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Fast-atom bombardment mass spectra of some pentapeptides related to natural enkephalins

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The mass spectral features of four diastereoisomeric pairs of peptides related to the enkephalins recorded under fast-atom bombardment conditions are presented and shown to provide evidence of molecular weight and amino acid content and sequence.

The technique of fast-atom bombardment (FAB) in mass spectrometry has proved a valuable aid to structural and analytical studies of biologically active peptides in its provision of molecular weight, and amino acid content and sequence data (Barber et al 1981a; Williams et al 1982). The natural enkephalins, leu- and met-enkephalin, were amongst the earliest peptides examined by the FAB procedure (Barber et al 1981b; Westmore et al 1982). Mass spectral data obtained using a FAB source are now reported for the eight synthetic enkephalin analogues **1–8** (four sets of diastereoisomers) which confirm the original promise of the procedure to the study of opioid peptides. The results emphasize the value of FAB mass spectrometry to pharmacologists who require sensitive and specific analytical aids for the detection and characterization of small peptides.

Peptide	Tyr–X–Gly–Phe–Y X	Y
1	D-Ala	D-Leu
2	D-Ala	L-Leu
3	D-Ala	D-Nle
4	D-Ala	L-Nle
5	D-Nle	D-Nle
6	D-Nle	L-Nle
7	D-Nle	D-NleS*
8	D-Nle	L-NleS*

* Analogue of Nle with CO_2H replaced by SO_3H.

Details of mass spectral features are given in Tables 1 and 2 which refer to positive and negative ions, respectively. Mass spectra were obtained using a 7070E VG Analytical instrument; samples were deposited in glycerol onto the probe tip. The *m/z* lines to which glycerol or its oligomers may contribute have been

Table 1. Masses of positive ions in the FAB mass spectra of peptides related to enkephalins (relative intensities in parentheses).

	Peptide							
Ion type	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
$[M+H]^+$	570 (15·6)	570 (13·7)	570 (15)	570 (25·7)	612 (17·9)	612 (15·7)	648 (1·7)	*abs.*
a_1	*abs.*	439 (0·8)	439 (1·0)	439 (1·1)	481 (1·3)	481 (1 1)	481 (4·1)	481 (3·9)
a_2	292 (7·4)	292 (6·4)	292 (9)	292 (6)	334 (7·2)	334 (6·5)	334 (10·4)	334 (6·7)
a_3	235 (7·4)	235 (7·2)	235 (11)	235 (7)	277 (7·6)	277 (6·9)	277 (7)	*abs.*
a_4	164 (1·3)	164 (1·0)	164 (1·9)	164 (1·8)	164 (2·3)	164 (2·9)	164 (1·9)	164 (2·4)
b_1	411 (2·3)	411 (2·0)	411 (2·4)	411 (2·3)	453 (2·4)	453 (1·7)	453 (1·9)	453 (2·7)
b_2	264 (0·6)	*abs.*	264 (1·3)	264 (0·9)	306 (0·7)	306 (0·9)	306 (1·2)	306 (0·8)
b_3	207 (7)	207 (6·9)	207 (8·9)	207 (7·7)	249 (12·2)	249 (11·3)	249 (7·8)	249 (8·5)
b_4	136 (57·4)	136 (59·3)	136 (60·5)	136 (65·6)	136 (87)	136 (86·1)	136 (45)	136 (68)
c_1	*abs.*	407 (1·5)	407 (1·5)	407 (1·3)	449 (1·4)	449 (1·2)	*abs.*	*abs.*
c_2	336 (3·1)	336 (1·8)	336 (4·2)	336 (3·7)	336 (4·8)	336 (2·9)	372 (1·5)	*abs.*
c_3	279 (11)	279 (6·9)	279 (13)	279 (10)	279 (10·4)	279 (6·4)	315 (2·6)	315 (0·6)
c_4	132 (11)	132 (1·4)	132 (11)	132 (10·9)	166 (1·7)	166 (1·9)	168 (2·5)	168 (1·9)
d_1	276 (2·1)	276 (2·4)	276 (3·4)	276 (2·8)	318 (2·4)	318 (1·8)	*abs.*	318 (1·4)
d_2	248 (1·5)	248 (1·1)	248 (2·6)	248 (1·8)	290 (1·6)	290 (1·3)	290 (3)	290 (2·3)
e_1	205 (7·4)	205 (6)	205 (8·3)	205 (5·7)	205 (7·7)	205 (6·2)	205 (4·1)	205 (3)
e_2	177 (9·7)	177 (11·7)	177 (15·1)	177 (10·8)	177 (14·9)	177 (11·9)	177 (5·9)	177 (5·5)
f	120 (100)	120 (100)	120 (100)	120 (100)	120 (100)	120 (100)	120 (55·6)	120 (70·5)
g	107 (12·2)	107 (17·6)	107 (13·15)	107 (16·1)	107 (25)	107 (28·7)	107 (21·2)	107 (23·3)
h	91 (18·6)	91 (23·1)	91 (26·4)	91 (21·9)	91 (35)	91 (34)	91 (25·3)	91 (29·6)
i	86 (27)	86 (20·9)	NR	NR	NR	NR	86·1 (100)	86·1 (100)
							566 (12.8)	566 (12·7)
							M-81	

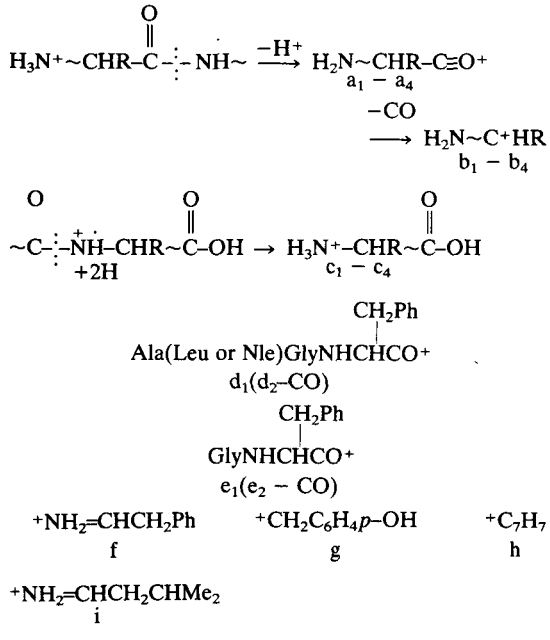
NR = Not recorded.

