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An improved scheme of leucine derivative fragmentation in mass spectrometry

C. Perier, J. Doumit, and **J. Frey**

Laboratoire de Biochimie, Faculté de Médecine, Saint-Etienne, France

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Summary. This study illustrates the contribution of stable isotopes to amino acid mass spectrometry. The mass spectra of natural leucine and 1 ^{13}C leucine were compared and the mass fragmentography pattern analysed. This analysis indicates that the position of the stable isotope in tracer molecules should be very dependent on the analytical procedure used for their determination.

Keywords: Amino acids – Stable isotope – Mass spectrometry

Introduction

Amino acids labelled with stable isotopes could be used for the dynamic investigation of protein metabolism in man (Bier et al., 1982; Carraro et al., 1991; Haliday et al., 1981). Labelled ^{13}C-leucine, an essential amino acid, has been used as tracer (Beaufrere et al., 1990; Meguid et al., 1988; Pelletier et al., 1991). The follow-up of this amino acid in blood is determined by isotopic enrichment based on the tracer/tracer + tracee ratio. This ratio is established from the abundance of specific mass fragments. These fragments for the natural amino acid and the labelled ^{13}C amino acid differ by one mass unit since the tracer molecule contains a ^{13}C atom. Stable isotopes can also be used to dermine the fragmentation scheme of biological compound in mass spectrometry. This report describes a new scheme for fragmentation of leucine derivative.

Material and methods

Natural L-leucine was purchased by Sigma, and labelled L-leucine (1 ^{13}C-leucine) was from Eurisotop, Saclay, France. Leucine has been derivatized as N-heptafluorobutyryl isobutyl ester before analysis by gas liquid chromatography and mass spectrometry (McKenzie and Hogge, 1977; Desgres et al., 1979). Gas liquid-mass spectrometry analysis was performed on a Hewlett Packard model 5890 gas chromatograph model 5989 mass spectrometer. The analytical capillary column was HP 1, 25 m lenght 0.2 mm ID, 0.33 μm film thickness. The analytical conditions were: carrier gas Helium flow rate 1.5 ml/min,

injection port temperature 250°C, detector 260°C, temperature programming 3°C/min from 90°C to 240°C. Source temperature was at 240°C and quadrupole 100°C. The ioniser voltage was 70 eV. The chromatographic and mass analytical data were recorded with HP chemstation software.

Results and discussion

The mass spectra of natural leucine and ^{13}C-leucine derivatives are shown in Fig. 1. The differences were in the fragments pairs 253/254, 310/311, 327/328,

