Analysis of Underivatised Peptide Mixtures by Collision-induced Dissociation of Negative Ions

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Summary Collision-induced decomposition of mass-selected negative ions has been used to provide useful sequence information from underivatised peptides and their mixtures

DETERMINATION of the amino acid sequence in peptides and peptide mixtures, by positive-ion electron-impact mass spectrometry, is well established.[1,2] Derivatisation is usually required and analysis of peptide mixtures often involves some physical separation of components. Reliable results are routinely obtained using these techniques although several disadvantages are inherent in the procedures used. Derivatisation is not always a high-yield process, may be time-consuming, and leads to increased molecular weights. Physical separation reduces sample size. Furthermore, electron-impact mass-spectra of peptides often yield relatively weak molecular ions.

A method is reported here for analysing peptide mixtures using OH^- negative ion chemical ionisation (NICI) to generate an abundant $[M-H]^-$ ion.[3] Separation of the peptides in the mixture is effected by mass selection using a reverse geometry mass spectrometer, the VG ZAB-2F.[4] Structural information is obtained by fragmentation of the $[M-H]^-$ ion using collision-induced dissociation and analysis of the fragment ions by mass-analysed-ion-kinetic-energy (MIKE) spectroscopy.[4]

The OH^- NICI mass spectra of a selection of di- and tripeptides and their mixtures have been recorded. In general, the $[M-1]^-$ ion forms the base peak and the *C*-terminal ion, (**3**), the major fragment. Thus, molecular weights are readily obtainable from the spectra but little structural information is available. After mass selection of the $[M-1]^-$ ion, collision-induced dissociation (CID), using argon as collision gas, yields a spectrum of fragment ions which are identified by MIKE spectroscopy leading to useful sequence information.[5] The underivatised peptides which have been recorded using this method obey a general fragmentation scheme which may be illustrated for a tripeptide as shown in Figure 1.

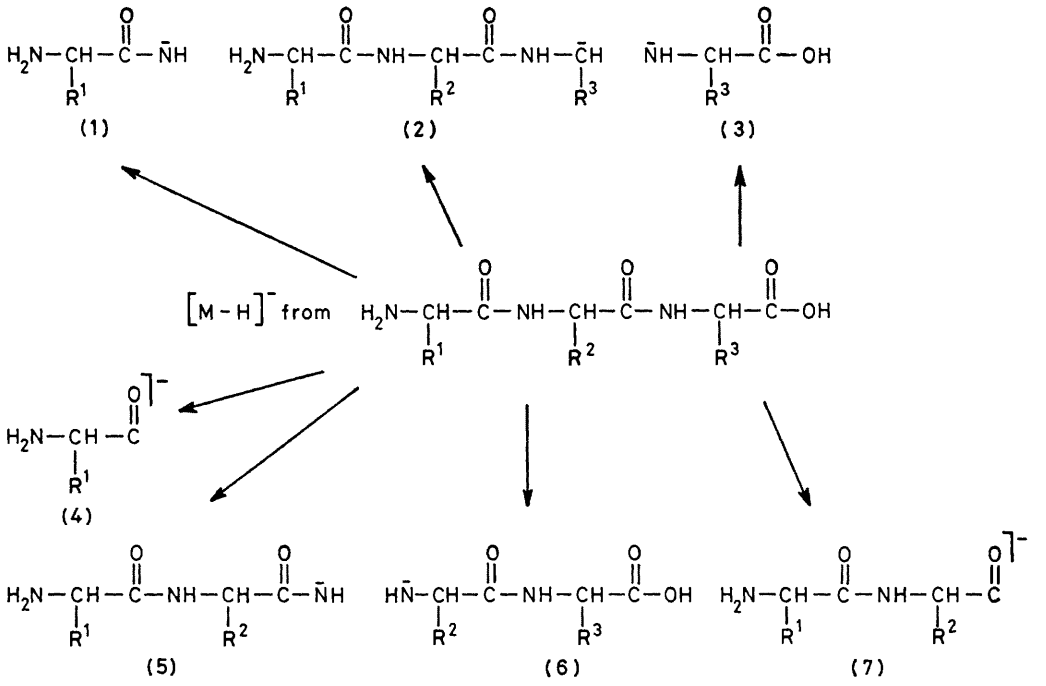

FIGURE 1. General fragmentation scheme of a tripeptide.

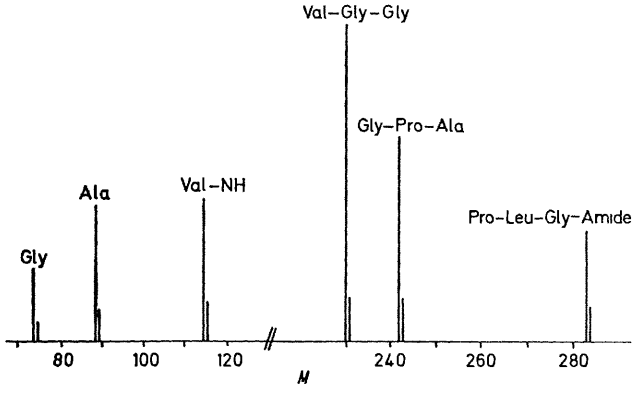

FIGURE 2. OH^- negative ion chemical ionisation (NICI) mass spectrum of a mixture of three tripeptides.

