Relative Abundances of Characteristic Ions in the APCI Mass Spectra of α -Amino Acids with Methylmandelate (MAM) as Resolving Agent

α -Amino acid M		MAM X	m/z (%)		A/B	A/A ^{a)} S-S/R-S
			$[M\cdots H\cdots X]^+$ (A)	MH^+ (B)		
Ala	R	S	256 (59.6)	90 (100)	0.60	1.4
	S	S	(83.2)	(100)	0.83	
Phe	R	S	332 (53.2)	166 (100)	0.53	1.6
	S	S	(85.2)	(100)	0.85	
Pgly	R	S	318 (52.3)	152 (100)	0.52	1.2
	S	S	(62.8)	(100)	0.63	
Met	R	S	316 (44.6)	150 (100)	0.45	1.7
	S	S	(74.0)	(100)	0.74	
Try	R	S	371 (43.5)	205 (100)	0.44	1.7
	S	S	(73.5)	(100)	0.74	
Aba	R	S	270 (60.4)	104 (100)	0.60	2.0
	S	S	(100)	(84.0)	1.18	

^{a)} Homo-chiral adduct ion/hetero-chiral adduct ion.

Table 3. Relative Abundances of Characteristic Ions in the APCI Mass Spectra of α -Amino Acids with 1-Phenylethylamine (PEA) as Resolving Agent

α -Amino acid M		PEA X	m/z (%)		A/B	A/A ^{a)}	
			$[M\cdots H\cdots X]^+$ A	MH^+ B		1) R-R/R-S 2) S-S/S-R	1) R-R/S-R 2) S-S/R-S
Phe	R	R	287 (100)	166 (18.2)	5.55		
	R	S	(100)	(75.2)	1.33	1) 4.2	1) 8.3
	S	R	(67.5)	(100)	0.67	2) 2.8	2) 1.4
	S	R	(100)	(54.1)	1.85		
Met	R	R	271 (100)	150 (38.6)	2.59		
	R	S	(100)	(55.0)	1.82	1) 1.4	1) 5.6
	S	R	(46.0)	(100)	0.46	2) 7.2	2) 1.8
	S	S	(100)	(30.1)	3.32		
Try	R	R	226 (100)	205 (68.5)	1.46		
	R	S	(46.8)	(100)	0.47	1) 3.1	1) 2.2
	S	R	(65.8)	(100)	0.66	2) 2.5	2) 2.8
	S	S	(100)	(59.9)	1.67		

^{a)} Homo-chiral adduct ion/hetero-chiral adduct ion.

formed the hydrogen bond faced another infinite chain, but these hydrogen bonding pattern of both crystal structures were obviously different. In case of the (S)-(S) crystal which faster precipitated from mother liquid. The faced Phe and (S)-MA were bonded by two hydrogen bonds; $OH_{(MA)}\cdots O=C_{(Phe)}$ and $NH_{(Phe)}\cdots O=C_{(MA)}$. In the case of (R)-(S)

Fig. 3. Packing diagrams of diastereomeric phenylalanine... (S)-mandelic acid complexes.
 a) (S)-Phe... (S)-MA; b) (R)-Phe... (S)-MA.

crystal which slowly precipitated faced (R)-Phe and (S)-MA were bonded by only one bifurcated hydrogen bond. The difference of these hydrogen bonding pattern is seem to lead to the difference of the binding force at the pair of Phe and MA, also lead to the difference of the length of the c-axis and β angles. The crystal structure obtained was in accordance with the stereochemical effects observed in APCIMS.

In LC/APCIMS under the chiral condition, the absolute configuration of the sample molecule may be determined by measurement of mass spectra of the target sample with R- and S-optically resolving agent. This method is also expected to play an important role in future applications to understand biological molecule recognition.

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