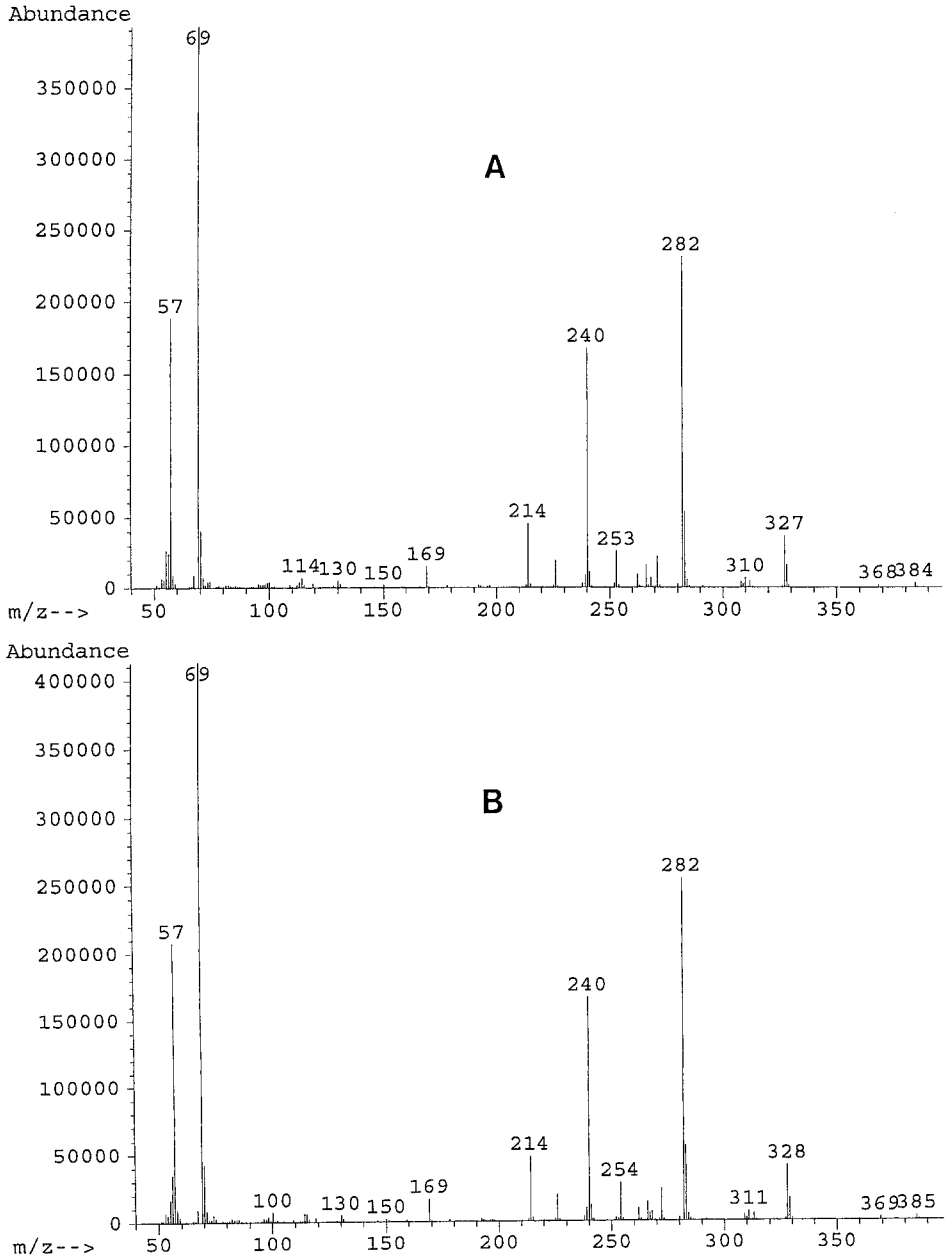

Fig. 1. Mass spectra of natural leucine (**A**) and 1 ^{13}C leucine derivatives (**B**). Analytical conditions are described in the text

368/369. These results are similar to those reported by McKenzie and Hogge (1977). However, there was an extra, undescribed mass fragment, 253 (natural leucine) and 254 (1 ^{13}C-leucine). This result suggests a scheme for the fragmentation of leucine derviative, that differs from the one previously described (McKenzie and Hogge, 1977). According to this scheme ion mass fragments 253 (natural leucine) and 254 (1 ^{13}C-leucine) came from the successive loss of groups C_4H_8 (ion mass fragment 327), OH (ion mass fragment 310) and C_4H_9 (Fig. 2). The ion mass fragment 214 could not result from loss of group C_3F_7, as previously described (McKenzie and Hogge, 1977), since it requires a mass fragment 215 of the same intensity in the mass fragmentation pattern of 1 ^{13}C-leucine derivative. A probable mass fragmentation scheme is proposed, involving from the ion mass fragment 310, a successive loss of CO (ion mass fragment 282), C_3H_6 (ion mass fragment 240) and C_2H_2 (Fig. 2). Thus, it appears that the introduction of stable isotope into amino acids is usefull to improve the mechanistic explanation of their fragmentation pattern in mass spectrometry. Moreover, this fragmentation must be checked before the choice of the molecular position of stable isotope, for metabolic investigation, with regard to specific mass spectra of the particular molecules and their metabolic derivatives.

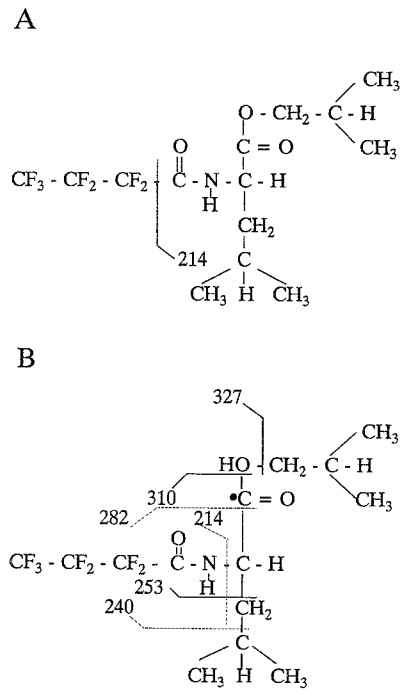

Fig. 2. Comparative fragmentation scheme of leucine derivative. **A** McKenzie, Hogge scheme; **B** Improved fragmentation scheme based on the position of the stable ^{13}C isotope (•) (—— successive loss from molecular ion up to ion mass fragment 253/254; ---- successive loss from ion mass fragment 310 up to ion mass fragment 214)

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Authors' address: Pr. J. Frey, Laboratoire de Biochimie, Faculté de Médecine, 15 rue A. Paré, F-42023 Saint Etienne Cédex 2, France.

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