Chiral recognition of amino acids by electrospray ionisation mass spectrometry/mass spectrometry

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Chiral recognition of 19 common amino acids is achieved by investigating the collision-induced dissociation spectra of protonated trimers formed by electrospray ionisation of amino acids in the presence of one of the following chiral selectors: L- and D-*N*-*tert*-butoxycarbonylphenylalanine (BPhe), L- and D-*N*-*tert*-butoxycarbonylproline (BPro) and L- and D-*N*-*tert*-butoxycarbonyl-*O*-benzylserine (BBSer).

Chiral recognition is an important topic in chemistry and biochemistry. A number of approaches[1] have been used for the chiral recognition of organic compounds, including polarimetry, circular dichroism, nuclear magnetic resonance, chromatography and capillary electrophoresis. Attentions has also been paid to the use of mass spectrometry[2,3]due to the many advantages of the technique, *e.g.* high sensitivity, short analysis time, ability to analyse mixtures by tandem mass spectrometry (MS/MS) or in combination with chromatography, and the ability to study the intrinsic properties of the chiral effect by isolating the interacting molecules in the gaseous phase.

Since the first observation of chiral effects in mass spectrometry by Fales and Wright,[4] about 50 papers have been published in this field.[3] In most of the studies, enantiomers were allowed to ionise in the presence of chiral selectors, and chiral recognition was observed by comparing the relative extent of the formation (MS) or fragmentation (MS/MS) of the diastereomeric complex ions. While chemical ionisation (CI)[5–8] was the main ionisation mode used in the early development of chiral mass spectrometry, significant results have been reported by applying the fast atom bombardment (FAB) mode.[9–14] A number of data evaluation methods are also available for the analysis, such as the relative peak intensity method (*i.e.* the internal standard method),[9] the deuterium-labelled method,[10] the stability constant method,[11] and the two-internal-standards method.[12] In spite of these efforts, only limited success has been achieved; so far, not even a single class of chiral compounds can be systematically recognised by mass spectrometry. In general, problems encountered in the systematic recognition of a class of chiral compounds include the limited recognition ability of chiral selectors and the poor reproducibility of mass spectra, as well as the requirement of high concentration or high volatility for the association between enantiomers and chiral selectors.

We now report the first systematic study of chiral recognition of 19 amino acids by comparing the relative extent of fragmentation (MS/MS signal intensities) of the diastereomeric protonated trimers formed by the amino acids and chiral selectors. Three new chiral selectors, namely, L- and D-*N*-*tert*-butoxycarbonylphenylalanine (BPhe), L- and D-*N*-*tert*-butoxycarbonylproline (BPro) and L- and D-*N*-*tert*-butoxycarbonyl-*O*-benzylserine (BBSer), were used in the present study. Electrospray ionisation (ESI)[15] was applied to provide complex ions of the amino acids and chiral selectors; a typical mass spectrum of the amino acid/chiral selector mixture is depicted in Fig. 1. Thus, the amino acid (X) combines with chiral selector (Y) to form protonated dimer XYH^+, protonated trimer XY_2H^+, protonated tetramer XY_3H^+ *etc*. The protonated trimers (XY_2H^+) were subsequently chosen for investigation because of their high intensities and large chiral discrimination toward most amino acids. The XY_2H^+ complex ion was fragmented by collision-induced dissociation (MS/MS) to form protonated dimers XYH^+ (Fig. 2). All amino acids were studied in four combinations with the chiral selectors, *i.e.* LD and DL (heterochiral) and LL and DD (homochiral), and the chiral recognition ratio *R* was defined according to eqn. (1).[14]

