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# Fast-atom-bombardment mass spectra of enkephalins

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The positive- and negative-ion mass spectra of [methionine]enkephalin and [leucine]enkephalin have been obtained by using a fast-atom-bombardment source described previously by Barber, Bordoli, Sedgwick & Tyler [(1981) *J. Chem. Soc. Chem. Commun.*, in the press]. This technique has allowed the spectra to be obtained without conversion of the enkephalins into volatile derivatives. The fast-atom-bombardment spectra show good pseudo-molecular-ion sensitivity and fragmentation that can be interpreted on the basis of the known molecular structure.

Mass spectrometry was used by Hughes *et al.* (1975) to demonstrate the presence of two discrete components in what was described as a low-molecular-mass peptide termed enkephalin. This was achieved by conversion of the pentapeptides into the *N*-acetyl-permethyl derivatives to impart sufficient volatility for ions to be produced using an electron-impact source. Morris *et al.* (1978) have described the molecular-ion region of the field-desorption spectrum of [leucine]enkephalin.

## Experimental

Samples of [leucine]enkephalin and [methionine]-enkephalin were purchased from Sigma (London) Chemical Co. and Calbiochem–Behring Corp. The samples were prepared as aqueous solutions of concentration $5 \times 10^{-4}$ mol/dm$^3$. The solution (1 $\mu$l) was deposited on the probe and diluted with glycerol (2 $\mu$l); the probe was then inserted via a vacuum lock into the modified source of a Vacuum Generator ZAB 1F mass spectrometer, where it intercepted the fast-atom beam. The spectra were recorded using a fast-response galvanometer recorder with u.v.-sensitive paper; the mass scale was applied manually. In all cases, spectra were recorded by magnetic scanning at an ion energy of 8000 eV.

## Results and discussion

The positive- and negative-ion mass spectra of [methionine]- and [leucine]-enkephalin are shown in Figs. 1 and 2 respectively. Significant structural information can be obtained by examination of the spectra, which all show prominent peaks in both the molecular-ion region and also at lower $m/z$ values. It is apparent that the FAB mass spectra of both polarities show good pseudomolecular ion intensities as $(M+H)^+$ and $(M-H)^-$ respectively.

*Positive-ion spectra* (*Figs. 1a and 2a*)

In addition to the intense protonated molecular ion, cationized species are observed in which the alkali-metal ions $Na^+$ or $K^+$ have been added to both the enkephalin molecules. These $(M+23)^+$ and $(M+39)^+$ peaks can only arise from alkali-metal ion impurities that are ubiquitous to such samples. Species of this type have been commonly observed in spectra from other soft-ionization methods, e.g. field desorption. The intensities of these adventitious 'impurity' peaks are not high, but may be enhanced by deliberate addition of suitable alkali-metal salts. This enhancement can thus be used as confirmatory evidence in molar mass determinations. Furthermore, these alkali-metal cationized species apparently have high intrinsic stability and do not in these cases lead to any detectable fragment ions retaining the alkali-metal ion. They can thus be ignored in any subsequent discussion of the fragmentation processes of these molecules.

Two types of fragmentation process can be considered for these molecules. One leads directly to sequencing of the amino acid units and hence involves cleavages of the peptide chain with minimal rearrangement. The fragment ions arising in such a way are defined as sequence ions. The second type of fragmentation process involves mechanisms specific to particular amino acids and may include complex rearrangements and side-chain cleavages.

The two enkephalins differ only in the *C*-terminal units of the peptide chain. Consequently sequence ions that arise by loss of a neutral *C*-terminal fragment are common to both molecules. Thus loss of the leucine residue from [leucine]enkephalin yields

Abbreviation used: FAB, fast-atom bombardment.



