Chiral discrimination of α-amino acids by the DNA triplet GCA†‡

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The DNA triplet GCA is successfully used for the first time as a chiral selector for the chiral discrimination and optical purity measurement of some α-amino acids by investigating the collision-induced dissociation spectra of the sodiated ternary complex ion formed by electrospray ionization.

Mass spectrometry has been used for differentiating enantiomers by probing the interactions with chiral references.[1–13] The MS based chiral recognition experiments are mainly (i) the measurement of the relative abundance of diastereomeric adduct ions formed between the analyte of interest and a chiral reference,[1–3] (ii) based on ion–molecule reactions; diastereomeric adducts (Host–Guest complexes) allowed to exchange the chiral analyte with a chiral or an achiral molecule, wherein chiral discrimination is possible as the rates of exchange depend on the chirality of guest,[4–6] and (iii) dissociation of diastereomeric adducts formed between the analyte and a chiral reference (the kinetic method) that results in distinct spectra. The kinetic method has been extensively used for the discrimination of chiral isomers, in which the abundance ratio of product ions formed during the dissociation of the diastereomeric complex ion is used for the discrimination. In the successful cases reported,[14–22] the diastereomeric ion $[A_xRef_2M–H]^+$ consisting of one analyte molecule of interest (A_x), two molecules of the chiral reference (Ref) and a metal ion (M) arranged in either a octahedral or tetrahedral fashion around the metal ion are generated in the source of a mass spectrometer and their dissociation studied. Many research groups have reported chiral discrimination and enantiomeric excess (ee) determination of amino acids using cyclodextrins, chiral crown ethers, sugars, modified amino acids, and peptides as selectors.[4,9,23–26] Recently, Filippi *et al.* reported exceptional chiral selectivity of chiral tetramide macrocycles for the ethyl ester/amide of phenylalanine and related compounds in the gas phase.[11] However, oligo deoxy nucleotides, which possess a variety of three-dimensional structures and offer innumerable combinations, have not been tested as chiral selectors for mass spectral work. In this communication we demonstrate the use of a trinucleotide as a selector for chiral recognition of a few amino acids.

We started with a set of trinucleotides,[27] DNA triplets AAA, AAG, AGA, AGG, ACA, ACG, CCA, CGA, CCG, GCA, GCC, CGC, CCC, CGG and GCG as chiral selectors for the differentiation of D and L-amino acids using a Q-STAR mass spectrometer under electrospray ionization (ESI) conditions. The negative ESI mass spectra of trinucleotides comprise of the dominant $[M - H]^-$ and $[M - 2H]^{2-}$ ions.[28] We selected a few amino acids from each class, *viz.*, neutral (alanine, proline, asparagine, phenylalanine, tryptophan, and tyrosine), acidic (aspartic acid and glutamic acid), and basic (lysine) amino acids as analytes. The spectra of a mixture of amino acid (X) and trinucleotide (Y) also predominantly show $[M - H]^-$ and $[M - 2H]^{2-}$ ions of the trinucleotide, however, they also include the $[X + Y - H]^-$ ion in considerable abundance. The ions appearing at the *m*/*z* value of the $[X + Y - H]^-$ ions are found to be a combination of two ions on the basis of the ^{13}C isotopic mass differences and isotopic pattern, in which $[2X + 2Y - 2H]^{2-}$ ion is predominant (70–80%) over the $[X + Y - H]^-$ ion. The source spectra obtained for two enantiomeric amino acids did not show significant differences among the D and L-isomers. But the collision induced dissociation (CID) spectra obtained for the adduct ion, $[2X + 2Y - 2H]^{2-}$ result in two fragment ions (loss of one and two amino acids) and the spectra are distinct for D and L-isomers when GCA is used as a selector. No such differences are observed with any other of the DNA triplets studied. Since there is no control over the extent of contribution of $[X + Y - H]^-$ ion in $[2X + 2Y - 2H]^{2-}$, the product ion spectra of the latter ion may not be highly suitable for chiral discrimination. Therefore, it became necessary to produce an ion that is similar to $[2X + 2Y - 2H]^{2-}$ without contribution from other ions of the same *m*/*z* value. One such ion could be generated by the replacement of a proton in the $[2X + 2Y - 2H]^{2-}$ ion with a sodium ion; indeed, the $[2X + 2Y - 3H + Na]^{2-}$ ion appeared in the pure form when the spectrum was recorded in the presence of NaCl. Hence, all the above performed CID experiments on $[2X + 2Y - 2H]^{2-}$ were repeated by selecting the $[2X + 2Y - 3H + Na]^{2-}$ ion. The CID spectra of $[2X + 2Y - 3H + Na]^{2-}$ ions (**a**) formed from all the combinations of the two provided doubly charged fragment ions, **b** and **c** corresponding to the loss of one and two amino acid units from the precursor ion, respectively. The results obtained from the CID experiments on $[2X + 2Y - 3H + Na]^{2-}$ ions are similar to those obtained for $[2X + 2Y - 2H]^{2-}$ ions.