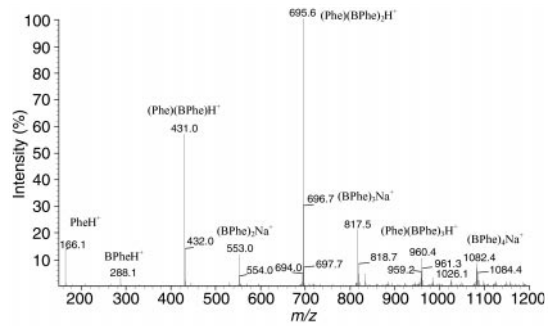

Fig. 1 ESI spectrum of a mixture of Phe (1 mM) and BPhe (1 mM). Major cluster ions are indicated. Conditions for ESI: heated capillary 50 °C, sheath gas 60 psi, spray voltage 4 kV, syringe pump 10 μL min^{-1}. Samples were run on a Finnigan LCQ mass spectrometer.

$$R = \frac{([XYH^+]/[XY_2H^+\})_{\text{hetero}}}{([XYH^+]/[XY_2H^+])_{\text{homo}}} = \frac{([XYH^+]/[XY_2H^+])_{\text{LD}} + ([XYH^+]/[XY_2H^+])_{\text{DL}}}{([XYH^+]/[XY_2H^+])_{\text{LL}} + ([XYH^+]/[XY_2H^+])_{\text{DD}}} \quad (1)$$

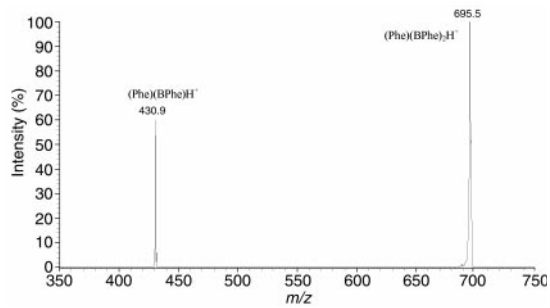

Fig. 2 MS/MS spectrum of protonated trimer from Phe and BPhe. Collision energy was 8%, isolation width 18 u, mass range 350–750 u.

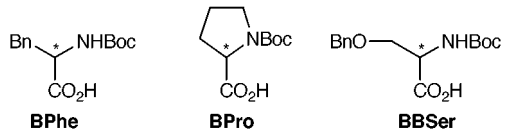

Table 1 Chiral discrimination of BPhe, BPro and BBSer toward amino acids as observed in the MS/MS spectra of the protonated trimers[a]

	BPhe			BPro			BBSer					
Amino acids	*R*	SD_R[b]	SD_R/R (%)	*R*	SD_R[b]	SD_R/R (%)	*R*	SD_R[b]	SD_R/R (%)	Suitable chiral selectors[c]		
Ala	1.044	0.006	0.59	1.074	0.006	0.56	1.143	0.012	1.05			BBSer
Arg[d]	1.416	0.007	0.49	2.027	0.015	0.74	0.640	0.005	0.73	BPhe,	BPro,	BBSer
Asn	0.938	0.003	0.34	1.163	0.004	0.34	0.992	0.011	1.06		BPro	
Asp	0.810	0.005	0.57	1.102	0.007	0.64	1.061	0.006	0.57	BPhe,	BPro	
Cys	1.054	0.006	0.55	1.122	0.005	0.45	1.262	0.009	0.71		BPro,	BBSer
Gln	1.905	0.013	0.66	2.042	0.009	0.44	2.953	0.039	1.32	BPhe,	BPro,	BBSer
Glu	0.855	0.003	0.32	1.752	0.010	0.57	0.615	0.006	0.99	BPhe,	BPro,	BBSer
His	1.738	0.008	0.45	2.107	0.015	0.71	4.316	0.027	0.63	BPhe,	BPro,	BBSer
Ile	1.025	0.007	0.68	1.060	0.006	0.57	1.572	0.012	0.76			BBSer
Leu	1.021	0.005	0.45	0.989	0.008	0.85	1.493	0.012	0.80			BBSer
Lys	0.840	0.005	0.57	0.945	0.004	0.45	0.378	0.004	1.03	BPhe,		BBSer
Met	1.152	0.005	0.45	1.470	0.017	1.16	1.277	0.010	0.78	BPhe,	BPro,	BBSer
Phe	0.812	0.004	0.46	1.096	0.004	0.36	0.928	0.008	0.83	BPhe	BPro[e]	
Pro	2.002	0.014	0.70	2.340	0.021	0.90	0.734	0.007	0.91	BPhe,	BPro,	BBSer
Ser	1.012	0.004	0.39	1.313	0.007	0.53	0.992	0.010	1.02		BPro	
Thr	1.158	0.006	0.52	1.166	0.005	0.43	1.078	0.009	0.83	BPhe,	BPro	
Trp	0.566	0.003	0.55	1.934	0.009	0.47	0.494	0.007	1.42	BPhe,	BPro,	BBSer
Tyr	0.894	0.006	0.62	1.137	0.006	0.53	0.963	0.010	0.99	BPhe,	BPro	
Val	1.046	0.009	0.83	1.050	0.008	0.76	1.527	0.014	0.92			BBSer