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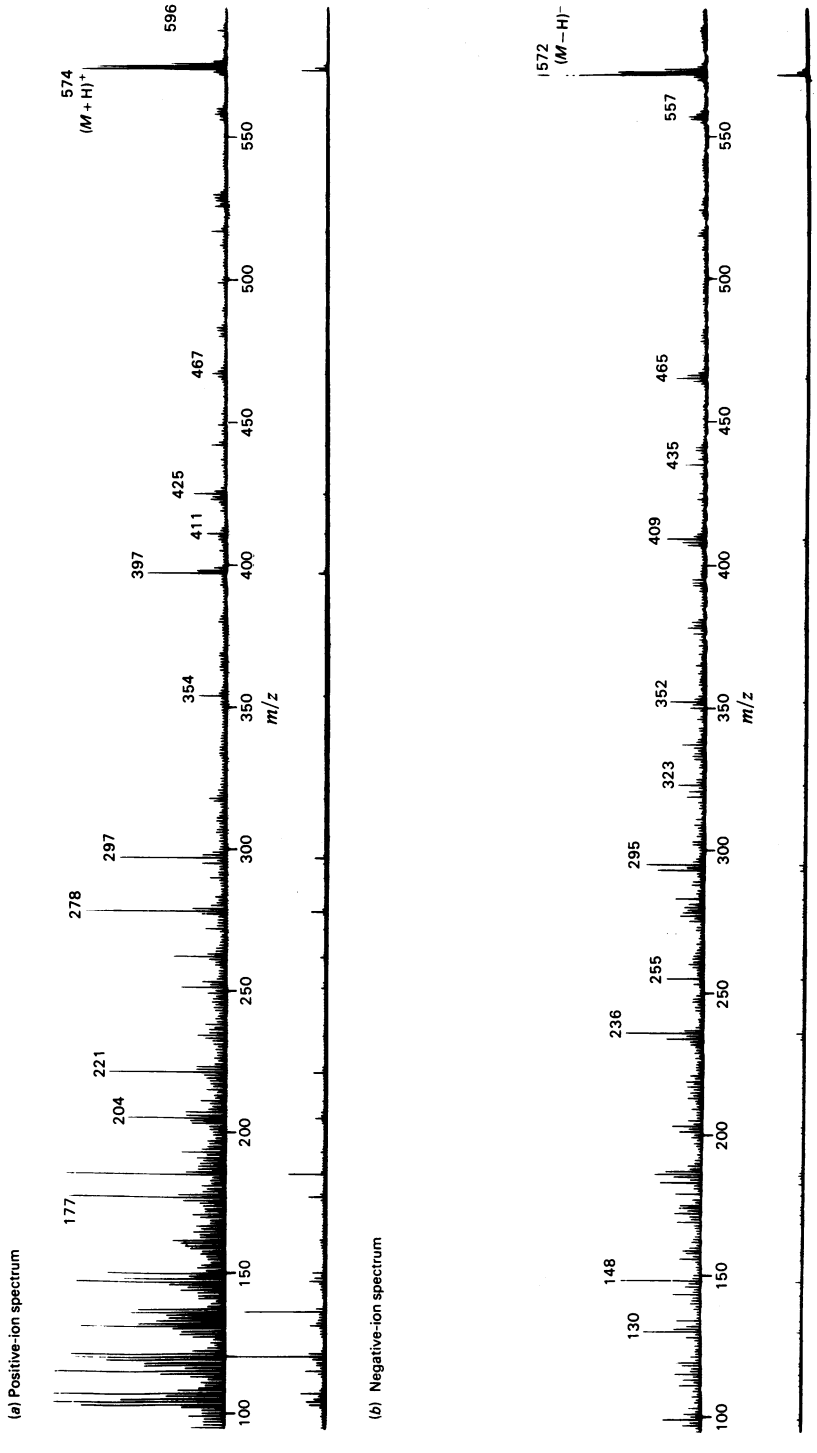



Fig. 1. *FAB mass spectra of* [*methionine*]*enkephalin* (Tyr-Gly-Gly-Phe-Met; $M_r = 573$)