We measured the R_{chiral} values by following the kinetic method and Chiral Recognition ratio (CR) method.[9,12,13] The R_{chiral} values are close to unity, though the spectra look very much distinct (Fig. 1). The CR values (Table 1), calculated by measuring the abundance ratio of precursor ion to either of the product, *i.e.*, CR = $(\mathbf{a/b})_D/(\mathbf{a/b})_L$ or $(\mathbf{a/c})_D/(\mathbf{a/c})_L$, show remarkable chiral recognition; and the CR values obtained from both the ways show same selectivity. Thus, the chiral discrimination can be seen only when the abundance of the precursor ion is considered for calculation (CR method). Hence, the stability of the adduct ion, $[2X + 2Y - 3H + Na]^{2-}$ must be playing a vital role in the chiral discrimination, rather than its dissociation. The results

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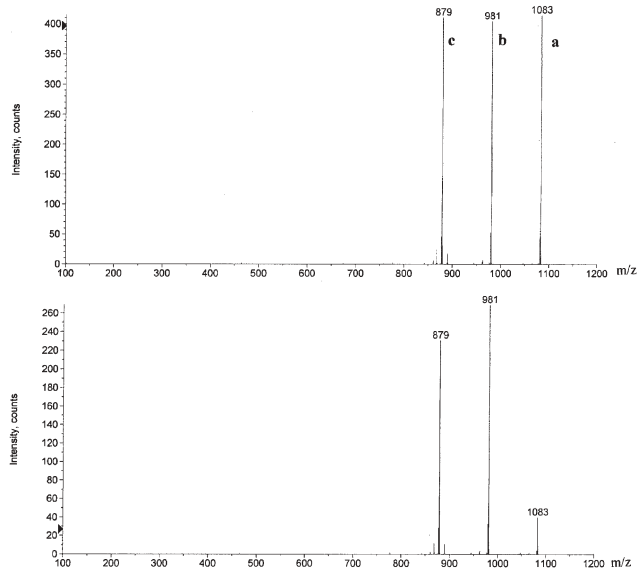

Fig. 1 CID spectra of $[2X + 2Y - 3H + Na]^{2-}$ ion (*m/z* 1083) formed from GCA and D-tryptophan (top) or L-typtophan (bottom); molecular masses of GCA and tryptophan are 869 and 204 Da, respectively.

Table 1 Calculated CR values from CID spectra of $[2X + 2Y - 3H + Na]^{2-}$ ion

	CR value	
Amino acid	$(a/b)_D/(a/b)_L$	$(a/c)_D/(a/c)_L$
Tryptophan	6.4	5.6
Phenylalanine	3.6	5.4
Glutamic acid	4.9	4.2
Aspartic acid	2.8	2.5
Asparagine	3.7	5
Tyrosine	2.5	2.1
Proline	1.2	1.5
Alanine	1.1	1.5