Fragmentation reactions which differ between specific amino acids have been observed. For example when the *C*-terminal acid is glycine, *i.e.*, when $R^3=H$, the $[M-CO_2H]^-$ ion is the most intense in the MIKE spectrum, whilst when R^3 is an alkyl group, the peak due to $[M-CO_2H]^-$ ions is considerably smaller. This may be attributed to fragmentation of structure (**2**) due to the alkyl groups R^3 in other amino acids such as valine, leucine, and alanine.

In the analysis of a mixture of peptides containing approximately equal amounts of three tripeptides, Val–Gly–Gly, Pro–Leu–Gly–Amide, and Gly–Pro–Ala, the OH^- NICI spectrum shown in Figure 2, provides useful molecular weight data but it is difficult to obtain any sequence information. However, the MIKE technique allows mass selection of the three $[M-1]^-$ ions and the CID–MIKE spectrum of each component may then be obtained. The

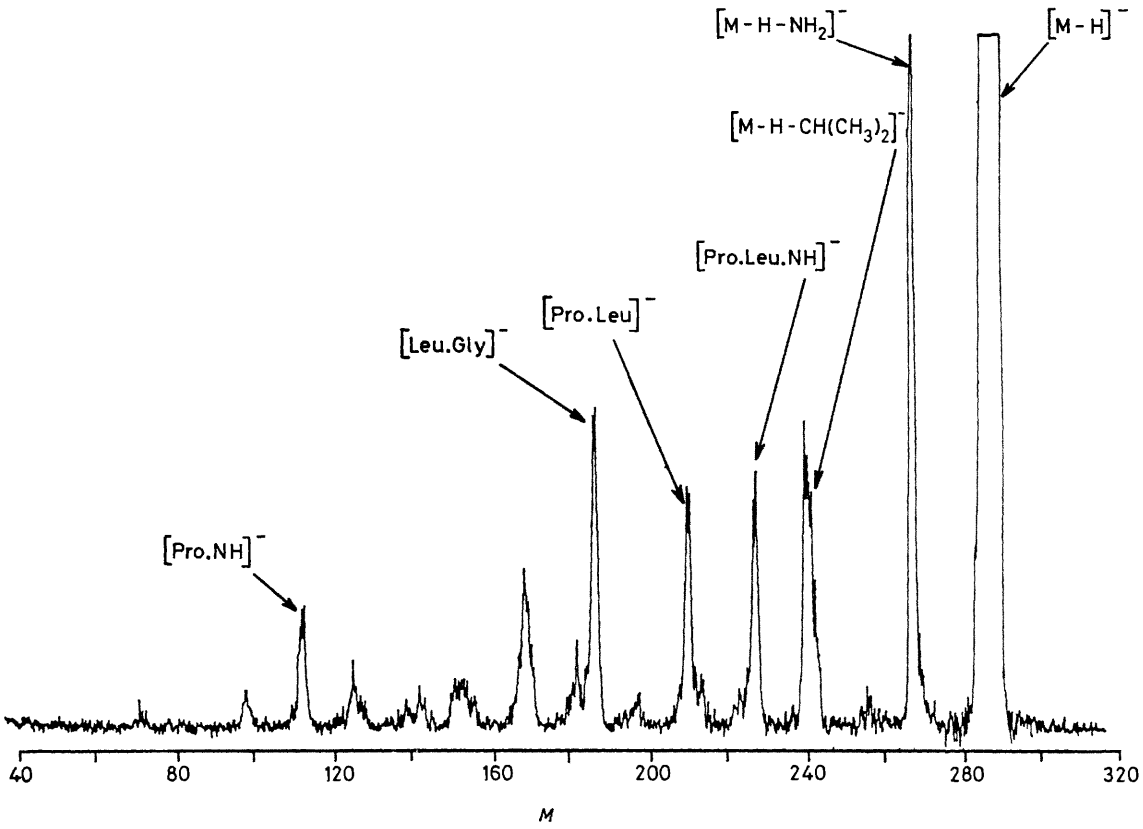

FIGURE 3. CID–MIKE spectrum of Pro–Leu-Gly-Amide. (CID = collision-induced dissociation, MIKE = mass-analysed-ion-kinetic-energy.)

tripeptide may then be unambiguously identified (see, for example in Figure 3 the CID–MIKE spectrum of the *m/z* 283 negative ion from this mixture). Sequence ions are formed according to the scheme shown in Figure 1 and are indicated on the MIKE spectrum. The ion formed by $CH(Me)_2$ loss is indicative of a leucine component.

The method discussed in this paper illustrates that concrete molecular weight and sequence information may be obtained on all components of an underivatised peptide mixture without prior physical separation. It is proposed to investigate the applicability of the technique to a wider range of peptides and to utilise laser radiation as an alternative to collision-induced dissociation.

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