Table 2. Masses of negative ions in the FAB mass spectra of peptides related to enkephalins (relative intensities in parentheses).

	Peptide							
Ion type	1	2	3	4	5	6	7	8
$[M-H]^-$	568 (100)	568 (100)	568 (100)	568 (100)	610 (100)	610 (100)	646 (37·1)	646 (100)
a_1 b_1	*abs.*	*abs.*	*abs.*	*abs.*	*abs.*	*abs.*	*abs.*	*abs.*
a_2 b_2	*abs.*	292 (3·8), *abs.*	*abs.*	*abs.*	*abs.*	334 (7·5), *abs.*	*abs.*	*abs.*
a_3 b_3	*abs.*	235 (4·9), *abs.*	*abs.*	*abs.*	277 (9·9), *abs.*	277 (6·2), *abs.*	*abs.* 249 (1·9)	*abs.*
a_4 b_4	*abs.*	164 (2·5), *abs.*	*abs.*	164 (5·9), *abs.*	*abs.*	*abs.*	164 (3·4), 136 (0·9)	164 (20), *abs.*
c_1	405 (8·7)	405 (14·1)	405 (9·7)	405 (8·5)	447 (7·3)	447 (5·1)	483 (2·5)	*abs.*
c_2	334 (7·4)	334 (12·7)	334 (13·6)	334 (11)	*abs.*	334 (7·5)	370 (4·8)	*abs.*
c_3	277 (9·6)	277 (16·9)	277 (15·6)	277 (13)	277 (9·9)	277 (6·1)	313 (10)	313 (8)
c_4	130 (16·4)	130 (64·1)	130 (26·2)	130 (25·9)	*abs.*	*abs.*	166 (6·4)	166 (30)
$[M-1]^-$–15	553 (7·6)	553 (14·8)	553 (16·6)	553 (35·8)	595 (22·3)	595 (13·6)	*abs.*	*abs.*
$(SO_3H)^-$							81 (100)*	81 (90)
$(SO_3)^-$							80 (45)	80 (48·7)
$(M-1)^-$–82							564 (10)	564 (65)

* Assignment confirmed by intensity of 81 + 2 line (3·9 for 7, 4·25 for 8).

disregarded. The data are analysed in terms of pseudo-molecular ions, sequence ions (a_1, b_1, c_1 etc.) and peaks diagnostic of specific amino acids (e–i).



Positive ion spectra (Table 1)

Molecular weights of the peptides are readily obtained from the $(M + 1)^+$ lines which are prominent (~15%) in all spectra except those of the sulphonic acids **7** and **8**. Sequence ions formed by removal from the C-terminus (a_1–a_4) are significant in all cases together with their associated ions (b_1–b_4) formed by loss of CO. The first member of the a series is usually of low intensity while the last of the b group ($^+NH_2{=}CHCH_2C_6H_4p{-}OH$) is the line of second highest intensity (~60%) in all spectra. The sequence ions (c_1–c_4) that contain the C-terminus and require uptake of 2H at the basic centre are also detectable in most spectra. Several *m/z* lines diagnostic of specific amino acids are found, notably b_4 (130 Tyr, see above), f(120 Phe, base peak), g(107 Tyr), h(91 Phe) and i(86 Leu and Nle) together with the fragments d_1 and e_1 plus related ions formed by loss of CO(d_2 and e_2). Spectra of the sulphonic acid analogues (**7** and **8**), while showing overall similarity to those of peptides **1**–**6**, differ in displaying prominent lines due to loss of the C-terminal acidic group ($M\text{-}SO_3H$), a feature presumably responsible for the generally low intensities of the C-terminus sequence ions c_1–c_4.

Negative ion spectra (Table 2)

$(M-1)$ ions are the base peaks of peptides **1**–**6** and **8**, and are prominent in **7** (37%). Sequence ions of the a series are absent or of low intensities while those of the c group (transfer of 2H does not occur) are prominent with a few exceptions. Spectra of the sulphonic acid peptides are unique in displaying prominent lines due to SO_3H (base peak for **7**) and SO_3 (45, 48·7%) and for ions formed by loss of 82 (H_2SO_3?) from the pseudo-molecular ion.

The FAB mass spectra of Ac–L–Phe–D–NleS, a dipeptide precursor of **7**, also displayed pseudomolecular ion peaks, $M + 1^+$ (357, 3%), $M-1^-$ (355, base peak), together with corresponding cluster ions $2M + 1^+$ (713, 1·5%) and $2M-1$ (711, 10%). Neither 70 eV electron impact nor chemical ionization (isoBu) spectra of the dipeptide showed molecular features; *m/z* 120(f) was the base peak of the EI and +ive FAB spectra and had a 30% intensity in the CI spectrum. Loss of H_2SO_3 (82) (*m/z* 274, 4%) was evident in the EI, and SO_3H (81) (*m/z* 275, 80%) in the CI spectra.

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