[a] **a** = $[2X + 2Y - 3H + Na]^{2-}$, **b** = [**a** − X], **c** = [**a** − 2X]

demonstrate that the GCA is preferentially binding with the D-isomer than the L-isomer irrespective of the amino acid used. The formation of $[2X + 2Y - 3H + Na]^{2-}$ in the source is found to be less for Ala, and Pro; and discrimination between D and L-isomers is also poor for these isomers. We could not obtain a good CID spectrum for the $[2X + 2Y - 3H + Na]^{2-}$ ion of lysine due to its poor abundance, hence the data are not included in Table 1. On the other hand, Phe, Trp, Tyr, Asn, Asp and Glu form abundant $[2X + 2Y - 3H + Na]^{2-}$ ions, and their CID spectra are distinct for the two isomers. Better selectivity for aromatic and acidic amino acids may be due to secondary interactions of aromatic ring/carboxylic group of amino acids with the trinucleotides, which is not understood at present. However, multipoint interactions always give better chiral recognition.[1] The experiments with GCA and tryptophan were also done with a LCQ quadrupole ion trap instrument and similar results were obtained.

Among the many trinucleotides selected, only GCA shows the discrimination of D and L-amino acids. The ACG, which has the same bases in the opposite direction to that of GCA does not show the selectivity. It implies that not only the presence of the bases but also the particular sequence is responsible for the remarkable discrimination by the GCA. Zhu *et al.* reported the formation of an unusual hairpin structure for CAATGCAATG based on NMR, X-ray and computational studies; this hairpin structure was explained by the formation of a GCA cap, involving a key G–A pairing between the G and A of the GCA sequence.[29] Such hairpin structures were not found for the nucleotides containing CAG or AGC. Similarly, in the present case, the cap like structure of GCA may be playing a crucial role in differential stability for the dimeric complexes with D and L-isomers of amino acids. In order to prove the importance of the sequence of GCA and the position of C in the middle we performed experiments by choosing three other trinucleotides keeping the position of G and A same, *viz*., GGA, GTA and GAA. With this set of trinucleotides abundant $[2X + 2Y - 3H + Na]^{2-}$ ions are also formed (using Glu and Trp isomers), but no chiral discrimination could be obtained for GTA and poor discrimination for GAA and GGA. The poor discrimination by GGA, GTA and GAA could be attributed to the failing of formation of a cap like structure involving G and A.

With a view to prove the importance of $–NH_2$ group of amino acid in chiral discrimination, we performed experiments with D and L- malic acid, whose structure is close to Asp with a –OH group instead of an $–NH_2$ group. Interestingly, the $[2X + 2Y - 3H + Na]^{2-}$ and $[2X + 2Y - 2H]^{2-}$ ions are negligible in the ESI mass spectrum recorded for the combination of GCA and malic acid; and the spectrum includes abundant $[2X + Y - H]^-$ ions. When CID experiments are performed on the $[2X + Y - H]^-$ ions, the spectra of D and L-malic acids are essentially similar with no chiral discrimination. It clearly illustrates a decisive role of $–NH_2$ group of amino acids in the formation of a 2 : 2 adduct ion of trinucleotide and amino acid, consequently leading to the discrimination of enantiomeric amino acids.

We extended the experiments to verify the suitability of the present method in measuring the ee of amino acids. The CR values obtained for the enantiomeric mixtures of Trp are plotted as a function of ee of the D-isomer and the plots are found to be linear with a correlation coefficient of 0.99. This demonstrates that the method can be used not only for the chiral discrimination of individual enantiomers but also for the determination of ee.

Thus, we demonstrate the efficacy of a DNA triplet, GCA, for chiral discrimination of amino acids as well as for measuring their optical purity. The use of GCA allows analysis under the negative ion mode, which opens another branch of research to explore for other multifunctional chiral analytes. Theoretical calculations studies would help in better understanding the multipoint interactions between the selector and analyte. We are exploring the utility of this system for other chiral substrates also.

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28 All the experiments were performed using QSTAR XL (Applied Biosystems/MDS Sciex, Foster city, USA): operating capillary voltage −4.0 kV; declustering potential, 50 V. Nitrogen was used as the curtain and collision gas. The collision energy was set to be 12 eV.
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