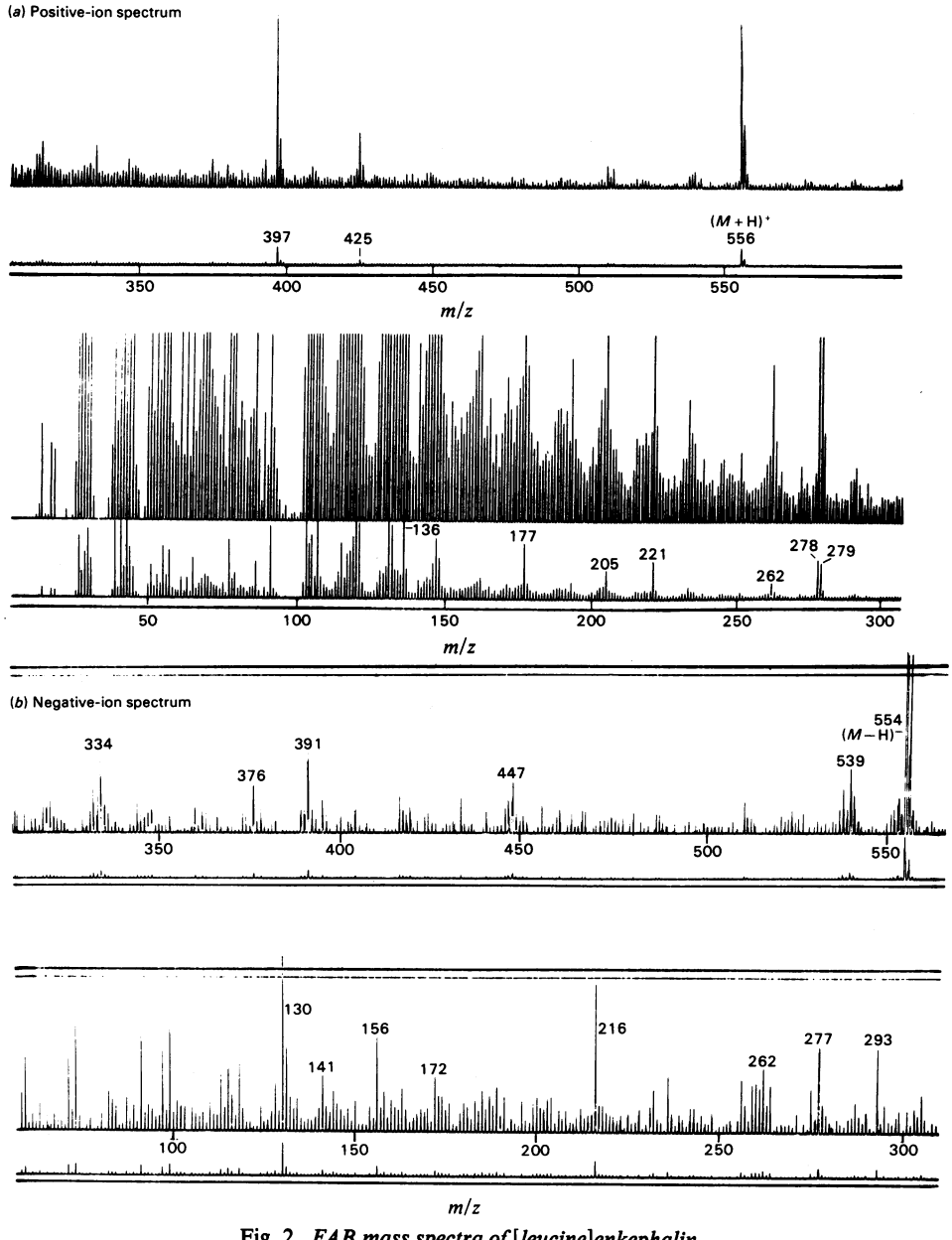



Fig. 2. *FAB mass spectra of* [*leucine*]*enkephalin* (Tyr-Gly-Gly-Phe-Leu; $M_r = 555$)

(Tyr-Gly-Gly-Phe)$^+$ at *m/z* 425, which also arises by loss of methionine from [methionine]enkephalin. Subsequent loss of phenylalanine and glycine from the *m/z* 425 ion yielded respectively (Tyr-Gly-Gly)$^+$ (*m/z* 278) and (Tyr-Gly)$^+$ (*m/z* 221). Other peaks observed in both spectra at *m/z* 262 and 205 have been interpreted as being due to (Gly-Gly-Phe)$^+$ and (Gly-Phe)$^+$ respectively. Loss of neutral *N*-terminal residues results in sequence ions that differ only by a methionine or leucine residue. The sequence ions produced by *N*-terminal fragmentation are listed in Table 1.

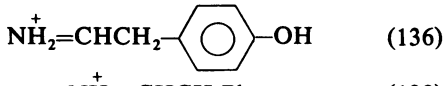
Identification of these sequence ions allows an unambiguous sequence of the amino acid residues to be deduced. Subsequent fragmentation of the sequence ions and fragmentations involving side-chain cleavages produces relatively small peaks that do not hinder interpretation of the primary sequence.

Subsequent fragmentation of the sequence ions occurs by, for example, loss of CO to give fragment

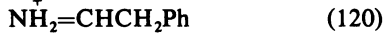
ions at $m/z$ values 28 less than the sequence ion value. Thus the fragmentations $m/z$ 425→397, 262→234, 221→193 and 205→177 occur in the FAB spectra of both enkephalins. The corresponding loss from $m/z$ 278, however, is not observed. This interpretation is preferred to that involving loss of 16 mass units (e.g. $NH_2$), which would require unstable odd electron species (e.g. $m/z$ 193→177).

The fragment ions observed at $m/z$ 499, 483 and 467 in the spectrum of [methionine]enkephalin (Fig. 1*a*) are interpreted as arising by side-chain cleavage of methionine, phenylalanine and tyrosine residues respectively in the parent species. Similar ions are observed at $m/z$ 499, 465 and 449 in the spectrum of [leucine]enkephalin (Fig. 2*a*) arising by side-chain loss from leucine, phenylalanine and tyrosine residues respectively. Similar side-chain cleavages are also observed in the sequence ions, e.g. cleavage of the tyrosine side-chain from $m/z$ 425 gives 318 and from 397 to 290 in the spectra of both enkephalins.