[a] All enantiomerically pure L- and D-BPhe, BPro, BBSer and amino acids were purchased from Sigma and used without further purification. [b] Standard deviation for *R*. [c] $R > 1.1$ or $R < 0.9$. [d] Protonated trimers in low intensities, thus the MS/MS spectra of protonated dimers were measured instead. [e] Should also be suitable.

The results (Table 1) indicated that the maximum relative standard deviation for the chiral recognition ratio *R* is 1.42%. Therefore at the 99% (or better) probability level, any chiral recognition ratio falling outside the range of 1.000 ± 0.081 *(i.e.* $1.000 \pm 9.92 \times 1.42\%/3^{0.5}$) would indicate a significant discrimination of chirality. In other words, chiral discrimination could be recognised by an *R* value larger than 1.1 or smaller than 0.9. On this basis, our data clearly show that each amino acid could be discriminated by at least one chiral selector, suggesting a successful chiral recognition of all 19 amino acids by mass spectrometry using BPhe, BPro and/or BBSer as chiral selectors.

In summary, we have developed a mass spectrometric method to recognise the chirality of common amino acids. The results indicate that ESI-MS/MS is a promising approach for chiral recognition. This method can provide abundant complex ions even when the samples are at low concentrations or bear large/labile groups. The continuous introduction of sample mixtures *via* a syringe pump and the averaging of MS/MS spectra ensure a high precision of the results. ESI-MS/MS can be used to study the chirality of compounds in a mixture, and it only requires minimal sample preparation.

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15 Solutions of D- and L-amino acids and D- and L-BPro (2 mM) were prepared in 50% MeOH containing 1% AcOH. Solutions of D- and L-BPhe and D- and L-BBSer (2 mM) were prepared in MeOH. The solutions of amino acid and chiral selector were mixed in a 1:1 ratio prior to mass spectrometric analysis. Mass spectrometry was carried out in a Finnigan LCQ instrument (San Jose, CA, USA) fitted with an ESI ion source. Conditions for ESI were: heated capillary 50 °C, sheath gas 60 psi, spray voltage 4 kV, syringe pump 10 μL min^{-1}. The LCQ was operated with the Automatic Gain Control (AGC) mode. The AGC target values were: Full MS—5e + 007, MS^n—2e + 007. The default maximum injection time was 500 ms with 5 micro scans. Helium was introduced and maintained a pressure of 1 mtorr for improving the trapping efficiency of the ion trap and for use as the collision gas during the MS/MS process. Typical MS/MS conditions were: relative collision energy 8%, isolation width 18 u and mass range 300–850 u when BBSer was used as the chiral selector; relative collision energy 6.5%, isolation width 15 u and mass range 250–700 u for BPro; and relative collision energy 8%, isolation width 18 u and mass range 300–800 u for BPhe.

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