Intense peaks at even $m/z$ values (136, 120) are common to both spectra (Figs. 1*a* and 2*a*). These peaks have been assigned the following structures:

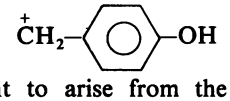


(136)



(120)

The peak at $m/z$ 86 in the spectrum of Fig. 2(*a*) has been assigned the structure $\overset{+}{N}H_2{=}CHCH_2CHMe_2$. These peaks have been interpreted as being uniquely diagnostic of the residues tyrosine, phenylalanine and leucine respectively. Similarly the peak at $m/z$ 107, which is also common to the spectra of both enkephalins, has been assigned the structure



and is thought to arise from the side chain of tyrosine. Diagnostic ions of particular amino acids have been observed in other peptides (M. Barber & R. D. Sedgwick, unpublished work).

*Negative-ion spectra (Figs. 1b and 2b)*

The negative-ion FAB mass spectra of both enkephalins are noticeably less complex than the positive-ion spectra. In the spectra of both enkephalins the pseudomolecular ion peaks at $m/z$ 572 and 554 are amongst the most intense peaks.

Generally the intensities of the fragment-ion peaks are lower than in the corresponding positive-ion spectra. Sequence ions that arise by cleavage of the peptide chain can be identified and, as in the positive-ion spectra, cleavage is observed from both ends of the chain.

Sequence ions that derive from cleavage at the *N*-termini generally appear to be dominant; this is in contrast with the pattern observed in the positive ion spectra. The sequence ions observed in the spectra of [methionine]enekphalin and [leucine]enkephalin are listed in Tables 2 and 3. Identification of these sequence ions again allows an unambiguous primary structure to be deduced for each of the enkephalins.

Table 1. *Sequence ions by N-terminal fragmentation*

| [Methionine]enkephalin (Fig. 1*a*) | | [Leucine]enkephalin (Fig. 2*a*) | |
|---|---|---|---|
| $(Gly\text{-}Gly\text{-}Phe\text{-}Met)^+$ | 411 | $(Gly\text{-}Gly\text{-}Phe\text{-}Leu)^+$ | 393 |
| $(Gly\text{-}Phe\text{-}Met)^+$ | 354 | $(Gly\text{-}Phe\text{-}Leu)^+$ | 336 |
| $(Phe\text{-}Met)^+$ | 297 | $(Phe\text{-}Leu)^+$ | 278 |

Table 2. *Sequence ions of [methionine]enkephalin*

| | *m/z* | | *m/z* |
|---|---|---|---|
| $(Gly\text{-}Gly\text{-}Phe\text{-}Met)^-$ | 409 | $(Tyr\text{-}Gly\text{-}Gly\text{-}PheNH)^-$ | 440 |
| $(Gly\text{-}Phe\text{-}Met)^-$ | 352 | $(Tyr\text{-}Gly\text{-}GlyNH)^-$ | 293 |
| $(Phe\text{-}Met)^-$ | 295 | $(Tyr\text{-}GlyNH)^-$ | 236 |
| $(Met)^-$ | 148 | | |
| $(Gly\text{-}Phe)^-$ | 203 | | |

Table 3. *Sequence ions of [leucine]enkephalin*

| | *m/z* | | *m/z* |
|---|---|---|---|
| $(Gly\text{-}Gly\text{-}Phe\text{-}Leu)^-$ | 391 | $(Tyr\text{-}Gly\text{-}Gly\text{-}PheNH)^-$ | 440 |
| $(Gly\text{-}Phe\text{-}Leu)^-$ | 334 | $(Tyr\text{-}Gly\text{-}GlyNH)^-$ | 293 |
| $(Phe\text{-}Leu)^-$ | 277 | $(Tyr\text{-}GlyNH)^-$ | 236 |
| $(Leu)^-$ | 130 | | |

## Conclusions

The positive- and negative-ion FAB mass spectra are complementary in the interpretation of an unambiguous amino acid sequence in both the pentapeptides studied. The technique of FAB mass spectroscopy lends itself to the elucidation of the primary structures of low-molar-mass peptides. The spectra are routinely obtained without recourse to time- and material-consuming chemical-derivative-formation processes or the delicate manipulations required for the preparation of field-desorption emitters.

Additional advantages in the investigation of materials, particularly of biological origin, are: (1) aqueous preparations can be used directly; (2) the presence of alkali-metal ions does not appear to be deleterious, and in fact may be advantageous in providing molar-mass information; (3) only microgram quantities of materials may be used, which compares favourably with other techniques.

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